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Fibrin Hydrogels for Articular Cartilage Tissue Engineering

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This Thesis is submitted to the
Faculty of Graduate and Postdoctoral Studies as a
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Hincke, Maxwell T.

Dr. Hincke was supervisor and project leader for all the manuscripts. He was involved in the overall design and strategy for all the work performed.

Abstract

Injury of articular cartilage due to trauma or pathological conditions is a major cause of disability worldwide, especially in North America. Due to inadequacies associated with routinely used repair approaches, the orthopaedic community has an increasing tendency to develop biological strategies such as tissue engineering. Tissue-engineered cartilage constructs represent a highly promising treatment option for knee injury as they mimic the biomechanical environment of the native cartilage and have superior integration capabilities. Current tissue engineering techniques utilize any combination of three critical components: a cellular component, a biocompatible and mechanically stable carrier vehicle/matrix scaffold and a bioactive component. Fibrin has been used extensively as a biopolymer scaffold in a variety of tissue engineering application since it combines some important advantages such as high seeding efficiency and uniform cell distribution. In addition, fibrin has adhesion capabilities. Further, it can be produced from the patient's own blood and used as an autologous scaffold without the potential risk of foreign body reaction or infection. We have evaluated the suitability of fibrin as a scaffolding matrix for tissue engineering of articular cartilage. In the first phase, a chondroprogenitor clonal cell line RCJ3.1C5.18 (C5.18) was used in combination with hydrogels from commercial fibrinogen as a model to guide the development of appropriate scaffolds for tissue engineering. However, rapid degradation of fibrin hydrogels was observed after encapsulation of C5.18 cells. Plasmin and matrix metalloproteinases (MMPs) were found to be responsible for fibrin gel breakdown; therefore, approaches to regulate their activity to control gel stability were developed. Aprotinin, a known serine protease inhibitor, and galardin (GM6001), a potent MMP inhibitor, in combination or separately, prevented the

breakdown of fibrin–C5.18 hydrogels, whereas only the combination of both promoted the accumulation of extracellular matrix. From this study it was concluded that plasmin and MMPs contribute independently to fibrin hydrogel breakdown, while either enzyme can achieve extracellular matrix breakdown. In order to move this research closer to clinical application, we next evaluated fibrin glue produced by the CryoSeal® FS System in combination with human bone marrow derived mesenchymal stem cells (hMSCs), since platelet rich fibrin glue can be prepared which releases a wide variety of growth factors upon activation by thrombin. We additionally tested the incorporation of heparin-binding delivery system (HBDS) into these fibrin matrices to immobilize endogenous growth factors as well as exogenous TGF-β_2. HBDS is composed of a bifunctional peptide, heparin and heparin binding growth factors. Strongly CD90+ and CD105+ hMSCs were encapsulated into fibrin (FG) and platelet-rich fibrin (PR-FG) glues with and without HBDS. Encapsulation in PR-FG resulted in a significant increase in collagen II expression at 2.5 weeks compared to other glues; however, no difference was detected between FG and PR-FG after 5 weeks. FG resulted initially in increased expression of aggrecan gene. Incorporation of HBDS in PR-FG resulted in lower collagen II gene expression at 2.5 weeks. In addition, incorporation of HBDS into either FG or PR-FG did not improve aggrecan gene expression. Both FG and PR-FG glues led to good accumulation of ECM components as indicated by alcian blue staining, while incorporation of HBDS into these glues resulted in slightly lower accumulation of the same ECM components. It was concluded from this study that FG and PR-FG produced by Cryoseal®-FS system are potential scaffolds for tissue engineering of articular cartilage; however, immobilizing growth factors inside fibrin scaffold with the HBDS system does not necessarily result in enhanced expression of the same markers. Our results indicate that stabilization of fibrin is

necessary to allow the accumulation of ECM components secreted from the encapsulated cell source. Furthermore, fibrin glue produced by the CryoSeal® FS system is a potential candidate for utilization in tissue engineering of articular cartilage. Further research should be conducted in order to enhance the mechanical properties of fibrin - based constructs and to gain a better understanding of cell signalling involved in chondrogenesis, in order to optimize the conditions for successful fibrin-based strategies to restore damaged articular cartilage.

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List of abbreviations

3D	Three dimensional
3DF	3D fiber deposition
AAc	Acrylic acid coacryloxysuccinimide
AAV	Adeno-associated virus
ACT (ACI)	Autologous chondrocytes transplantation
AMIC	Autologous matrix-induced chondrogenesis
ANOVA	Analysis of variance
BM-hMSCs	Bone marrow-derived human mesenchymal stem cells
BMP	Bone morphogenetic protein
BSA	Bovine serum albumin
Ca^{2+}	Calcium ion
CAIS	Cartilage autograft implantation system
CCM	Complete culture media
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CM	Compression-molding
DAPI	4',6-diamidino-2- phenylindole stain
DMEM	Dulbecco's modified Eagle medium
DNA	Deoxyribonucleic acid
DRGs	Dorsal root ganglia
E-64	trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane
ECL	Enhanced chemiluminescence

ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
FDA	Food and Drug Administration
FDPs	Fibrin degradation products
FG	Fibrin glue
FGF	Fibroblast growth factor
FMBs	Fibrin microbeads
FV	Foamyvirus
GAGS	Glycosaminoglycans
GAPDH	Glyceraldehyde-3 phosphate dehydrogenase
H&E	Haematoxylin and Eosin
H^+	Hydrogen ion
HA	Hyaluronic acid
HBDS	Heparin-based (binding) delivery system
HBGFs	Heparin-binding growth factors
HSV	Herpes simplex virus
HTO	High tibial osteotomy
IGF	Insulin-like growth factor
kDa	kiloDalton
Ki	Inhibition constant
LCST	Lower critical solution temperature
LIUS	Low intensity ultrasound
MACI	Matrix-induced autologous chondrocyte implantation

MMP	Matrix metalloproteinase
MoMLV	Moloney murine leukemia virus
MPa	megaPascal
MRI	Magnetic resonance imaging
NGF	Nerve growth factor
NSAIDs	Non-steroidal anti-inflammatory drugs
NT-3	Neurotrophin-3
OCT	Optimum cutting temperature
OPF	Oligo(poly(ethylene glycol) fumarate
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PBT	Poly(butylene) terephthalate
PCL	Polycaprolactone
PD-EGF	Platelet-derived epidermal growth factor
PDGF	Platelet-derived growth factor
PDS	Polydioxanone
PEG	Poly(ethylene) glycol
PEGDA	Poly(ethylene glycol) diacrylate
PEGDM	Poly(ethylene glycol) dimethacrylate
PEGT	Poly(ethylene glycol)-terephthalate
PGA	polyglycolic acid
PH4B	Polyhydroxybutyrate
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)

PNIPAAm	Poly(N-isopropylacrylamide)
PR-FG	Platelet-rich fibrin glue
PTHrP	Parathyroid hormone related protein
RGD	arginine-glycine-aspartic acid sequence
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase - polymerase chain reaction
SD	Standard Deviation
SDS	Sodium dodecyl sulfate
SE	Standard Error
SOX-9	SRY (sex determining region Y) - box 9
TBS	Tris-buffered saline
TGF	Transforming growth factor
TIMPs	Tissue inhibitors of MMPs
TKA	Total knee arthroplasty
t-PA	Tissue-type plasminogen activator
TPD	Thrombin processing device
UKA	Unicompartmental knee arthroplasty
u-PA	Urokinase plasminogen activator
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
αMEM	Alpha minimum essential medium
β-TCP	β-tricalciumphosphate

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I. INTRODUCTION

A wide variety of conditions including trauma and pathological insults can lead to injury of knee articular cartilage (Ahmed et al., 2007). As the number of individuals suffering from knee-related disabilities increases mainly due to an increase in the prevalence of arthritis, obesity and old age in North America, the economic burden concomitantly increases (Mehrotra et al., 2005). Although a wide range of strategies are used currently for the treatment of cartilage-associated disabilities including palliative and restorative techniques (Detterline et al., 2005, and Siparsky et al., 2007), they fail to restore fully the structure and functions of native cartilage.

The goal of tissue engineering, a rapidly growing and promising field in regenerative medicine, is to restore the function of damaged tissues and organs. Tissue engineering constructs have superior integration capabilities and are predicted to have a great impact on the future of orthopaedics (Kerker et al., 2008). Tissue engineering requires scaffolding materials in which a suitable cell source can proliferate and differentiate to form a specific tissue type under the influence of bioactive factors (Ahmed et al., 2008).

Fibrin has been investigated extensively as a bio-polymeric scaffold during development of tissue engineering-based strategies to restore structure and function (Ahmed et al., 2008). It has numerous advantageous over other materials: high seeding efficiency, uniform cell distribution, great adhesion capabilities, high cost effectiveness, easy manipulability, and the ability to be prepared in autologous form. Therefore, fibrin has been used extensively for many tissue engineering applications to restore the function of many tissues, including knee cartilage (Ahmed et al., 2008). However, fibrin has two major disadvantages: rapid degradation of the scaffold before the proper formation of

cartilaginous constructs, and low mechanical stiffness (Ahmed et al., 2007, 2008). One option is to enhance the final mechanical properties of fibrin hydrogel; for example, by using a natural cross-linker (genipin) that does not affect cell viability (Dare et al., 2009).

II. HYPOTHESES

We hypothesize that proteases secreted from the chondrogenic cells (C5.18) after encapsulation into fibrin gels are mainly responsible for the observed rapid degradation of fibrin-cell constructs *in vitro*. We also hypothesize that bone marrow - derived human mesenchymal stem cells (BM-hMSCs), that can be obtained autologously, in combination with different autologous fibrin glues produced by Cryoseal®-FS system, can promote the formation of cartilaginous tissue for potential use in the clinic to replace knee cartilage after injury.

III. THESIS OBJECTIVES

The first objective of this thesis study was to characterize and to identify the enzymes responsible for fibrin hydrogel breakdown after encapsulation of chondrogenic cells in order to control gel stability. RCJ 3.1C5.18 (C5.18) were used as a model in this study. The second objective was to evaluate BM-MSCs as alternative cell source to chondrocytes, after encapsulation into the different fibrin glues produced by Cryoseal®-FS system for the development of articular cartilage substitute, since chondrocyte utilization in tissue engineering- based strategies has some limitation after expansion for long time in monolayer culture (Temenoff et al., 2000). Both bone marrow-derived mesenchymal/stromal cells (BM-hMSCs) and fibrin gel precursors can be obtained autologously; therefore, we hypothesized that hMSCs encapsulated into autologous fibrin glue produced by the Cryoseal®-FS system (in contrast to gels fabricated from purified

fibrinogen) can be used as an alternative cell source to chondrocytes. Although commercially produced fibrin glues are available in standardized quality, autologous fibrin glue has two theoretical advantages: reduced possibility of viral transmission and prion infection, already very low with respect to the commercial pooled allogeneic product, and lowered cost. Such advances in clinical application will minimize the surgeries required to restore the function of cartilage.

This thesis is composed of five chapters that independently contribute significantly to the field of tissue engineering for orthopaedic medicine. Chapter one is a review article which summarizes the most widely used strategies and approaches, over past decades, for current procedures that repair and restore the function of articular cartilage. The review also summarizes most of the scaffolding matrices, cell sources, bioactive factors, gene therapy and physical stimulants that are utilized during the development of tissue engineering-based strategies.

Chapter two is a second review article that summarizes the advantages, drawbacks, different forms, different manipulations, strategies and tissue engineering applications of fibrin scaffolds.

Chapter three presents the sole study that has yet been conducted to characterize and identify the enzymes secreted from a chondrogenic cell source after encapsulation into the fibrin hydrogel during development of tissue engineering constructs suitable for articular cartilage repair. The study demonstrated how to control enzyme activity so that both gel stability and extracellular matrix accumulation can be controlled.

Chapter four evaluates the utilization of hMSCs after encapsulation into fibrin glue produced by the CryoSeal® FS system (Stabilized by the addition of tranexamic acid, a known plasmin inhibitor), as an alternative to chondrocytes, for development of tissue engineering-based cartilage replacements.

Finally, in chapter five, the major contributions of this thesis study as well as approaches for future consideration are discussed.

IV. CHAPTER 1. STRATEGIES FOR ARTICULAR CARTILAGE LESION REPAIR AND FUNCTIONAL RESTORATION

Articular cartilage has very limited capacity for self-repair and regeneration due to its avascular nature (Ahmed et al., 2010). Therefore, injury of articular cartilage represents a major problem in orthopaedic medicine; with an ever-aging population there is a continuously increasing economic burden. A wide range of techniques are established to reduce pain and restore function, including conservative treatment, nonrestorative nonreparative strategies, marrow stimulation techniques, high tibial osteotomy, knee replacement approaches, osteochondral grafting and autologous chondrocyte transplantation. However, most of them fail to restore completely the function and structure of native cartilage and are usually associated with significant limitations (Ahmed et al., 2010). Tissue engineering-based constructs show improved capability to integrate with the adjacent native cartilage; moreover, this approach reduces the number of required surgeries to a single one, which is a minimally invasive procedure.

In this review, we discuss the most widely used current strategies for treatment of knee cartilage injury and their associated limitations, with special emphasis on tissue engineering approaches since they will have a tremendous impact on the future of orthopaedic medicine. The review was entirely written by myself.

Manuscript 1

Strategies for Articular Cartilage Lesion Repair and Functional Restoration

Tamer A. E. Ahmed, M.Sc., and Maxwell T. Hincke, Ph.D.

Submitted to Tissue Engineering Part B (Reviews), September 2, 2009, revised version submitted October 28, 2009. Epub ahead of print, 2010.

1. ABSTRACT

Injury of articular cartilage due to trauma or pathological conditions is the major cause of disability worldwide, especially in North America. The increasing number of patients suffering from joint-related conditions leads to a concomitant increase in the economic burden. In this review article, we focus on strategies to repair and replace knee joint cartilage, since knee-associated disabilities are more prevalent than any other joint. Due to inadequacies associated with widely used approaches, the orthopaedic community has an increasing tendency to develop biological strategies, which include transplantation of autologous (*i.e.* mosaicplasty) or allogeneic osteochondral grafts, autologous chondrocytes (ACT), or tissue-engineered cartilage substitutes. Tissue-engineered cartilage constructs represent a highly promising treatment option for knee injury as they mimic the biomechanical environment of the native cartilage and have superior integration capabilities. Currently, a wide range of tissue engineering – based strategies are established and investigated clinically as an alternative to the routinely used techniques (i.e. knee replacement and ACT). Tissue engineering-based strategies include implantation of autologous chondrocytes in combination with collagen I, collagen I/III (matrix – induced autologous chondrocyte implantation, MACI), HYAFF® 11 (Hyalograft® C), and fibrin

glue (Tissucol®) or implantation of minced cartilage in combination with copolymers of polyglycolic acid along with polycaprolactone PGA/PCL (cartilage autograft implantation system, CAIS), and fibrin glue (DeNovo NT graft). Tissue-engineered cartilage replacements show better clinical outcomes in the short-term, and with advances that have been made in orthopaedics they can be introduced arthroscopically in a minimally invasive fashion. Thus, the future is bright for this innovative approach to restore function.

Running title: Strategies for Cartilage Repair and Replacement.

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2. INTRODUCTION

Articular cartilage is a highly resilient connective tissue that covers the surfaces at the ends of long bones.[1-3] The knee consists of two types of articular surfaces: tibiofemoral and patellofemoral. The medial and lateral compartments of the tibiofemoral joint along with the patellofemoral joint comprise the three knee compartments (FIG.1).[4,5] In a typical synovial joint, articular cartilage serves as a lubricating surface to promote frictionless movements.[1-3] This hyaline type cartilage composed mainly of chondrocytes surrounded by extracellular matrix (ECM).[1-3,6,7] ECM is synthesized and secreted by the chondrocytes. It is composed mainly of collagen fibrils, proteoglycans, and water.[2,6,7] The biomechanical properties of articular cartilage are largely dependent on the composition and the integrity of its ECM.[1,6] Morphologically, articular cartilage can be subdivided into different zones named superficial, middle, deep and calcified.[1] The different knee compartments, knee structure, and the organization of articular cartilage are shown in FIG.1.

Articular cartilage injuries are a major dilemma in the field of orthopaedic medicine.[7,8] Due to its avascular nature, articular cartilage has very low innate capability for self repair and regeneration. Consequently, injury to cartilage usually heals through scar tissue formation composed mainly of fibrocartilage.[2,3,7-11] Fibrocartilage has inferior mechanical and biological properties compared to hyaline cartilage and gradually degenerates with time resulting in permanent loss of structure and function leading to severe pain. These factors in concert lead to disability.[1,7,10,11] A wide variety of pathological conditions such as osteoarthritis and rheumatoid arthritis lead to articular cartilage injury. In addition, many traumatic conditions either direct such as intra-articular fracture or indirect such as cartilage insult following a ligament injury have been shown to cause damage of the articular cartilage.[1,2,6,7,11]

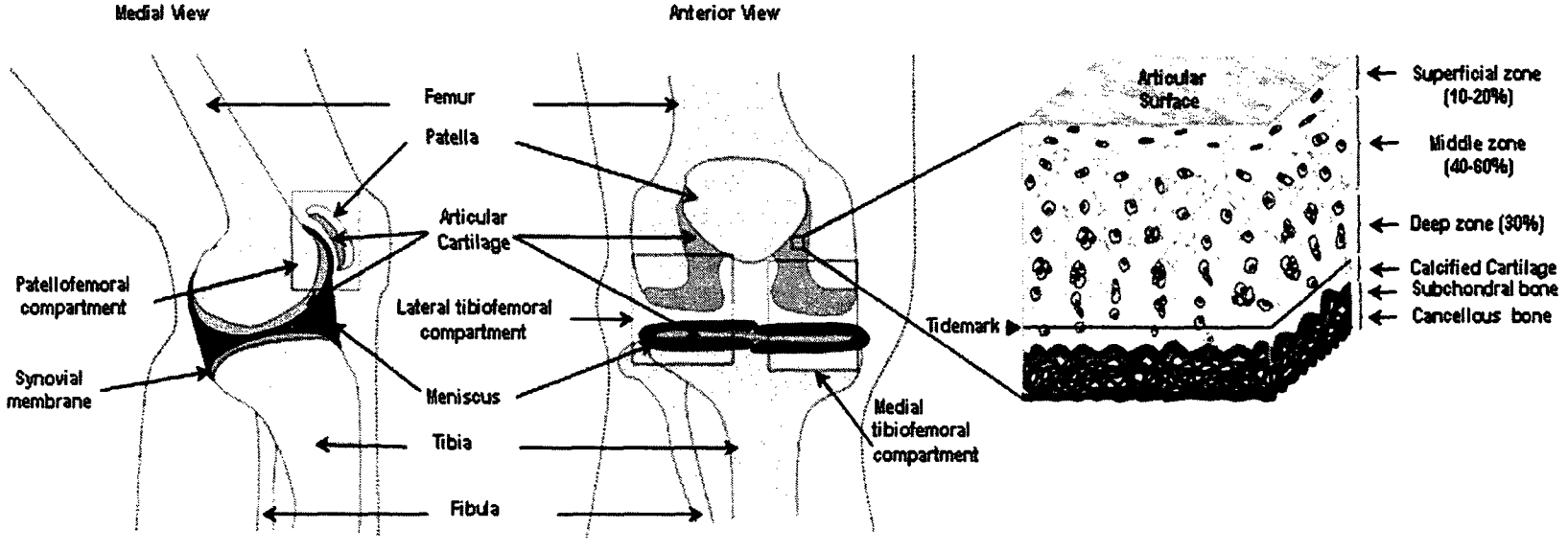

FIG.1. The structural and compartmental organization of the knee joint. The zonal organization of the articular cartilage is illustrated as follows: superficial zone composed of flattened chondrocytes and collagen fibers that are arranged parallel to the articular surface, middle zone composed of collagen fibers arranged obliquely to the articular surface and spherical chondrocytes, deep zone composed of spherical chondrocytes and collagen fibers that are arranged in a columnar pattern perpendicular to the articular surface, and finally calcified cartilage zone which facilitates the integration of cartilage with subchondral bone composed of ellipsoid chondrocytes along with perpendicular collagen fibers.

Articular cartilage injury due to arthritis is the major causes of disability in middle-aged and elderly people in Canada and USA.[7,12-14] It has been shown that the total number of people suffering from arthritis will increase to 6.5 million in Canada by 2031 (approximately 16% of population). In addition, arthritis-associated disability in the population aged 15+ is estimated to increase up to 1.13 million by 2031.[12] In 2005 arthritis affected approximately 70 million USA adults. It is sobering that in 2000, the estimated economic burden of arthritis in USA was $60 billion and it is expected to increase to $100 billion by 2020.[14]

Articular cartilage defects fall into two categories, partial and full thickness defects (FIG.2). Partial thickness defects are limited to the cartilage; therefore they have no access to bone marrow-derived stem cells and subsequently lack the ability to repair spontaneously.[3,7,11] Usually, this type of defect gradually deteriorates with time and can lead to a variety of problems, for instance, swelling and mechanical symptoms mediated by irritation of the synovial lining by enlarged cartilaginous flaps in addition to knee locking mediated by detachment of this excess cartilage.[15] On the contrary, full thickness cartilage defects extend to the subchondral bone; in these cases bone marrow - derived stem cells lead to spontaneous healing through the formation of fibrocartilaginous tissue.[3,7,11]

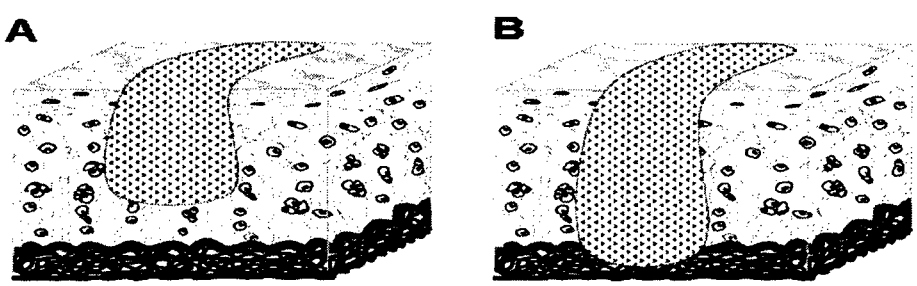

FIG.2. Different types of cartilage lesions; **A**: partial thickness cartilage lesion, **B**: full thickness cartilage lesion.

A wide variety of strategies have been widely developed to restore the structure and functions of injured cartilage. These strategies range from reducing the pain and swelling, to repair of cartilage through the formation of fibrocartilage (*i.e.* Reparative procedures), and to a variety of restorative procedures including tissue engineering-based strategies.[2,10,15] Herein we will briefly summarize some of the most commonly used procedures to address damaged cartilage of the knee:

3. PALLIATIVE STRATEGIES

3.1. Conservative treatment

Conservative procedures are usually the primary approach to treat symptoms of knee lesions and these include physiotherapy, weight loss, systemic pain relief medications, intra-articular injections, and orthotic interventions.[16] It is important to point out that none of the conservative treatment strategies lead to cartilage healing.[15]

Physiotherapy will maintain range of motion and strengthen the affected limb which might improve the symptoms.[15,16] Weight loss helps decrease the forces exerted on the knee.[16] A wide variety of medications have been used to relieve pain including analgesics such as acetaminophen, tramadol, and opioids. Combination of analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to relieve pain in both the short and long term. However, they have adverse systemic effects. Intra-articular injection includes injection of analgesics along with steroids which will relieve pain.[15,16] In addition, viscosupplements such as hyaluronate or nutraceuticals such as glucosamine and chondroitin sulfate can be injected intra-articularly.[15] Injection of hyaluronate promotes enhanced lubrication and decreases friction on joint surfaces, and has been shown to slightly improve the clinical outcome compared to placebo.[16]

3.2. Nonreparative nonrestorative procedures

Nonreparative nonrestorative techniques can be conducted independently to relieve pain or as a complementary procedure to other arthroplastic techniques to promote enhanced integration of the newly formed repair tissue with the surrounding native cartilage.[9,17-20] Nonreparative nonrestorative procedures can also be done arthroscopically in a minimally invasive procedure to minimize the pain and to improve the mobility, but not to restore completely the structure and functions of diseased cartilage.[19,21,22] One major drawback of nonreparative nonrestorative techniques is that, the long term beneficial outcomes are dramatically reduced due to loss of chondrocytes at the border between healthy and damaged cartilage after mechanical removal of the injured cartilage.[23]

3.2.1. Debridement

Debridement is a commonly used orthopaedic procedure that serves to remove all the debris resulted from the arthritis-mediated joint damage. The debridement procedure was established by Magnuson in 1941, as cited by Day.[24] It involves removal of inflammatory cells and other fragments, for instance, unstable chondral flaps, osteophytes, superfluous synovia, degenerated menisci, and torn ligaments.[17,18,20] Debridement is usually carried out by mechanical instruments; however, other methods such as electrocautery, lasers or radiofrequency can also be utilized.[18] The debridement technique is intended to eliminate the biochemical and mechanical factors causing symptoms of arthritis with subsequent relief of pain and improvement of functions. However, due to the intrinsic degenerative nature of the disease, arthritis-associated symptoms are expected to return shortly after debridement.[17,20,25,26] In addition, the technique cannot be used routinely for the treatment of all types of arthritis.[17,19]

3.2.2. Chondral shaving

The procedure of excising damaged cartilage to relieve pain was initially developed in 1908 by Büdinger, as cited by Fithian, to treat traumatic cartilage lesions of the patella.[27] Chondral shaving utilizes a motorized shaver to excise the damaged area of cartilage, converting the fibrillated surface into a smooth surface.[20,28] However, it has been shown macroscopically that mechanical shaving causes a rough surface with grooves. In contrast, previous studies revealed that hydro jet and thermal energy produces smoother chondral surface than that from mechanical shaving.[29] However, it has also been shown that chondral shaving has adverse biological effects on the cells and matrix of articular cartilage.[26] Deleterious effects of shaving and debridement have been attributed to the heat developed from the frictional resistance of the patients' cartilage upon application of the excising arthroscopic tools.[30] Exposure of chondrocytes to uncontrolled heat can cause cell death, as chondrocytes die at temperatures of 45 °C to 55 °C.[31,32] Finally, chondral shaving can cause chondral tears that do not heal leading to a progressive degeneration of the remaining cartilage.[28,29]

3.2.3. Knee Joint lavage

Knee joint lavage is usually performed when conservative treatment of knee osteoarthritis is inadequate and knee replacement is not yet indicated.[33] The effect of simply rinsing the joint was reported in North America by R. Jackson (the pioneer of arthroscopy) in 1988, as cited by Nehrer.[34] Joint lavage is usually carried out in combination with debridement.[35] In some instances, lavage is considered as a component of the debridement procedure.[21] Joint lavage includes rinsing the knee with physiological fluid such as Ringer's solution to wash out degradation products of cartilage, meniscus, and synovium along with inflammatory cells and degradative enzymes (i.e. agents that cause

mechanical irritation and pain) followed by a suction step.[21,33,36,37] It has been shown that intra-articular lavage of the arthritic knee joint gave satisfactory outcomes.[36] However, the irrigation fluid can potentially harm cartilage.[33] Moreover, arthroscopic lavage and debridement do not alter disease progression and are no longer recommended for routine treatment of the osteoarthritic knee.[38]

3.3. Reparative procedures

Reparative strategies aim at initiating bleeding from the subchondral bone which will permit the migration of bone marrow stem cells to the site of injury along with blood clot formation leading to repair tissue formation composed mainly of fibrocartilage (FIG.3 A-F).[9,34,39] This is why reparative procedures are commonly referred to as marrow stimulation techniques. Fibrocartilage has inferior mechanical properties to that of hyaline cartilage. However, it covers the exposed underlying bone, which subsequently reduces pain and swelling.[15]

3.3.1. Arthroscopic abrasion arthroplasty

Arthroscopic abrasion arthroplasty was established three decades ago by Johnson as an alternative to total knee replacement for older patients with degenerative arthritis.[40] It is a one-stage arthroscopic procedure and is considered as a modification of the house cleaning procedure (*i.e.* Magnusson) that involves multiple tissue debridement carried out with an automated burr.[34,40] It involves burring of the exposed sclerotic bone to access the vasculature of the subchondral plate, which promotes the formation of blood clot in the defect and subsequent healing via fibrocartilage formation (FIG.3 A,B,F).[34,40,41] Arthroscopic abrasion arthroplasty has two major advantages, in that it can be performed in a minimally invasive procedure; in addition, it is less traumatic compared to the other arthroscopic techniques. However, because of the multiple tissue involvement in the

abrasion procedure, its clinical benefits are unclear.[40] It has been shown that approximately half of middle-aged and older patients improved after abrasion arthroplasty procedure in the short term follow-up.[34] It has been also shown that the fibrocartilaginous repair tissue maintains integrity for up to 6 years when the joint is properly protected. However, in other studies breakdown of the repair tissue has been reported to start within 1 year.[41]

3.3.2. Microfracture

The microfracture procedure was developed by Steadman in 1997, as cited by Kocheta, to enhance chondral resurfacing.[42] It is the most widely used one-stage arthroscopic procedure that involves a preparative debridement step to remove the damaged cartilage to expose the subchondral bone, followed by a V- shaped piercing of the exposed bone using a bent awl.[34,39,43] The formed fractures should be 2 mm in diameter and 3 mm apart. The subchondral piercing results in bleeding from bone marrow followed by adherence of the formed blood clot to the exposed bone surface and subsequent fibrocartilage-based repair tissue formation (FIG.3 A,C,F). In general, the clinical outcomes of microfracture are largely dependent on patient age and the size of the defect. In younger active patients, microfracture is the recommended procedure and has the best long term result for cartilage defects that are smaller than 2.5 cm^2.[34,39] It has been reported that the microfracture procedure resulted in pain relief and restored knee function in 75% of patients with deep subchondral defects in long term follow-up.[34] In contrast, it has been shown in another study that the clinical outcome begins to decline at approximately 2 years postoperatively, especially in older patients.[39,43] The major drawbacks of microfracture include the poor biomechanical nature of the repair tissue, partial defect filling, and abnormal bone growth in the lesion area.[34,39]

3.3.3. Subchondral drilling

Subchondral drilling is also a one-stage arthroscopic procedure[34] that involves drilling into the subchondral bone to establish bleeding channels.[34,44] The technique was originally established by Pridie in 1959, as cited by Mitchell, according to the assumption that generating numerous holes in the subchondral plate would promote the formation of hyaline cartilage that will spread out of the holes to resurface the exposed bone (FIG.3 A,D,F).[45] It was reported that injured knee treated by this procedure resulted in satisfactory long term outcomes in 85% of the patients, especially when associated with high tibial osteotomy.[34] However, it has been demonstrated that the typical morphology of newly formed hyaline cartilage was lost within 1 year.[45] In addition, the drilling process can lead to subchondral bone damage due to the generated heat or the development of subchondral hematoma.[34]

3.3.4. Spongialization

Spongialization was introduced by Ficat in 1979, as cited by Bhosale and Galois as a modification of debridement and drilling. It involves the removal of the entire injured cartilage with the underneath subchondral bone which subsequently exposes the spongy bone, also known as spongiosia (*i.e.* cancellous bony bed) (FIG.3 A,E,F).[46,47] The removal of the subchondral plate in spongialization has some advantages over subchondral drilling, which include primarily eliminating the origin of the pain since the subchondral plate is highly innervated, and secondarily promoting better healing in the absence of the weight-bearing load exerted by the opposite cartilage surface.[48] Experimental data regarding the spongialization technique are not available, suggesting that the technique has been applied directly to human patients. Spongialization led to good to excellent results in 70-80% of patients in terms of joint functionality and pain relief. Although no serious drawbacks was

reported during the procedure, it has not gained great popularity.[49] However, it has been shown that using powered instruments may cause thermal necrosis of the cells that one is hoping to stimulate.[42] Spongialization is still used by some surgeons for patellar resurfacing during total knee replacement arthroplasty.[50]

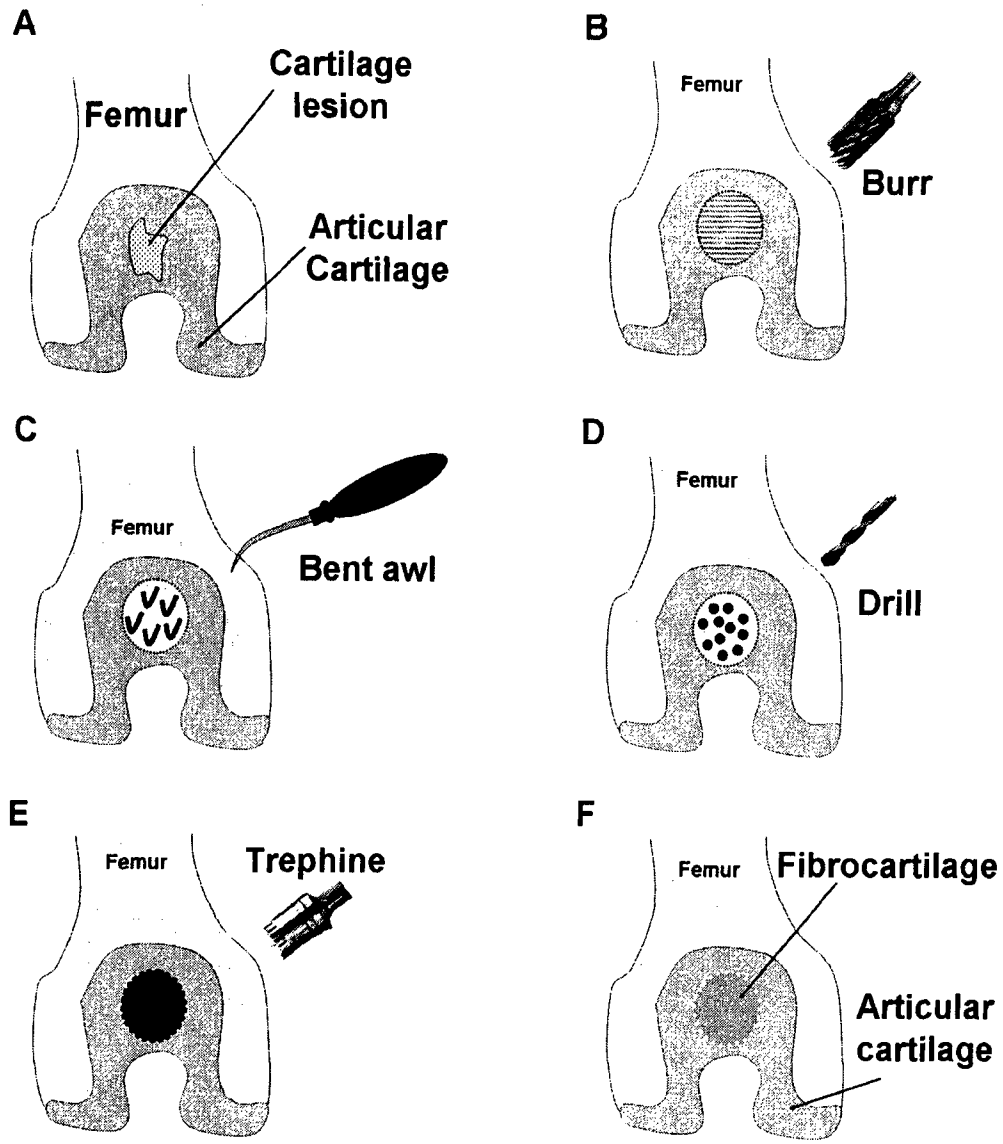

FIG.3. Different marrow stimulation techniques used for the treatment of cartilage lesions and the associated tool of operation. **A**: typical femoral cartilage lesion, **B**: abrasion arthroplasty carried out by automated burr, **C**: microfracture carried out by bent awl, **D**: subchondral drilling carried out by automated driller, **E**: spongialization carried out by trephine, and **F**: repair via fibrocartilage formation after marrow stimulation.

4. RESTORATIVE STRATEGIES

4.1. High tibial osteotomy (HTO)

Osteochondral lesions can arise in one of the three joint compartments medial, lateral, or patellofemoral.[5] Since, reparative procedures such as microfracture and some restorative procedures such as osteochondral grafting do not have satisfactory outcomes in the treatment of symptomatic cartilage degeneration in the medial compartment of the varus knee[51], HTO is considered for the treatment of this condition in carefully selected patients.[5,51-53] The technique of HTO was first introduced in 1958 by Jackson, as cited by Wright.[53] The aim of HTO is to unload the damaged medial compartment and transfer weight bearing to the intact lateral compartment which will subsequently relieve pain and prevent progression of osteochondral damage.[5,51] HTO is the procedure of choice for younger and more active varus knee patients rather than joint replacement arthroplasty.[5] HTO showed satisfactory clinical outcomes in approximately 80% of patients at 5 years and 60% at 10 years.[52] However, HTO has two major disadvantages: first, it cannot be utilized in bicompartmental or tricompartmental lesions[53], and second, the clinical outcomes deteriorate with time.[52,53] These drawbacks have made knee replacement the technique of choice, since it has more reliable long-term outcomes.[53]

4.2. Knee replacement

Knee arthroplasty involves removal of sufficient bone from the femoral and tibial surfaces followed by resurfacing with prosthetic implants. Knee arthroplasty can be subdivided into unicompartmental knee arthroplasty (UKA) and total knee arthroplasty (TKA).[54,55]

4.2.1. Unicompartmental knee arthroplasty (UKA)

UKA as a procedure lies between osteotomy and total knee arthroplasty (TKA) in the treatment of the knee lesions.[56] The concept of unicompartmental replacement, was originally reported in 1957 by McKeever, as cited by Bert.[57] If the knee lesions are located in either the medial or lateral compartment, UKA can be utilized in which the femoral condyle and the tibial surface of the affected compartment are replaced.[16,54] However, it has been suggested that HTO is recommended for treatment of lesions in the medial compartment whereas UKA is recommended for lesions in the lateral compartment.[56] UKA sometimes referred as pre - TKA, since failed UKA can be revised by TKA.[58] In addition, UKA can be done after HTO procedures.[59] Prostheses for UKA are composed of metallic femoral and tibial components. The tibial component is attached to a polyethylene disk. The prosthetic components can be cemented in place or porous-coated (porous-ingrowth) (FIG.4 A).[54] Since only one compartment is replaced and the other two are preserved, patient satisfactions is higher in UKA compared to TKA.[5] Similar to HTO, UKA should be preformed in carefully selected patients[16], typically younger patients.[5,58] UKA has many advantages over TKA including less invasiveness, better patient mobility, lower morbidity, lower cost, and shorter recovery time.[16,58,60] In addition, UKA delays the need for invasive TKA at least 10 years.[58] Furthermore, revision of UKA to TKA has similar success rate to that of primary TKA.[5] Despite the fact that survival rate of UKA at 10 years follow-up was 98%, cartilage progressively deteriorates in the other compartments due to osteoarthritis as revealed by radiographic analysis.[60]

4.2.2. Total knee arthroplasty (TKA)

TKA has been shown to be an effective and durable procedure to relieve pain and to improve mobility in people suffering from end-stage knee lesions.[5,16,61,62] TKA is

indicated when conservative treatments have failed.[16,62] When both the medial and lateral compartments have deteriorated, TKA should be performed which may include resurfacing of the patella. Prostheses for TKA are unconstrained, partially constrained, or fully constrained depending on the stability that they promote in the knee. The total condylar knee prosthesis was developed in 1974 at the Hospital for Special Surgery (HSS), New York by Insall, Ranawat, and Scott, as cited by Pavone.[63] The TKA prosthesis is composed of two components, a metallic femoral component also known as the condylar component and a metallic tibial component.[54] The condylar component is either cemented or porous- ingrowth, whereas the tibial component is usually cemented. The tibial component is attached to a disk-shaped polyethylene piece that articulates with the condylar component (FIG.4 B).[16,54,64] Some TKA procedures involve patellar resurfacing. The patellar prosthesis is composed of a cemented polyethylene component, usually without a metallic support. During the TKA procedure, the anterior cruciate ligament (ACL) is always removed while the posterior cruciate ligament (PCL) is either kept, substituted, or removed.[54] It has been reported that TKA is an excellent procedure in elderly patients (>70 years). However, TKA failure rate is higher in younger patients (<50 years) compared to the elderly patient at 10 years follow-up.[5] Therefore, HTO and UKA are recommended for younger patients.[5,58] It has been shown in many studies that patient satisfaction ranges between 90% and 95% after TKA.[65] In addition, the implant survival at 10-15 years is over 90% .[54,65] Other reports have shown 98% implant survival at 20 years.[54] Although, the rate of TKA-associated complications is low, the complications can be fatal.[16] Complications can be minimized and the outcomes improved when the TKA is performed in specialty hospitals rather than general hospitals.[5] Complications of TKA are either confined to the prosthesis itself such as stiffness[66], instability, aseptic loosening, infection,

prosthesis failure and mal-alignment[54], or general complications that usually take place within three months after TKA procedures, for instance deep vein thrombosis, pneumonia, myocardial infarction, pulmonary embolism, wound infections, and death.[16,67]

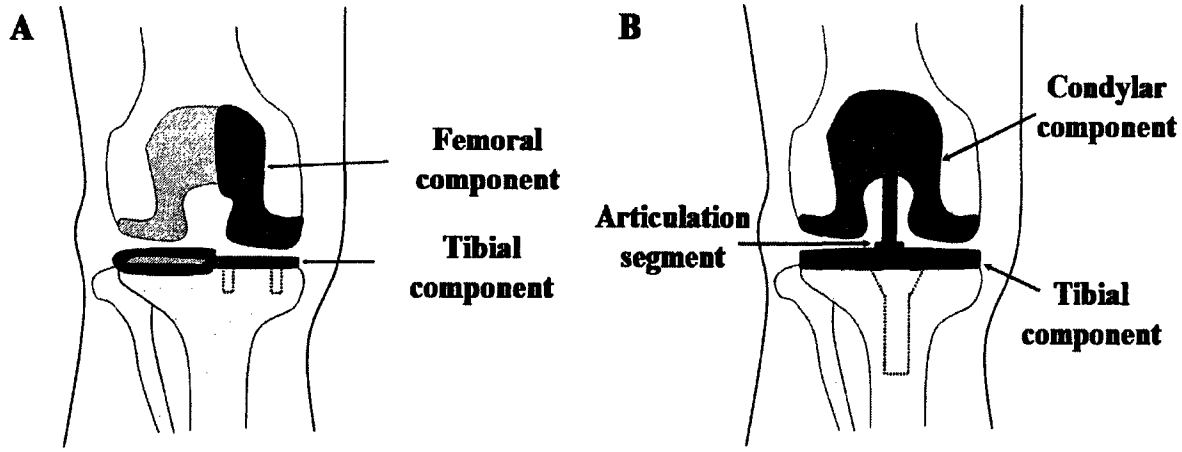

FIG.4. Different prostheses used in knee replacement surgeries either: **A.** unicompartmental knee arthroplasty (UKA), or **B.** total knee arthroplasty (TKA).

In conclusion, microfracture is the recommended procedure for localized lesions affecting any of the knee compartments, while HTO and UKA are recommended for lesions affecting varus knee medial compartment and lateral compartment, respectively, in younger active patient. Although microfracture, HTO, and UKA can be carried out in a minimally invasive fashion with subsequent function restoration, they fail to restore native structure of articular cartilage. For elderly patients, TKA is the currently recommended technique to restore functions for the entire knee after articular cartilage damage. In addition, TKA can be performed as revision surgery for the aforementioned arthroplastic procedures. However, it is more invasive. Therefore, current strategies should be developed or replaced by strategies that restore both the structural and functional elements of the articular

cartilage, which can be achieved through transplantation of viable substitute, including tissue engineering-based ones, since viable substitutes represent the future of the orthopaedic field.

4.3. Transplantation

Currently, the orthopaedic community has greater tendency to utilize biological approaches to treat cartilage lesions.[68] Biological approaches include autologous chondrocyte transplantation (ACT) and osteochondral grafting, which have been widely used in clinical practice to restore cartilage function and structure after injury.[69,70] Restorative strategies have been developed to promote healing of injured cartilage through the formation of tissue that resembles native cartilage.[15]

4.3.1. Osteochondral transplantation (Osteochondral grafting)

Osteochondral transplantation can be subdivided into autologous osteochondral transplantation (mosaicplasty) and allograft osteochondral transplantation (FIG.5 A-F).[70]

4.3.1.1. Mosaicplasty

Mosaicplasty is a single-stage arthroscopic procedure consisting of three steps.[71] The technique was first described by Matsusue in 1993, as cited by Bhosale.[46] The first step is the preparation of the recipient site which involves removal of the residual cartilage fragments from the chondral defect down to the subchondral bone[69,70,72] followed by creation of 15 mm deep holes spaced 1 mm apart. The recipient hole diameter should be smaller than the donor graft diameter by 0.1 mm to promote press-fit transplantation of the osteochondral cylinder.[72] The hole should be also perpendicular to the cartilage surface.[71] The second step is harvesting the cylindrical osteochondral grafts that are 10-15 mm long and of varying size from non-weight-bearing articular surfaces such as margin of the medial and lateral femoral condyle using tubular chisels.[69-72] Again, the harvested grafts

should be taken perpendicular to the cartilage surface.[71] The last step is the insertion of osteochondral plugs into the recipient site. Since, the donor cartilage is thinner than the excised cartilage; the grafts should be inserted congruently and should be supported at the base of the bone tunnels. It has been shown that different graft sizes such as 5.5 mm, 6.5 mm, and 8.5 mm can be utilized in mosaicplasty.[71,72] In fact, excellent congruent surface and filling rate can be achieved by combining different graft sizes.[69] A range of 3-6 grafts can be used for mosaicplasty.[72] During healing, the space between the grafts in the recipient site will be filled with a fibrocartilaginous grout produced by the subchondral bone, whereas the donor holes will be filled with cancellous bone covered by fibrocartilage (FIG.5 A-D).[69,70] Mosaicplasty is indicated for small and medium size focal chondral and osteochondral defects of the femoral condyle.[43,70] It has been shown that mosaicplasty promotes pain relief and joint function improvement.[43] In addition, around 77 % of the femoral condyle transplants showed good to excellent results in the long-term follow-up.[71] However, mosaicplasty has some drawbacks; first, although the donor site is non weight-bearing surface, disability can occur as a result of graft harvesting (*i.e.* donor site morbidity), second, the optimum orientation of the graft into the recipient site necessary to restore the contour of the femoral condyle is difficult to achieve[70], third, marginal chondrocyte death can lead to degeneration of graft and subsequently graft failure[43]; and finally, there is a risk of cartilage and bone collapse.[3]

4.3.1.2. Allograft osteochondral transplantation

When the osteochondral lesions are very large, mosaicplasty is no longer indicated.[43,70] Allograft osteochondral transplantation might be indicated for large osteochondral defects that affect almost the entire femoral condyle[70,73],when all the previously performed less invasive techniques have failed to relieve pain and restore

functions.[70] The first allograft osteochondral transplantation was performed in 1907 by Lexer, as cited by Kocheta.[42] The technique involves replacing the injured cartilage along with its underlying subchondral bone by a matching, fresh or fresh frozen cartilage segment obtained from an organ donor.[70,73] A cylindrical hole is created in the recipient site by a power reamer, then a cylindrical graft is harvested from the donor tissue using a core reamer after being properly demarcated. The bony portion of the allograft will be trimmed depending on the degree of bone involvement in the recipient site. The allograft height ranges between 8-15 mm and it should be 0.5 mm wider than the recipient hole to facilitate press-fit fixation (FIG.5 A,E,F).[73] Alternatively, the graft can be fixed with screws.[70] It has been shown that transplantation of fresh femoral osteochondral allografts resulted in 85% and 74% graft survival rate at 10 and 15 years respectively.[74,75] Limitations of this technique include graft availability, possible disease transmission and technical difficulty.[3] In addition, it has been reported that chondrocyte viability and biomechanical integrity of the donor graft deteriorate after two weeks of storage. Therefore, fresh osteochondral allograft is recommended for the procedure.[73]Finally, revision by TKA after failure of allograft osteochondral transplantation is associated with many technical difficulties.[75]

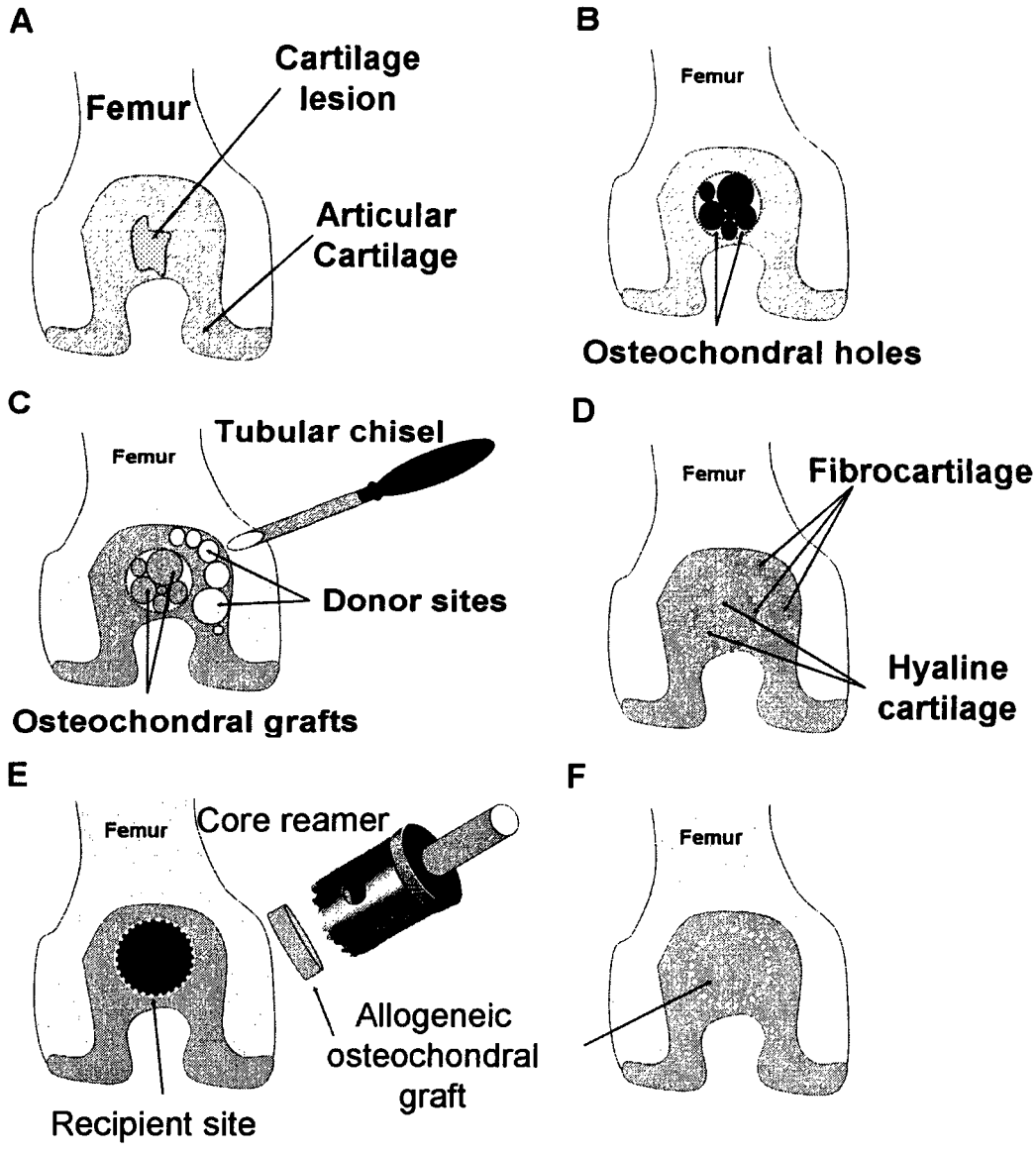

FIG.5. Different osteochondral grafting techniques used for the treatment of cartilage lesions and the associated tool of operation. **A**: typical femoral cartilage lesions, **B-D**: autologous osteochondral grafting (mosaicplasty) and the resulting repair tissue, and **E, F**: allogeneic osteochondral grafting and the resulting repair tissue.

4.3.2. Autologous chondrocyte transplantation (ACT)

ACT is a two-staged cell-based procedure used to relieve pain and to restore function of knees affected by either chondral or osteochondral defects.[3,76,77] The first human ACT was made by a Swedish team (Brittberg, Lindahl and Peterson) in 1994, as

cited by Bhosale.[46] The first stage of ACT involves arthroscopic harvesting of chondrocytes from the femoral intercondylar notch or trochlear border followed by cell culturing for approximately 6 weeks, while the second stage involves transplantation of the cultured cells by open surgery.[9,43,70,77] The lesion site is debrided and covered by a periosteal flap harvested from the tibia or femur. This periosteal cover is then sealed at the edge of the defect using fibrin glue and the cultured cells are injected into the defect site underneath this flap (FIG.6 A-D).[9,70,77] Autologous chondrocyte transplantation is indicated for inconsistent medium to large size defects (*i.e.* 2-10 cm^2)[70,78] with clinical results that last for up to 10 years.[76] It has been shown that ACT promoted the formation of hyaline-like repair tissue and the clinical outcome including pain relief along with function improvement were satisfactory in 80% - 90% of the patients.[70,79] In contrast, it has been reported that the repair tissue still contains a considerable proportion of fibrocartilage.[80] In retrospective study, good to excellent clinical outcomes were observed in 92% of patients with isolated femoral condyle lesions at 2-9 years follow-up.[43,70,81] In addition, it is reported that integration of the newly formed repair tissue with the surrounding cartilage was satisfactory in approximately 90% of the patients at 5 years follow-up.[79] However, ACT has many disadvantages including donor site morbidity, requirement for two surgeries, possible leakage of chondrocytes form the recipient site, uneven distribution of the cells in the defect, chondrocyte dedifferentiation in monolayer culture, long recovery time after operation, and finally periosteal hypertrophy which is the major cause of failure after ACT.[9,43,76,77,82] A new improved variation of ACT known as "characterized chondrocyte implantation" is currently in clinical use. Characterized chondrocyte implantation is similar to ACT, however, the technique depends on selecting the chondrocyte populations having greater capability to produce hyaline-like cartilage.

Chondrocytes are characterized by a ChondroCelect product introduced to the market by Tigenix (Leuven, Belgium).[83] The company uses chondral markers to sort potential chondrocytes harvested from patients.[84] A randomized control study comparing characterized chondrocyte implantation to microfracture techniques was published in 2008. Clinical results after 18 months revealed that characterized chondrocyte implantation led to clinical outcomes similar to microfracture.[83] Other possible tissue types have been investigated for transplantation at the experimental and clinical level, including perichondrium, periosteum, and sternal cartilage, and showed promising clinical outcomes. However, the procedures are associated with morbidity, specially for larger cartilage defects.[9]

4.4. Tissue Engineering

Although all the aforementioned strategies promote pain relief and enhanced joint functions, they fail to restore the native structure and biomechanical properties of articular cartilage.[43] In addition, some of the currently used strategies are associated with technical difficulties and adverse results; therefore, investigators have focused on reconstructing cartilage *in vitro* using tissue engineering strategies.[77] Current tissue engineering strategies exploit a combination of three crucial components: appropriate cell source, stable scaffolding/carrier matrix, and bioactive agents.[68,77,85] Tissue - engineered cartilage should have similar biphasic properties as native cartilage (*i.e.* Fluid phase (80% water, <1% electrolytes) and solid phase (10-20 % collagen II, 5-10% proteoglycans)) after remodelling.[7,10] In addition, the regenerated tissue should occupy entirely the defect site and integrate optimally with the surrounding native cartilage to withstand *in vivo* mechanical forces (FIG.6 A,E,F).[9] In the following section we will summarize the most

widely used scaffolding materials, cell sources, and bioactive molecules that are used either experimentally or clinically to develop tissue-engineered cartilage substitute:

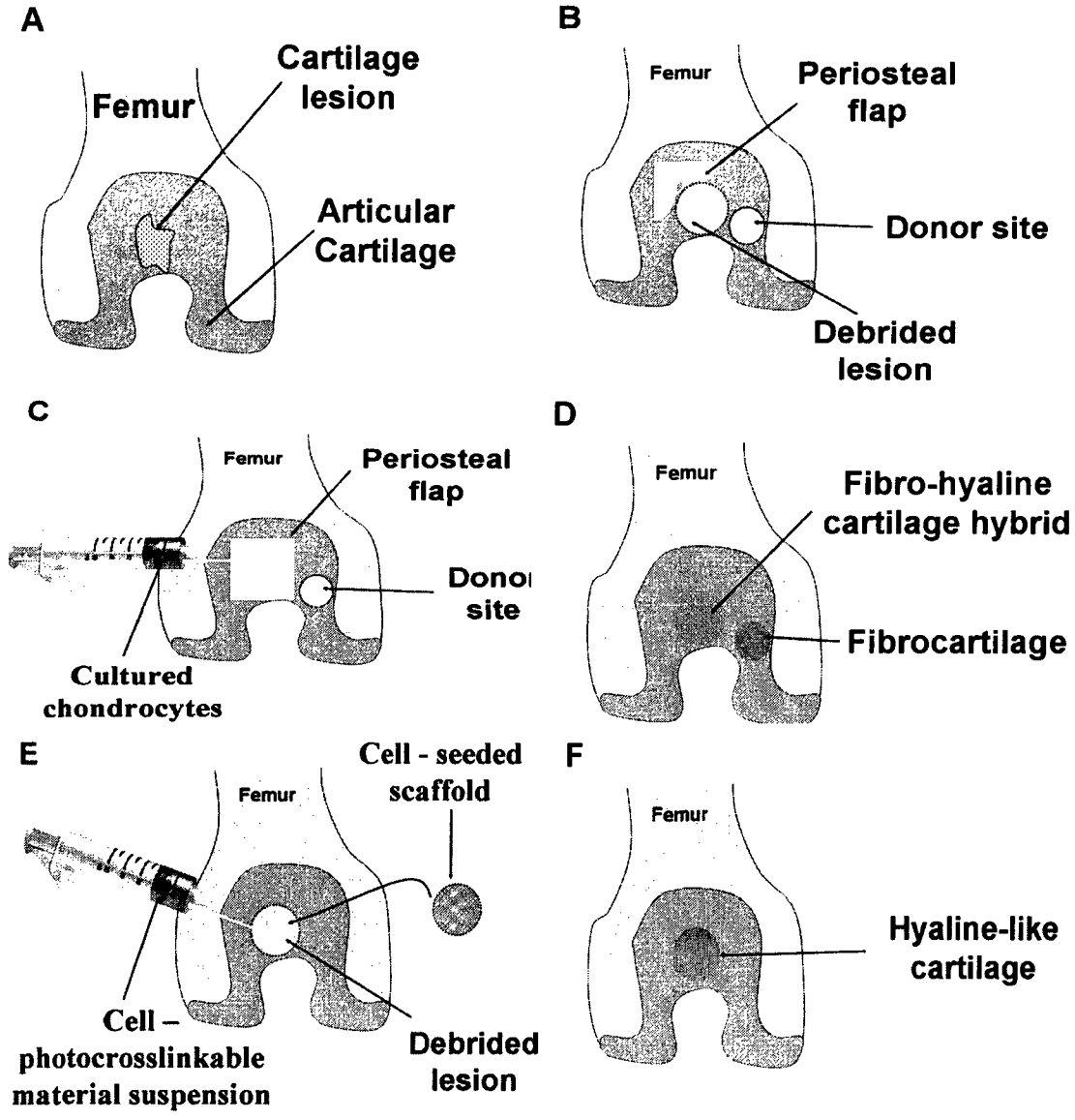

FIG.6. Autologous chondrocyte transplantation (ACT) versus tissue-engineered cartilage substitute. **A**: typical femoral cartilage lesions, **B-D**: ACT and the resulting repair tissue, and **E, F**: tissue engineering-mediated cartilage repair.

4.4.1. Scaffolding/carrier matrix

In comparison to ACT, the combination of chondrocytes with a supporting 3D scaffolding material greatly facilitates the transplantation procedure, so it can be carried out

arthroscopically in a minimally invasive fashion; in addition, harvesting of the periosteal flap is not required.[77,79] Ideally, scaffolds for articular cartilage tissue engineering should mimic the effect of native cartilage ECM on cell proliferation, differentiation and cell-to-cell interaction. Good examples of this type of scaffolds are hyaluronate, collagen, gelatin, and chondroitin sulfate. In general, the scaffold should be biocompatible and stable for an appropriate length of time. They should promote uniform cell distribution and optimum integration with the surrounding native cartilage. The scaffold should also be replaced gradually by cartilage-like ECM secreted from the cells (*i.e.* remodelling) while maintaining the cells at the lesion site.[10,68] In addition, the scaffold should have sufficient mechanical properties to withstand *in vivo* forces.[68] It has been shown that the nature of the scaffolding material and its porosity have tremendous effects on the proliferation and phenotype of chondrocytes.[10,86] The observed dedifferentiation of chondrocytes in monolayer culture during the ACT procedure can be prevented by embedding cells within a three dimensional scaffolding material.[77,79] The typical phenotype of chondrocytes can be maintained for up to 8 months in 3D alginate scaffold.[87] It has been also reported that chondrocytes have better proliferation and ECM secretion in scaffolds with pore size between 250 and 500 μm.[86] Scaffolding materials utilized for articular cartilage tissue engineering can be subdivided into natural and artificial:

4.4.1.1. Natural polymers

4.4.1.1.1. Carbohydrate - based scaffolds

Because of the stimulatory effect of glycosaminoglycans (GAGs), a long unbranched carbohydrate, on chondrogenesis, the utilization of carbohydrates - based scaffolds is a relevant strategy to induce chondrogenesis.[88]

4.4.1.1.1.1. Agarose

Chondrocyte-seeded agarose is a useful model for the evaluation of cell-associated matrix assembly.[89] It has been reported that chondrocyte-seeded agarose cultures promoted the production of functional extracellular matrix (ECM) and maintained the chondrocyte phenotype.[89,90] It has also been shown that the mechanical environment can alter the metabolic activity and the biomechanical properties of articular cartilage. The accumulation of proteoglycans was significantly enhanced in chondrocyte-seeded agarose subjected to dynamic loading.[91,92] Other factors such as the extracellular pH, the addition of collagen hydrolysate to the culture media, and the seeding density have great impact on the accumulation of ECM components in chondrocyte-seeded agarose.[93-95]

4.4.1.1.1.2. Alginate

Alginate has been widely utilized for microencapsulation of tissues and cells.[96] Expansion of chondrocytes into alginate beads significantly increased the accumulation of cartilage markers such as GAGs and collagen II[97]; however, the continuous and interconnected collagen/proteoglycan network of native cartilage could not be achieved.[98] Encapsulation of chondrocytes into alginate beads and subsequent culturing in bone morphogenetic protein-2 (BMP-2), insulin-like growth factor-1 (IGF-1), Osteogenic protein-1 (BMP-7) or platelet supernatant - supplemented media has been shown to enhance expression of the aforementioned markers.[99-102] It has been also reported that increasing the medium volume and glucose concentration significantly increased the production of GAGs in alginate-chondrocyte constructs.[103] In addition, exposure of chondrocyte and human mesenchymal stem cells (hMSCs) - seeded alginate beads to low intensity ultrasound (LIUS) resulted in enhanced accumulation of collagen II and proteoglycans.[104,105] Similar to agarose, increasing the cell density in alginate-seeded

constructs resulted in increased accumulation of collagen II and sulphated GAGs[106]; however, cell and matrix distribution was uneven, with greater densities of both observed in the periphery of the constructs.[107] A common practice in tissue engineering is to combine two or more biomaterials to incorporate advantages of the different materials.[85]Alginate has been combined with hyaluronate and fibrin to optimize the retention of proteoglycan inside alginate beads.[108] In addition, mixing alginate with chitosan enhanced the adhesion properties of chondrocytes, maintained the typical morphology of the cells, and regenerated the pattern of dense collagen II fibres compared with alginate alone.[88] Culturing and maintaining hMSCs-seeded alginate constructs with either transforming growth factor β_3 (TGF-β_3) or BMP-2 promotes the accumulation of collagen II and GAGs.[109,110] A recent review by Lee et al. (2009) summarizes the properties of various polysaccharides including alginate, chitin, chitosan, and hyaluronate, which are either currently being used or have potential to be used for electrospinning. Electrospun polysaccharides and their derivatives have great potential for utilization in the field of regenerative medicine as scaffolds.[111]

4.4.1.1.1.3. Chitosan and chitin

Chitosan is the deacetylated derivative of chitin. Chitosan alone or in combination with a wide variety of scaffolds has been used extensively in the tissue engineering of articular cartilage.[112] The seeding of chondrocytes on chitosan - coated coverslips maintained the spherical chondrocyte morphology and promoted the expression of collagen II and aggrecan.[113] In addition, chitosan can serve as a cytocompatible space filling scaffold that can gelate and adhere to cartilage when injected *in situ* in combination with chondrocytes.[114] In contrast, it has been shown that the rate of cell proliferation, the typical chondrocyte morphology, and the production of collagen II diminished in chitosan scaffold compared to chitosan - alginate hybrid after two weeks of culturing.[115]

Hybridizing chitosan with hyaluronate improved the adhesion of the chondrocytes to the scaffold, increased the proliferation rate while maintaining the round morphology of chondrocytes, and enhanced the accumulation of aggrecan and collagen II, compared to chitosan alone.[116] In addition, combining chitosan with both alginate and hyaluronate and the subsequent modification with arginine-glycine-aspartic acid sequence (RGD)-containing protein led to enhanced cell proliferation and increased production of GAGs along with collagen II.[117] Furthermore, lactose-modified chitosan promoted production of collagen II and aggrecan to a greater degree than chitosan alone.[118] Although combining chitosan with collagen I and chondroitin sulfate led to good adhesion properties, proper proliferation, enhanced production of cartilage markers, and stable round morphology of chondrocytes[119], the incorporation of TGF-β_1-loaded chitosan microspheres in the copolymer aforementioned resulted in a further significant increase in the proliferation rate and production of GAGs from chondrocytes, compared to the copolymer alone.[120] However, combining chitosan with other scaffolds does not necessarily result in desirable effects; for instance, combining chitosan with chondroitin sulfate and dermatan sulfate can deleteriously affect the production of cartilage markers and the morphology of the cells while preserving the mechanical and adhesion properties of the copolymer.[121] Encapsulation of chondrocytes into genipin-crosslinked chitin/chitosan hybrid has been shown to increase the proliferation rate along with improving the production of cartilage markers. In addition, increasing the ratio of chitin to chitosan resulted in a smaller pore diameter, a greater porosity, and a larger effective surface area of the construct.[122] Alternatively, chondrocytes can be seeded on the surface of bioresorbable β-chitin sponge with subsequent formation of a cartilage-like layer on the surface of the sponge that is similar to hyaline cartilage in terms of round morphology of the chondrocytes and secretion

of the ECM components.[123] MSCs are a promising substitute to chondrocytes for tissue engineering of articular cartilage. When encapsulated into a copolymer of water soluble-chitosan and thermosensitive poly(N-isopropylacrylamide), MSCs have been shown to differentiate into chondrocytes *in vitro* and this construct can be injected *in vivo* below the lower critical solution temperature (LCST) which then can gelate and produce demonstrating expression of chondrocytic markers (i.e. GAGs and collagen II) especially collagen II.[124] Different applications and future directions for chitosan in articular cartilage tissue engineering have been recently discussed.[112] A scaffold composed of chitosan and β-glycerophosphate known as BST-CarGel is used as adjunct to microfracture to stabilize the blood clot and retain MSCs in the cartilage lesion. BST-CarGel is liquid at room temperature and solidifies at human body temperature. It was introduced for clinical application by Biosyntech (Quebec, Canada).[84] The technique involves mixing fresh autologous blood with BST-CarGel in the operation room which then delivered to the holes created by microfracture and the surrounding prepared defect. Interim 6-months data showed better reparative tissue formation in comparison to microfracture alone.[68,82]

4.4.1.1.1.4. Hyaluronate and HYAFF®11

Due to its multiple functions in regulating and stabilizing the internal environment of cartilage, hyaluronate (hyaluronic acid, HA), is a promising scaffold to promote cartilage repair.[125] Chondrocytes treated with hyaluronate has been shown to have better proliferation both in micromass and rotation cultures. However, cell differentiation in terms of cartilage-specific proteoglycans was enhanced only in rotation culture.[126] Alternatively, encapsulation of chondrocytes in a photo-crosslinkable methacrylated form of HA has been reported to maintain the chondrocyte morphology along with promoting matrix accumulation *in vitro*. In addition, HA injected into a rabbit osteochondral defect model

integrated well with the native tissue and promoted the migration of cells into the hydrogel along with the production of cartilage-specific matrix.[127] Further, HA-chondrocyte hydrogels subjected to mechanical loading significantly increased the gene expression of articular chondrocytic markers.[128] Likewise, transplantation of bone marrow-derived hMSCs – seeded hyaluronate sponge into osteochondral defects in rabbit resulted in considerable regeneration of cartilage in the defect which was very similar in nature to the surrounding cartilage, especially when HA hydrogel was loaded with fibroblast growth factor-2 (FGF-2).[129] In contrast, combining bone marrow with fibronectin-coated hyaluronan-based sponge (ACP™, Fidia Advanced Biopolymers Srl, Abano Terme, Italy) does not have a remarkable effect on the medium and long-term outcomes of the regeneration process when compared to bone marrow-free ACP™. However, the outcomes of osteochondral defects filled with ACP™ hydrogel were superior to that of non-treated defects.[130] Recent reviews discuss huyaluronate as a potential scaffolding material for tissue engineering applications.[131,132]

HYAFF® 11 is the esterified derivative of hyaluronate and when combined with autologous articular chondrocytes it forms Hyalograft® C (Fidia Advanced Biopolymers, Abano Terme, Italy).[68,77,125] In terms of the quality of regenerated cartilage and production of chondrocytic markers, trials with Hyalograft® C yielded comparable results to that of autologous chondrocyte transplantation (ACT) in animal models and in humans.[125,133] The long term clinical outcomes of Hyalograft® C grafting in human was similar to that of ACT as indicated by magnetic resonance imaging (MRI), objective and subjective knee scoring systems. However, one advantage of Hyalograft® grafting over ACT is that it can be carried out using a less-complicated and minimally - invasive surgical procedure.[68,82,125,134,135] Rabbit MSCs seeded on HYAFF® 11 showed good adhesion and proliferative properties

along with elaboration of the marker collagen II *in vitro*. In addition, implantation of cell - seeded or un-seeded HYAFF® 11 scaffolds into osteochondral lesions did not elicit any immune reaction and promoted the healing process compared to non-treated lesions.[136] In a similar study, tissue that regenerated after implantation of the MSCs- seeded HYAFF® 11 sponge showed better cellular density and better integration with the surrounding cartilage than the cell - free sponge.[137] The theoretical and experimental evidence leading to the development of Hyalograft® C and its application for articular cartilage defects have been discussed by Tognana and coworkers.[138]

4.4.1.1.2. Protein - based scaffolds

4.4.1.1.2.1. Collagen

Collagen-based hydrogels have been extensively used in tissue engineering applications because of their low immunogenicity and easy manipulability.[47,139] In addition, collagen can achieve high seeding efficiency and good cell adhesion due to its hydrophilicity and the presence of bioactive domains in its structure.[140,141] Furthermore, as a component of ECM, collagen has been reported to regulate the chondrocytic phenotype and chondrogenesis both *in vitro* and *in vivo*.[47,139] However, collagen hydrogels cannot withstand the *in vivo* forces and fails to maintain the required shape *in vitro* due to its poor mechanical properties.[140,141] It has been shown that seeding of chondrocytes into collagen I or collagen I/III scaffolds maintained the cell viability and morphology, and promoted the elaboration of articular chondrocytic markers under standard and serum-free conditions.[142,143] The same outcomes were also observed when chondrocyte-seeded collagen I/III was maintained under stirring conditions.[143] In addition, implantation of either collagen I, collagen I/III, or collagen I/II/III hydrogels along with chondrocytes into rabbit chondral defects led to satisfactory hyaline cartilage regeneration as indicated by

histological and biomechanical assessment.[144-146] Furthermore, combining collagen I with GAGs and chitosan or fibrin has been shown to maintain the chondrocyte proliferation, morphology, and expression of chondrocytic markers.[119,147] Moreover, impregnation of collagen I - chondrocyte constructs in FGF-2-containing media prior to implantation resulted in rapid regeneration and significant production of GAGs along with maintenance of the typical chondrocytic phenotype.[148] In a similar study, seeding of chondrocytes into heparin-containing polystyrene - coated type I collagen after incorporation of either FGF-2 or TGF-β_1 has been shown to increase the proliferation rate and to maintain stable phenotype, respectively.[149] In contrast, applying platelet-derived growth factor (PDGF) has been shown to alter chondrocyte morphology[150,151] while, applying static compression resulted in reduced production of cartilage-specific markers.[150,151] Alternatively, *in vitro* seeding of MSCs into collagen I scaffold preserves the cell viability and morphology along with elaboration of the chondrocytic markers.[152] In addition, implantation of MSCs in combination with bi-layered collagen I/II/III into ovine chondral defects promoted better repair and formation of hyaline-like tissues.[153] A bilayered collagen I-III scaffold in combination with autologous chondrocytes is used clinically in the last decade and is considered as a modification of conventional ACT.[77] The technique is referred to as matrix-induced autologous chondrocyte implantation (MACI) in which autologous chondrocytes are seeded into bilayered porcine collagen before implantation then secured to the pre-prepared chondral defect using FG.[68,77,82] This technique resulted in clinical improvements comparable to that of ACT or microfracture after 1 year.[68,82] In addition, it showed good to excellent clinical outcomes in 82% of the patients at 4 years follow-up.[68] The MACI technique was introduced for clinical use by Genzyme (Cambridge, MA). However, other products utilizing the same principle and

biomaterial are also available: Carticel® produced also by Genzyme for application in USA; and Chondro-Gide produced by Geistlich (Wolhusen, Switzerland) for application in Europe.[83,84] Chondro-Gide can also be utilized clinically in a single stage procedure after microfracture and this known as autologous matrix-induced chondrogenesis (AMIC). Chondro-Gide membrane in this case provides the scaffold for growth and multiplication of cells released after microfracture procedure.[84] Satisfactory outcomes have been reported after 2 years follow-up during the first clinical assessment of 32 patients treated with AMIC in combination with microfracture, in terms of defect filling, functional improvement, pain reduction and patient satisfaction.[39] Collagen I scaffold in combination with fresh autologous chondrocytes is also used clinically and showed good to excellent clinical outcomes along with complete defect filling in 79% and 84% of patients respectively at 2 years follow-up. [82] The different applications for collagen during development of tissue engineering- based stratgeies have been recently summarized [154,155] Chajra H et al. [156] review collagen utilization as a tissue engineering tool for the treatment of osteochondral defects. Bovine collagen I in combination with bioadhesive is currently used clinically and known as Neocart; this material is produced by Histogenics (Waltham, MA). The harvested cartilage samples are processed and chondrocytes grown into collagen; the construct is then applied to the lesion and secured by bioadhesive.[84] Another similar product known as CaReS® has been introduced by Arthro Kinetics (Esslingen, Germany) and is also available for clinical application. CaReS® utilizes rat tail collagen I, instead of bovine collagen (Neocart). VeriCart, another product of Histogenics, has been introduced to the market as an adjuvant to microfracture. VeriCart relies upon the same principle as BST-CarGel; however, a double- structured collagen scaffold is utilized in VeriCart instead of the chitosan and β-glycerophosphate scaffold utilized in BST-CarGel.

As a major component of hyaline cartilage, collagen II is a potential alternative to collagen I.[157-165] Although collagen II shows similar effect on the chondrocyte phenotype compared to that of collagen I after seeding the cells in low density[157], implantation of collagen II alone into the chondral defects has been shown to significantly induce the chondrocytic phenotype in the migrating cells compared to collagen I.[158] Similar to collagen I, implantation of chondrocytes in combination with collagen II into a canine model of chondral defects resulted in adequate cartilage regeneration; however, the compressive stiffness of the regenerated tissue is far lower than that of native cartilage.[159] In another approach, *in vitro* application of dynamic compression to chondrocyte-seeded collagen II promoted increased biosynthesis of total collagen and GAGs. However, static compression decreased the synthesis of collagen and GAGs in the same constructs.[160] Collagen II can be manipulated through electrospinning to form specific fiber diameters resembling those of native cartilage, which was found to facilitate adherence, proliferation and infiltration of seeded chondrocytes.[161] In addition, factors such as cross-linking density and FGF-2 have been shown to induce chondrogenesis and cell proliferation when chondrocytes are seeded into collagen II-GAGs hybrids.[162,163] It has been also shown that combining both GAGs and hyaluronate with collagen II showed a similar effect to that of cross-linking density and FGF-2 on chondrogenesis and cell proliferation.[164] In contrast, another study reported that combining GAGs with collagen II did not have any impact on the elaboration of chondrocytic markers or proliferation rate compared to collagen II alone.[165]

4.4.1.1.2.2. Fibrin

Fibrin hydrogel combine some important characteristics such as high seeding efficiency, uniform cell distribution, and great adhesion capabilities. In addition, it can be

produced from the patient's own blood and used as an autologous scaffold without the potential risk of foreign body reaction or infection. Because of these advantages, fibrin scaffolds has been widely used for tissue engineering applications.[85] It has been reported that injecting nasal chondrocytes in combination with fibrin glue (FG) into an osteochondral defect model resulted in hyaline-like tissue formation along with maintenance of the chondrocyte morphology and chondrocytic marker expression.[166] It has also been shown that applying oscillatory compression to chondrocyte-seeded fibrin hydrogels resulted in significant softening of the gels and inhibition of GAG accumulation. However, incubation with static compression did not significantly alter the mechanical stiffness and biochemical content of the hydrogels.[167] FG combined with alginate allowed adequate chondrocyte proliferation and maintained cell differentiation to establish a stable matrix structure *in vitro*.[168,169] Combining fibrin with a either tri-copolymer of gelatin/hyaluronate/ chondroitin-6-sulfate or polyurethane has been shown to enhance the final mechanical properties of the constructs, while promoting uniform distribution and high seeding efficiency of chondrocytes into the hydrogels along with proper accumulation of ECM components.[170-172] In addition, encapsulation of chondrocytes into fibrin combined with either hyaluronate or polyurethane scaffolds followed by subcutaneous implantation in nude mice promoted long-term stabilization of the constructs, the formation of cartilage-like tissue, remarkable production of the GAGs, and uniform distribution of the ECM components.[172,173] FG are used extensively to secure other tissue-engineered cartilage in clinical settings.[77] FG has also been used in combination with autologous chondrocytes for the treatment of deep chondral defects in human.[174-176] When combined with commercial FG (Tissucol®, Baxter, Deerfield, IL), autologous chondrocytes showed better clinical outcomes during the treatment of deep chondral defects compared to abrasive

techniques after 1 years in terms of subjective scores.[176] Alternatively, minced cartilage in combination with fibrin glue known as Denovo NT grafts (Zimmer, Warsaw, IN/ISTO, St Louis, MO) is currently under clinical investigation, where cartilage pieces obtained from juvenile allograft donor joint are aseptically minced and mixed intra-operatively with fibrin glue then implanted in the prepared lesions.[3] Juvenile cartilage is chosen depending on the assumption that it would have higher anabolic capability and better expandability. Disease transmission and limited supply is the drawbacks of this technique.[82] A similar product known as DeNovo ET graft (ISTO technologies, St. Louis, MO) is generated from juvenile allogeneic cartilage cells which implanted into the prepared lesion site and secured via fibrin glue. The graft is hyaline-like and implantable in a single stage procedure.[82,84] Finally, photopolymerizable poly(ethylene glycol) modified fibrinogen, known as PEGylated fibrinogen or Gelrin C (Regentis Biomaterials, Haifa, Israel), that crosslinks *in situ* after exposure to UV light is now in the stage of preclinical trails. [82] The development of PEGylated derivative of fibrin leads to the formation of fibrin gels that have good mechanical strength. In addition, PEG of different molecular weights can be utilized to develop fibrin hydrogels with different mechanical properties.[177,178] Gelrin C is characterized as having innate chondrogenic and osteoconductive potential, while it is nonimmunogenic as indicated from the *in vitro* study. Implantation of Gelrin C into ovine osteochondral defects showed enhanced production of cartilage-specific markers compared to empty defects which show scar formation composed mainly of fibrocartilage.[82] Gelrin C is considered as an adjunct to microfracture or osteochondral defect filler and its rate of degradation depends on the degree of PEGylation.[82] Our recent review summarized different applications of fibrin for tissue engineering purposes.[85]

4.4.1.1.2.3. Gelatin

Gelatin is produced commercially through boiling and hydrolysis of collagen-containing raw material such as bones and skins, mainly of cows and pigs.[179] In comparison to collagen, gelatin has lower immunogenicity and cost. However, it maintains some of the positive collagen properties such as adhesion, differentiation and proliferation capabilities.[86] Gelatin has been widely used for cartilage tissue engineering especially in combination with other scaffolding materials to combine the advantages of both.[180-186] It has been shown that seeding of chondrocytes into genipin-crosslinked gelatin increased the chondrocyte growth rate and the production of chondrocytic markers along with maintaining the chondrocyte morphology, especially at larger gelatin pore sizes.[86] In addition, seeding chondrocytes into resorbable gelatin sponge in the presence of high molecular weight hyaluronate promoted the retention of cells inside gelatin, chondrocyte proliferation, and the production of cartilage-specific ECM components.[180] Similar to chitosan, suspension of chondrocytes within solutions of poly(N-isopropylacrylamide)-grafted gelatin facilitated the *in situ* injection of the construct, which then rapidly solidified at 37 °C.[181] In such constructs, cell viability and the round chondrocyte morphology were maintained in concert with development of mechanical properties and expression of chondrocytic markers that resembled native cartilage. However, accidental temperature decreases (during or shortly after the operation) and mechanical loading can lead to leakage and deformation of the construct, respectively.[181,182] Alternatively, chondrocytes mixed with photopolymerizable styrenated gelatin is another approach for *in situ* injection of tissue - engineered cartilage substitutes.[183] Gelatin–calcium phosphate biphasic scaffold is a promising approach to facilitate the integration of the tissue - engineered cartilage into the host subchondral bone, where the chondrocyte-seeded gelatin serves as the cartilaginous

component while the osteoblasts-seeded calcium phosphate served as the bony element.[184] Finally, mixing gelatin with both hyaluronate and chondroitin-6-sulfate promote the formation of uniform pore sizes and chondrocyte distribution while maintaining the chondrocyte morphology and expression of collagen II, especially in spinner flask culture.[185] In an *in vivo* study, the implantation of chondrocytes along with gelatin/hyaluronate/chondroitin-6-sulfate into pig full thickness cartilage defects resulted in a satisfactory repair process through the formation of hyaline and/or fibrocartilage and good integration with the host tissue after 36 weeks.[186] The application of gelatin as a delivery vehicle for the controlled release of bioactive molecules for tissue engineering, therapeutic angiogenesis, gene therapy, and drug delivery has been recently summarized.[187]

4.4.1.2. Artificial biodegradable polymers

Synthetic biodegradable polymers combine some important characteristics such as controllable degradation rate, high reproducibility, high mechanical strength, and easy manipulation into specific shapes.[140,188] However, the cell recognition signals are missing in such scaffolds. In addition, they posses relatively hydrophobic surfaces which is not ideal for efficient cell seeding.[140,141]

4.4.1.2.1. Polyglycolic acid (PGA)

PGA is a highly crystalline, hydrophobic linear polyester.[189] It has been shown that the seeding of chondrocytes into nonwoven fibrous PGA disks maintains the chondrocyte morphology and promotes the formation of ECM resembling normal cartilage in terms of water content and percentage of type II collagen under static culture conditions. However, applying dynamic fluid pressure did not significantly enhance the cartilage matrix formation in such constructs.[190] Similarly, seeding the chondrocytes into PGA disks under serum free conditions promoted significant synthesis of aggrecan and expression of

collagen II along with maintained rounded cell morphology compared to PLGA (Poly(lactic-co-glycolic acid)) disks. In addition, implantation of chondrocyte-seeded PGA produced cartilage-like tissue in terms of cartilage specific markers as indicated by histological and biochemical findings.[189] Recently, a copolymer of PGA/PLA and polydioxanone (PDS) in combination with autologous chondrocytes dispersed into fibrin glue has been introduced into clinical use and is referred to as Bio-Seed®-C (Biotissue Technologies, Freiburg, Germany).[77] The expanded chondrocytes are loaded onto the mechanically stable porous matrix using fibrin glue for even distribution, and then implanted arthroscopically.[191] Evaluation of midterm results of ACT compared to Bio-Seed®-C revealed that they are equally effective as a treatment option for focal degenerative chondral lesions.[192] Good clinical outcomes have been reported with Bio-Seed®-C over a period of 4 years.[193] A new system has also been developed recently and known as cartilage autograft implantation system (CAIS), utilizes resorbable copolymer of PGA and polycaprolactone (PCL) reinforced with PDS mesh that is combined with minced cartilage as a source of viable chondrocytes.[82] The system composed of harvester, disperser, scaffold, and staples. Cartilage pieces are harvested from the non-weight bearing area using the harvester then minced into small cartilage fragments using the disperser.[3] Minced cartilage is then evenly distributed and stabilized into the PGA/PCL copolymer using fibrin glue then fixed into the lesion site using the resorbable PDS staples.[3,82] CAIS has been studied *in vitro* and in animal models but still in the early phase of clinical trials in humans.[82] Early clinical trials using CAIS showed promising results in comparison to microfracture. However, long term and randomized human trials have not been conducted yet.[3]

4.4.1.2.2. Polylactic acid (PLA)

PLA is a linear polyester, which is less crystalline but more hydrophobic than PGA due to the presence of an extra methyl group.[189] It has been reported that the implantation of perichondrocyte - seeded PLA composite within rabbit osteochondral defects results in a repair tissue that is similar histologically and biochemically to the surrounding cartilage tissue.[194] However, more recent work showed that implantation of chondrocyte – seeded PLA scaffolds into animal cartilage lesions did not promote successful cartilage repair as the scaffolds were mechanically too hard.[195] To overcome this drawback, PLA scaffold can be combined with gelatin through a porogen - leaching technique, with gelatin particles as the porogen. This strategy allowed the fabrication of a PLA-based scaffold with controllable microstructure and mechanical properties.[196] It has also been shown that implantation of PLA mixed with PEG (poly(ethylene glycol)) and interconnected porous hydroxyapatite together with recombinant human BMP-2 (rhBMP-2) into rabbit full thickness cartilage defects promoted the migration of MSCs from bone marrow with subsequent formation of cartilage - like repair tissue as indicated from the formation of mature ECM and appropriate chondrocyte organization.[197]

4.4.1.2.3. Poly(lactic-co-glycolic acid); PLGA

Combining lactic acid with glycolic acid to form PLGA has been shown to prolong the degradation time of the hybrid, probably because glycolic acid is more hydrophilic than lactic acid.[198] It has been reported that chondrocytes delivered to a cylindrical mold with PLGA microspheres promoted the formation of cartilage-like tissue that has stable shape and collagen type II expression along with improved total tissue mass and GAGs production, especially when higher molecular weight PLGA was used.[199] Similar findings have been observed when chondrocytes were mixed with PLGA microspheres and injected

immediately into athymic mouse subcutaneous sites or rabbit osteochondral lesions, as indicated from histological and biochemical analyses.[200,201] Likewise, transplantation of MSCs-seeded PLGA into rabbit osteochondral defects showed significant cartilage regeneration as indicated by gross morphology, mechanical properties, histological examination, and the accumulation of chondrocytic markers.[202] Application of intermittent hydrostatic pressure to cell-seeded PLGA nanofiber scaffolds fabricated by the electrospinning technique increased chondrocyte proliferation and ECM accumulation.[198] In addition, modifying PLGA by NaOH through chemical etching techniques enhanced chondrocyte functions including adhesion, growth, differentiation, and ECM formation.[203] Furthermore, combining PLGA nanophase produced by this technique with nanophase titania provided promising alternatives in the design of prostheses at the bone–cartilage interface.[204] Moreover, combining PLGA with fibrin[188,205], collagen I[141], or hyaluronate[206] led to the formation of cartilage-like tissue in terms of GAG and collagen II accumulation, both *in vitro* and *in vivo* models, when chondrocytes were used as a cell source. This enhanced accumulation of cartilage-specific markers might be due to the positive features of combining a natural polymer with PLGA polymer: promotion of efficient cell seeding, good cell distribution, better adhesion, and improved differentiation.[141,188,205,206] It has also been shown that the implantation of fibrin hydrogel-mediated chondrocyte seeding within methoxypoly(ethyleneglycol) - PLGA scaffold into full thickness cartilage defect resulted in significant cartilage repair as compared to empty defects, osteochondral drilling and fibrin hydrogel with chondrocytes.[207] Finally, encapsulation of chondrocytes into a PLGA/PLA blend modified with type II collagen has been shown to enhance cell proliferation along with improvement in GAGs and collagen content. Subcutaneous implantation of PLGA/PLA/collagen II construct after encapsulation

of chondrocytes did not exhibit infiltration by host tissue or capsule formation, and satisfactory repair tissue was observed after implantation into rabbit cartilage defects. Further modification of PLGA/PLA/collagen II constructs with RGD- containing proteins has adverse effects on cell proliferation and chondrocytic markers production.[208] One major drawback of using PLGA as a scaffolding material, that its degradation products, e.g. glycolic acid and lactic acid, might cause intense inflammatory reaction after *in vivo* implantation.[186,208] A resorbable porous composite, TruFit bone substitute plugs (Smith & Nephew, Andover, MA), composed of mechanically stable, cylindrical shaped, bilayered PLGA and calcium sulfate is used clinically nowadays to facilitate the ingrowth of new healing tissue to restore osteochondral defects.[68] The plugs are designed to degrade within a years and is commonly used for back-filling femoral donor site during mosaicplasty.[82] They promote cancellous bone replacement in the subchondral region and formation of fibrocartilage at the surface. TruFit use in patients with chondral lesions was shown to be stable without failure or revision surgery after 2 years.[209] In addition, improved subjective scores without any complications at average follow-up of 39 months were observed after application of the plugs to either back-filling or osteochondral defects.[68] The recent successes of PLGA-based nanotechnologies and tools in medicine-related applications including tissue engineering have been recently reviewed.[210]

4.4.1.2.4. Poly(ethylene glycol); PEG

PEG is a synthetic polymer that has wide biotechnological applications. It is a highly biocompatible material and used widely for many medicinal purposes.[177] The neutral and non-interactive nature of PEG facilitates ECM secretion from cells after their encapsulation.[211] Another advantage of the PEG-based scaffold is that it can be laminated by adding a second layer before complete crosslinking of the first layer, which creates

zonal patterns resembling the organization of articular cartilage.[178] It has been shown that the mechanical properties of PEG - based scaffolds are dependent on the mesh size. Different mesh sizes can be achieved by changing the PEG molecular weight, concentration or the crosslinking density.[178,212,213] Poly(ethylene glycol) dimethacrylate (PEGDM) and poly(ethylene glycol) diacrylate (PEGDA) are the most widely used PEG derivative as they can crosslink via photoinitiator under the effect of UV light (*i.e.* photopolymerizable PEG). One advantage of the photocrosslinkabe derivatives is that, they can be used for *in* situ application in a minimally invasive procedure such as liquid injection.[212-217] It has been shown that encapsulation of chondrocytes or MSCs into photopolymerizable PEG resulted in uniform distribution of cells and enhanced accumulation of chondrocytic markers.[216,217] However, encapsulation of chondrocytes in a highly crosslinked photopolymerizable PEG scaffold led to decreased cell proliferation and GAGs production, along with alteration of the cell morphology in the presence or absence of compressive loading.[212,214] It has also been shown that changing the PEGDM polymer concentration, along with polymer chemistry alterations via incorporation of rapidly-degrading PLA-b-PEG-b-PLA moieties, enables the control of both ECM accumulation and scaffold integrity: this approach generates gels that can withstand the *in vivo* mechanical loads while simultaneously facilitating the formation of new cartilage.[213,218] Further, the incorporation of negatively-charged molecules such as chondroitin sulphate into PEG resulted in better mechanical properties as indicated from the compressive modulus results.[211] It has been reported that the architecture of the PEG scaffold plays a critical role in elaboration of the ECM components into the engineered cartilaginous constructs. For example, scaffolds of poly(ethylene glycol)-terephthalate/poly(butylene terephthalate) (PEGT/PBT) co-polymers prepared by 3D fiber deposition (3DF) technique creates an environment that enhances

cartilaginous matrix deposition when encapsulated chondrocytes are subcutaneously implanted in nude mice, compared to scaffolds prepared by compression-molding/particulate-leaching (CM) method.[219] Another derivative of PEG is oligo(poly(ethylene glycol) fumarate), OPF, which can be manipulated to form two layered constructs that can solve the problem of bone–cartilage interface. Here the top layer serves as the cartilage forming layer and the bottom one serves as the bone forming layer. Implantation of this construct in a rabbit osteochondral defects resulted in formation of cartilaginous tissue in the top layer and a mix of trabecular and compact bone in the bottom layer which integrated with the surrounding cartilage and subchondral bone respectively.[220] Finally, the PEG-based 4-armed crosslinker (*i.e.* pentaerythritol poly(ethylene glycol) ether tetrasuccinimidyl glutarate) has been used to crosslink alkali treated, naturally-derived polymer (i.e. atelocollagen) to promote *in situ* polymerization after encapsulation of chondrocytes.[221] A photopolymerizable hydrogel composed of PEG in combination with chondroitin sulphate (bioadhesive) are now utilized in the clinical setting (ChonDux, introduced to the market by Cartilix (Foster City, CA)). The product is applied in conjunction with microfracture to enhance the cartilage repair process through promotion of chondrogenic differentiation and cartilage tissue formation by BM-MSCs. The product is applied in a liquid from that solidifies upon exposure to UVA light.[84] Different photopolymerizable scaffolding materials that are utilized for tissue engineering applications have been reviewed.[215,222]

4.4.2. Clinically approved matrices

Although a wide range of products have been introduced to the market since 2000 for the treatment of osteochondral lesions, only a few of them, including Tissucol® and Carticel®, are approved for clinical use in USA. The remaining products are still in

different phases of clinical trials in Europe and USA. MACI and Chondro-Gide are the versions of Carticel® available in Europe, Asia, and Australia. Gelrin C, is in the preclinical stage of development. A wide range of products are still undergoing or have completed phase I clinical trials including VeriCart, CAIS, and Denovo ET graft. Neocart is in phase II clinical trials. Hyalograft® C, ChondroCelect, and BST-CarGel are either in or completed phase III clinical trials. DeNovo NT is in phase IV.[3,39,68,76,77,82-85,191] The clinical trial phase is unclear for other products including Bio-Seed®-C, AMIC, MACI, ChonDux, CaReS®, and TruFit. However, it can be deduced from various peer-reviewed publications that the AMIC product is approved, and that Trufit and Bio-Seed®-C are either in or have completed phase II. Chondux and MACI are either in or have completed phase III clinical trials.[68,82,84,191,192] CaReS® product has pharmaceutical manufacturing approval and US IND status as posted in the manufacturer's website. The different scaffolding/carrier matrices used experimentally or clinically during development of tissue engineering strategies for the treatment of knee cartilage injury and different clinical phase of the products utilized clinically are summarized in table 1.

Scaffolding/carrier matrix			Reference number	In clinical Application	Clinical phase	Region/Country
Natural	Carbohydrate-based	Agarose	(89-95)	BST-CarGel*	II/III	USA/Canada
		Alginate	(5,88,96-111,115,117,168,169)			
		Chitosan	(68,82,88,111-122,124)			
		Chitin	(111,122,123)			
		Hyaluronate	(108,111,116,117,125-130,164, 170,173,180,185,186,206)			
		HYAFF®11	(68,77,125,133-137)	Hyalograft® C*	III	Europe/Asia
	Protein-based	Collagen I/III	(10,47,68,77,82,119,139-156,221)	MACI**	III	Europe
				Carticel®*	Approved	USA
				Chondro-Gide*	Approved	Europe
				AMIC**	Approved	Europe
				Neocart*	II	USA
				CaReS®***	PreI	Europe/USA
		Collagen II	(146,153-165,208)	Vericart*	I	USA
		Fibrin	(8,9,68,77,82,85,108,147,166-177,188,205,207)	Tissucol®*	Approved	USA
				Denovo NT graft*	Postlaunch/IV	USA
				DeNovo ET graft*	I/II	USA
		Gelatin	(10,86,170,179-187,196)	Gelrin C*	Pre I	Europe
Artificial		PGA	(3,77,82,186,189-191,193,208)	Bio-Seed®-C**	I/II	Europe
				CAIS scaffolds*	I/II	USA
		PLA	(77,186,189,194-197,208,213,218)	Bio-Seed®-C**	I/II	Europe
		PLGA	(68,82,140,186,198-208,210)	TruFit bone substitute plugs**	I	Europe
				Bio-Seed®-C**	I/II	Europe
		PEG	(82,177,178,197,211-222)	Gelrin C*	Pre I	Europe
				ChonDux**	II/III	Europe

* Data reprted in peer-reviewed publications [3,39,68,76,77,82-85,191], ** Date deduced from peer-reviewed publications [68,82,84,191,192], *** Data posted in the company websites.

4.4.3. Cell source

Finding the best cell source is a key element during tissue engineering of articular cartilage. The cellular component should be healthy, viable, easily accessible, manipulable, nonimmunogenic, and non-tumorigenic.[85,223] The cells should also have stable phenotype and should respond appropriately to the bioactive factors with subsequent production of the typical chondrocytic markers.[68] Since, obtaining sufficient number of cells is problematic in cartilage tissue engineering[9], the cellular component should be easily expandable to provide a sufficient quantity of tissue for the regeneration process.[223] Chondrocytes[88,97-108,166-176,216] and MSCs[109,124,130,137,152,197,217] are the most widely used cellular component during tissue engineering of articular cartilage. They can be obtained autologously without harmful immune response and disease transmission. They can be also obtained allogeneically to eliminate donor site morbidity and to maximize availability.[68,87] Articular chondrocytes are a relevant source of cells to be used in cartilage tissue engineering, since they are expressing the chondrocytic markers normally.[224] Similar to ACT, chondrocytes for tissue engineering are harvested from the non-weight bearing area of healthy knee cartilage.[125,135] However, as mentioned earlier, maintaining chondrocytes in *in vitro* culture without a supporting 3D matrix results in dedifferentiation of the cells.[77,79] Other sources of chondrocytes such as auricular cartilage, costal cartilage, and nasoseptal cartilage have been tested for cartilage tissue engineering and compared to articular chondrocytes.[128,225] It has been demonstrated that non-articular chondrocytes can secrete articular ECM components after implantation into articular cartilage defect.[87] However, they exhibit different behaviour during their *in vitro* testing for development of tissue-engineered cartilage.[128,225] Alternatively, MSCs can be utilized in the formation of tissue-engineered cartilage substitute.[68] MSCs utilized for articular cartilage tissue engineering can be

obtained from a wide variety of sources including bone marrow, adipose tissue, blood, periosteum, synovial membrane, trabecular bone, dermis, and muscle.[226] It has been shown that MSCs of bone marrow, periosteum, synovium have superior chondrogenic potential to those from adipose tissue or muscle.[224] MSCs have some advantages over chondrocytes, in that they have multi-differentiation potential,[109,152] which enables the use of the same cell source to develop constructs containing more than one cell type; for example, constructs needed at the cartilage-bone interface, and to develop ligament and meniscus substitutes. However, optimizing the culture condition to induce MSCs differentiation into pure population of chondrocytes is an absolute requirement during the development of tissue-engineered cartilage substitute. Another advantage of MSCs that they have very low tendency to dedifferentiate in monolayer culture.[223] It has been reported that BM-MSCs-seeded collagen I hydrogels have improved mechanical stability, cell viability, chondrocytic marker elaboration compared to chondrocyte-seeded hydrogels as indicated by *in vitro* testing.[152]

4.4.4. Bioactive agents

Bioactive agents should have the capability to induce the cellular machinery of tissue regeneration. Ideal bioactive factors should promote proliferation, differentiation, and maturation of the cellular component. In addition, it should have chemotactic activity and should maintain suitable ECM production by the cells.[85,227] A wide range of bioactive agents have been investigated during the development of tissue-engineered cartilage substitutes and were shown to regulate chondrogenesis in chondrocytes and MSC cell types including TGF-β_1[98,120,139,149,152,162,220,228-230], β_2[110,228,230-232], β_3[103,109,124,152,164,202,230,233-238], BMP-2[99,110,197,228,230,235,237], 4[235,236], 6[228,235,236,238], 7[102,228,230], FGF-2[148,149,162,163,217,232,235], IGF-1[100,163,230,231,233,235,238], and parathyroid hormone related

protein (PTHrP)[234,235]. TGF-β_1 and TGF- β_3 are the most widely applied bioactive molecules during the development of cartilaginous substitutes and have been shown to promote chondrogenesis[98,124,164,230] in 3-D cultures (Chondrogenesis is facilitated in the 3-D culture).[232] BMP-2 can induce chondrogenic differentiation of adult stem cells effectively in a comparable way to the TGF-β family members in both monolayer and 3D cultures.[110,237] In a related study in alginate matrix, BMP-2 and BMP-7 have been shown to induce chondrogenic differentiation of MSCs better than TGF- β_1.[230] In addition, a combination of TGF-β_2 and BMP-7 has been shown to promote chondrogenesis of MSCs in pellet culture better than a combination of TGF-β_2 and BMP-2, a combination of TGF-β_2 and BMP-6 or each growth factor separately.[228] Alternatively, it has been shown that a combination of TGF-β_3 and BMP-2 induce chondrogenesis in hMSCs pellet culture better than a combination of TGF-β_3 and BMP-4, a combination of TGF-β_3 and BMP-6, or TGF-β_3 alone.[236] However, a combination of TGF-β_3 and BMP-6[235,238] or TGF-β_3 and IGF-1[238] were shown to be effective inducers of chondrogenesis in MSCs pellet[238] and monolayer[233] cultures as indicated by gene expression.[233,238] It has also been revealed that FGF-2 increased, while TGF-β_2 decreased, MSCs proliferation in monolayer culture.[232] Furthermore, FGF-2 significantly improved the proliferation of chondrocytes in high - performance ECM compared to TGF-β_1.[149] Similar findings were observed when chondrocytes encapsulated in thermoreversible gelation polymer under the effect of TGF-β_2 and IGF-1 in combination or IGF-1 alone.[231] Moreover, FGF-2 promoted improved expression of the chondrocytic markers in chondrocytes seeded into collagen matrix. However, when FGF-2 was combined with IGF-1, the production of GAGs did not significantly improve in the same setting.[163] In contrast, it has been shown that combining IGF-1 with TGF-β_1 induced chondrogenic differentiation of MSCs into chondrocyte-like

cells and promoted the expression of chondrocytic markers in monolayer culture.[229] Alternatively, combining IGF-1 with TGF-β_2 resulted in increased production of proteoglycans in MSCs pellet culture compared to TGF- β_2 alone.[232] It has also been revealed that combining TGF-β_3 with PTHrP promoted chondrogenesis of MSCs and resulted in decreased expression of collagen X (hypertrophic marker), collagen I (fibrocartilage marker), and alkaline phosphatase (bone marker) without affecting the other hyaline cartilage markers (i.e. collagen II and aggrecan).[234] Finally, inhibitors of the retinoic acid receptor such as LE135 can induce chondrogenesis in monolayer and PGA-seeded MSCs in a comparable way to TGF-β_3.[239] The different growth factors and their commonly used concentrations are illustrated in table 2.

Table 2. Different growth factors evaluated experimentally as inducers of chondrogenesis during tissue engineering of articular cartilage

Growth factor	Concentration range	Commonly used concentration
TGF-β_1	1-160 ng/ml	10 ng/ml
TGF-β_2	0.05-50 ng/ml	10 ng/ml
TGF-β_3	10-20 ng/ml	10 ng/ml
FGF-2	5-160 ng/ml	5,10 ng/ml
BMP-2	10-500 ng/ml	10,50 ng/ml
BMP-4	10-500 ng/ml	10,500 ng/ml
BMP-6	10-500 ng/ml	500 ng/ml
BMP-7	10-200 ng/ml	10,100,200 ng/ml
IGF-1	1-200 ng/ml	10,100 ng/ml
PTHrP	1,10 μM	1,10 μM
LE135	10 ng/ml	10 ng/ml
FBS/FCS	1-20 %	10 %

4.4.5. Gene therapy

To further improve the quality of the regeneration tissue, new genetic information can be delivered to the cellular component, which subsequently regulates the regeneration process at the cellular and molecular levels. The genetic information can be delivered to the

cells of interest through viral or nonviral vectors.[240] The most widely used viral vectors include adenovirus, adeno-associated virus (AAV), foamyvirus (FV), herpes simplex virus (HSV), lentivirus, and Moloney murine leukemia virus (MoMLV).[240,241] The nonviral vectors include naked DNA, plasmid DNA in liposomes, and DNA delivered via Ca/P precipitation, electroporation, gene gun, or injection.[241] Viral vectors have higher transfection efficiency compared to the nonviral vectors. In addition, the delivered gene is expressed longer in the viral versus non viral vectors. However, non viral vectors are easier to synthesize and have lower immunogenicity compared to the viral vectors.[240,242] *IGF-1*, *TGF-β*, *BMP-2*, *BMP-7*, and *FGF-2* genes are the best candidates for gene therapy during development of tissue-engineered cartilage substitute.[240-242] It has been shown that implantation of *BMP-7*[243] and *TGF-β_1*[244] overexpressing- MSCs, in combination with a 3-D supporting matrix, into a chondral defect led to satisfactory production of GAGs and the formation of hyaline-like regeneration tissue.[243,244] In addition, overexpression of *TGF-β_3*[245], *BMP-2*[246], *SOX-9*[247], and endostatin[248] in MSCs resulted in enhanced production of cartilage specific markers. However, overexpression of *IGF-1* in MSCs did not result in significant improvement in the expression of cartilage-specific markers.[246] Similarly, implantation of chondrocytes overexpressing *TGF-β_1*[249] alone or overexpressing *TGF-β_1* along with *PTHrP*[250] simultaneously into chondral defect resulted in hyaline - like repair tissue formation showing significant production of the known chondrocytic markers.[249,250] Furthermore, overexpression of *TGF-B_3*[245], *BMP-2*[251], *BMP-7*[252], *IGF-1*[253], and *SOX-9*[247] in chondrocytes promoted the production of cartilage-specific ECM.

4.4.6. Physical stimulants

Since the knee joint is a mechanical tissue, physical stimulants play a crucial role during the normal development and homeostasis of knee cartilage. However, excessive

physical forces can lead to cartilage damage.[254] The cartilage is normally subjected to physical pressure ranging between 1 and 20 megapascal (MPa) at a frequency of 0-1 Hz.[255] It has been shown that static compression reduces chondrocyte proliferation, decreases the synthesis of cartilge - associated ECM, and alters the chondrocyte phenotype in agarose, alginate, collagen scaffolds.[151,160,167,255] In contrast, applying dynamic compression results in enhanced accumulation of collagen II and GAGs in the aforementioned scaffolds.[91,92,95,160,167,255] However, it has been demonstrated in a related study that applying dynamic fluid compression on chondrocytes - seeded PGA did not result in improved accumulation of cartilage-specific matrix components.[190] Similarly, MSCs pellet subjected to intermittent hydrostatic pressure in presence of TGF-β_3 showed enhanced production of cartilage – associated ECM molecules.[256] In addition, application of continuous cyclic tensile loading to MSCs-seeded collagen I-GAG scaffold led to increased synthesis of GAGs, while application of uniaxial static mechanical constraint resulted in reduced production of GAGs.[257] Furthermore, TGF- β_3 – induced chondrogenesis of MSCs pellet and the subsequent expression of chondrocytic markers were both improved under the effect of low-intensity ultrasound (LIUS).[258,259] LIUS has been shown to enhance the accumulation of collagen II and proteoglycans in chondrocyte cultures. Moreover, it inhibited apoptosis that was observed when MSCs were seeded into alginate scaffold with TGF-β_1.[259]

This review of the vast and diverse literature leads to some predictions for optimal tissue engineering of articular cartilage. The most promising cell source would be MSCs, either normal or genetically modified to express member(s) of the TGF superfamily. Photopolymerizable PEGylated fibrinogen is a promising scaffold as it has good initial mechanical properties and can gelate *in siu* under the effect of UVA light. A good

combination of growth factors to promote chondrogenesis and induce the expression of cartilage-specific markers would be TGF (β_2, β_3) plus BMP-2 or -7 in combination with IGF-1. Finally, static mechanical constraint, cyclic tensile loading, or low intensity ultrasound are promising physical stimulants to promote the maturation of a tissue engineering-based cartilage substitute.

5. CONCLUSIONS

Although a wide range of surgical procedures are available for the treatment of articular cartilage lesions, and have been shown to be successful at short and long terms follow-ups, none of them are yet able to restore the function and structure of injured cartilage completely to its original state. Moreover, many of the currently used surgical approaches require revision after deterioration of the initially successful clinical outcome. Ideally, successful cartilaginous constructs should meet the following criteria. First, it should promote the synthesis of the cartilage specific ECM components that will assemble to provide the same architecture as native cartilage ECM. Second, it should integrate completely with the surrounding native tissue. Third, it should have mechanical properties comparable to those of native cartilage to withstand *in vivo* forces. Finally, it should be introduced in a minimally invasive fashion. Of all the aforementioned orthopaedic strategies, tissue engineering is the most promising approach to fulfill these goals. MSCs are a promising cell source, since they can be obtained autologously in a minimally invasive procedure and eliminate the need for primary surgery to harvest chondrocytes. In addition, the phenotype of chondrocytes harvested from the osteoarthritic knee might still reflect the disease state and could lead to degeneration of the tissue-engineered cartilage substitute, while MSCs can be obtained from a young healthy donor. In addition, MSCs can be genetically manipulated to express a wide variety of growth factors and ECM molecules.

Of all the scaffolding materials, photocrosslinkable materials, for instance PEGylated fibrin scaffold (Gelrin C) are the most promising scaffolding material as it can be combined with MSCs and gelate *in situ* under the effect of UV-A light in a minimally invasive procedure. A better knowledge of cell signalling during chondrogenesis is necessary to determine the correct combination of bioactive factors that will promote the proliferation and maturation of the cellular component, initiate the secretion of proper ECM component, and stimulate secretion of enzymes to remodel the supporting matrix. Finally, appropriate mechanical conditions should be determined to promote maturation of the construct *in vivo*, if applicable.

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7. REFERENCES

1. Aigner, T., and Stove, J. Collagens--major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. Adv Drug Deliv Rev.**55**:1569, 2003.

2. Rosowski, M., Falb, M., Tschirschmann, M., and Lauster, R. Initiation of mesenchymal condensation in alginate hollow spheres--a useful model for understanding cartilage repair? Artif Organs **30**:775, 2006.

3. McCormick, F., Yanke, A., Provencher, M.T., and Cole, B.J. Minced articular cartilage-basic science, surgical technique, and clinical application. Sports Med Arthrosc.**16**:217, 2008.

4. Blackburn, T.A., and Craig, E. Knee anatomy: a brief review. Phys Ther.**60**:1556, 1980.

5. Richmond, J.C. Surgery for osteoarthritis of the knee. Rheum Dis Clin North Am.**34**:815, 2008.

6. Bastiaansen-Jenniskens, Y.M., Koevoet, W., de Bart, A.C., van der Linden, J.C., Zuurmond, A.M., Weinans, H., Verhaar, J.A., van Osch, G.J., and Degroot, J. Contribution of collagen network features to functional properties of engineered cartilage. Osteoarthritis Cartilage **16**:359, 2008.

7. Temenoff, J.S., and Mikos, A.G. Review: tissue engineering for regeneration of articular cartilage. Biomaterials **21**:431, 2000.

8. Ahmed, T.A., Griffith, M., and Hincke, M. Characterization and inhibition of fibrin hydrogel-degrading enzymes during development of tissue engineering scaffolds. Tissue Eng.**13**:1469, 2007.

9. Peretti, G.M., Xu, J.W., Bonassar, L.J., Kirchhoff, C.H., Yaremchuk, M.J., and Randolph, M.A. Review of injectable cartilage engineering using fibrin gel in mice and swine models. Tissue Eng.**12**:1151, 2006.

10. Zehbe, R., Libera, J., Gross, U., and Schubert, H. Short-term human chondrocyte culturing on oriented collagen coated gelatine scaffolds for cartilage replacement. Biomed Mater Eng.**15**:445, 2005.

11. Beris, A.E., Lykissas, M.G., Papageorgiou, C.D., and Georgoulis, A.D. Advances in articular cartilage repair. Injury **36 Suppl 4**:S14, 2005.

12. Badley, E.M., and Wang, P.P. Arthritis and the aging population: projections of arthritis prevalence in Canada 1991 to 2031. J Rheumatol.**25**:138, 1998.

13. Wang, P.P., and Badley, E.M. Consistent low prevalence of arthritis in quebec: findings from a provincial variation study in Canada based on several canadian population health surveys. J Rheumatol.**30**:126, 2003.

14. Mehrotra, C., Remington, P.L., Naimi, T.S., Washington, W., and Miller, R. Trends in total knee replacement surgeries and implications for public health, 1990-2000. Public Health Rep.**120**:278, 2005.

15. Detterline, A.J., Goldberg, S., Bach, B.R., Jr., and Cole, B.J. Treatment options for articular cartilage defects of the knee. Orthop Nurs.**24**:361, 2005.

16. Rosneck, J., Higuera, C.A., Tadross, N., Krebs, V., and Barsoum, W.K. Managing knee osteoarthritis before and after arthroplasty. Cleve Clin J Med.**74**:663, 2007.

17. Li, X., Shah, A., Franklin, P., Merolli, R., Bradley, J., and Busconi, B. Arthroscopic debridement of the osteoarthritic knee combined with hyaluronic acid (Orthovisc(R)) treatment: A case series and review of the literature. J Orthop Surg.**3**:43, 2008.

18. Laupattarakasem, W., Laopaiboon, M., Laupattarakasem, P., and Sumananont, C. Arthroscopic debridement for knee osteoarthritis. Cochrane Database Syst Rev.CD005118, 2008.

19. Siparsky, P., Ryzewicz, M., Peterson, B., and Bartz, R. Arthroscopic treatment of osteoarthritis of the knee: are there any evidence-based indications? Clin Orthop Relat Res.**455**:107, 2007.

20. Clarke, H.D., and Scott, W.N. The role of debridement: through small portals. J Arthroplasty **18**:10, 2003.

21. Stuart, M.J., and Lubowitz, J.H. What, if any, are the indications for arthroscopic debridement of the osteoarthritic knee? Arthroscopy **22**:238, 2006.

22. Altman, R.D., and Gray, R. Diagnostic and therapeutic uses of the arthroscope in rheumatoid arthritis and osteoarthritis. Am J Med.**75**:50, 1983.

23. Hunziker, E.B., and Quinn, T.M. Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge. J Bone Joint Surg Am.**85-A Suppl 2**:85, 2003.

24. Day, B. The indications for arthroscopic debridement for osteoarthritis of the knee. Orthop Clin North Am.**36**:413, 2005.

25. McLaren, A.C., Blokker, C.P., Fowler, P.J., Roth, J.N., and Rock, M.G. Arthroscopic debridement of the knee for osteoarthrosis. Can J Surg.**34**:595, 1991.

26. Burkart, A.C., Schoettle, P.B., and Imhoff, A.B. [Surgical therapeutic possibilities of cartilage damage]. Unfallchirurg **104**:798, 2001.

27. Fithian, D.C. A historical perspective of anterior knee pain. Sports Medicine and Arthroscopy Review **9**:273, 2001.

28. Kim, H.K., Moran, M.E., and Salter, R.B. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. J Bone Joint Surg Am.**73**:1301, 1991.

29. Spahn, G., Frober, R., and Linss, W. Treatment of chondral defects by hydro jet. Results of a preliminary scanning electron microscopic evaluation. Arch Orthop Trauma Surg.**126**:223, 2006.

30. Uribe, J.W. The use of radiofrequency devices for chondral debridernent. Sports Medicine and Arthroscopy Review **11**:214, 2003.

31. David, T.S., and Shields, C.L. Radiofrequency and articular cartilage. Tech Knee Surg.**3**:193, 2004.

32. Vangsness, C.T. Radiofrequency use on articular cartilage lesions. Orthop Clin N Am.**36**:427, 2005.

33. Hempfling, H. Intra-articular hyaluronic acid after knee arthroscopy: a two-year study. Knee Surg Sports Traumatol Arthrosc.**15**:537, 2007.

34. Nehrer, S., and Minas, T. Treatment of articular cartilage defects. Invest Radiol.**35**:639, 2000.

35. Jackson, R.W., and Dieterichs, C. The results of arthroscopic lavage and debridement of osteoarthritic knees based on the severity of degeneration: a 4- to 6-year symptomatic follow-up. Arthroscopy **19**:13, 2003.

36. Tanaka, N., Sakahashi, H., Sato, E., Hirose, K., and Ishii, S. Effects of needle-arthroscopic lavage with different volumes of fluid on knee synovitis in rheumatoid arthritis. Clin Rheumatol.**21**:4, 2002.

37. Fu, X., Lin, L., Zhang, J., and Yu, C. Assessment of the efficacy of joint lavage in rabbits with osteoarthritis of the knee. J Orthop Res.**27**:91, 2009.

38. Lutzner, J., Kasten, P., Gunther, K.P., and Kirschner, S. Surgical options for patients with osteoarthritis of the knee. Nat Rev Rheumatol.**5**:309, 2009.

39. Steinwachs, M.R., Guggi, T., and Kreuz, P.C. Marrow stimulation techniques. Injury **39 Suppl 1**:S26, 2008.

40. Johnson, L.L. Arthroscopic abrasion arthroplasty: a review. Clin Orthop Relat Res.S306, 2001.

41. Johnson, L.L. Arthroscopic abrasion arthroplasty historical and pathologic perspective: present status. Arthroscopy **2**:54, 1986.

42. Kocheta, A., Toms, A. Isolated chondral injuries of the knee (diagnosis and treatment). Curr Orthopaed.**18**:154, 2004.

43. Kessler, M.W., Ackerman, G., Dines, J.S., and Grande, D. Emerging technologies and fourth generation issues in cartilage repair. Sports Med Arthrosc.**16**:246, 2008.

44. Anderson, A.F., Richards, D.B., Pagnani, M.J., and Hovis, W.D. Antegrade drilling for osteochondritis dissecans of the knee. Arthroscopy **13**:319, 1997.

45. Mitchell, N., and Shepard, N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg Am.**58**:230, 1976.

46. Bhosale, A.M., and Richardson, J.B. Articular cartilage: structure, injuries and review of management. Brit Med Bull.**87**:77, 2008.

47. Galois, L., Freyria, A.M., Grossin, L., Hubert, P., Mainard, D., Herbage, D., Stoltz, J.F., Netter, P., Dellacherie, E., and Payan, E. Cartilage repair: surgical techniques and tissue engineering using polysaccharide- and collagen-based biomaterials. Biorheology **41**:433, 2004.

48. Ficat, R.P., Ficat, C., Gedeon, P., and Toussaint, J.B. Spongialization: a new treatment for diseased patellae. Clin Orthop Relat Res.74, 1979.

49. Hunziker, E.B. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. Osteoarthritis Cartilage **10**:432, 2002.

50. Grelsamer, R.P., and Stein, D.A. Patellofemoral arthritis. J Bone Joint Surg Am.**88**:1849, 2006.

51. Mina, C., Garrett, W.E., Jr., Pietrobon, R., Glisson, R., and Higgins, L. High tibial osteotomy for unloading osteochondral defects in the medial compartment of the knee. Am J Sports Med.**36**:949, 2008.

52. Tang, W.C., and Henderson, I.J. High tibial osteotomy: long term survival analysis and patients' perspective. Knee **12**:410, 2005.

53. Wright, J.M., Crockett, H.C., Slawski, D.P., Madsen, M.W., and Windsor, R.E. High tibial osteotomy. J Am Acad Orthop Surg.**13**:279, 2005.

54. Miller, T.T. Imaging of knee arthroplasty. Eur J Radiol.**54**:164, 2005.

55. Bert, J.M. Unicompartmental knee replacement. Orthop Clin North Am.**36**:513, 2005.

56. Antoniou, J., Hadjipavlou, A., Enker, P., and Antoniou, A. Unicompartmental knee arthroplasty with patelloplasty. Int Orthop.**20**:94, 1996.

57. Bert, J.M. Unicompartmental arthroplasty for unicompartmental knee arthritis. Tech Knee Surg.**7**:51, 2007.

58. Meek, R.M., Masri, B.A., and Duncan, C.P. Minimally invasive unicompartmental knee replacement: rationale and correct indications. Orthop Clin North Am.**35**:191, 2004.

59. Rees, J.L., Price, A.J., Lynskey, T.G., Svard, U.C., Dodd, C.A., and Murray, D.W. Medial unicompartmental arthroplasty after failed high tibial osteotomy. J Bone Joint Surg Br.**83**:1034, 2001.

60. Berger, R.A., Meneghini, R.M., Jacobs, J.J., Sheinkop, M.B., Della Valle, C.J., Rosenberg, A.G., and Galante, J.O. Results of unicompartmental knee arthroplasty at a minimum of ten years of follow-up. J Bone Joint Surg Am.**87**:999, 2005.

61. O'Neill, T., Jinks, C., and Ong, B.N. Decision-making regarding total knee replacement surgery: a qualitative meta-synthesis. BMC Health Serv Res.**7**:52, 2007.

62. Buly, R.L., and Sculco, T.P. Recent advances in total knee replacement surgery. Curr Opin Rheumatol.**7**:107, 1995.

63. Pavone, V., Boettner, F., Fickert, S., and Sculco, T.P. Total condylar knee arthroplasty: a long-term followup. Clin Orthop Relat Res.**18**, 2001.

64. Rorabeck, C.H., Bourne, R.B., and Nott, L. The cemented kinematic-II and the non-cemented porous-coated anatomic prostheses for total knee replacement. A prospective evaluation. J Bone Joint Surg Am.**70**:483, 1988.

65. Sharkey, P.F., Hozack, W.J., Rothman, R.H., Shastri, S., and Jacoby, S.M. Insall Award paper. Why are total knee arthroplasties failing today? Clin Orthop Relat Res.7, 2002.

66. Gonzalez Della Valle, A., Leali, A., and Haas, S. Etiology and surgical interventions for stiff total knee replacements. Hss J.**3**:182, 2007.

67. Mahomed, N.N., Barrett, J., Katz, J.N., Baron, J.A., Wright, J., and Losina, E. Epidemiology of total knee replacement in the United States Medicare population. J Bone Joint Surg Am.**87**:1222, 2005.

68. Kerker, J.T., Leo, A.J., and Sgaglione, N.A. Cartilage repair: synthetics and scaffolds: basic science, surgical techniques, and clinical outcomes. Sports Med Arthrosc.**16**:208, 2008.

69. Hangody, L., Vasarhelyi, G., Hangody, L.R., Sukosd, Z., Tibay, G., Bartha, L., and Bodo, G. Autologous osteochondral grafting--technique and long-term results. Injury **39 Suppl 1**:S32, 2008.

70. Recht, M., White, L.M., Winalski, C.S., Miniaci, A., Minas, T., and Parker, R.D. MR imaging of cartilage repair procedures. Skeletal Radiol.**32**:185, 2003.

71. Marcacci, M., Kon, E., Delcogliano, M., Filardo, G., Busacca, M., and Zaffagnini, S. Arthroscopic autologous osteochondral grafting for cartilage defects of the knee: prospective study results at a minimum 7-year follow-up. Am J Sports Med.**35**:2014, 2007.

72. Gudas, R., Stankevicius, E., Monastyreckiene, E., Pranys, D., and Kalesinskas, R.J. Osteochondral autologous transplantation versus microfracture for the treatment of articular cartilage defects in the knee joint in athletes. Knee Surg Sports Traumatol Arthrosc.**14**:834, 2006.

73. Williams, R.J., 3rd, Ranawat, A.S., Potter, H.G., Carter, T., and Warren, R.F. Fresh stored allografts for the treatment of osteochondral defects of the knee. J Bone Joint Surg Am.**89**:718, 2007.

74. Aubin, P.P., Cheah, H.K., Davis, A.M., and Gross, A.E. Long-term followup of fresh femoral osteochondral allografts for posttraumatic knee defects. Clin Orthop Relat Res.S318, 2001.

75. Morag, G., Kulidjian, A., Zalzal, P., Shasha, N., Gross, A.E., and Backstein, D. Total knee replacement in previous recipients of fresh osteochondral allograft transplants. J Bone Joint Surg Am.**88**:541, 2006.

76. Hettrich, C.M., Crawford, D., and Rodeo, S.A. Cartilage repair: third-generation cell-based technologies--basic science, surgical techniques, clinical outcomes. Sports Med Arthrosc.**16**:230, 2008.

77. Iwasa, J., Engebretsen, L., Shima, Y., and Ochi, M. Clinical application of scaffolds for cartilage tissue engineering. Knee Surg Sports Traumatol Arthrosc.**17**:561, 2009.

78. Jobanputra, P., Parry, D., Fry-Smith, A., and Burls, A. Effectiveness of autologous chondrocyte transplantation for hyaline cartilage defects in knees: a rapid and systematic review. Health Technol Assess.**5**:1, 2001.

79. Ferruzzi, A., Buda, R., Faldini, C., Vannini, F., Di Caprio, F., Luciani, D., and Giannini, S. Autologous chondrocyte implantation in the knee joint: open compared with arthroscopic technique. Comparison at a minimum follow-up of five years. J Bone Joint Surg Am.**90 Suppl 4**:90, 2008.

80. McNickle, A.G., L'Heureux, D.R., Yanke, A.B., and Cole, B.J. Outcomes of autologous chondrocyte implantation in a diverse patient population. Am J Sports Med.**37**:1344, 2009.

81. Peterson, L., Minas, T., Brittberg, M., Nilsson, A., Sjogren-Jansson, E., and Lindahl, A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. Clin Orthop Relat Res.212, 2000.

82. McNickle, A.G., Provencher, M.T., and Cole, B.J. Overview of existing cartilage repair technology. Sports Med Arthrosc.**16**:196, 2008.

83. Clair, B.L., Johnson, A.R., and Howard, T. Cartilage repair: current and emerging options in treatment. Foot Ankle Spec.**2**:179, 2009.

84. Cascio, B.M., and Sharma, B. The future of cartilage repair. Oper Tech Sports Med.**16**:221, 2008.

85. Ahmed, T.A., Dare, E.V., and Hincke, M. Fibrin: A Versatile Scaffold for Tissue Engineering Applications. Tissue Eng Part B Rev. 2008.

86. Lien, S.M., Ko, L.Y., and Huang, T.J. Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering. Acta Biomater.**5**:670, 2009.

87. Gerard, C., Catuogno, C., Amargier-Huin, C., Grossin, L., Hubert, P., Gillet, P., Netter, P., Dellacherie, E., and Payan, E. The effect of alginate, hyaluronate and hyaluronate derivatives biomaterials on synthesis of non-articular chondrocyte extracellular matrix. J Mater Sci Mater Med.**16**:541, 2005.

88. Iwasaki, N., Yamane, S.T., Majima, T., Kasahara, Y., Minami, A., Harada, K., Nonaka, S., Maekawa, N., Tamura, H., Tokura, S., Shiono, M., Monde, K., and Nishimura, S. Feasibility of polysaccharide hybrid materials for scaffolds in cartilage tissue engineering: evaluation of chondrocyte adhesion to polyion complex fibers prepared from alginate and chitosan. Biomacromolecules **5**:828, 2004.

89. Quinn, T.M., Schmid, P., Hunziker, E.B., and Grodzinsky, A.J. Proteoglycan deposition around chondrocytes in agarose culture: construction of a physical and biological interface for mechanotransduction in cartilage. Biorheology **39**:27, 2002.

90. Ng, K.W., Wang, C.C., Mauck, R.L., Kelly, T.A., Chahine, N.O., Costa, K.D., Ateshian, G.A., and Hung, C.T. A layered agarose approach to fabricate depth-dependent inhomogeneity in chondrocyte-seeded constructs. J Orthop Res.**23**:134, 2005.

91. Chowdhury, T.T., Bader, D.L., Shelton, J.C., and Lee, D.A. Temporal regulation of chondrocyte metabolism in agarose constructs subjected to dynamic compression. Arch Biochem Biophys.**417**:105, 2003.

92. Mauck, R.L., Soltz, M.A., Wang, C.C., Wong, D.D., Chao, P.H., Valhmu, W.B., Hung, C.T., and Ateshian, G.A. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J Biomech Eng.**122**:252, 2000.

93. Wu, M.H., Urban, J.P., Cui, Z.F., Cui, Z., and Xu, X. Effect of extracellular ph on matrix synthesis by chondrocytes in 3D agarose gel. Biotechnol Prog.**23**:430, 2007.

94. Ng, K.W., Saliman, J.D., Lin, E.Y., Statman, L.Y., Kugler, L.E., Lo, S.B., Ateshian, G.A., and Hung, C.T. Culture duration modulates collagen hydrolysate-induced tissue remodeling in chondrocyte-seeded agarose hydrogels. Ann Biomed Eng.**35**:1914, 2007.

95. Mauck, R.L., Seyhan, S.L., Ateshian, G.A., and Hung, C.T. Influence of seeding density and dynamic deformational loading on the developing structure/function relationships of chondrocyte-seeded agarose hydrogels. Ann Biomed Eng.**30**:1046, 2002.

96. Kuo, S.M., Wang, Y.J., Weng, C.L., Lu, H.E., and Chang, S.J. Influence of alginate on type II collagen fibrillogenesis. J Mater Sci Mater Med.**16**:525, 2005.

97. Lee, D.A., Reisler, T., and Bader, D.L. Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared to conventional monolayer techniques. Acta Orthop Scand.**74**:6, 2003.

98. Stevens, M.M., Qanadilo, H.F., Langer, R., and Prasad Shastri, V. A rapid-curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. Biomaterials **25**:887, 2004.

99. Grunder, T., Gaissmaier, C., Fritz, J., Stoop, R., Hortschansky, P., Mollenhauer, J., and Aicher, W.K. Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. Osteoarthritis Cartilage **12**:559, 2004.

100. Yoon, D.M., and Fisher, J.P. Effects of exogenous IGF-1 delivery on the early expression of IGF-1 signaling molecules by alginate embedded chondrocytes. Tissue Eng Part A.**14**:1263, 2008.

101. Gaissmaier, C., Fritz, J., Krackhardt, T., Flesch, I., Aicher, W.K., and Ashammakhi, N. Effect of human platelet supernatant on proliferation and matrix synthesis of human

articular chondrocytes in monolayer and three-dimensional alginate cultures. Biomaterials **26**:1953, 2005.

102. Masuda, K., Pfister, B.E., Sah, R.L., and Thonar, E.J. Osteogenic protein-1 promotes the formation of tissue-engineered cartilage using the alginate-recovered-chondrocyte method. Osteoarthritis Cartilage **14**:384, 2006.

103. Heywood, H.K., Bader, D.L., and Lee, D.A. Glucose concentration and medium volume influence cell viability and glycosaminoglycan synthesis in chondrocyte-seeded alginate constructs. Tissue Eng.**12**:3487, 2006.

104. Choi, B.H., Woo, J.I., Min, B.H., and Park, S.R. Low-intensity ultrasound stimulates the viability and matrix gene expression of human articular chondrocytes in alginate bead culture. J Biomed Mater Res A.**79**:858, 2006.

105. Lee, H.J., Choi, B.H., Min, B.H., Son, Y.S., and Park, S.R. Low-intensity ultrasound stimulation enhances chondrogenic differentiation in alginate culture of mesenchymal stem cells. Artif Organs **30**:707, 2006.

106. Williams, G.M., Klein, T.J., and Sah, R.L. Cell density alters matrix accumulation in two distinct fractions and the mechanical integrity of alginate-chondrocyte constructs. Acta Biomater.**1**:625, 2005.

107. Heywood, H.K., Sembi, P.K., Lee, D.A., and Bader, D.L. Cellular utilization determines viability and matrix distribution profiles in chondrocyte-seeded alginate constructs. Tissue Eng.**10**:1467, 2004.

108. Lindenhayn, K., Perka, C., Spitzer, R., Heilmann, H., Pommerening, K., Mennicke, J., and Sittinger, M. Retention of hyaluronic acid in alginate beads: aspects for *in vitro* cartilage engineering. J Biomed Mater Res.**44**:149, 1999.

109. Xu, J., Wang, W., Ludeman, M., Cheng, K., Hayami, T., Lotz, J.C., and Kapila, S. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Eng Part A.**14**:667, 2008.

110. Park, Y., Sugimoto, M., Watrin, A., Chiquet, M., and Hunziker, E.B. BMP-2 induces the expression of chondrocyte-specific genes in bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. Osteoarthritis Cartilage **13**:527, 2005.

111. Lee, K.Y., Jeong, L., Kang, Y.O., Lee, S.J., and Park, W.H. Electrospinning of polysaccharides for regenerative medicine. Adv Drug Deliv Rev.**61**:1020, 2009.

112. Di Martino, A., Sittinger, M., and Risbud, M.V. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials **26**:5983, 2005.

113. Lahiji, A., Sohrabi, A., Hungerford, D.S., and Frondoza, C.G. Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. J Biomed Mater Res.**51**:586, 2000.

114. Hoemann, C.D., Sun, J., Legare, A., McKee, M.D., and Buschmann, M.D. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. Osteoarthritis Cartilage **13**:318, 2005.

115. Li, Z., and Zhang, M. Chitosan-alginate as scaffolding material for cartilage tissue engineering. J Biomed Mater Res A.**75**:485, 2005.

116. Yamane, S., Iwasaki, N., Majima, T., Funakoshi, T., Masuko, T., Harada, K., Minami, A., Monde, K., and Nishimura, S. Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering. Biomaterials **26**:611, 2005.

117. Hsu, S.H., Whu, S.W., Hsieh, S.C., Tsai, C.L., Chen, D.C., and Tan, T.S. Evaluation of chitosan-alginate-hyaluronate complexes modified by an RGD-containing protein as tissue-engineering scaffolds for cartilage regeneration. Artif Organs **28**:693, 2004.

118. Donati, I., Stredanska, S., Silvestrini, G., Vetere, A., Marcon, P., Marsich, E., Mozetic, P., Gamini, A., Paoletti, S., and Vittur, F. The aggregation of pig articular chondrocyte and synthesis of extracellular matrix by a lactose-modified chitosan. Biomaterials **26**:987, 2005.

119. Yan, J., Qi, N., and Zhang, Q. Rabbit articular chondrocytes seeded on collagen-chitosan-GAG scaffold for cartilage tissue engineering *in vivo*. Artif Cells Blood Substit Immobil Biotechnol.**35**:333, 2007.

120. Lee, J.E., Kim, K.E., Kwon, I.C., Ahn, H.J., Lee, S.H., Cho, H., Kim, H.J., Seong, S.C., and Lee, M.C. Effects of the controlled-released TGF-beta 1 from chitosan microspheres on chondrocytes cultured in a collagen/chitosan/glycosaminoglycan scaffold. Biomaterials **25**:4163, 2004.

121. Chen, Y.L., Lee, H.P., Chan, H.Y., Sung, L.Y., Chen, H.C., and Hu, Y.C. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. Biomaterials **28**:2294, 2007.

122. Kuo, Y.C., and Lin, C.Y. Effect of genipin-crosslinked chitin-chitosan scaffolds with hydroxyapatite modifications on the cultivation of bovine knee chondrocytes. Biotechnol Bioeng.**95**:132, 2006.

123. Abe, M., Takahashi, M., Tokura, S., Tamura, H., and Nagano, A. Cartilage-scaffold composites produced by bioresorbable beta-chitin sponge with cultured rabbit chondrocytes. Tissue Eng.**10**:585, 2004.

124. Cho, J.H., Kim, S.H., Park, K.D., Jung, M.C., Yang, W.I., Han, S.W., Noh, J.Y., and Lee, J.W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. Biomaterials **25**:5743, 2004.

125. Marcacci, M., Berruto, M., Brocchetta, D., Delcogliano, A., Ghinelli, D., Gobbi, A., Kon, E., Pederzini, L., Rosa, D., Sacchetti, G.L., Stefani, G., and Zanasi, S. Articular cartilage engineering with Hyalograft C: 3-year clinical results. Clin Orthop Relat Res.96, 2005.

126. Banu, N., and Tsuchiya, T. Markedly different effects of hyaluronic acid and chondroitin sulfate-A on the differentiation of human articular chondrocytes in micromass and 3-D honeycomb rotation cultures. J Biomed Mater Res A.**80**:257, 2007.

127. Nettles, D.L., Vail, T.P., Morgan, M.T., Grinstaff, M.W., and Setton, L.A. Photocrosslinkable hyaluronan as a scaffold for articular cartilage repair. Ann Biomed Eng.**32**:391, 2004.

128. Chung, C., Erickson, I.E., Mauck, R.L., and Burdick, J.A. Differential behavior of auricular and articular chondrocytes in hyaluronic acid hydrogels. Tissue Eng Part A.**14**:1121, 2008.

129. Kayakabe, M., Tsutsumi, S., Watanabe, H., Kato, Y., and Takagishi, K. Transplantation of autologous rabbit BM-derived mesenchymal stromal cells embedded in hyaluronic acid gel sponge into osteochondral defects of the knee. Cytotherapy **8**:343, 2006.

130. Solchaga, L.A., Gao, J., Dennis, J.E., Awadallah, A., Lundberg, M., Caplan, A.I., and Goldberg, V.M. Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. Tissue Eng.**8**:333, 2002.

131. Allison, D.D., and Grande-Allen, K.J. Review. Hyaluronan: a powerful tissue engineering tool. Tissue Eng.**12**:2131, 2006.

132. Almond, A. Hyaluronan. Cell Mol Life Sci.**64**:1591, 2007.

133. Grigolo, B., Roseti, L., Fiorini, M., Fini, M., Giavaresi, G., Aldini, N.N., Giardino, R., and Facchini, A. Transplantation of chondrocytes seeded on a hyaluronan derivative (hyaff-11) into cartilage defects in rabbits. Biomaterials **22**:2417, 2001.

134. Trattnig, S., Pinker, K., Krestan, C., Plank, C., Millington, S., and Marlovits, S. Matrix-based autologous chondrocyte implantation for cartilage repair with HyalograftC: two-year follow-up by magnetic resonance imaging. Eur J Radiol.**57**:9, 2006.

135. Nehrer, S., Domayer, S., Dorotka, R., Schatz, K., Bindreiter, U., and Kotz, R. Three-year clinical outcome after chondrocyte transplantation using a hyaluronan matrix for cartilage repair. Eur J Radiol.**57**:3, 2006.

136. Radice, M., Brun, P., Cortivo, R., Scapinelli, R., Battaliard, C., and Abatangelo, G. Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. J Biomed Mater Res.**50**:101, 2000.

137. Gao, J., Dennis, J.E., Solchaga, L.A., Goldberg, V.M., and Caplan, A.I. Repair of osteochondral defect with tissue-engineered two-phase composite material of injectable calcium phosphate and hyaluronan sponge. Tissue Eng.**8**:827, 2002.

138. Tognana, E., Borrione, A., De Luca, C., and Pavesio, A. Hyalograft C: hyaluronan-based scaffolds in tissue-engineered cartilage. Cells Tissues Organs **186**:97, 2007.

139. Stark, Y., Suck, K., Kasper, C., Wieland, M., van Griensven, M., and Scheper, T. Application of collagen matrices for cartilage tissue engineering. Exp Toxicol Pathol.**57**:305, 2006.

140. Chen, G., Sato, T., Ushida, T., Hirochika, R., Shirasaki, Y., Ochiai, N., and Tateishi, T. The use of a novel PLGA fiber/collagen composite web as a scaffold for engineering of articular cartilage tissue with adjustable thickness. J Biomed Mater Res A.**67**:1170, 2003.

141. Chen, G., Sato, T., Ushida, T., Ochiai, N., and Tateishi, T. Tissue engineering of cartilage using a hybrid scaffold of synthetic polymer and collagen. Tissue Eng.**10**:323, 2004.

142. Yates, K.E., Allemann, F., and Glowacki, J. Phenotypic analysis of bovine chondrocytes cultured in 3D collagen sponges: effect of serum substitutes. Cell Tissue Bank.**6**:45, 2005.

143. Freyria, A.M., Cortial, D., Ronziere, M.C., Guerret, S., and Herbage, D. Influence of medium composition, static and stirred conditions on the proliferation of and matrix protein expression of bovine articular chondrocytes cultured in a 3-D collagen scaffold. Biomaterials **25**:687, 2004.

144. Wakitani, S., Goto, T., Young, R.G., Mansour, J.M., Goldberg, V.M., and Caplan, A.I. Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. Tissue Eng.**4**:429, 1998.

145. Willers, C., Chen, J., Wood, D., Xu, J., and Zheng, M.H. Autologous chondrocyte implantation with collagen bioscaffold for the treatment of osteochondral defects in rabbits. Tissue Eng.**11**:1065, 2005.

146. Dorotka, R., Bindreiter, U., Macfelda, K., Windberger, U., and Nehrer, S. Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair. Osteoarthritis Cartilage **13**:655, 2005.

147. Malicev, E., Radosavljevic, D., and Velikonja, N.K. Fibrin gel improved the spatial uniformity and phenotype of human chondrocytes seeded on collagen scaffolds. Biotechnol Bioeng.**96**:364, 2007.

148. Fujisato, T., Sajiki, T., Liu, Q., and Ikada, Y. Effect of basic fibroblast growth factor on cartilage regeneration in chondrocyte-seeded collagen sponge scaffold. Biomaterials **17**:155, 1996.

149. Sato, M., Ishihara, M., Ishihara, M., Kaneshiro, N., Mitani, G., Nagai, T., Kutsuna, T., Asazuma, T., Kikuchi, M., and Mochida, J. Effects of growth factors on heparin-carrying polystyrene-coated atelocollagen scaffold for articular cartilage tissue engineering. J Biomed Mater Res B Appl Biomater.**83**:181, 2007.

150. Weiser, L., Bhargava, M., Attia, E., and Torzilli, P.A. Effect of serum and platelet-derived growth factor on chondrocytes grown in collagen gels. Tissue Eng.**5**:533, 1999.

151. Hunter, C.J., Imler, S.M., Malaviya, P., Nerem, R.M., and Levenston, M.E. Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels. Biomaterials **23**:1249, 2002.

152. Schulz, R.M., Zscharnack, M., Hanisch, I., Geiling, M., Hepp, P., and Bader, A. Cartilage tissue engineering by collagen matrix associated bone marrow derived mesenchymal stem cells. Biomed Mater Eng.**18**:S55, 2008.

153. Dorotka, R., Windberger, U., Macfelda, K., Bindreiter, U., Toma, C., and Nehrer, S. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials **26**:3617, 2005.

154. Cen, L., Liu, W., Cui, L., Zhang, W., and Cao, Y. Collagen tissue engineering: development of novel biomaterials and applications. Pediatr Res.**63**:492, 2008.

155. Glowacki, J., and Mizuno, S. Collagen scaffolds for tissue engineering. Biopolymers **89**:338, 2008.

156. Chajra, H., Rousseau, C.F., Cortial, D., Ronziere, M.C., Herbage, D., Mallein-Gerin, F., and Freyria, A.M. Collagen-based biomaterials and cartilage engineering. Application to osteochondral defects. Biomed Mater Eng.**18**:S33, 2008.

157. Freyria, A.M., Ronziere, M.C., Cortial, D., Galois, L., Hartmann, D., Herbage, D., and Mallein-Gerin, F. Comparative phenotypic analysis of articular chondrocytes cultured within type I or type II collagen scaffolds. Tissue Eng Part A.**15**:1233, 2009.

158. Buma, P., Pieper, J.S., van Tienen, T., van Susante, J.L., van der Kraan, P.M., Veerkamp, J.H., van den Berg, W.B., Veth, R.P., and van Kuppevelt, T.H. Cross-linked type I and type II collagenous matrices for the repair of full-thickness articular cartilage defects--a study in rabbits. Biomaterials **24**:3255, 2003.

159. Lee, C.R., Grodzinsky, A.J., Hsu, H.P., and Spector, M. Effects of a cultured autologous chondrocyte-seeded type II collagen scaffold on the healing of a chondral defect in a canine model. J Orthop Res.**21**:272, 2003.

160. Lee, C.R., Grodzinsky, A.J., and Spector, M. Biosynthetic response of passaged chondrocytes in a type II collagen scaffold to mechanical compression. J Biomed Mater Res A.**64**:560, 2003.

161. Shields, K.J., Beckman, M.J., Bowlin, G.L., and Wayne, J.S. Mechanical properties and cellular proliferation of electrospun collagen type II. Tissue Eng.**10**:1510, 2004.

162. Vickers, S.M., Squitieri, L.S., and Spector, M. Effects of cross-linking type II collagen-GAG scaffolds on chondrogenesis *in vitro*: dynamic pore reduction promotes cartilage formation. Tissue Eng.**12**:1345, 2006.

163. Veilleux, N., and Spector, M. Effects of FGF-2 and IGF-1 on adult canine articular chondrocytes in type II collagen-glycosaminoglycan scaffolds *in vitro*. Osteoarthritis Cartilage **13**:278, 2005.

164. Ko, C.S., Huang, J.P., Huang, C.W., and Chu, I.M. Type II collagen-chondroitin sulfate-hyaluronan scaffold cross-linked by genipin for cartilage tissue engineering. J Biosci Bioeng.**107**:177, 2009.

165. Pieper, J.S., van der Kraan, P.M., Hafmans, T., Kamp, J., Buma, P., van Susante, J.L., van den Berg, W.B., Veerkamp, J.H., and van Kuppevelt, T.H. Crosslinked type II collagen matrices: preparation, characterization, and potential for cartilage engineering. Biomaterials **23**:3183, 2002.

166. Vinatier, C., Gauthier, O., Masson, M., Malard, O., Moreau, A., Fellah, B.H., Bilban, M., Spaethe, R., Daculsi, G., and Guicheux, J. Nasal chondrocytes and fibrin sealant for cartilage tissue engineering. J Biomed Mater Res A.**89**:176, 2009.

167. Hunter, C.J., Mouw, J.K., and Levenston, M.E. Dynamic compression of chondrocyte-seeded fibrin gels: effects on matrix accumulation and mechanical stiffness. Osteoarthritis Cartilage **12**:117, 2004.

168. Perka, C., Spitzer, R.S., Lindenhayn, K., Sittinger, M., and Schultz, O. Matrix-mixed culture: new methodology for chondrocyte culture and preparation of cartilage transplants. J Biomed Mater Res.**49**:305, 2000.

169. Almqvist, K.F., Wang, L., Wang, J., Baeten, D., Cornelissen, M., Verdonk, R., Veys, E.M., and Verbruggen, G. Culture of chondrocytes in alginate surrounded by fibrin gel: characteristics of the cells over a period of eight weeks. Ann Rheum Dis.**60**:781, 2001.

170. Chou, C.H., Cheng, W.T., Kuo, T.F., Sun, J.S., Lin, F.H., and Tsai, J.C. Fibrin glue mixed with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular

cartilage tissue engineering: the results of real-time polymerase chain reaction. J Biomed Mater Res A.**82**:757, 2007.

171. Lee, C.R., Grad, S., Gorna, K., Gogolewski, S., Goessl, A., and Alini, M. Fibrin-polyurethane composites for articular cartilage tissue engineering: a preliminary analysis. Tissue Eng.**11**:1562, 2005.

172. Eyrich, D., Wiese, H., Maier, G., Skodacek, D., Appel, B., Sarhan, H., Tessmar, J., Staudenmaier, R., Wenzel, M.M., Goepferich, A., and Blunk, T. *In vitro* and *in vivo* cartilage engineering using a combination of chondrocyte-seeded long-term stable fibrin gels and polycaprolactone-based polyurethane scaffolds. Tissue Eng.**13**:2207, 2007.

173. Park, S.H., Park, S.R., Chung, S.I., Pai, K.S., and Min, B.H. Tissue-engineered cartilage using fibrin/hyaluronan composite gel and its *in vivo* implantation. Artif Organs.**29**:838, 2005.

174. Handl, M., Trc, T., Hanus, M., Stastny, E., Fricova-Poulova, M., Neuwirth, J., Adler, J., Havranova, D., and Varga, F. [Therapy of severe chondral defects of the patella by autologous chondrocyte implantation]. Acta Chir Orthop Traumatol Cech.**73**:373, 2006.

175. Visna, P., Pasa, L., Hart, R., Kocis, J., Cizmar, I., and Adler, J. [Treatment of deep chondral defects of the knee using autologous chondrocytes cultured on a support--results after one year]. Acta Chir Orthop Traumatol Cech.**70**:356, 2003.

176. Visna, P., Pasa, L., Cizmar, I., Hart, R., and Hoch, J. Treatment of deep cartilage defects of the knee using autologous chondrograft transplantation and by abrasive techniques--a randomized controlled study. Acta Chir Belg.**104**:709, 2004.

177. Barker, T.H., Fuller, G.M., Klinger, M.M., Feldman, D.S., and Hagood, J.S. Modification of fibrinogen with poly(ethylene glycol) and its effects on fibrin clot characteristics. J Biomed Mater Res.**56**:529, 2001.

178. Temenoff, J.S., Athanasiou, K.A., LeBaron, R.G., and Mikos, A.G. Effect of poly(ethylene glycol) molecular weight on tensile and swelling properties of oligo(poly(ethylene glycol) fumarate) hydrogels for cartilage tissue engineering. J Biomed Mater Res.**59**:429, 2002.

179. Redwan el, R.M. Animal-derived pharmaceutical proteins. J Immunoassay Immunochem.**30**:262, 2009.

180. Goodstone, N.J., Cartwright, A., and Ashton, B. Effects of high molecular weight hyaluronan on chondrocytes cultured within a resorbable gelatin sponge. Tissue Eng.**10**:621, 2004.

181. Ibusuki, S., Fujii, Y., Iwamoto, Y., and Matsuda, T. Tissue-engineered cartilage using an injectable and *in situ* gelable thermoresponsive gelatin: fabrication and *in vitro* performance. Tissue Eng.**9**:371, 2003.

182. Ibusuki, S., Iwamoto, Y., and Matsuda, T. System-engineered cartilage using poly(N-isopropylacrylamide)-grafted gelatin as *in situ*-formable scaffold: *in vivo* performance. Tissue Eng.**9**:1133, 2003.

183. Hoshikawa, A., Nakayama, Y., Matsuda, T., Oda, H., Nakamura, K., and Mabuchi, K. Encapsulation of chondrocytes in photopolymerizable styrenated gelatin for cartilage tissue engineering. Tissue Eng.**12**:2333, 2006.

184. Chang, C.H., Lin, F.H., Lin, C.C., Chou, C.H., and Liu, H.C. Cartilage tissue engineering on the surface of a novel gelatin-calcium-phosphate biphasic scaffold in a double-chamber bioreactor. J Biomed Mater Res B Appl Biomater.**71**:313, 2004.

185. Chang, C.H., Liu, H.C., Lin, C.C., Chou, C.H., and Lin, F.H. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. Biomaterials.**24**:4853, 2003.

186. Chang, C.H., Kuo, T.F., Lin, C.C., Chou, C.H., Chen, K.H., Lin, F.H., and Liu, H.C. Tissue engineering-based cartilage repair with allogenous chondrocytes and gelatin-chondroitin-hyaluronan tri-copolymer scaffold: A porcine model assessed at 18, 24, and 36 weeks. Biomaterials **27**:1876, 2006.

187. Young, S., Wong, M., Tabata, Y., and Mikos, A.G. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J Control Release **109**:256, 2005.

188. Munirah, S., Kim, S.H., Ruszymah, B.H., and Khang, G. The use of fibrin and poly(lactic-co-glycolic acid) hybrid scaffold for articular cartilage tissue engineering: an *in vivo* analysis. Eur Cell Mater.**15**:41, 2008.

189. Zwingmann, J., Mehlhorn, A.T., Sudkamp, N., Stark, B., Dauner, M., and Schmal, H. Chondrogenic differentiation of human articular chondrocytes differs in biodegradable PGA/PLA scaffolds. Tissue Eng.**13**:2335, 2007.

190. Lu, L., Zhu, X., Pederson, L.G., Jabbari, E., Currier, B., O'Driscoll, S., and Yaszemski, M. Effects of dynamic fluid pressure on chondrocytes cultured in biodegradable poly(glycolic acid) fibrous scaffolds. Tissue Eng.**11**:1852, 2005.

191. Kon, E., Delcogliano, M., Filardo, G., Montaperto, C., and Marcacci, M. Second generation issues in cartilage repair. Sports Med Arthrosc.**16**:221, 2008.

192. Erggelet, C., Kreuz, P.C., Mrosek, E.H., Schagemann, J.C., Lahm, A., Ducommun, P.P., and Ossendorf, C. Autologous chondrocyte implantation versus ACI using 3D-bioresorbable graft for the treatment of large full-thickness cartilage lesions of the knee. Arch Orthop Trauma Surg. 2009.

193. Kreuz, P.C., Muller, S., Ossendorf, C., Kaps, C., and Erggelet, C. Treatment of focal degenerative cartilage defects with polymer-based autologous chondrocyte grafts: four-year clinical results. Arthritis Res Ther.**11**:R33, 2009.

194. Chu, C.R., Coutts, R.D., Yoshioka, M., Harwood, F.L., Monosov, A.Z., and Amiel, D. Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study. J Biomed Mater Res.**29**:1147, 1995.

195. Pulliainen, O., Vasara, A.I., Hyttinen, M.M., Tiitu, V., Valonen, P., Kellomaki, M., Jurvelin, J.S., Peterson, L., Lindahl, A., Kiviranta, I., and Lammi, M.J. Poly-L-D-lactic acid scaffold in the repair of porcine knee cartilage lesions. Tissue Eng.**13**:1347, 2007.

196. Gong, Y., Zhou, Q., Gao, C., and Shen, J. *In vitro* and *in vivo* degradability and cytocompatibility of poly(l-lactic acid) scaffold fabricated by a gelatin particle leaching method. Acta Biomater.**3**:531, 2007.

197. Tamai, N., Myoui, A., Hirao, M., Kaito, T., Ochi, T., Tanaka, J., Takaoka, K., and Yoshikawa, H. A new biotechnology for articular cartilage repair: subchondral implantation of a composite of interconnected porous hydroxyapatite, synthetic polymer (PLA-PEG), and bone morphogenetic protein-2 (rhBMP-2). Osteoarthritis Cartilage **13**:405, 2005.

198. Shin, H.J., Lee, C.H., Cho, I.H., Kim, Y.J., Lee, Y.J., Kim, I.A., Park, K.D., Yui, N., and Shin, J.W. Electrospun PLGA nanofiber scaffolds for articular cartilage reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation *in vitro*. J Biomater Sci Polym Ed.**17**:103, 2006.

199. Mercier, N.R., Costantino, H.R., and Tracy, M.A., Bonassar, L.J. Poly(lactide-co-glycolide) microspheres as a moldable scaffold for cartilage tissue engineering. Biomaterials **26**:1945, 2005.

200. Kang, S.W., Jeon, O., and Kim, B.S. Poly(lactic-co-glycolic acid) microspheres as an injectable scaffold for cartilage tissue engineering. Tissue Eng.**11**:438, 2005.

201. Kang, S.W., Yoon, J.R., Lee, J.S., Kim, H.J., Lim, H.W., Lim, H.C., Park, J.H., and Kim, B.S. The use of poly(lactic-co-glycolic acid) microspheres as injectable cell carriers for cartilage regeneration in rabbit knees. J Biomater Sci Polym Ed.**17**:925, 2006.

202. Han, S.H., Kim, Y.H., Park, M.S., Kim, I.A., Shin, J.W., Yang, W.I., Jee, K.S., Park, K.D., Ryu, G.H., and Lee, J.W. Histological and biomechanical properties of regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold *in vivo*. J Biomed Mater Res A.**87**:850, 2008.

203. Park, G.E., Pattison, M.A., Park, K., and Webster, T.J. Accelerated chondrocyte functions on NaOH-treated PLGA scaffolds. Biomaterials **26**:3075, 2005.

204. Savaiano, J.K., and Webster, T.J. Altered responses of chondrocytes to nanophase PLGA/nanophase titania composites. Biomaterials **25**:1205, 2004.

205. Sha'ban, M., Kim, S.H., Idrus, R.B., and Khang, G. Fibrin and poly(lactic-co-glycolic acid) hybrid scaffold promotes early chondrogenesis of articular chondrocytes: an *in vitro* study. J Orthop Surg.**3**:17, 2008.

206. Yoo, H.S., Lee, E.A., Yoon, J.J., and Park, T.G. Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. Biomaterials **26**:1925, 2005.

207. Lind, M., Larsen, A., Clausen, C., Osther, K., and Everland, H. Cartilage repair with chondrocytes in fibrin hydrogel and MPEG polylactide scaffold: an *in vivo* study in goats. Knee Surg Sports Traumatol Arthrosc.**16**:690, 2008.

208. Hsu, S.H., Chang, S.H., Yen, H.J., Whu, S.W., Tsai, C.L., and Chen, D.C. Evaluation of biodegradable polyesters modified by type II collagen and Arg-Gly-Asp as tissue engineering scaffolding materials for cartilage regeneration. Artif Organs **30**:42, 2006.

209. Smith, R.C., Spalding, T., and Masri, B.A. Trufit polymer scaffolds for the management of cartilage defects of the knee. Tech Knee Surg.;**8**:136, 2009.

210. Lu, J.M., Wang, X., Marin-Muller, C., Wang, H., Lin, P.H., Yao, Q., and Chen, C. Current advances in research and clinical applications of PLGA-based nanotechnology. Expert Rev Mol Diagn.**9**:325, 2009.

211. Bryant, S.J., Arthur, J.A., and Anseth, K.S. Incorporation of tissue-specific molecules alters chondrocyte metabolism and gene expression in photocrosslinked hydrogels. Acta Biomater.**1**:243, 2005.

212. Bryant, S.J., Chowdhury, T.T., Lee, D.A., Bader, D.L., and Anseth, K.S. Crosslinking density influences chondrocyte metabolism in dynamically loaded photocrosslinked poly(ethylene glycol) hydrogels. Ann Biomed Eng.**32**:407, 2004.

213. Bryant, S.J., Bender, R.J., Durand, K.L., and Anseth, K.S. Encapsulating chondrocytes in degrading PEG hydrogels with high modulus: engineering gel structural changes to facilitate cartilaginous tissue production. Biotechnol Bioeng.**86**:747, 2004.

214. Bryant, S.J., Anseth, K.S., Lee, D.A., and Bader, D.L. Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain. J Orthop Res.**22**:1143, 2004.

215. Nguyen, K.T., and West, J.L. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials **23**:4307, 2002.

216. Elisseeff, J., McIntosh, W., Anseth, K., Riley, S., Ragan, P., and Langer, R. Photoencapsulation of chondrocytes in poly(ethylene oxide)-based semi-interpenetrating networks. J Biomed Mater Res.**51**:164, 2000.

217. Buxton, A.N., Zhu, J., Marchant, R., West, J.L., Yoo, J.U., and Johnstone, B. Design and characterization of poly(ethylene glycol) photopolymerizable semi-

interpenetrating networks for chondrogenesis of human mesenchymal stem cells. Tissue Eng.**13**:2549, 2007.

218. Bryant, S.J., Durand, K.L., and Anseth, K.S. Manipulations in hydrogel chemistry control photoencapsulated chondrocyte behavior and their extracellular matrix production. J Biomed Mater Res A.**67**:1430, 2003.

219. Malda, J., Woodfield, T.B., van der Vloodt, F., Wilson, C., Martens, D.E., Tramper, J., van Blitterswijk, C.A., and Riesle, J. The effect of PEGT/PBT scaffold architecture on the composition of tissue engineered cartilage. Biomaterials **26**:63, 2005.

220. Holland, T.A., Bodde, E.W., Baggett, L.S., Tabata, Y., Mikos, A.G., and Jansen, J.A. Osteochondral repair in the rabbit model utilizing bilayered, degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds. J Biomed Mater Res A.**75**:156, 2005.

221. Taguchi, T., Xu, L., Kobayashi, H., Taniguchi, A., Kataoka, K., and Tanaka, J. Encapsulation of chondrocytes in injectable alkali-treated collagen gels prepared using poly(ethylene glycol)-based 4-armed star polymer. Biomaterials **26**:1247, 2005.

222. Ifkovits, J.L., and Burdick, J.A. Review: photopolymerizable and degradable biomaterials for tissue engineering applications. Tissue Eng.**13**:2369, 2007.

223. Song, L., Baksh, D., and Tuan, R.S. Mesenchymal stem cell-based cartilage tissue engineering: cells, scaffold and biology. Cytotherapy **6**:596, 2004.

224. Kessler, M.W., and Grande, D.A. Tissue engineering and cartilage. Organogenesis **4**:28, 2008.

225. Isogai, N., Kusuhara, H., Ikada, Y., Ohtani, H., Jacquet, R., Hillyer, J., Lowder, E., and Landis, W.J. Comparison of different chondrocytes for use in tissue engineering of cartilage model structures. Tissue Eng.**12**:691, 2006.

226. Tuan, R.S., Boland, G., and Tuli, R. Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res Ther.**5**:32, 2003.

227. Leo, A.J., and Grande, D.A. Mesenchymal stem cells in tissue engineering. Cells Tissues Organs **183**:112, 2006.

228. Kim, H.J., and Im, G.I. Combination of transforming growth factor-beta2 and bone morphogenetic protein 7 enhances chondrogenesis from adipose tissue-derived mesenchymal stem cells. Tissue Eng Part A.**15**:1543, 2009.

229. Xiang, Y., Zheng, Q., Jia, B.B., Huang, G.P., Xu, Y.L., Wang, J.F., and Pan, Z.J. Ex vivo expansion and pluripotential differentiation of cryopreserved human bone marrow mesenchymal stem cells. J Zhejiang Univ Sci B.**8**:136, 2007.

230. Kurth, T., Hedbom, E., Shintani, N., Sugimoto, M., Chen, F.H., Haspl, M., Martinovic, S., and Hunziker, E.B. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. Osteoarthritis Cartilage **15**:1178, 2007.

231. Yasuda, A., Kojima, K., Tinsley, K.W., Yoshioka, H., Mori, Y., and Vacanti, C.A. *In vitro* culture of chondrocytes in a novel thermoreversible gelation polymer scaffold containing growth factors. Tissue Eng.**12**:1237, 2006.

232. Im, G.I., Jung, N.H., and Tae, S.K. Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. Tissue Eng.**12**:527, 2006.

233. Takagi, M., Umetsu, Y., Fujiwara, M., and Wakitani, S. High inoculation cell density could accelerate the differentiation of human bone marrow mesenchymal stem cells to chondrocyte cells. J Biosci Bioeng.**103**:98, 2007.

234. Kafienah, W., Mistry, S., Dickinson, S.C., Sims, T.J., Learmonth, I., and Hollander, A.P. Three-dimensional cartilage tissue engineering using adult stem cells from osteoarthritis patients. Arthritis Rheum.**56**:177, 2007.

235. Hennig, T., Lorenz, H., Thiel, A., Goetzke, K., Dickhut, A., Geiger, F., and Richter, W. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol.**211**:682, 2007.

236. Sekiya, I., Larson, B.L., Vuoristo, J.T., Reger, R.L., and Prockop, D.J. Comparison of effect of BMP-2, -4, and -6 on *in vitro* cartilage formation of human adult stem cells from bone marrow stroma. Cell Tissue Res.**320**:269, 2005.

237. Schmitt, B., Ringe, J., Haupl, T., Notter, M., Manz, R., Burmester, G.R., Sittinger, M., and Kaps, C. BMP2 initiates chondrogenic lineage development of adult human mesenchymal stem cells in high-density culture. Differentiation **71**:567, 2003.

238. Indrawattana, N., Chen, G., Tadokoro, M., Shann, L.H., Ohgushi, H., Tateishi, T., Tanaka, J., and Bunyaratvej, A. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. Biochem Biophys Res Commun.**320**:914, 2004.

239. Kafienah, W., Mistry, S., Perry, M.J., Politopoulou, G., and Hollander, A.P. Pharmacological regulation of adult stem cells: chondrogenesis can be induced using a synthetic inhibitor of the retinoic acid receptor. Stem Cells **25**:2460, 2007.

240. Kim, H.T., Zaffagnini, S., Mizuno, S., Abelow, S., and Safran, M.R. A peek into the possible future of management of articular cartilage injuries: gene therapy and scaffolds for cartilage repair. J Orthop Sports Phys Ther.**36**:765, 2006.

241. Steinert, A.F., Noth, U., and Tuan, R.S. Concepts in gene therapy for cartilage repair. Injury.**39 Suppl 1**:S97, 2008.

242. Saraf, A., and Mikos, A.G. Gene delivery strategies for cartilage tissue engineering. Adv Drug Deliv Rev.**58**:592, 2006.

243. Grande, D.A., Mason, J., Light, E., and Dines, D. Stem cells as platforms for delivery of genes to enhance cartilage repair. J Bone Joint Surg Am.**85-A Suppl 2**:111, 2003.

244. Guo, X., Zheng, Q., Yang, S., Shao, Z., Yuan, Q., Pan, Z., Tang, S., Liu, K., and Quan, D. Repair of full-thickness articular cartilage defects by cultured mesenchymal stem cells transfected with the transforming growth factor beta1 gene. Biomed Mater.**1**:206, 2006.

245. Hao, J., Yao, Y., Varshney, R.R., Wang, L., Prakash, C., Li, H., and Wang, D.A. Gene transfer and living release of transforming growth factor-beta3 for cartilage tissue engineering applications. Tissue Eng Part C Methods **14**:273, 2008.

246. Palmer, G.D., Steinert, A., Pascher, A., Gouze, E., Gouze, J.N., Betz, O., Johnstone, B., Evans, C.H., and Ghivizzani, S.C. Gene-induced chondrogenesis of primary mesenchymal stem cells *in vitro*. Mol Ther.**12**:219, 2005.

247. Babister, J.C., Tare, R.S., Green, D.W., Inglis, S., Mann, S., and Oreffo, R.O. Genetic manipulation of human mesenchymal progenitors to promote chondrogenesis using "bead-in-bead" polysaccharide capsules. Biomaterials **29**:58, 2008.

248. Sun, X.D., Jeng, L., Bolliet, C., Olsen, B.R., and Spector, M. Non-viral endostatin plasmid transfection of mesenchymal stem cells via collagen scaffolds. Biomaterials **30**:1222, 2009.

249. Song, S.U., Cha, Y.D., Han, J.U., Oh, I.S., Choi, K.B., Yi, Y., Hyun, J.P., Lee, H.Y., Chi, G.F., Lim, C.L., Ganjei, J.K., Noh, M.J., Kim, S.J., Lee, D.K., and Lee, K.H.

Hyaline cartilage regeneration using mixed human chondrocytes and transforming growth factor-beta1- producing chondrocytes. Tissue Eng.**11**:1516, 2005.

250. Goomer, R.S., Deftos, L.J., Terkeltaub, R., Maris, T., Lee, M.C., Harwood, F.L., and Amiel, D. High-efficiency non-viral transfection of primary chondrocytes and perichondrial cells for ex-vivo gene therapy to repair articular cartilage defects. Osteoarthritis Cartilage **9**:248, 2001.

251. Chen, H.C., Sung, L.Y., Lo, W.H., Chuang, C.K., Wang, Y.H., Lin, J.L., and Hu, Y.C. Combination of baculovirus-expressed BMP-2 and rotating-shaft bioreactor culture synergistically enhances cartilage formation. Gene Ther.**15**:309, 2008.

252. Kaps, C., Bramlage, C., Smolian, H., Haisch, A., Ungethum, U., Burmester, G.R., Sittinger, M., Gross, G., and Haupl, T. Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction. Arthritis Rheum.**46**:149, 2002.

253. Madry, H., Padera, R., Seidel, J., Langer, R., Freed, L.E., Trippel, S.B., Vunjak- and Novakovic, G. Gene transfer of a human insulin-like growth factor I cDNA enhances tissue engineering of cartilage. Hum Gene Ther.**13**:1621, 2002.

254. Thibault, M., Poole, A.R., and Buschmann, M.D. Cyclic compression of cartilage/bone explants *in vitro* leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. J Orthop Res.**20**:1265, 2002.

255. Quinn, T.M., Maung, A.A., Grodzinsky, A.J., Hunziker, E.B., and Sandy, J.D. Physical and biological regulation of proteoglycan turnover around chondrocytes in cartilage explants. Implications for tissue degradation and repair. Ann N Y Acad Sci.**878**:420, 1999.

256. Miyanishi, K., Trindade, M.C., Lindsey, D.P., Beaupre, G.S., Carter, D.R., Goodman, S.B., Schurman, D.J., and Smith, R.L. Effects of hydrostatic pressure and transforming growth factor-beta 3 on adult human mesenchymal stem cell chondrogenesis *in vitro*. Tissue Eng.**12**:1419, 2006.

257. McMahon, L.A., Reid, A.J., Campbell, V.A., and Prendergast, P.J. Regulatory effects of mechanical strain on the chondrogenic differentiation of MSCs in a collagen-GAG scaffold: experimental and computational analysis. Ann Biomed Eng.**36**:185, 2008.

258. Ebisawa, K., Hata, K., Okada, K., Kimata, K., Ueda, M., Torii, S., and Watanabe, H. Ultrasound enhances transforming growth factor beta-mediated chondrocyte differentiation of human mesenchymal stem cells. Tissue Eng.**10**:921, 2004.

259. Lee, H.J., Choi, B.H., Min, B.H., and Park, S.R. Low-intensity ultrasound inhibits apoptosis and enhances viability of human mesenchymal stem cells in three-dimensional alginate culture during chondrogenic differentiation. Tissue Eng.**13**:1049, 2007.

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V. CHAPTER 2. FIBRIN: A VERSATILE SCAFFOLD FOR TISSUE ENGINEERING APLLICATIONS.

In chapter 1, the different and most widely applied strategies to repair and restore the function of knee articular damage after injury were discussed. We also discussed tissue engineering strategies, where three essential components are required for successful tissue engineering-based cartilage replacement including the productive component (cell source), conductive component (carrier/scaffolding matrix), and inductive component (bioactive factors). Finding the optimum carrier/scaffolding matrix that combines the desired features is a critical requirement for any given tissue engineering application. Alternatively, researchers have evaluated carrier-free approaches to eliminate complications associated with the carrier matrix such as integration and rate of degradation, in order to promote optimum tissue regeneration, particularly for cartilage restoration (Jubel A et al., 2008). Fibrin gels, as a promising tissue engineering carrier matrix, combine many advantages; however, they also have several disadvantages.

Chapter two provides the reader with in-depth details about fibrin as a bio-polymeric scaffold including its origin and formation, its important characteristics as a scaffolding material, different techniques used to enhance its features, different manipulation techniques that maximize its utilization and its tissue engineering application for a wide variety of soft and hard tissues. The review was written by myself with significant contribution of my colleague Emma V. Dare, Ph.D. who is currently a post doctoral fellow in Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto.

Manuscript 2

Fibrin: A Versatile Scaffold for Tissue Engineering Applications

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1. ABSTRACT

Tissue engineering combines cell and molecular biology with materials and mechanical engineering to replace damaged or diseased organs and tissues. Fibrin is a critical blood component responsible for hemostasis, which has been used extensively as a biopolymer scaffold in tissue engineering. In this review we summarize the latest developments in organ and tissue regeneration using fibrin as the scaffold material. Commercially available fibrinogen and thrombin are combined to form a fibrin hydrogel. The incorporation of bioactive peptides and growth factors via a heparin-binding delivery system improves the functionality of fibrin as a scaffold. New technologies such as inkjet printing and magnetically influenced self-assembly can alter the geometry of the fibrin structure into appropriate and predictable forms. Fibrin can be prepared from autologous plasma, and is available as glue or as engineered microbeads. Fibrin alone or in combination with other materials has been used as a biological scaffold for stem or primary cells to regenerate adipose tissue, bone, cardiac tissue, cartilage, liver, nervous tissue, ocular tissue, skin, tendons, and ligaments. Thus, fibrin is a versatile biopolymer, which shows a great potential in tissue regeneration and wound healing.

Running title: Fibrin scaffolds for tissue engineering.

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2. INTRODUCTION

The loss or damage of organs and tissue due to aging or pathological conditions is a major human health problem. Tissue engineering, a new field in biomedical sciences, combines cellular and molecular biology on one hand and material and mechanical engineering on the other to provide an alternative to organ and tissue transplants that face a limited supply of donor organs.[1,2]

Current tissue engineering techniques utilize any combination of three critical components: a cellular component, biocompatible and mechanically stable carrier vehicle/matrix scaffold, and a bioactive component. The cellular component should consist of healthy, viable cells that are accessible, manipulable, and nonimmunogenic. The carrier component has a dual function, acting as both a delivery vehicle and a matrix scaffold. The bioactive component should promote differentiation and maturation of the cellular component.[3–6] Three approaches have been established to regenerate tissues by tissue engineering. The first approach is to deliver a bioactive factor/scaffold combination that induces migration, proliferation, and differentiation of cells from the surrounding tissue.[7] The second approach is to transplant cells microencapsulated within a semipermeable membrane with subsequent partial restoration of tissue functions.[8] The third approach, which is most widely used now, aims to transplant cells into a three-dimensional supporting biodegradable matrix, which becomes capable of replacing the functions of the pathologically altered tissues.[9,10]

Tissue engineering generally requires an artificial extracellular matrix (ECM) (scaffold) in which the cells can proliferate and differentiate with subsequent new tissue generation. The scaffold must be biodegradable and allow reasonable cell adhesion. It should also provide sufficient mechanical support to withstand *in vivo* forces.[11] A broad

array of synthetic polymers (polyglycolic acids (PGA), polylactic acid (PLA), copolymers of glycolic and lactic acids, polyurethanes, polyhydroxybutyrate (PH4B), polyanhydrides and polyortho esters), and natural polymers (collagen, fibrin, glycosaminoglycans, and chitosan) have been used as scaffolds in tissue engineering.[2,7,12,13]

Hydrogels are a class of biomaterials that have great scaffolding potential in many tissue engineering applications due to their high tissue-like water content, high biocompatibility in general, mechanical properties that parallel the properties of soft tissues, efficient transport of nutrients and waste, powerful ability to uniformly encapsulate cells, and ability to be injected as a liquid that gels *in situ*.[13-15] Hydrogels, which are based on water-soluble components, are either chemically or physically cross-linked; depending on their chemistry, they can be either degradable or nondegradable.[14] Chemically cross-linked hydrogels can be polymerized using a chemical initiator[16] or photoinitiator. The photoinitiated hydrogels use initiators that are inactive until they are exposed to light of a particular wavelength.[17] In comparison to the most widely used scaffolds, fibrin gels combine some important advantages such as high seeding efficiency and uniform cell distribution.[18] In addition, fibrin has adhesion capabilities.[11] Further, it can be produced from the patient's own blood and used as an autologous scaffold without the potential risk of foreign body reaction or infection.[2]

Fibrin is a biopolymer of the monomer fibrinogen. The fibrinogen molecule is composed of two sets of three polypeptide chains named Aα, Bβ, and γ, which are joined together by six disulfide bridges.[19] Fibrin is formed after thrombin-mediated cleavage of fibrinopeptide A from the Aα chains and fibrinopeptide B from the Bβ chains,[20] with subsequent conformational changes and exposure of polymerization sites. This generates the fibrin monomer that has a great tendency to self-associate and form insoluble fibrin.

Further, the blood coagulation factor XIIIa is a transglutaminase that rapidly cross-links γ chains in the fibrin polymer[21] by introducing intermolecular ξ-(γ-glutamyl) lysine covalent bonds between the lysine of one γ-chain and glutamine of the other.[15] This covalent cross-linking produces a stable fibrin network that is resistant to protease degradation.[22] This effect can be reinforced by introducing chemical cross-linker such as genipin.[23]

Fibrin and fibrinogen have critical roles in blood clotting, fibrinolysis, cellular and matrix interactions, the inflammatory response, wound healing, and neoplasia.[20] Fibrin has been used clinically as a hemostatic agent in cardiac, liver, and spleen surgery. In addition, it can be used in surgery for patients with hemophilia. Further, fibrin is useful as a sealant in a variety of clinical applications, including procedures such as colonic anastomosis as well as in seroma prevention following soft tissue dissection. Moreover, it has been used to reduce suture vascular and intestinal anastomosis, to promote fistula closure, and in laparoscopic/endoscopic procedures.[24] A number of allogeneic fibrin sealants such as Tisseel®, Evicel™, and Crosseal™ have been approved by the Food and Drug Administration (FDA) for clinical use as hemostatic agents. However, this review will be restricted to discussion of the manipulation of fibrin for tissue engineering applications. The most widely used forms of fibrin scaffolds are fibrin hydrogels, fibrin glue, and fibrin microbeads (FMBs).

3. FIBRIN HYDROGEL

Fibrin hydrogels are constructed from commercially purified allogeneic fibrinogen and purified thrombin.[25] Fibrin hydrogels have been used widely in the last decade in a variety of tissue engineering applications, and these include tissue engineering of adipose,[26] cardiaovascular,[2,27-30] ocular,[31-33] muscle,[34-37] liver,[38,39] skin,[40,41] cartilage,[42-47] and bone tissues.[48,49] In addition, fibrin hydrogels have applications for promoting angiogenesis[50-52]

and enhancing neurite extension.[22,53,54] The fibrin hydrogel as a potential scaffold has three major disadvantages: the shrinkage of the gel that happens during the formation of flat sheets, low mechanical stiffness, and its rapid degradation before the proper formation of tissue-engineered structures.[2,28] Gel shrinkage can be prevented by incorporating a fixing agent such as poly-L-lysine into the fibrin gel during the culturing period.[2] In order to improve the low mechanical stiffness for some tissue engineering applications, fibrin hydrogels can be combined with other scaffold materials to obtain constructs with desired mechanical strength. Examples of materials used for preparing composite scaffolds are polyurethane,[55] polycaprolactone-based polyurethane,[56] polycaprolactone,[57] β-tricalciumphosphate (β-TCP),[58] β-tricalciumphosphate/ploycaprolactone (β-TCP/PCL),[59] and polyethylene glycol.[60]

Most commercial preparations of fibrinogen usually contain other plasma proteins such as fibronectin, growth factors, enzymes, enzyme inhibitors, and proenzymes.[61] In addition, serum plasminogen is usually a component of the *in vitro* culture medium.[2] Further, plasminogen and matrix metalloproteinases may be secreted from the encapsulated cells inside the fibrin hydrogel.[25] These are all factors that contribute to the rapid degradation of the fibrin hydrogel, which is usually observed. The stability of the fibrin hydrogel can be prolonged using a number of strategies. One approach is to concomitantly optimize pH and the concentrations of fibrinogen and calcium ion (Ca^{2+}).[42] Another strategy is to use FMBs, a highly cross-linked, dense, and denatured three dimensional fibrin matrix.[62,63] Alternatively, fibrin can be modified with a molecule such as polyethylene glycol, which renders the fibrin structure more stable.[64] The fourth strategy is to reduce the cell density.[46] And finally protease inhibitors that are specific for plasmin and matrix metalloproteinases are often added to the *in vitro* culturing media,[2,25] or

alternatively, the plasmin inhibitor, aprotinin, can be immobilized into the fibrin hydrogel.[65] An allied strategy is to modify the fibrin hydrogel to introduce additional characteristics that enhance its use in tissue engineering applications. This approach is described in more detail in the following section.

3.1. Incorporation of biologically active peptides into fibrin hydrogels

The usefulness of the fibrin hydrogels in many tissue engineering applications can be extended further by incorporating the biological activity of other proteins, such as those derived from the ECM proteins—fibronectin, vitronectin, laminin, and collagen. The peptide domains responsible for the biological activity of these proteins (bioactive domains) can be synthesized for covalent cross-linking to the fibrin hydrogel through a transglutaminase-catalyzed reaction.[22] The bioactive domain peptide is coupled to a transglutaminase substrate sequence (NQEQVSP) to generate a bifunctional peptide or bi-domain peptide. A variety of bioactive adhesion domains have been synthesized to incorporate the transglutaminase substrate sequence: these include IKVAV, YIGSR or RNIAEIIKDI derived from laminin, DGEA derived from collagen, and RGD derived from many ECM proteins (Fig. 1).[22,66] By extension of this concept, a heparin-binding domain can be synthesized with addition of the transglutaminase substrate sequence for subsequent crosslinking to fibrin. The heparin-binding domain is derived from antithrombin III (K(βA)FAKLAARLYRKA), modified antithrombin III (R(βA)FARLAARLYRRA), neural cell adhesion molecule (KHKGRDVILKKDVR), or platelet factor-4 (YKKIIKKL). The heparin-binding domain binds heparin, which is then available to bind a wide variety of heparin-binding growth factors (HBGFs). This constitutes the heparin-binding delivery system, which enables sequestered growth factors to be slowly released by a cell-mediated degradation of the reservoir, such that release occurs primarily in response to cellular

activity instead of by simple diffusion (Fig. 1). The release of growth factors is regulated by heparinase and plasmin enzyme activities.[53,67] Heparin binds a wide variety of growth factors, which are selected to provide suitable inductive cues, including acidic and basic fibroblast growth factor (aFGF, bFGF), transforming growth factor-β1,β2 (TGF-β1,β2), neurotrophin-3 (NT-3), brain-derived neurotrophic factor, and nerve growth factor (NGF).[53,67–70] Although some growth factors such as NGF bind heparin with lower affinity than bFGF, NGF delivered from heparin-binding delivery system is more effective than soluble NGF in promoting neurite extension of dorsal root ganglia (DRGs).[67,69]

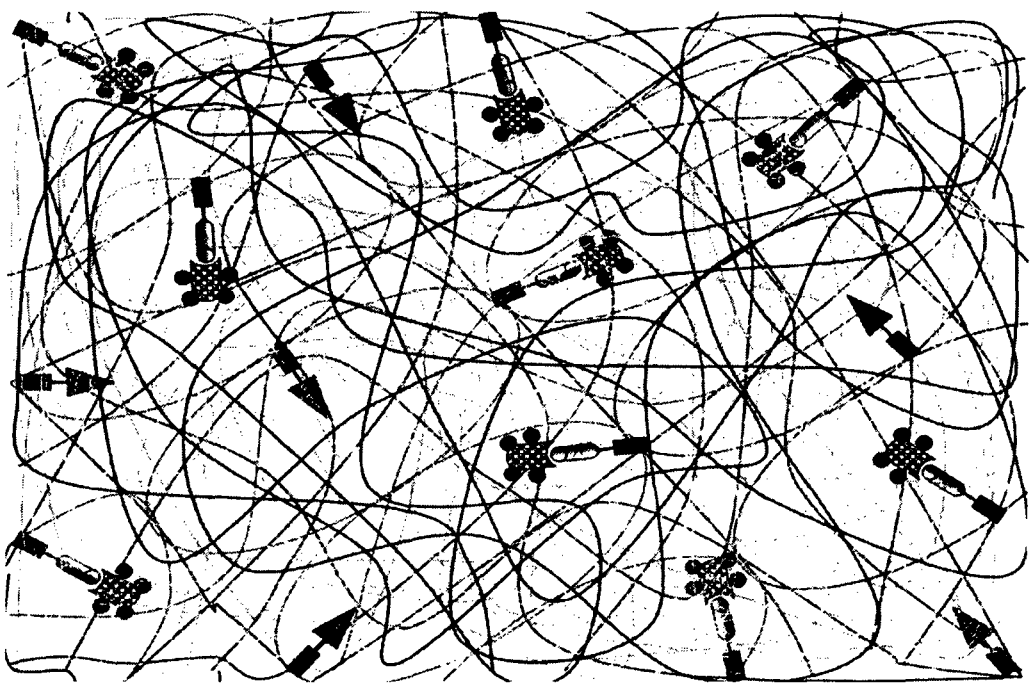

FIG. 1. Cartoon depicting incorporation of active biological peptides into fibrin gel.

3.2. Fibrin hydrogel inkjet printing

Inkjet printing technology has been used recently in tissue engineering, as it offers a practical and efficient tool to dependably handle and dispense biological and/or material elements in a tunable manner to generate cellular and tissue structures, or even organ

analogs.[71,72] Proteins,[73,74] growth factors,[75] and even whole viable cells[71,76] can be deposited with the inkjet printing method mainly with the aim of constructing cell patterns. In this technique, fibrin is used as a printable hydrogel to build three-dimensional constructs. Printing of thrombin onto fibrinogen leads to geometric-specific cross-linking and enables the rapid construction of three-dimensional fibrin scaffolds with specific structures and forms.[71]

3.3. Magnetically guided self-assembled fibrin hydrogel

Fibrin gels with defined architecture on the nanometer scale can be fabricated using magnetic forces. Active thrombin is chemically cross-linked to the surface of magnetic microbeads that are positioned in a defined two-dimensional array by a magnetic field. The geometric patterns guide the self-assembly of fibrin fibrils through localized catalytic cleavage of soluble fibrinogen substrate (Fig. 2).[77]

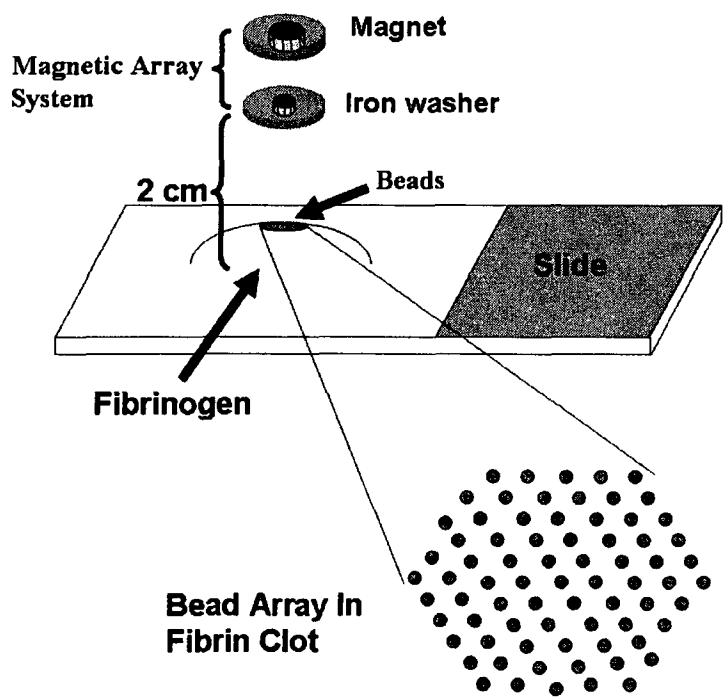

FIG. 2. Magnetically guided self-assembly of fibrin hydrogel using active thrombin that is chemically cross-linked to the surface of magnetic microbeads.

4. FIBRIN GLUE

Fibrin glue is clearly distinguished from fibrin hydrogels that are prepared from purified fibrinogen and thrombin. Fibrin glue, also referred to as fibrin sealant or fibrin tissue adhesive, has been widely used as a helper tool in many surgical fields within the last three decades because of its capability to promote hemostasis and tissue bonding.[78] Further, fibrin glue accelerates wound healing, reduces blood loss, and protects against bacterial infection.[79] Fibrin glue is usually obtained after the fractionation of pooled plasma and consists primarily of two components-enriched fibrinogen (also known as cryoprecipitate) and thrombin.[78] The cryoprecipitate often contains factor XIIIa and fibronectin in addition to fibrinogen, while the thrombin component sometimes contains $CaCl_2$ and antifibrinolytic drugs.[80] Fibrin glue can be prepared either from allogeneic pooled plasma such as commercially available Tissucol/Tisseel®, Beriplast®, and Quixil™, or from autologous plasma that is collected from the patient prior to surgery and processed using devices such as Cryoseal®-FS or Vivostat®.[79,81,82] Although commercially produced fibrin glue is available in standardized quality, autologous fibrin glue has two major advantages: the possibility of viral transmission and prion infection is greatly reduced, and the cost is lower.[81-85] The total cost of 10 mL autologous fibrin glue obtained by cryoprecipitation, including the material cost, was estimated in 2005 to be around $50, while 1 mL of commercial fibrin glue costs approximately $200.[86]

Fibrinogen is the major component of fibrin glue, and several techniques have been developed for its isolation. The first technique is the cryoprecipitation of fresh-frozen plasma or stored plasma. After freezing at -80 °C for at least 12 h, then thawing for several hours at 4 °C, plasma is centrifuged and the supernatant is discarded. The remaining yellow precipitate of fibrinogen is resuspended in small volume of the plasma supernatant.[87,88] The

second technique depends on the Cell Saver equipment, which is a centrifugal blood concentrator that concentrates the blood by removing plasma from whole blood.[89,90] The physical separation of cellular components by the centrifugal field is based on the differences in density and particle size.[91] The third technique is chemical precipitation of fibrinogen using agents such as ethanol, polyethylene glycol, or ammonium sulfate.[92] The last technique is the chromatographic method in which plasma is passed through a column containing ligand immobilized to a matrix such as agarose.[93] The fibrinogen in the plasma binds to the ligand with high affinity and then is selectively eluted from the column. Many ligands have high affinity for fibrinogen such as heparin,[94] protamine,[95] insolubilized fibrin, and fibrin monomer.[96] Cryoprecipitation, chemical precipitation, and chromatographic separation can be conducted in the laboratory.

Fibrin glue for tissue engineering applications has two functions: it serves as a delivery vehicle and as a scaffolding matrix.[4-6] Fibrin glue in combination with an appropriate cell source has been used in a variety of tissue engineering applications, including maxillofacial bone,[97,98] periodontal bone,[99] bone,[100-103] ear cartilage,[104-106] cartilage,[107-109] cornea,[110] heart,[111] blood vessel,[112] tendon, and ligament regeneration.[113-115] In addition, fibrin glue has been used to promote the healing of severe burns and chronic wounds.[116-118] Further, fibrin glue is widely used to affix a variety of tissue-engineered constructs at the implantation site due to its adhesive properties.[119-123] The two components of fibrin glue can be mixed and processed in different ways.[80,104,114,124] The spray application is more powerful in the treatment of extensive wounds, where the two components are injected separately into a continuous compressed air jet (Fig. 3).[124] The spray system delivers an even and fine layer of rapidly polymerized fibrin onto the wound surface.[125] The co-application of fibrin glue with cells using the spray system has many

advantages. It creates a thin and homogenous film of fibrin glue, coating extensive surfaces with a small amount of fibrin glue and localizing the proliferating cells to the wound.[124,126]

Fibrin glue can be modified to enhance its mechanical strength by incorporating other polymers such as gelatin, hyaluronic acid, and chondroitin-6-sulfate.[127] Further, heparin can be incorporated to potentiate the effects of HBGFs on cell proliferation and migration. Heparin binds HBGFs and sequesters them inside the fibrin glue with subsequent slow release of growth factors. Binding of HBGFs to heparin protects them from thrombin-induced degradation.[128-131]

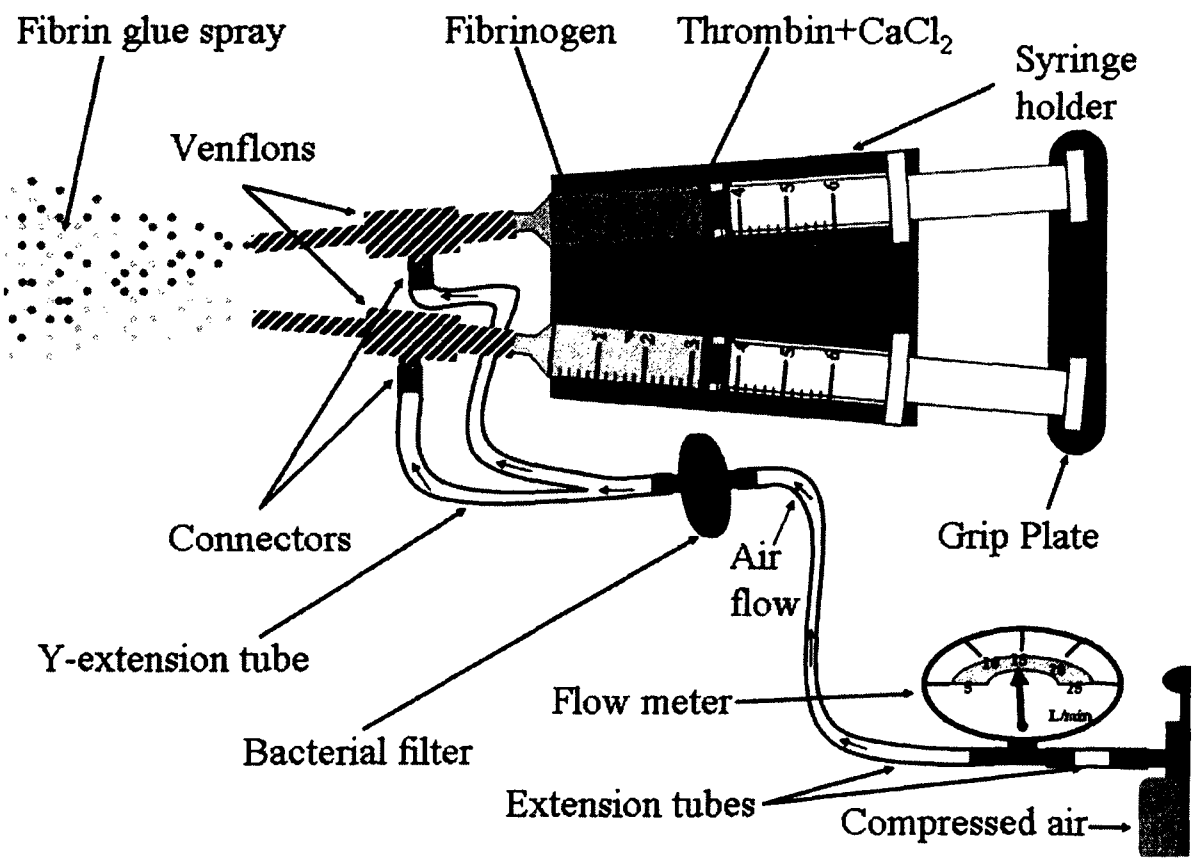

FIG. 3. Spray applicator of fibrin glue. The two components of the fibrin glue at room temperature are injected separately into continuous compressed air jet to form a thin even layer of cross-linked fibrin.

5. FIBRIN MICROBEADS

FMBs are small spherical dense beads with a diameter ranging from 50 to 250 microns that consist of highly condensed and cross-linked fibrin. FMBs are produced from plasma fibrinogen obtained by fractionation.[132] The fibrinogen is mixed with thrombin, and the activated fibrin is immediately and quickly stirred in a heated oil emulsion (corn oil: isooctane, 1:1; 75 °C) to yield spherical droplets that are further cross-linked into solid beads (Fig. 4).[62,133] Fibrinogen denatures above 50 °C due to the instability of the D-domain, whereas factor XIIIa is much more stable and can cross-link proteins at higher temperatures. Denatured fibrinogen in the FMB is greatly haptotactic to mesenchymal-type cells, such as endothelial cells, smooth muscle cells, and fibroblasts.[133] FMBs have been used widely to isolate and grow mesenchymal stem cells from both bone marrow and blood.[62,63,132,134] Kidney gene and cell therapy has been tested *in vitro* using FMBs as a three-dimensional platform, since a variety of transduced renal cells grow and differentiate in this material.[134] Further, FMBs in combination with the appropriate cell source can be used in bone regeneration and wound healing.[132,135]

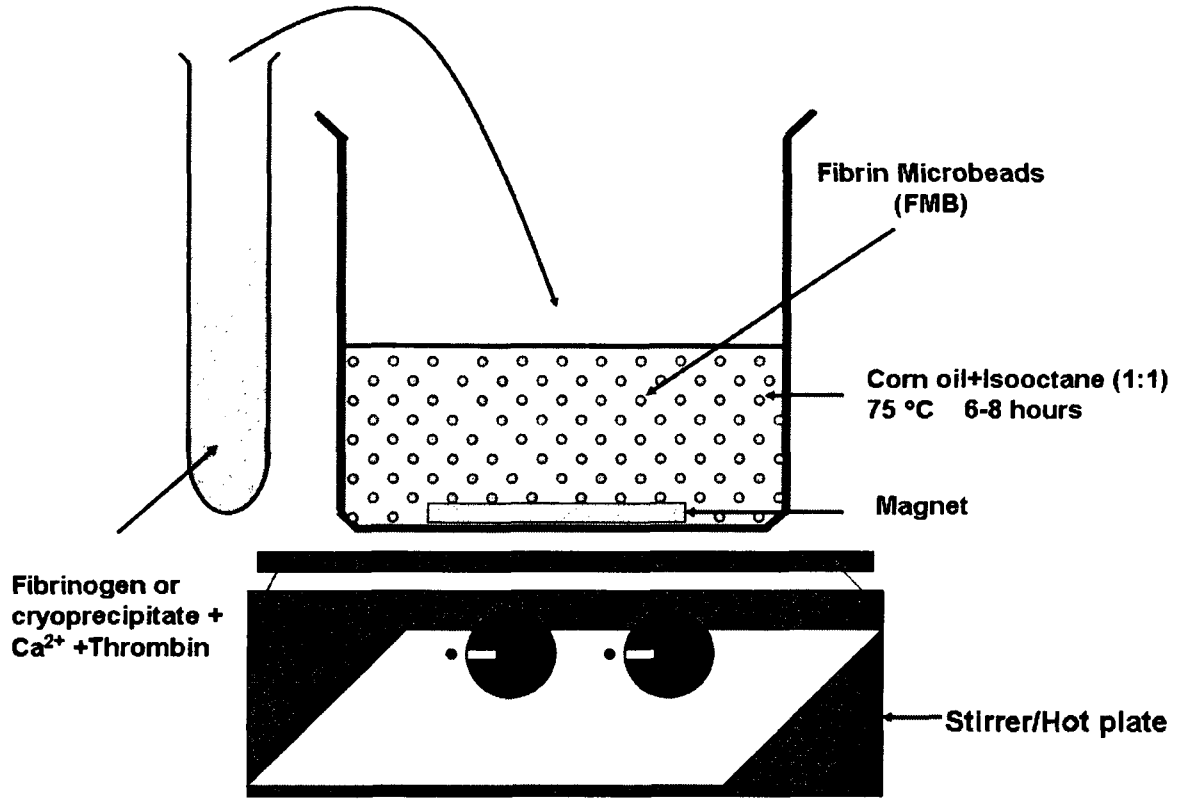

FIG. 4. Formation of fibrin microbeads (FMBs). Fibrinogen, in conjugation with factor XIIIa, is mixed with thrombin, and the activated fibrin is immediately and quickly stirred in heated oil emulsion (75 °C) to yield spherical denatured droplets that are further cross-linked into solid beads by heat-stable transglutaminase.

6. TISSUE ENGINEERING APPLICATIONS OF FIBRIN

6.1. Adipose tissue

Many synthetic and natural materials have been evaluated clinically for reconstruction of missing soft tissue due to mastectomy or lumpectomy in plastic and reconstructive surgery, but the results are frequently not satisfactory. For examples, silicone may elicit immune reactions in the recipient tissue, and collagen scaffolds shrink *in vivo*. Autologous adipose tissue has been used as a replacement; however, this strategy failed to maintain a stable volume.[136] In the tissue engineering approach to regenerate

adipose tissue, fibrin hydrogels have been evaluated using commercial plasminogen-free fibrinogen, factor XIIIa, and thrombin to encapsulate preadipocytes, which are a feasible cell source for adipose tissue regeneration.[26,136] Although, scaffolds such as poly (lactic-co-glycolic acid) and collagen have been used in combination with preadipocytes to regenerate adipose tissue in rodent models, the volume of the regenerated tissue failed to be maintained over a long implantation period due to a rapid and unpredictable resorption rate.[137-139] Fat tissue is a highly vascularized tissue; therefore, the formation of an efficient microcirculatory network in a cell–fibrin graft should allow the *in vivo* engineering of persistent three-dimensional implants. In support of this concept, highly vascularized fibrin matrix containing preadipocytes cells in a chorioallantoic membrane experimental model was volume stable.[140] Basic FGF was found to enhance both adipogenesis and neovascularization of the newly formed tissue in fibrin-preadipocyte constructs cultured in adipogenic media prior to implantation into athymic mice.[26] This finding suggests that the heparin-binding delivery system approach could yield superior results. Another approach is to mechanically stabilize the space, for example, using a dome-shaped support structure for suitable regeneration of volume-stable adipose tissue. Preadipocytes mixed with fibrin matrix (space-filling matrix) were injected under such a dome structure fabricated from PGA and poly(L-lactic acid) and implanted subcutaneously in athymic mice. The volume of the newly formed adipose tissues was maintained for at least 6 weeks. This study demonstrated that volume-stable adipose tissues can be engineered *in vivo* using mechanical support structures, and it offers the potential for augmentation of adipose tissues with volume conservation.[136] In another strategy to address the problem, fibrin-preadipocyte constructs were implanted into a pre-prepared capsule in the recipient rats. The capsule was created by implanting a silicone block into the recipient tissue, which

induced capsule formation prior to the implantation of fibrin-adipocyte constructs. Volume retention was demonstrated in implanted areas up to 1 year after implantation. Using a prefabricated capsule pouch as the recipient site and fibrin glue as a transport vehicle seems to be a reliable and safe source of material for adipose tissue augmentation.[139]

6.2. Bone tissue

In the field of orthopedics, tissue engineering has been used in attempts to treat a variety of bone defects that are a result of traumatic injury, as well as those that arise during bone development. Fibrin has been used alone or in combination as a scaffold for bone regeneration. In promising animal studies, rabbit autologous fibrin beads were used to treat defects created in the proximal tibial physis of New Zealand white rabbits.[141] The fibrin beads were created by combining fibrin with alginate. After gel formation the alginate was removed and the construct was seeded with perichondrial cells and implanted into the defect. After 12 weeks it was found that the bones with untreated tibial defects were shorter and more deformed than those treated with the fibrin bead implant. This represents a possible future treatment for partial arrest of physeal growth. Platelet-rich fibrin glue has also been used as a scaffold for bone tissue engineering.[101] Platelet-rich fibrin gels have a high concentration of platelets that release growth factors and bioactive proteins to initiate and accelerate tissue repair and regeneration.[142] When the platelet-rich fibrin glue was mixed with bone marrow mesenchymal stem cells and bone morphogenic protein-2 and injected subcutaneously into nude mice, nodules containing bone tissue formed after 12 weeks.[101] Platelet-rich plasma mixed with fibrin glue and mesenchymal stem cells has been implanted into mandible bone defects along with dental implants to augment bone volume in dogs.[99] Much greater osseointegration was observed with the fibrin glue implant than in controls without any graft material. The results of these studies

indicate that platelet-rich fibrin glue may be a suitable scaffold to enhance healing and integration of implants with bone. Fibrin glue has also been combined with other materials, such as hyaluronic acid and hydroxyapatite, for improved mechanical, adhesive, and biological properties of cellular scaffolds.[143,144] The combination of calcium phosphate granules with fibrin glue leads to a porous, mineralized ultrastructure, which may provide favorable osteogenic properties for rapid healing of bone defects. The combination of these materials is predicted to improve mechanical properties compared to the ceramic alone, and enhances angiogenesis, cell attachment, and proliferation.[145] Another approach, autologous bone fragments seeded into fibrin glue and mixed with β-tricalcium phosphate, efficiently regenerated cranial bone *in situ* in dogs.[146]

6.3. Cardiac tissue

Congestive heart failure can be treated by several therapeutic strategies; however, ischemic or scarred myocardium resulting from congestive heart failure can lead to abnormal heart function.[147] Cardiac tissue engineering may provide an alternative treatment to replace the damaged tissue. Fibrin has been used as a scaffold for the regeneration of myocardial tissue and heart valves. In one approach, a neonatal cardiac rat myocytes were suspended within a fibrin gel and implanted into silicone tubing. The construct was surgically placed around the femoral artery and vein of adult rats. After 3 weeks *in vivo*, the tissue within the silicone chamber resembled normal myocardial tissue.[27] Tissue-engineered heart valves have many advantages over mechanical and bioprosthetic valves such as the ability to adapt to a growing patient, better durability, and low thrombogenicity.[148] Williams et al.[149] encapsulated human dermal fibroblasts or porcine valve interstitial cells into adherent fibrin gel disks. After 5 weeks of *in vitro* culture both cell types were able to remodel fibrin and deposit collagen and elastin. Mechanical

stimulation of moulded fibrin gel scaffolds seeded with arterial cells was shown to enhance deposition of ECM proteins, including type I and III collagen. This study indicated that dynamic conditioning of autologous fibrin-based scaffolds may be necessary for tissue engineering of heart valves.[150] Fibrin as a cell carrier has also been used in combination with polycaprolactone for aortic valve regeneration. Fibrin with knitted polycaprolactone provided good load-bearing properties and permitted cellular ingrowth. Both materials were found to be biocompatible and durable.[57] Therefore, fibrin scaffolds show promise for this application, since the complex valve shape can be cast from fibrin using an injection moulding technique.[30,151]

6.4. Cartilage tissues

Fibrin has successfully been used as a scaffold and adhesive to improve healing and regeneration of fibrocartilage, elastic cartilage, craniofacial cartilage, and articular cartilage. For fibrocartilage regeneration, mesenchymal stem cells have been seeded into fibrin glue and implanted into meniscal defects in rats. Meniscal healing in the avascular zone was observed after 8 weeks.[152] It has also been shown that incorporation of a fibrin clot derived from whole blood into a meniscus repair site resulted in a reduction in failure rate from 61% to 8%.[153,154] Regeneration of elastic cartilage by tissue engineering using fibrin has also been investigated. Ruszymah et al.[155] seeded pediatric auricular chondrocytes into fibrin and implanted the constructs into nude mice. It was found that the tissue-engineered cartilage resembled native elastic cartilage. In combination with polytetrafluoroethylene as an artificial perichondrium, fibrin gel-seeded auricular chondrocytes regenerated flexible tissue that returned to its original shape without fracture after rigorous torsion.[156] Fibrin glue has also been used along with autologous concha cartilage to reconstruct the middle ear canal wall in the chinchilla.[157]

Tissue engineering approaches to articular cartilage repair offer potentially important advantages over modern metallic and plastic joint prostheses.[158] Fibrin gel has been widely investigated for tissue engineering of cartilage,[42-47,159] often in combination with other biomaterials to incorporate the advantages of both biomaterials.[160,161] Fibrin glue as scaffolding material has been used for the treatment of deep chondral defects in human.[162,163] Genipin is a natural product that can be used to cross-link fibrin hydrogels. It has been shown to enhance the scaffold mechanical properties, and also induces chondrogenic differentiation of human primary articular chondrocytes.[23] Genipin is significantly less toxic than other chemical cross-linkers such as glutaraldehyde.[164] In other approaches, fibrin hydrogels can be mixed with a PGA mesh to form a solid cell delivery device that is easily manipulated *in vitro*.[165] Fibrin gels mixed with hyaluronic acid provide a favorable environment for chondrocytes to maintain stable phenotype and to synthesize cartilage ECM in nude mice.[161] Further, fibrin hydrogels with collagen allow stable graft fixation in a chicken model of cartilage repair.[166] Similarly, fibrin glue can be mixed with alginate to allow initial cell proliferation and subsequent preservation of differentiated cells to establish a stable matrix structure *in vitro* and in rabbit.[160,167,168] Further, fibrin glue has been combined with a polycaprolactone-based polyurethane scaffold to establish a long-term construct with a stable shape after implantation in mice.[56] Moreover, when a tri-copolymer of gelatin, hyaluronic acid, and chondroitin- 6-sulfate is combined with fibrin glue, it promotes ECM secretion and inhibits ECM degradation *in vitro*.[127] Chondrocytes have been used widely as a productive cue after encapsulation in fibrin during articular cartilage tissue engineering.[42–44,47,56,127,160,161,165,166,168–173] However, many other cell lines are also used for cartilage tissue engineering, including periosteal cells,[167] bone marrow stromal cells,[174] adipose tissue-derived mesenchymal stem cells,[175] and bone marrow–

derived mononuclear cells.[176] When added to the culture media, members of TGF-β family serve as inductive cues for the fibrin glue–encapsulated cells.[159,167] However, other factors can alternatively be used, such as insulin-like growth factor I (IGF-I)[171] and FGF-2.[177] Bone morphogenic protein-2 and IGF-I genes have been transferred to mesenchymal cells through adenovirus-mediated transfection for use with fibrin glue for cartilage restoration in experimental partial thickness cartilage lesions.[178]

6.5. Liver tissue

Fibrin matrix supports three-dimensional cell organization and new tissue formation *in vitro*; in addition, it is a robust carrier for hepatocytes transplantation. Liver transplantation can be orthotopic where liver cells are transplanted into the rats spleen or portal vein,[38] or heterotopic where hepatocytes are transplanted either underneath the kidney capsules[179] or intraperitoneally.[180] Fibrin hydrogel has been used as a scaffold for orthotopic extravascular transplantation of hepatocytes, where fibrin-hepatocyte constructs were fabricated by mixing commercial fibrinogen with thrombin.[38] This study showed that the injection of fibrin gel-immobilized hepatocytes into the rat liver parenchyma is technically feasible without significant safety problems or harmful side effects. Further, the transplanted cells are actively integrated into the recipient liver parenchyma. The fibrin matrix is resorbed by the donor cells, and its degradation products do not affect hepatocyte survival and differentiation.[38] Microencapsulation of hepatocytes in combination with a fibrinogen-collagen mixture, by extrusion into a terpolymer solution, and submerging the resultant microcapsules into a thrombin solution, induced the formation of an insoluble fibrin network inside the microcapsules. The formation of a fibrin network inside the microcapsules not only increased the rates of urea and albumin synthesis by hepatocytes but also enhanced the mechanical strength in the interior of the microcapsules. This

modification enabled microcapsules to withstand the large compressive and shear forces found in bioreactors and resulted in uniform perfusion.[39,181]

6.6. Nervous tissue

Tissue-engineered neural structures may present an alternative strategy to treat serious clinical conditions of the nervous system, such as brain and spinal cord injuries and neurodegenerative diseases, in which functional neural cells are often lost or degenerate within the nervous system.[71,182] Fibrin-based scaffolds have been used to treat both central and peripheral nerve injury in rat and chicken models.[67,68] Fibrin gels within nerve guide tubes implanted in rat sciatic nerve injury models facilitate axonal regeneration and cell migration in short-gap nerve injuries.[183] The regenerated axons and associated glia invaded and grew inside the fibrin. In the absence of fibrin, no nerve generation occurred. Neurite penetration in fibrin occurs by plasmin-mediated fibrinolysis localized to the neurite tip, since addition of plasmin inhibitors such as aprotinin decrease neurite extension dramatically in embryonic chicken DRGs.[184] However, when murine embryonic stem cells are used as a cell source, aprotinin is required to optimize fibrin conditions for nerve regeneration.[185] It has been shown that fibrin hydrogel alone does not allow proper nerve regeneration and fibrin must be covalently modified to incorporate exogenous heparin-binding domains, a class of adhesion domains that bind heparin and other sulfated glycosaminoglycans and promote nerve regeneration. Four different heparin-binding peptides, derived from antithrombin III, modified antithrombin III, neural cell adhesion molecule, and platelet factor-4 were covalently bound to fibrin separately through a bi-domain peptide. These peptides were found to enhance the degree of neurite extension from embryonic chicken DRGs.[54] In a similar study, fibrin hydrogels modified through a bi-domain peptide with four laminin-derived oligopeptides—RGD, IKVAV, YIGSR, and

RNIAEIIKDI in combination resulted in an induction of neurite extension from DRGs in the same model.[66] A third group examined the feasibility of using a fibrin-based drug delivery system, which was designed to provide controlled release of neurotrophin-3 (NT-3), basic fibroblast growth factor (bFGF), brain-derived neurotrophin factor, and βNGF *in vitro* and *in vivo*. Therapy for nerve injury was successful using a heparin-based delivery system, where a linker peptide contained a factor XIIIa substrate was covalently linked to fibrin during polymerization. The linker captured heparin within the fibrin hydrogel, and the immobilized heparin bound NT-3 and βNGF, preventing their loss from the scaffold by diffusion. The result of these studies demonstrated that the incorporation of a delivery system providing controlled release of growth factors across short nerve gaps led to enhanced central and peripheral nerve regeneration in chicken and rat models, and represents a feasible method to enhance nerve generation in DRGs.[53,67-69] Fibrin glue has also been used to bridge gaps between injured spinal cord segments and to secure dorsal root stumps after dorsal column injury in a rat model.[186,187] Recently, fibrin was used as a printable hydrogel to build a three-dimensional neural structure. Complex cellular patterns and structures were created by automated and direct inkjet printing of rat primary embryonic hippocampal and cortical neurons onto fibrin. The printed fibrin-based neural constructs provide suitable cell or tissue sources for clinical treatments of serious neural injuries and degenerative diseases.[71]

6.7. Ocular tissue

Tissue engineering of the cornea could overcome the disadvantages of corneal transplantation such as immune rejection and a shortage of donor supply.[188] Fibrin-based matrices have been used extensively for the treatments of many corneal diseases, including corneal perforation and total limbal cell deficiency.[189,190] Transplantation of fibrin glue-

cultured limbal stem cell constructs permanently restored corneal integrity in patients with total limbal deficiency.[190] In addition, fibrin glue in combination with amniotic membrane transplant allowed total reepithelialization and subsequent repair of the corneal perforation associated with loss of the stroma in human.[189] Encapsulation of corneal epithelial stem cells into an autologous fibrin gel rich in fibronectin produces a flexible and easily manipulated material *in vitro*, which is a potential source of a bioengineered ocular surface. It should be pointed out that aprotinin (plasmin inhibitor) should be added to the conditioned medium to prevent fibrin degradation.[33] Fibrin glue is an effective scaffold for creating carrier-free transplantable corneal epithelial sheets in rabbit model. Carrier-free sheets are more differentiated than amniotic membranes and retain similar levels of colony-forming progenitor cells.[110] However, fibrin has been combined with agarose to obtain better biomechanical properties, and has been used to create a full-thickness corneal model in rabbit, in which the three corneal cell types (endothelial, stromal, and epithelial) were layered sequentially within the fibrin-agarose scaffold.[31] Fibrin has also been combined with collagen to create an *in vitro* model of the cornea with adjacent sclera. Fibrin cross-linked with poly(N-isopropylacrylamide)-co-acrylic acid coacryloxysuccinimide (PNiPAAmco-AAc) together with laminin and NGF was used for constructing sclera, and collagen plus chondrotin 6-sulfate cross-linked with glutaraldehyde was used for constructing the cornea. This approach led to complete innervation and vascularization.[32]

6.8. Skin tissue

In the past few years, skin grafting has evolved from the initial autograft and allograft preparations to biosynthetic and tissue-engineered living skin replacements.[191] Cultured keratinocytes have been used widely for the treatment of a variety of skin defects such as acute partial or full-thickness burns and chronic wounds such us ulcers in

human.[117] However, keratinocytes alone do not attach properly to the wound, and the presence of fibroblasts is essential for keratinocytes to maintain their function in mice.[41] Clinically, fibrin glue is a common carrier and matrix vehicle for keratinocytes. Cultured keratinocyte suspensions in fibrin glue are used for the reepithelialization of deep, partial, and full-thickness wounds in both animal models and in humans.[192,193] In addition, fibrin glue has been shown to improve the skin graft success rate, especially when incorporated into difficult grafting sites or sites associated with unavoidable movement. Further, when fibrin is associated with fibronectin, it supports keratinocyte and fibroblast growth, both *in vivo* and *in vitro*. Constructs of fibrin glue and keratinocytes plus fibroblasts provide similar advantages to those demonstrated with conventional skin grafts.[118] Similarly, fibrin hydrogels seeded with keratinocytes and fibroblasts resulted in proper epidermal structure formation and enhanced migration of vascular endothelial cells in comparison to collagen in a mice model.[41] Moreover, the fibrin gel increased the consistency of the wound healing response, reduced the time for keratinocyte activation, and promoted more complete reepithelialization in skin equivalent incisional wounds.[194] Implantation in pig excisional wounds of fibroblasts encapsulated into FMBs resulted in high fibroblast proliferation, more angiogenesis, and granulation tissue formation, especially when platelet-derived growth factor-BB (PDGF-BB) was included in the FMBs.[132] A more effective technique was the co-application to wounds of fibrin glue with either keratinocytes or mesenchymal stem cells using a fibrin glue spray system with a double-barreled syringe. This resulted in enhancement of the healing and subsequently accelerated repair processes in acute and chronic cutaneous wounds of animal models and human patients.[125,126,195] On the contrary, poor physical properties such as low mechanical strength and rapid degradation rates are the major disadvantages of fibrin-based cell cultures. To overcome these disadvantages,

synthetic polymeric membrane such as polycaprolactone, which is characterized by its biodegradability, biocompatibility, high tensile strength, slow degradation kinetics, and good barrier properties, can be coated with fibrin glue, which then serves as a biomimetic surface for the adherence and proliferation of keratinocytes *in vitro*.[196] Although protease inhibitors such as aprotinin are usually added to the fibrin component to delay its degradation rate, aprotinin decreases the regeneration of granulation tissue and was not particularly beneficial to the wound healing process in rat.[197]

6.9. Tendons and ligaments

Tendon and ligament tears are amongst the most common orthopaedic injuries. However, incomplete regeneration of the tissue often occurs during healing.[198] Hankemeier et al.[113] combined human bone marrow stem cells (BMSCs) with liquid fibrin glue and injected the mixture into patellar tendon defects of immunodeficient rats. An advantage of using fibrin as a matrix is that it can be injected as a liquid for *in situ* gelation. Injection of the fibrin/cell material leads to formation of tissue that is histologically more mature; however, biomechanical measurements will be important for determining the functionality of fibrin for this application. Similarly, fibrin glue has been used as a carrier for BMSCs and injected into transected rabbit Achilles tendons along with repair by suturing.[114] It was found that fibrin/ BMSC therapy following primary repair led to an improvement of histological and biomechanical parameters at only the early stages of healing.

Table 1 summarizes the diverse tissue engineering applications of fibrin scaffolds.

TABLE 1. Tissue engineering applications of fibrin scaffolds.

Engineered Tissue	Types of Fibrin Scaffold (reference numbers)		
	Fibrin Hydrogel	Fibrin Glue	Fibrin Microbeads
Adipose tissue	26,136	139,140	-
Bone	48,49,58-60,141,174	97-103,121,143-146,177	62,63,135
Cardiac tissue	2,27-30,57,149-151	111	-
Cartilage	9,23,25,42-47,156,159, 161, 165, 166,170,171, 173-175	55,56,104-109,121,127,152,155, 157,160,162,163,167-169,172, 176-178	-
Liver	38,39	-	-
Muscle tissue	34-37	-	-
Nervous tissue	22,53,54,66-69,71,184, 185	186,187	-
Ocular tissue	31-33	110,189,190	-
Skin	40,41,194,197	116-118,125,126,191-193,195	132
Tendons and ligaments	-	113-115	-
Vascular tissue	12,18,50-52,128,130	129	-

7. CONCLUSIONS

In this review we have summarized many important characteristics that make fibrin a robust hydrogel scaffold for tissue engineering. Fibrin can be manipulated as gel, glue, or microbeads to increase its utility in tissue engineering applications. In addition, it can be modified to incorporate several biologically active peptides to increase the local concentrations of growth factors and mimic the natural milieu surrounding the cells. The incorporation of these active peptides facilitates adhesion of the cells to the scaffold, promotes migration of the cells inside the gel, and enhances cell-cell interaction. Further, fibrin gels can be fabricated with defined architecture on the nanometer scale under the influence of magnetic forces. Fibrin can also be utilized as a printable hydrogel to establish specific cell patterns in the three-dimensional construct using inkjet-printing techniques. Fibrin as an ideal scaffold has a significant disadvantage: the gradual disintegration of the gel with subsequent loss of shape and volume before the proper formation of tissue-engineered constructs. This disadvantage can be overcome by optimizing the concentrations of fibrinogen, calcium ion (Ca^{2+}), and pH, by using a lower cell density or

by adding specific protease inhibitors. Stability can also be enhanced by using highly denatured densely cross-linked FMBs or by combining fibrin with an artificial supporting polymer. Fibrin gel shrinkage and its low mechanical stiffness represent other disadvantages of fibrin scaffolds in some tissue engineering applications, which can be controlled by cross-linking or by combining fibrin with other artificial scaffolding material. Due to its numerous advantages, fibrin is used extensively to support three-dimensional scaffolds in tissue engineering for adipose, bone, cardiac, cartilage, liver, nervous, ocular, skin, tendon, and ligament tissues.

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9. REFERENCES

1. Risbud, M. Tissue engineering: implication in the treatment of organ and tissues defects (review). Biogerontology **2**, 117, 2001.

2. Jockenhoevel, S., Zund, G., Hoerstrup, S.P., Chalabi, K., Sachweh, J.S., Demircan, L., Messmer, B.J., and Turina, M. Fibrin gel—advantages of a new scaffold in cardiovascular tissue engineering. Eur. J. Cardiothorac. Surg. **19**, 424, 2001.

3. Song, L., Baksh, D., and Tuan, R.S. Mesenchymal stem cell-based cartilage tissue engineering: cells, scaffold and biology. Cytotherapy **6**, 596, 2004.

4. Leo, A.J., and Grande, D.A. Mesenchymal stem cells in tissue engineering. Cells Tissues Organs **183**, 112, 2006.

5. Gruber, H.E., Leslie, K., Ingram, J., Norton, H.J., and Hanley, E.N. Cell-based tissue engineering for the intervertebral disc: *in vitro* studies of human disc cell gene expression and matrix production within selected cell carriers. Spine J. **4**, 44, 2004.

6. Ringe, J., Kaps, C., Burmester, G.R., and Sittinger, M. Stem cells for regenerative medicine: advances in the engineering of tissues and organs. Naturwissenschaften **89**, 338, 2002.

7. Whang, K., Tsai, D.C., Nam, E.K., Aitken, M., Sprague, S.M., Patel, P.K., and Healy, K.E. Ectopic bone formation via rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. J. Biomed. Mater. Res. **42**, 491, 1998.

8. Lanza, R.P., Jackson, R., Sullivan, A., Ringeling, J., McGrath, C., Kuhtreiber, W., and Chick, W.L. Xenotransplantation of cells using biodegradable microcapsules. Transplantation **67**, 1105, 1999.

9. Hunter, C.J., Mouw, J.K., and Levenston, M.E. Dynamic compression of chondrocyte-seeded fibrin gels: effects on matrix accumulation and mechanical stiffness. Osteoarthritis Cartilage **12**, 117, 2004.

10. Risbud, M.V., and Sittinger, M. Tissue engineering advances in *in vitro* cartilage generation (review). Trends Biotechnol. **20**, 351, 2002.

11. Rosso, F., Marino, G., Giordano, A., Barbarisi, M., Parmeggiani, D., and Barbarisi, A. Smart materials as scaffolds for tissue engineering (review). J. Cell Physiol. **203**, 465, 2005.

12. Cummings, C.L., Gawlitta, D., Nerem, R.M., and Stegemann, J.P. Properties of engineered vascular constructs made from collagen, fibrin, and collagen-fibrin mixtures. Biomaterials **25**, 3699, 2004.

13. Williams, C.G., Kim, T.K., Taboas, A., Malik, A., Manson, P., and Elisseeff, J. *In vitro* chondrogenesis of bone marrowderived mesenchymal stem cells in photopolymerizing hydrogel. Tissue Eng. **9**, 679, 2003.

14. Lavik, E., and Langer, R. Tissue engineering: current state and perspectives (mini-review). Appl. Microbiol. Biotechnol. **65**, 1, 2004.

15. Ramaswamy, S., Wang, D.A., Fishbein, K.W., Elisseeff, J.H., and Spencer, R.G. An analysis of the integration between articular cartilage and nondegradable hydrogel using magnetic resonance imaging. J. Biomed. Mater. Res. B. Appl. Biomater. **77**, 144, 2006.

16. Stammen, J.A., Williams, S., Ku, D.N., and Guldberg, R.E. Mechanical properties of a novel PVA hydrogel in shear and unconfined compression. Biomaterials **22**, 799, 2001.

17. Nguyen, K.T., and West, J.L. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials **23**, 4307, 2002.

18. Swartz, D.D., Russel, J.A., and Andreadis, S.T. Engineering of fibrin-based functional and implantable small-diameter blood vessels. Am. J. Physiol. Heart. Circ. Physiol. **288**, H1451, 2005.

19. Mosesson, M.W. Fibrinogen and fibrin structure and functions (review). J. Thromb. Haemost. **3**, 1894, 2005.

20. Mosesson, M.W., Siebenlist, K.R., and Meh, D.A. The structure and biological features of fibrinogen and fibrin. Ann. N.Y. Acad. Sci. **936**, 11, 2001.

21. Horan, J.T., and Francis, C.W. Fibrin degradation products, fibrin monomer and soluble fibrin in disseminated intravascular coagulation. Semin. Thromb. Hemost. **27**, 657, 2001.

22. Schense, J.C., and Hubbell, J.A. Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. Bioconjug. Chem. **10**, 75, 1999.

23. Dare, E.V., Griffith, M., Poitras, P., Kaupp, J.A., Waldman, S.D., Carlsson, D.J., Dervin, G., Mayoux, C., and Hincke, M.T. Genipin cross-linked fibrin hydrogels for *in vitro* human articular cartilage tissue engineered regeneration (submitted).

24. Spotnitz, W.D., and Prabhu, R. Fibrin sealant tissue adhesive- review and update. J. Long-Term Eff. Med. Implants **15**, 245, 2005.

25. Ahmed, T.A., Griffith, M., and Hincke, M. Characterization and inhibition of fibrin hydrogel-degrading enzymes during development of tissue engineering scaffolds. Tissue Eng. **13**, 1469, 2007.

26. Cho, S.W., Kim, I., Kim, S.H., Rhie, J.W, Choi, C.Y., and Kim, B.S. Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. Biochem. Biophys. Res. Commun. **345**, 588, 2006.

27. Birla, R.K., Borschel, G.H., Dennis, R.G., and Brown, D.L. Myocardial engineering *in vivo*: formation and characterization of contractile, vascularized three-dimensional cardiac tissue. Tissue Eng. **11**, 803, 2005.

28. Mol, A., van Lieshout, M.I., Dam-de Veen, C.G., Neuenschwander, S., Hoerstrup, S.P., Baaijens, F.P., and Bouten, C.V. Fibrin as a cell carrier in cardiovascular tissue engineering applications. Biomaterials **26**, 3113, 2005.

29. Long, J.L., and Tranquillo, R.T. Elastic fiber production in cardiovascular tissue-equivalents. Matrix Biol. **22**, 339, 2003.

30. Ye, Q., Zund, G., Benedikt, P., Jochenhoevel, S., Hoerstrup, S.P., Sakyama, S., Hubbell, J.A., and Turina, M. Fibrin gel as a three-dimensional matrix in cardiovascular tissue engineering. Eur. J. Cardiothorac. Surg. **17**, 587, 2000.

31. Alaminos, M., Del Carmen Sanchez-Quevedo, M., Munoz-Avila, J.I., Serrano, D., Medialdea, S., Carreras, I., and Campos, A. Construction of a complete rabbit cornea substitute using fibrin-agarose scaffold. Invest. Ophthalmol. Vis. Sci. **47**, 3311, 2006.

32. Suuronen, E.J., Muzakare, L., Doillon, C.J., Kapila, V., Li, F., Ruel, M., and Griffith, M. Promotion of angiogenesis in tissue engineering: developing multicellular matrices with multiple capacities. Int. J. Artif. Organs **29**, 1148, 2006.

33. Han, B., Schwab, I.R., Madsen, T.K., and Isseroff, R.R. A fibrin-based bioengineered ocular surface with human corneal epithelial stem cells. Cornea **21**, 505, 2002.

34. Nieponice, A., Maul, T.M., Cumer, J.M., Soletti, L., and Vorp, D.A. Mechanical stimulation induces morphological and phenotypic changes in bone marrow-derived progenitor cell within a three-dimensional fibrin matrix. J. Biomed. Mater. Res. A. **81**, 523, 2007.

35. Rowe, S.L., Lee, S., and Stegmann, J.P. Influence of thrombin concentration on the mechanical and morphological properties of cell-seeded fibrin hydrogels. Acta Biomater. **3**, 59, 2007.

36. Huang, Y.C., Dennis, R.G., Larkin, L., and Baar, K. Rapid formation of functional muscle *in vitro* using fibrin gels. J. Appl. Physiol. **98**, 706, 2005.

37. Hecker, L., Baar, K., Dennis, R.G., and Bitar, K.N. Development of a three-dimensional physiological model of the internal and sphincter bioengineered *in vitro* from isolated smooth muscle cells. Am. J. Physiol. Gastrointest. Liver. Physiol. **289**, G188, 2005.

38. Bruns, H., Kneser, U., Holzhuter, S., Roth, B., Kluth, J., Kaufmann, P.M., Kluth, D., and Fiegel, H.C. Injectable liver: a novel approach using fibrin as a matrix for culture and intrahepatic transplantation of hepatocytes. Tissue Eng. **11**, 1718, 2005.

39. Sun, T., Chan, M.L., Quek, C.H., and Yu, H. Improving mechanical stability and density distribution of hepatocytes microcapsules by fibrin clot and gold nanoparticles. J. Biotechnol. **111**, 169, 2004.

40. Balestrini, J.L., and Billiar, K.L. Equibiaxial cyclic stretch stimulates fibroblasts to rapidly remodel fibrin. J. Biochem. **39**, 2983, 2006.

41. Hojo, M., Inokuchi, S., Kidokoro, M., Fukuyama, N., Tanaka, E., Tsuji, C., Miyasaka, M., Tanino, R., and Nakazawa, H. Induction of vascular endothelial growth factor by a fibrin as dermal substrate for cultured skin substitutes. Plast. Reconstr. Surg. **111**, 1638, 2003.

42. Eyrich, D., Brandle, F., Appel, B., Wiese, H., Maier, G., Wenzel, M., Staudenmaier, R., Goepferich, A., and Blunk, T. Long-term stable fibrin gels for cartilage engineering. Biomaterials **28**, 55, 2007.

43. Mesa, J.M., Zaporojan, V., Weinand, C., Johnson, T.S., Bonassar, L., Randolph, M.A., Yaremchuk, M.J., and Butler, P.E. Tissue engineering cartilage with aged articular chondrocytes *in vivo*. Plast. Reconstr. Surg. **118**, 41 (discussion 50–53), 2006.

44. Peretti, G.M., Xu, J.W., Bonassar, L.J., Kirchhoff, C.H., Yaremchuk, M.J., and Randolph, M.A. Review of injectable cartilage engineering using fibrin gel in mice and swine models. Tissue Eng. **12**, 1151, 2006.

45. Connelly, J.T., Vanderploeg, E.J., and Levenston, M.E. The influence of cyclic tension amplitude on chondrocyte matrix synthesis: experimental and finite element analyses. Biorheology **41**, 377, 2004.

46. Passaretti, D., Silverman, R.P., Huang, W., Kirchhoff, C.H., Ashiku, S., Randolph, M.A., and Yaremchuk, M.J. Cultured chondrocytes produce injectable tissue-engineered cartilage in hydrogel polymer. Tissue Eng. **7**, 805, 2001.

47. Johnson, T.S., Xu, J.W., Zaporojan, V.V., Mesa, J.M., Weinand, C., Randolph,M.A., Bonassar, L.J., Winograd, J.M., and Yaremchuk, M.J. Integrative repair of cartilage with articular and nonarticular chondrocytes. Tissue Eng. **10**, 1308, 2004.

48. Chung, Y.I., Ahn, K.M., Jeon, S.H., Lee, S.Y., Lee, J.H., and Tae, G. Enhanced bone regeneration with BMP-2 loaded functional nanoparticles-hydrogel complex. J. Control. Release **121**, 91, 2007.

49. Weinand, C., Pomerantseva, I., Neville, C.M., Gupta, R., Weinberg, E., Madisch, I., Shapiro, F., Abukawa, H., Troulis, M.J., and Vacanti, J.P. Hydrogel-β-TCP scaffolds and stem cells for tissue engineering bone. Bone **38**, 555, 2006.

50. Liu, J.Y., Swartz, D.D., Peng, H.F., Gugino, S.F., Russell, J.A., and Andreadis, S.T. Functional tissue-engineered blood vessels from bone marrow progenitor cells. Cardiovasc. Res. **75**, 618, 2007.

51. Dietrich, F., and Lelkes, P.I. Fine-tuning of a threedimensional microcarrier-based angiogenesis assay for the analysis of endothelial-mesenchymal cell co-cultures in fibrin and collagen gels. Anginonesis **9**, 111, 2006.

52. Urech, L., Bitterman, A.G., Hubbell, J.A. and Hall, H. Mechanical properties, proteolytic degradability and biological modifications affect angiogenic process extension into native and modified fibrin matrices *in vitro*. Biomaterials **26**, 1369, 2005.

53. Sakiyama-Elbert, S.E., and Hubbell, J.A. Development of fibrin derivatives for controlled release of heparin-binding growth factors. J. Control. Release **65**, 389, 2000.

54. Sakiyama, S.E., Schense, J.C., and Hubbell, J.A. Incorporation of heparin-binding peptide into fibrin gels enhances neurite extension: an example of designer matrices in tissue engineering. FASEB J. **13**, 2214, 1999.

55. Lee, C.R., Grad, S., Gorna, K., Gogoleswski, S., Goessl, A., and Alini, M. Fibrin-polyurethane composites for articular cartilage tissue engineering: a preliminary analysis. Tissue Eng. **11**, 1562, 2005.

56. Eyrich, D., Wiese, H., Maier, G., Skodacek, D., Appel, B., Sarhan, H., Tessmar, J., Staudenmaier, R., Wenzel, M.M., Goepferich, A., and Blunk, T. *In vitro* and *in vivo* cartilage engineering using a combination of chondrocytes-seeded long-term stable fibrin gels and polycaprolactone-based polyurethane scaffolds. Tissue Eng. **13**, 2207, 2007.

57. Van Lieshout, M., Peters, G., Rutten, M., and Baaijens, F. A knitted, fibrin-covered polycaprolactone scaffold for tissue engineering of the aortic valve. Tissue Eng. **12**, 481, 2006.

58. Weinand, C., Gupta, R., Huang, A.Y., Weinberg, E., Madisch, I., Qudsi, R.A., Neville, C.M., Pomerntseva, I., and Vacanti, J.P. Comparison of hydrogels in the *in vivo* formation of tissue-engineered bone using mesenchymal stem cells and beta-tricalcium phosphate. Tissue Eng. **13**, 757, 2007.

59. Weinand, C., Gupta, R., Weinberg, E., Madisch, I., Jupiter, J.B., and Vacanti, J.P. Human shaped thumb bone tissue engineered by hydrogel-beta-tricalcium/poly-epsiloncarpolactone scaffolds and magnetically sorted stem cells. Ann. Plast. Surg. **59**, 46, 2007.

60. Peled, E., Boss, J., Bejar, J., Zinman, C., and Seliktar, D. A novel poly (ethylene glycol)-fibrinogen hydrogel for tibial segmental defect repair in rat model. J. Biomed. Mater. Res. A. **80**, 874, 2007.

61. Clark, R.A. Fibrin is a many splendored thing. J. Invest. Dermatol. **121**, xxi, 2003.

62. Rivkin, R., Ben-Ari, A., Kassis, I., Zangi, L., Gaberman, E., Levdansky, L., Marx, G., and Gorodetsky, R. High-yield isolation, expansion, and differentiation of murine bone

marrow-derived mesenchymal stem cells using fibrin microbeads (FMB). Cloning Stem Cells **9**, 157, 2007.

63. Kassis, I., Zangi, L., Rivkin, R., Levdansky, L., Samuel, S., Marx, G., and Gorodetsky, R. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant. **37**, 967, 2006.

64. Dikovsky, D., Bianco-Peled, H., and Seliktar, D. The effect of structural alterations of PEG-fibrinogen hydrogel scaffolds on 3d-cellular morphology and cellular migration. Biomaterials **27**, 1496, 2006.

65. Smith, J.D., Chen, A., Ernst, L.A., Waggoner, A.S., and Campbell, P.G. Immobilization of aprotinin to fibrinogen as a novel method for controlling degradation of fibrin gels. Bioconjug. Chem. **18**, 695, 2007.

66. Schense, J.C., Bloch, J., Aebischer, P., and Hubbell, J.A. Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. Nat. Biotechnol. **18**, 415, 2000.

67. Lee, A.C., Yu, V.M., Lowe, J.B., Brenner, M.J., Hunter, D.A., Mackinnon, S.E., and Sakiyama-Elbert, S.E. Controlled release of nerve growth factor enhances sciatic nerve regeneration. Exp. Neurol. 184, 295, 2003.

68. Taylor, S.J., McDonald, J.W., and Sakiyama-Elbert, S.E. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J. Control. Release **98**, 281, 2004.

69. Sakiyama-Elbert, S.E., and Hubbell, J.A. Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. J. Control. Release **69**, 149, 2000.

70. Lyon, M., Rushton, G., and Gallagher, J.T. The interaction of the transforming growth factor-βs with heparin/heparn sulfate is isoforms-specific. J. Biol. Chem. **272**, 18000, 1997.

71. Xu, T., Gregory, C.A., Molnar, P., Cui, X., Jolata, S., Bhaduri, S.B., and Boland, T. Viability and electrophysiology of neural cell structures generated by the inkjet printing method. Biomaterials **27**, 3580, 2006.

72. Mironov, V., Boland, T., Trusk, T., Forgacs, G., and Markwald, R.R. Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol. **21**, 157, 2003.

73. Roth, E.A., Xu, T., Das, M., Gregory, C., Hickman, J.J., and Boland, T. Inkjet printing for high-throughput cell patterning. Biomaterials **25**, 3707, 2004.

74. Sanjana, N.E., and Fuller, S.B. A fast flexible ink-jet printing method for patterning dissociated neurons in culture. J. Neurosci. Methods **136**, 151, 2004.

75. Watanabe, K., Miyazaki, T., and Matsuda, R. Growth factor array fabrication using a color ink jet printer. Zool. Sci. **20**, 429, 2003.

76. Xu, T., Jin, J., Gregory, C., Hickman, J.J., and Boland, T. Inkjet printing of viable mammalian cells. Biomaterials **26**, 93, 2005.

77. Alsberg, E., Feinstein, E., Joy, M.P., Prentiss, M., and Ingber, D.E. Magnetically guided self-assembly of fibrin matrices with ordered nano-scale structure for tissue engineering. Tissue Eng. **12**, 3247, 2006.

78. Marx, G. Evolution of fibrin glue applicators. Transfus. Med. Rev. **17**, 287, 2003.

79. Buchta, C., Dettke, M., Funovics, P.T., Hocker, P., Knobl, P., Macher, M., Quehenberger, P., Treitl, C., and Worel, N. Fibrin sealants produced by the cryoseal FS system: product chemistry, material properties and possible preparation in the autologous preoperative setting. Vox San. **86**, 257, 2004.

80. Carless, P.A., Anthony, D.M., and Henry, D.A. Systematic review of the use of fibrin sealant to minimize perioperative allogeneic blood transfusion. Br. J. Surg. **89**, 695, 2002.

81. Buchta, C., Hedrich, H.S., Macher, M., Hocker, P., and Redl, H. Biochemical characterization of autologous fibrin sealants produced by Cryoseal and Vivostat in comparison to homologous fibrin sealants produced Tissucol/Tisseel. Biomaterials **26**, 6233, 2005.

82. Jackson, M.R. Fibrin sealants in surgical practice: an overview. Am. J. Surg. **182S**, 1, 2001.

83. Evatt, B., Austin, H., Leon, G., Ruiz-Saez, A., and de Bosch, N. Hemophilia treatment. Predicting the long-term risk of HIV exposure by cryoprecipitate. Haemophilia **6**, 128, 2000.

84. Kawamura, M., Sawafuji, M., Watanabe, M., Horinouchi, H., and Kobayashi, K. Frequency of transmission of human parvovirus B 19 infection in fibrin sealant used during thoracic surgery. Ann. Thorac. Surg. **73**, 1098, 2002.

85. Hunter, N., and Houston, F. Can prion diseases be transmitted between individuals via blood transfusion: evidence from sheep experiments. Dev. Biol. (Basel) **108**, 93, 2002.

86. Nakamura, H., Matsuyama, Y., Yoshihara, H., Sakai, Y., Katayama, Y., Nakashima, S., Takamatsu, J., and Ishiguro, N. The effect of autologous fibrin tissue adhesive on postoperative cerebrospinal fluid leak in spinal cord surgery: a randomized controlled trial. Spine **30**, E347, 2005.

87. Dresdale, A., Rose, E.A., Jeevanandam, V., Reemtsma, K., Bowman, F.O., and Malm, J.R. Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery **97**, 750, 1985.

88. Spotnitz, W.D., Mintz, P.D., Avery, N., Bithell, T.C., Kaul, S., and Nolan, S.P. Fibrin glue from stored human plasma. An inexpensive and efficient method for local blood bank preparation. Am. Surg. **53**, 460, 1987.

89. Nakamura, Y., Masuda, M., Toshima, Y., Asou, T., Oe, M., Kinoshita, K., Kawachi, Y., Tanaka, J., and Tokunaga, K. Comparative study of cell saver and ultrafiltration nontransfusion in cardiac surgery. Ann. Thorac. Surg. **49**, 973, 1990.

90. Sirieix, D., Chemla, E., Castier, Y., Massonnet-Castel, S., Fabiani, J.N., and Baron, J.F. Comparative study of different biological glues in an experimental model of surgical bleeding in anesthetized rats: platelet-rich and -poor plasma-based glue with and without aprotinin versus commercial fibrinogen-based glue. Ann. Vasc. Surg. **12**, 311, 1998.

91. Koster, A., Sanger, S., Kno¨rig, F.J., Kuppe, H., Hetzer, R., and Loebe, M. Autologous plasma and platelet sequestration at the beginning of cardiopulmonary bypass: a pilot investigation in five patients undergoing extended vascular surgery in deep hypothermia. ASAIO J. **48**, 106, 2002.

92. Silver, F.H., Wang, M.C., and Pins, G.D. Preparation of fibrin glue: a study of chemical and physical methods. J. Appl. Biomater. **6**, 175, 1995.

93. Burnouf, T., and Radosevich, M. Affinity chromatography in the industrial purification of plasma proteins for therapeutic use. J. Biochem. Biophys. Methods **49**, 575, 2001.

94. Mohri, H., and Ohkubo, T. Fibrinogen binds to heparin: the relationship of the binding of other adhesive proteins to heparin. Arch. Biochem. Biophys. **303**, 27, 1993.

95. Dempfle, C.E., and Heene, D.L. Purification of human plasma fibrinogen by chromatography on protamine-agarose. Thromb. Res. **46**, 19, 1987.

96. Matthias, F.R., Hocke, G., and Lasch, H.G. Isolation of fibrinogen from plasma by affinity chromatography on insolubilized fibrinogen (FG-ag) and insolubilized fibrinmonomer (FM-ag). Thromb. Res. **7**, 861, 1975.

97. Follmar, K.E., Prichard, H.L., DeCroos, F.C., Wang, H.T., Levin, L.S., Klitzman, B., Olbrich, K.C., and Erdmann, D. Combined bone allograft and adipose-derived stem cells

autograft in a rabbit model. Ann. Plast. Surg. **58**, 561, 2007.

98. Groger, A., Klaring, S., Merten, H.A., Holste, J., Keps, C., and Sittinger, M. Tissue engineering of bone for mandibular augmentation in immunocompetent minipigs: preliminary study. Scand. J. Plast. Reconstr. Surg. Hand. Surg. **37**, 129, 2003.

99. Ito, K., Yamada, Y., Naiki, T., and Ueda, M. Simultaneous implant placement and bone regeneration around dental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and platelet-rich plasma. Clin. Oral.Implants Res. **17**, 579, 2006.

100. Lechner, S., and Huss, R. Bone engineering: combining smart biomaterials and application of stem cells. Artif. Organs **30**, 770, 2006.

101. Zhu, S.J., Choi, B.H., Jung, J.H., Lee, S.H., Huh, J.Y., You, T.M., Lee, H.J., and Li, J. A comparative histologic analysis of tissue-engineered bone using platelet-rich plasma and platelet-enriched fibrin glue. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. **102**, 175, 2006.

102. Zhu, S.J., Choi, B.H., Huh, J.Y., Jung, J.H., Kim, B.Y., Lee, H.J., and Li, J. A comparative qualitative histological analysis of tissue-engineered bone using bone marrow mesenchymal stem cells, alveolar bone cells, and periosteal cells. Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod. **101**, 164, 2006.

103. Lee, O.K., Coathup, M.J., Goodship, A.E., and Blunn, G.W. Use of mesenchymal stem cells to facilitate bone regeneration in normal and chemotherapy-treated rats. Tissue Eng. **11**, 1727, 2005.

104. Neumeister, M.W., Wu, T., and Chambers, C. Vascularized tissue-engineered ears. Plast. Reconstr. Surg. **117**, 116, 2006.

105. Westreich, R., Kaufman, M., Gannon, P., and Lawson, W. Validating the subcutaneous model of injectable autologous cartilage using a fibrin glue scaffold. Laryngoscope **114**, 2154, 2004.

106. Neovius, E.B., and Kratz, G. Tissue engineering by cocultivating human elastic chondrocytes and keratinocytes. Tissue Eng. **9**, 365, 2003.

107. Chang, J., Rasamny, J.J., and Park, S.S. Injectable tissue engineered cartilage using a fibrin sealant. Arch. Facial. Plast. Surg. **9**, 161, 2007.

108. Dragoo, J.L., Carlson, G., McCormick, F., Khan-Farooqi, H., Zhu, M., Zuk, P.A., and Benhaim, P. Healing fullthickness cartilage defects using adipose-derived stem cells. Tissue Eng. **13**, 1615, 2007.

109. Meinhart, J., Fussenegger, M., and Hobling, W. Stabilization of fibrin-chondrocyte constructs for cartilage reconstruction. Ann. Plast. Surg. **42**, 673, 1999.

110. Higa, K., Shimmura, S., Kato, N., Kawakita, T., Miyashita, H., Itabashi, Y., Fukuda, K., Shimazaki, J., and Tsubota, K. Proliferation and differentiation of transplantable rabbit epithelial sheets engineered with or without an amniotic membrane carrier. Invest. Ophthalmol. Vis. Sci. **48**, 597, 2007.

111. Kofidis, T., Lenz, A., Boublik, J., Akhyari, P., Wachsmann, B., Muller-Stahl, K., Hofmann, M., and Haverich, A. Pulsatile perfusion and cardiomyocytes viability in a solid three-dimensional matrix. Biomaterials **24**, 5009, 2003.

112. Arkudas, A., Tjiawi, J., Bleiziffer, O., Grabinger, L., Polykandriotis, E., Beier, J.P., Sturzel, M., Horch, R.E., and Kneser, U. Fibrin gel-immobilized VEGF and bFGF efficiently stimulate angiogenesis in the AV loop model. Mol. Med. **13**, 480, 2007.

113. Hankemeier, S., van Griensven, M., Ezechieli, M., Barkhausen, T., Austin, M., Jagodzinski, M., Meller, R., Bosch, U., Krettek, C., and Zeichen, J. Tissue engineering of

tendons and ligaments by human bone marrow stromal cells in a liquid fibrin matrix in immunodeficient rats: results of a histologic study. Arch. Orthop. Trauma. Surg. **127**, 815,2007.

114. Chong, A.K., Ang, A.D., Goh, J.C., Hui, J.H., Lim, A.Y., Lee, E.H., and Lim, B.H. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit Achilles tendon model. J. Bone Joint. Surg. Am. **89**, 74, 2007.

115. Soon, M.Y., Hassan, A., Hui, J.H., Goh, J.C., and Lee, E.H. An analysis of soft tissue allograft anterior cruciate ligament reconstruction in a rabbit model: a short-term study of the use of mesenchymal stem cells to enhance tendon osteointegration. Am. J. Sports Med. **35**, 962, 2007.

116. Johnsen, S., Ermuth, T., Tanczos, E., Bannasch, H., Horch, R.E., Zschocke, I., Peschen, M., Schopf, E., Vanscheidt, W., and Augustin, M. Treatment of therapy-refractive ulcera cruris of various origins with autologous keratinocytes in fibrin sealant. Vasa **34**, 25, 2005.

117. Kopp, J., Jeschke, M.G., Bach, A.D., Kneser, U., and Horch, R.E. Applied tissue engineering in the closure of severe burns and chronic wounds using cultures human autologous keratinocytes in a natural fibrin matrix. Cell. Tissue. Bank. **5**, 89, 2004.

118. Currie, L.J., Sharpe, J.R., and Martin, R. The use of fibrin glue in skin grafts and tissue-engineered skin replacements: a review. Plast. Reconstr. Surg. **108**, 1713, 2001.

119. Marlovits, S., Striessnig, G., Kutscha-Lissberg, F., Resinger, C., Aldrian, S.M., Vecsei, V., and Trattnig, S. Early postoperative adherence of matrix-induced autologous chondrocyte implantation for the treatment of full-thickness cartilage defects of the femoral condyle. Knee Surg. Sports Traumatol. Arthrosc. **13**, 451, 2005.

120. Kanemaru, S., Nakamura, T., Omori, K., Magrufov, A., Yamashita, M., and Ito, J. Regeneration of mastoid air cells in clinical applications by *in situ* tissue engineering. Laryngoscope **115**, 253, 2005.

121. Oshima, Y., Watanabe, N., Matsuda, K., Takai, S., Kawata, M., and Kubo, T. Behavior of transplanted bone marrow derived GFP mesenchymal cells in osteochondral defect as a simulation of autologous transplantation. J. Histochem. Cytochem. **53**, 207, 2005.

122. Oshima, Y., Watanabe, N., Matsuda, K., Takai, S., Kawata, M., and Kubo T. Fate of transplanted bone marrow-derived mesenchymal cells during osteochondral repair using transgenic rats to stimulate autologous transplantation. Osteoarthritis Cartilage **12**, 811, 2004.

123. Ronga, M., Grassi, F.A., and Bulgheroni, P. Arthroscopic autologous chondrocyte implantation for the treatment of a chondral defects in the tibial plateau of the knee. Arthroscopy **20**, 79, 2004.

124. Neuman, A.R., Weinberg, A., Benmeir, P., Eldad, A., and Wexler, M.R. Spray application of fibrin glue by a simple device. Plast. Reconstr. Surg. **88**, 922, 1991.

125. Falanga, V., Iwamoto, S., Chartier, M., Yufit, T., Butmarc, J., Kouttab, N., Shrayer, D., and Carson, P. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng. **13**, 1299, 2007.

126. Grant, I., Warwick, K., Marshall, J., Green, C., and Martin, R. The co-application of sprayed cultured autologous keratinocytes and autologous fibrin sealant in a porcine wound model. Br. J. Plast. Surg. **55**, 219, 2002.

127. Chou, C.H., Cheng, W.T., Kuo, T.F., Sun, J.S., Lin, F.H., and Tsai, J.C. Fibrin glue with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular cartilage tissue engineering: the results of real-time polymerase chain reaction. J. Biomed. Mater. Res. A. **82**, 757, 2007.

128. Uriel, S., Brey, E.M., and Greisler, H.P. Sustained low levels of fibroblast growth factor-1 promote persistent microvascular network formation. Am. J. Surg. **192**, 604, 2006.

129. Erzurum, V.Z., Bian, J.F., Husak, V.A., Ellinger, J., Xue, L., Burgess, W.H., and Greisler, H.P. R136K fibroblast growth factor-1 mutant induces heparin-independent migration of endothelial cells through fibrin glue. J. Vasc. Surg. **37**, 1075, 2003.

130. Shireman, P.K., Hampton, B., Burgess, W.H., and Greisler, H.P. Modulation of vascular cell growth kinetics by local cytokine delivery from fibrin glue suspensions. J. Vasc. Surg. **29**, 852, 1999.

131. Greisler, H.P., Gosselin, C., Ren, D., Kang, S.S., and Kim, D.U. Biointeractive polymers and tissue engineered blood vessels. Biomaterials **17**, 329, 1996.

132. Gorodetsky, R., Clark, R.A., An, J., Gailit, J., Levdansky, L., Vexler, A., Berman, E., and Marx, G. Fibrin microbeads (FMB) as a biodegradable carriers for culturing cells and for accelerating wound healing. J. Invest. Dermatol. **112**, 866, 1999.

133. Marx, G., Mou, X., Hotovely-Salomon, A., Levdansky, L., Gaberman, E., Belenky, D., and Gorodetsky, R. Heat denaturation of fibrinogen to develop a biomedical matrix. J. Biomed. Mater. Res. B. Appl. Biomater. **84**, 49, 2008.

134. Shimony, N., Gorodetsky, R., Marx, G., Gal, D., Rivkin, R., Ben-Ari, A., Landsman, A., and Haviv, Y.S. Fibrin microbeads (FMB) as a 3D platform for kidney gene and cell therapy. Kidney Int. **69**, 625, 2006.

135. Gurevich, O., Vexler, A., Marx, G., Prigozhina, T., Levdansky, L., Slavin, S., Shimeliovich, I., and Gorodetsky, R. Fibrin microbeads for isolating and growing bone marrowderived progenitor cells capable of forming bone tissue.Tissue Eng. **8**, 661, 2002.

136. Cho, S.W., Kim, S.S., Rhie, J.W., Cho, H.M., Choi, C.Y., and Kim, B.S. Engineering of volume-stable adipose tissue. Biomaterials **26**, 3577, 2005.

137. Patrick, C.W., Zheng, B., Johnston, C., and Reece, J.P. Long-term implanation of preadipocytes-seeded PLGA scaffolds. Tissue Eng. **8**, 283, 2002.

138. von Heimburg, D., Zachariah, S., Heschel, I., Kuhling, H., Schoof, H., Hafemann, B., and Pallua, N. Human preadipocytes seeded on freeze-dried collagen scaffolds investigated *in vitro* and *in vivo*. Biomaterials **22**, 429, 2001.

139. Wechselberger, G., Russell, R.C., Neumeister, M.W., Schoeller, T., Piza-katzer, H., and Rainer, C. Successful transplantation of three tissue-engineered cell types using capsule induction techniques and fibrin glue as a delivery vehicle. Plast. Reconstr. Surg. **110**, 123, 2002.

140. Borges, J., Mueller, M.C., Padron, N.T., Tegtmeier, F., Lang, E.M., and Stark, G.B. Engineered adipose tissue supplied by functional microvessels. Tissue Eng. **9**, 1263, 2003.

141. Yoo, W.J., Choi, I.H., Chung, C.Y., Cho, T.-J., Kim, I.-O., and Kim, C.J. Implantation of perichondrium-derived chondrocytes in physeal defects of rabbit tibiae. Acta. Orthop. **76**, 628, 2005.

142. Altmeppen, J., Hansen, E., Bonnla¨nder, G.L., Horch, R.E., and Jeschke, M.G. Composition and characteristics of an autologous thrombocyte gel. J. Surg. Res. **117**, 202, 2004.

143. Wittkampf, A.R. Fibrin glue as cement for HA-granules. J. Craniomaxillofac. Surg. **17**, 179, 1989.

144. Nakamura, K., Koshino, T., and Saito, T. Osteogenic response of the rabbit femur to a hydroxyapatite thermal decomposition product-fibrin glue mixture. Biomaterials **19**, 1901, 1998.

145. Le Nihouannen, D., Le Guehennec, L., Rouillon, T., Pilet, P., Bilban, M., Layrolle, P., and Daculsi, G. Microarchitecture of calcium granules and fibrin glue composites for bone tissue engineering. Biomaterials **27**, 2716, 2006.

146. Umeda, H., Kanemaru, S.-I., Yamashita, M., Kishimoto, M., Tamura, Y., Nakamura, T., Omori, K., Hirano, S., and Ito, J. Bone regeneration of canine skull using bone marrowderived stromal cells and β-tricalcium phosphate. Laryngoscope **117**, 997, 2007.

147. Stevenson, L.W., Warner, S.L., Steimyle, A.E., Fonarow, G.C., Hamilton, M.A., Moriguchi, J.D., Kobashigawa, J.A., Tissisch, J.H., Drinkwater, D.C., and Laks, H. The impending crisis awaiting cardiac transplantation: modeling a solution based on selection. Circulation **89**, 450, 1994.

148. Schoen, F.J. Future directions in tissue heart valves: impact of recent insights from biology and pathology. J. Heart Valve Dis. **8**, 350, 1999.

149. Williams, C., Johnson, S.L., Robinson, P.S., and Tranquillo, R.T. Cell sourcing and culture conditions for fibrin-based valve constructs. Tissue Eng. **12**, 1489, 2006.

150. Flanagan, T.C., Cornelissen, C., Koch, S., Tschoeke, B., Sachweh, J.S., Schmitz-Rode, T., and Jockenhoevel, S. The *in vitro* development of autologous fibrin-based tissue-engineered heart valves through optimised dynamic conditioning. Biomaterials **28**, 3388, 2007.

151. Jockenhoevel, S., Chalabi, K., Sachweh, J.S., Groesdonk, H.V., Demircan, L., Grossman, M., Zund, G., and Messmer, B.J. Tissue engineering: complete autologous valve conduit–a new moulding technique. Thorac. Cardiovasc. Surg. **49**, 287, 2001.

152. Izuta, Y., Ochi, M., Adachi, N., Deie, M., Yamasaki, T., and Shinomiya, R. Meniscal repair using bone marrow-derived mesenchymal stem cells: experimental study using green fluorescent protein transgenic rats. Knee **12**, 217, 2005.

153. Henning, C.E., Lynch, M.A., Yearout, K.M., Vequist, S.W., Stallbaumer, R.J., and Decker, K.A. Arthroscopic meniscal repair using an exogenous fibrin clot. Clin. Orthop. Relat. Res. **252**, 64, 1990.

154. Swenson, T.M. The use of exogenous fibrin clot to supplement meniscal surgery techniques. Orthopedics **30**, 718, 2007.

155. Ruszymah, B.H., Lokman, B.S., Asma, A., Munirah, S., Chua, K., Mazlyzam, A.L., Isa, M.R., Fuzina, N.H., and Aminuddin, B.S. Pediatric auricular chondrocytes gene expression analysis in monolayer culture and engineered elastic cartilage. Int. J. Pediatr. Otorhinolaryngol. **71**, 1225, 2007.

156. Jian-Wei, X., Randolph, M.A., Peretti, G.M., Nazzal, J.A., Roses, R.E., Morse, K.R., and Yaremchuk, M.J. Producing a flexible tissue-engineered cartilage framework using expanded polytetrafluoroethylene membrane as a pseudoperichondrium. Plast. Reconstr. Surg. **116**, 577, 2005.

157. Siedentop, K.H., O'Grady, K., Bhattacharyya, T.K., and Shah, A. Fibrin tissue adhesive and autologous concha cartilage for reconstruction of the posterior-superior canal wall of the chinchilla middle ear. Otol. Neurotol. **25**, 220, 2004.

158. Kim, H.W., and Han, C.D. An overview of cartilage tissue engineering. Yonsei Med. J. **41**, 766, 2000.

159. Dare, E.V., Vascotto, S.G., Carlsson, D., Hincke, M.T., and Griffith, M. Differentiation of fibrin gel encapsulated chondrogenic cell line. Int. J. Artif. Organs **30**, 619, 2007.

160. Perka, C., Spitzer, R.S., Lindenhayn, K., Sittinger, M., and Schultz, O. Matrix-mixed culture: new methodology for chondrocyte culture and preparation of cartilage transplants. J. Biomed. Mater. Res. **49**, 305, 2000.

161. Park, S.H., Park, S.R., Chung, S.I., Pai, K.S., and Min, B.H. Tissue-engineered cartilage using fibrin/hyaluronan composite gel and its *in vivo* implantation. Artif. Organs **29**, 838, 2005.

162. Handl, M., Trc, T., Hanus, M., Stastný, E., Fricová-Poulová, M., Neuwirth, J., Adler, J., Havranová, D., and Varga, F. Therapy of severe chondral defects of the patella by autologous chondrocyte implantation. Acta. Chir. Orthop. Traumatol. Cech. **73**, 373, 2006.

163. Visna, P., Pasa, L., Hart, R., Kocis, J., Cizmár, I., and Adler, J. Treatment of deep chondral defects of the knee using autologous chondrocytes cultured on a support-results after one year. Acta. Chir. Orthop. Traumatol. Cech. **70**, 356, 2003.

164. Bedran-Russo, A.K., Pereira, P.N., Duarte, W.R., Drummond, J.L., and Yamauchi, M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J. Biomed. Mater. Res. B. Appl. Biomater. **80**, 268, 2007.

165. Ameer, G.A., Mahmood, T.A., and Langer, R. A biodegradable composite scaffold for cell transplantation. J. Orthop. Res. **20**, 16, 2002.

166. Perka, C., Schultz, O., Lindenhayn, K., Spitzer, R.S., Muschik, M., Sittinger, M., and Burmester, G.R. Joint cartilage repair with transplantation of embryonic chondrocytes embedded in collagen-fibrin matrices. Clin. Exp. Rheumatol. **18**, 13, 2000.

167. Perka, C., Schultz, O., Spitzer, R.S., and Lindenhayn, K. The influence of transforming growth factor beta1 on mesenchymal cell repair of full-thickness cartilage defects. Biomed. Mater. Res. **52**, 543, 2000.

168. Almqvist, K.F., Wang, L., Wang, J., Baeten, D., Cornelissen, M., Verdonk, R., Veys, E.M., and Verbruggen, G. Culture of chondrocytes in alginate surrounded by fibrin gel: characteristics of the cells over a period of eight weeks. Ann. Rheum. Dis. **60**, 781, 2001.

169. Sims, C.D., Butler, P.E., Cao, Y.L., Casanova, R., Randolph, M.A., Black, A., Vacanti, C.A., and Yaremchuk, M.J. Tissue engineered neocartilage using plasma derived polymer substrates and chondrocytes. Plast. Reconstr. Surg. **101**, 1580, 1998.

170. Silverman, R.P., Passaretti, D.M., Huang, W.M., Randolph, M.A., and Yaremchuk, M.J. Injectable tissue-engineered cartilage using a fibrin glue polymer. Plast. Reconstr. Surg. **103**, 1809, 1999.

171. Fortier, L.A., Mohammed, H.O., Lust, G., and Nixon, A.J. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. J. Bone. Joint. Surg. Br. **84**, 276, 2002.

172. Malicev, E., Radosavljevic, D., and Velikonja, N.K. Fibrin gel improved the spatial uniformity and phenotype of human chondrocytes seeded on collagen scaffolds. Biotechnol. Bioeng. **96**, 364, 2007.

173. Peretti, G.M., Campo-Ruiz, V., Gonzalez, S., Randolph, M.A., Wei, X.J., Morse, K.R., Roses, R.E., and Yaremchuk, M.J. Tissue engineered cartilage integration to live and devitalized cartilage: a study by reflectance mode confocal microscopy and standard histology. Connect. Tissue. Res. **47**, 190, 2006.

174. Xu, X.L., Lou, J., Tang, T., Ng, K.W., Zhang, J., Yu, C., and Dai, K. Evaluation of different scaffolds for BMP-2 genetic orthopedic tissue engineering. J. Biomed. Mater. Res. B. Appl. Biomater. **75**, 289, 2005.

175. Im, G.I., Shin, Y.W., and Lee, K.B. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells?.Osteoarthritis Cartilage **13**, 845, 2005.

176. Chang, F., Ishii, T., Yanai, T., Mishima, H., Akaogi, H., Ogawa, T., and Ochiai, N. Repair of large full-thickness articular cartilage defects by transplantation of autologous uncultured bone-marrow-derived mononuclear cells. J. Orthop. Res. **26**, 18, 2008.

177. Dragoo, J.L., Samimi, B., Zhu, M., Hame, S.L., Thomas, B.J., Lieberman, J.R., Hedrick, M.H., and Benhaim, P. Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads. J. Bone Joint Surg. Br. **85**, 740, 2003.

178. Gelse, K., von der Mark, K., Aigner, T., Park, J., and Schneider, H. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum. **48**, 430, 2003.

179. Xiangdong, W., Ar'Rajab, A., Ahren, B., Andersson, R., and Bengmark, S. The effect of pancreatic islets on transplanted hepatocytes in the treatment of acute liver failure in rats. Res. Exp. Med. (Berl.) **191**, 429, 1991.

180. Demetriou, A.A., Whiting, J.F., Feldman, D., Levenson, S.M., Chowdhury, N.R., Moscioni, A.D., Kram, M., and Chowdhury, J.R. Replacement of liver function in rats by transplantation of microcarrier attached hepatocytes. Science **233**, 1190, 1986.

181. Chia, S.M., Leong, K.W., Li, J., Xu, X., Zeng, K., Er, P.N., Gao, S., and Yu, H. Hepatocyte encapsulation for enhanced cellular functions. Tissue Eng. **6**, 481, 2000.

182. Taylor, J.P., Hardy, J., and Fischbeck, K.H. Toxic proteins in neurodegenerative disease. Science **296**, 1991, 2002.

183. Williams, L.R., Longo, F.M., Powell, H.C., Lundborg, G., and Varon, S. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. J. Comp. Neurol. **218**, 460, 1983.

184. Herbert, C.B., Bittner, G.D., and Hubbell, J.A. Effects of fibrinolysis on neurite growth from dorsal root ganglia cultured in two and three-dimensional fibrin gels. J. Comp. Neurol. **365**, 380, 1996.

185. Willerth, S.M., Arendas, K.J., Gottlieb, D.I., and Sakiyama-Elbert, S.E. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. Biomaterials **27**, 5990, 2006.

186. Cheng, H., Cao, Y., and Olson, L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science **273**, 510, 1996.

187. Iwaya, K., Mizoi, K., Tessler, A., and Itoh, Y. Neurotrophic agents in fibrin glue mediate adult dorsal root regeneration into spinal cord. Neurosurgery **44**, 589 (discussion 595), 1999.

188. Nishida, K. Tissue engineering of the cornea. Cornea **22**, S28, 2003.

189. Duchesne, B., Tahi, H., and Galand, A. Use of human fibrin glue and amniotic membrane transplant in corneal perforation. Cornea **20**, 230, 2001.

190. Rama, P., Bonini, S., Lambiase, A., Golisano, O., Paterna, P., de Luca, M., and Pellegrini, G. Autologous fibrincultured limbal stem cells permanently restore the corneal surface of patients with total limbal cell deficiency. Transplantation **72**, 1478, 2001.

191. Horch, R.E., Kopp, J., Kneser, U., Beier, J., and Bach, A.D. Tissue engineering of cultured skin substitutes. J. Cell. Mol. Med. **9**, 592, 2005.

192. Hafemann, B., Hettich, R., Ensslen, S., Kowol, B., Zuhlke, A., Ebert, R., Konigs, M., and Kirkpatrick, C.J. Treatment of skin defects using suspension of *in vitro* cultured keratinocytes. Burns **20**, 168, 1994.

193. Kaiser, H.W., Stark, G.B., Kopp, J., Balcerkiewicz, A., Spilker, G., and Kreysel, H.W. Cultured autologous keratinocytes in fibrin glue suspension, exclusively and combined

with STS-allograft (preliminary clinical and histological report of a new technique). Burns **20**, 23, 1994.

194. Geer, D.J., Swartz, D.D., and Andreadis, S.T. Fibrin promotes in a three-dimensional *in vitro* model of wound regeneration. Tissue Eng. **8**, 787, 2002.

195. Siedler, S., and Schuller-Petrovic, S. Wound healing enhancement in leg ulcers: a case report. Cell Tissue Bank **3**, 25, 2002.

196. Khor, H.L., Ng, K.W., Htay, A.S., Schanz, J.T., Teoh, S.H., and Hutmacher, D.W. Preliminary study of a polycaprotone membrane utilized as epidermal substrate. J. Mater. Sci. Mater. Med. **14**, 113, 2003.

197. Marx, G., and Mou, X. Characterizing fibrin glue performance as modulated by heparin, aprotinin, and factor XIII. J. Lab. Clin. Med. **140**, 152, 2002.

198. Provenzano, P.P., Hurschler, C., and Vanderby, R., Jr. Microstructural morphology in the transition region between scar and intact residual segments of a healing rat medial collateral ligament. Connect. Tissue Res. **42**, 123, 2001.

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VI. CHAPTER 3. CHARACTERIZATION AND INHIBITION OF FIBRIN HYDROGEL - DEGRADING ENZYMES DURING DEVELOPMENT OF TISSUE ENGINEERING SCAFFOLDS.

In chapter 1, I have discussed different strategies which are currently in use for articular cartilage lesion repair and functional restoration, which include tissue engineering that utilizes a wide variety of scaffolding materials. Amongst the naturally occurring materials, fibrin is a protein-based matrix that combines important features which make it the scaffold of choice for tissue engineering. In chapter 2, I discussed the different approaches to maximize the benefits of fibrin in a wide variety of tissue engineering applications. It is clear, however, that fibrin has two major disadvantages: its rapid degradation rate and poor mechanical properties. In this chapter, I report my experimental work with the chondroprogenitor clonal cell line RCJ 3.1C5.18 (C5.18) to characterize and identify the different enzymes responsible for fibrin hydrogel degradation, in order to prolong scaffold life span until the development of proper cartilaginous tissue. This cell line was chosen since it was evaluated earlier to develop chondrogenic conditions for culture of fibrin-encapsulated cells (Dare et al., 2007). We hypothesize that inhibiting the proteases secreted from the encapsulated chondrogenic cell line (i.e. C5.18 cells), especially plasmin and MMPs, will prolong the lifespan of fibrin hydrogel and promote adequate ECM accumulation. In this study, we detect and characterize the different enzymes responsible for fibrin gel hydrogel breakdown, and control their activity in order to regulate gel stability. The experimental work and writing the first draft of this paper was done entirely by myself.

Manuscript 3

Characterization and Inhibition of Fibrin Hydrogel–Degrading Enzymes during Development of Tissue Engineering Scaffolds

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1. ABSTRACT

The goal of articular cartilage tissue engineering is to provide cartilaginous constructs to replace abnormal cartilage. We have evaluated the chondroprogenitor clonal cell line RCJ3.1C5.18 (C5.18) as a model to guide the development of appropriate scaffolds for tissue engineering. Rapid degradation of fibrin hydrogels was observed after encapsulation of C5.18 cells. The enzymes responsible for this fibrin gel breakdown were characterized to control their activity and regulate gel stability. Western blotting, confirming zymography, revealed bands due to matrix metalloproteinases (MMP-2, MMP 3) that are secreted concomitantly with fibrin hydrogels breakdown. High plasmin activity was detected in conditioned media during hydrogel breakdown but not in the confluent cells before encapsulation. Reverse transcriptase polymerase chain reaction indicated the expression of MMP-2, -3, and -9 and plasminogen in the cells. MMP-9 was 100 times higher at day 1, whereas MMP-2 started to increase and reached its maximum level by day 7. Aprotinin, a known serine protease inhibitor, and galardin (GM6001), a potent MMP inhibitor, in combination or separately, prevented the breakdown of fibrin–C5.18 hydrogels, whereas only the combination of both promoted the accumulation of extracellular matrix. These findings suggest that plasmin and MMPs contribute

independently to fibrin hydrogel breakdown, but that either enzyme can achieve extracellular matrix breakdown.

Running title: Inhibition of fibrin hydrogel-degrading enzymes during tissue engineering

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2. INTRODUCTION

The loss of articular cartilage function due to injury or pathological conditions is a major problem in orthopedic medicine.[1] Although osteochondral defects repair spontaneously, the tissue formed is of variable nature. It ranges from fibrous tissue through fibrocartilaginous character to a hyaline-like cartilaginous nature; however, the original hyaline cartilage is not regenerated.[2,3] The development of tissue-engineered replacements are therefore the focus of intensive research.

Although a range of synthetic to naturally occurring polymers has been investigated, we focused on the use of fibrin, which can be isolated autologously from patients and fabricated into a hydrogel scaffold.[4] Other features of fibrin scaffolds include biodegradability and biocompatibility. In addition, they can achieve high seeding efficiency and uniform cell distribution.[5] Although fibrin is a promising scaffold for the regeneration of cartilage, fibrin–chondrocyte constructs have been observed to degrade before the proper formation of cartilaginous tissue.[6] We therefore hypothesized that stabilization of fibrin-chondrocyte constructs is important for its use in regeneration of cartilaginous tissue. Matrix metalloproteinases (MMPs) are a large family of structurally related zinc- and calcium-dependent proteases that have the combined ability to degrade the major components of the extracellular matrix (ECM).[7] MMPs are involved in the normal turnover of connective tissue matrix that takes place during growth and development. MMPs are also responsible for pathological destruction of tissue in a variety of diseases.[8] The catalytic activity of MMPs is regulated at multiple levels, including transcription, secretion, activation, and inhibition.[9] MMPs are secreted in latent form that must be cleaved to be activated, either by plasmin or membrane-type MMPs.[10] Tissue inhibitors of MMPs (TIMPs) can inhibit MMPs physiologically.[11] Galardin (GM6001), a synthetic peptidyl

hydroxamate inhibitor capable of inhibiting several members of MMPs with Ki values in the nMolar range for MMP -1, -2, -3, -8 and -9,[12] can also inhibit MMPs. It can also prevent the activation of latent matrix metalloproteinases (proMMPs).[13]

Plasminogen activators (t-PA and u-PA) convert another proenzyme, plasminogen, to the active enzyme plasmin.[14] Plasmin is a broad-spectrum protease that can directly degrade multiple ECM components in parallel with other ECM-degrading enzymes, including members of MMPs family.[15] Plasmin degrades fibrin *in vivo* and can convert proMMPs into active MMPs, which in turn degrade fibrinogen, cross-linked fibrin (thrombin induced), and ECM. u-PA, or other MMPs, may also activate ProMMPs directly.[14] Aprotinin is a 6.5 kDa protein that can inhibit plasmin *in vivo* with inhibition constant Ki of nM.[16]

The objectives of this study were to identify the proteases responsible for fibrin hydrogel breakdown and control the activity of these proteases so that gel stability can be regulated. RCJ 3.1C5.18 (C5.18) cells, a rat chondroprogenitor clonal cell line isolated from rat calvaria cells, were used in this study to test the suitability of fibrin hydrogels as a scaffold for the regeneration of articular cartilage.

3. MATERIALS AND METHODS

3.1. Chondrocyte–fibrin constructs

C5.18 cells, a gift from Dr. Jane Aubin, University of Toronto, were grown to confluence in alpha minimum essential medium (Invitrogen, Burlington, ON, Canada) supplemented with 15% fetal bovine serum (Sigma, Toronto, ON, Canada), 100U/mL penicillin, 100 mg/mL streptomycin (Invitrogen), and 10^{-7} M dexamethasone (Sigma).[17] Cells were then dissociated using 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA; Invitrogen), centrifuged, and washed 2 times using phosphate buffered saline (pH 7.4) to

remove traces of trypsin. Fibrin–C5.18 hydrogels were constructed using porcine fibrinogen (Sigma), porcine thrombin (Enzyme Research Laboratories, South Bend, IN), and C5.18 cells. Fibrinogen (14%) was prepared in Dulbecco's modified Eagle medium (DMEM; high glucose, Sigma) supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin, then filtered using a 0.45-μm cellulose acetate filter (Corning, Nepean, ON, Canada). The C5.18 cells were mixed with 14% fibrinogen and 100 IU/mL thrombin to give a final concentration of 10% fibrinogen, 2.2 IU/mL thrombin and 6.4 x 10^6 cells/mL. Preliminary mechanical testing results revealed that 10% fibrin hydrogel is stiff and may be more suitable for implantation. Furthermore, in terms of degradability, it had a longer half-life than lower concentrations such as 5% and 8%. Polymerization of fibrinogen was induced using thrombin and completed within a few minutes. The fibrin C5.18 hydrogels were cultured in chondrogenic DMEM (high glucose, Sigma) supplemented with 10 ng/mL transforming growth factor-beta3 (R&D Systems, Minneapolis, MN, USA), 10^{-7} M dexamethasone, 50 μg/mL ascorbic acid-2- phosphate (Fluka, Toronto, ON, Canada), 100 μg/mL sodium pyruvate (Sigma), 40 μg/mL L-proline (Sigma), 1% insulin-transferrin selenous acid-plus premix (BD Bioscience Pharmingen, Mississauga, ON, Canada), 100 U/mL penicillin, and 100 mg/mL streptomycin as control. For inhibition assays, galardin 25 μM[12] (GM6001, Chemicon, Mississauga, ON, Canada) and aprotinin 20 μg/mL[4] (Sigma) were added to the chondrogenic media separately or in combination. Conditioned media from control and inhibitor-treated hydrogels were collected for analysis using zymography, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), western blotting, and plasmin detection during incubation (5 weeks). Hydrogel samples were collected at various time points for analysis using quantitative RT-PCR, histology, and immunostaining. To study the effect of different proteases on the gel

breakdown, fibrin hydrogels were constructed without cells and subjected to degradation by purified human plasmin 4 μg/mL (Sigma) or MMPs (Chemicon) separately or in combination. To study the effect of galardin on plasmin-mediated degradation, cell-free fibrin hydrogels were constructed and maintained in DMEM containing 4 μg/mL plasmin plus galardin (5, 10, 15, 20, 25 μM) or 20 μg/mL aprotinin.

3.2. Zymography

Proteolytic activity was identified in the samples using 8% fibrin, gelatin, and casein zymography.[18,19]

3.3. SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli.[20] Samples were analyzed on 8% gels using Mini-Protean III electrophoresis cell (Bio-Rad Laboratories, Mississauga, ON, Canada).

3.4. Western blotting

SDS-PAGE was performed (8% poly-acrylamide gels), followed by electrotransfer to nitrocellulose membrane. Table 1 indicates the antibodies used in western blot. After incubation with the primary and secondary antibodies and thorough washing steps, the blot was processed with an enhanced chemiluminescence western blot analysis system (ECL Kit, Amersham, Baie d'Urfe, QC, Canada). The immunoreactive bands were visualized by exposure of the membrane to Bioflex-MSI films (Clonex, Markham, ON, Canada).[21]

3.5. Plasmin

Plasmin activity was detected using a modification of an established method.[22] Samples (10 μL) were added to 35 μL of 50 mM Tris-hydrochloric acid (HCl), pH 7.4, and incubated for 10 min at 37 °C. After incubation, 30 μL of 0.5 mg/mL S-2251 substrate solution (D-Val-Leu-Lys p-nitroanilide dihydrochloride, Sigma) dissolved in 50 mM Tris-

HCl, pH 7.4, was added. After incubation (4 h, 37 °C), the reaction was quenched by the addition of 30 µL of 20% acetic acid. The absorbance at 405 nm was measured using a microplate AutoReader (Bio-Tek Instruments, Winooski, VT). A standard curve was prepared using purified plasmin (human).

Table 1. Antibodies used in western and immunostaining MMP, matrix metalloproteinase

No	*Antibody*	*Host*	*Type*	*Final concentration*	*Company and Catalog No.*
1	Anti-MMP-2 Antibody	Mouse	Monoclonal	1 µg/mL	Calbiochem-IM33L
2	Anti-MMP-3 Antibody	Rabbit	Polyclonal	0.5–2 µg/mL	Anaspec-29576
3	Anti-Collagen II Antibody	Rabbit	Polyclonal	1:40	Chemicon-AB2036
4	Anti-Aggrecan Antibody	Rabbit	Polyclonal	1:40	Chemicon-AB1031
5	Horseradish peroxidase-conjugated Anti-mouse antibody	Sheep	Polyclonal	1:5000	Amersham-NA931
6	Horseradish peroxidase-conjugated Anti-rabbit antibody	Donkey	Polyclonal	1:5000	Amersham-NA934
7	Anti-rabbit IgG antibody,Cy2 conjugate (green)	Goat	Polyclonal	1:200	Amersham Pharmacia-PA42004
8	Anti-rabbit IgG antibody,Cy3 conjugate (red)	Sheep	Polyclonal	1:400	Sigma-C2306

3.6. RT-PCR of MMP-2, -3, -9 and plasminogen

Total ribonucleic acid (RNA) was extracted from fibrin-C5.18 hydrogels using the manufacturer's protocol for the RNeasy-Fibrous Tissue Mini kit (Qiagen, Mississauga, ON, Canada). Total RNA from C5.18-degraded hydrogels was isolated using the Trizol reagent (Invitrogen). Complementary deoxyribonucleic acid (cDNA) was generated from total RNA using superscript II reverse transcriptase and random primers and following the manufacturer's instructions (Invitrogen). The reaction mixture for PCR was heated for 2 min at 95 °C for pre-denaturation and then subjected to 40 cycles, each cycle consisting of denaturation at 95 °C for 45 s, annealing for 45 s, and extension at 72 °C for 1 min, followed by incubation for 3 min at 72 °C to complete the extension. Amplicons were analyzed using 2% agarose gel electrophoresis. Primer sequences, annealing RT-temperatures and product sizes are summarized in Table 2.

Table 2. Polymerase chain reaction primer sequence MMP, matrix metalloproteinase

Target gene & Accession no.		*Primers*	*Annealing T°C*	*Sequence position and Product size*
Rat MMP-2	Forward	5'AGG ATG GAG GCA CGA TTG GTC T 3'	64°C	471–879
NM_031054	Reverse	5'CCA GGT CAG GTG TGT AAC CAA TGA 3'		409 BP
Rat MMP-3	Forward	5'CCT AAA AGC ATT CAC ACT CTG GGC 3'	62°C	1168–1656
NM_133523	Reverse	5'CAG TGC TTC TGA ATG TCC TTC GAC 3'		489 BP
Rat MMP-9	Forward	5'CTA AGG CTC CTC TTT TGC TTC AGG 3'	64°C	2129–2686
NM_031055	Reverse	5'GAT GAG TGG ATA GCT CGG TGG TGT 3'		558 BP
Rat Plasminogen	Forward	5'GGG GAC TCA CTG GAT GGC TAT GTA 3'	60°C	95–561
AJ242649	Reverse	5'TCA TAT CTC TGG TCC GGG TCT GTT 3'		467 BP

3.7. DNA sequencing

Amplicons were cut from the agarose gel and DNA extracted using Wizard SV gel and PCR clean-up system following the manufacturer's recommended protocol (Promega, Madison, WI) and sequenced using Hitachi 3100 genetic analyzer (AB Applied Biosystems, Hornby, ON, Canada).

3.8. Real-time PCR

Rat-specific primers for real-time RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), MMP-2, MMP-3, and MMP-9 were obtained from SuperArray (Bioscience Corporation, Hornby, ON, Canada). PCR amplification was performed using a BioRad iCycler thermocycler with the following conditions: an initial denaturing at 95 °C for 3 min; 45 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 seconds; and a final extension at 72 °C for 10 min. A melt curve analysis using SYBR green was performed as follows: 95 °C for 1 min, 55 °C for 1 min, and 55 °C for 10 s, with an increase of 0.5 °C at each successive cycle for 80 cycles.

The results were analyzed using the comparative C_T method. Expression levels for C5.18 cells at confluence were used as a baseline for each gene, which was normalized to GAPDH housekeeping expression. Baseline expression levels were therefore assigned a value of 1.0.

3.9. Histology

Fibrin–C5.18 hydrogels were fixed in 4% paraformaldehyde overnight, maintained in 30% sucrose for 24 h, and then frozen in 1:1 30% sucrose: OCT for storage at -80 °C. The tissue blocks were cut in 10-μm sections using a Shandon cryotome FSE (Thermo Electron Corporation, Nepean, ON, Canada). Sections were stained with hematoxylin and eosin or Alcian blue (pH 2.5) using standard protocols.

3.10. Live/dead stain

Confluent C5.18 cells were maintained for 3 days in chondrogenic medium supplemented with different inhibitors then stained with calcein green (live cells) and ethidium homodimer-1 red (dead cells) according to manufacturer's protocol (Live/Dead Viability/Cytotoxicity Kit, Molecular Probes, Eugene, OR).

3.11. Immunohistochemistry

After frozen sections were brought to room temperature, excess water was removed and slides then washed once in Tris-buffered saline (TBS) (100 mM Tris-HCl, 150mM sodium chloride, pH 7.7). The sections were digested in 2% hyaluronidase (Sigma) in TBS for 30 min, followed by 3 washing steps in TBS of 3 min each. The slides were then placed in blocking buffer (4% fetal bovine serum, 1% bovine serum albumin, 0.3% triton X-100) for 30 min, washed again 3 times in TBS, and maintained in the primary antibody (prepared in blocking buffer) overnight. After incubation with primary antibody overnight, the slides were washed 3 times in TBS and incubated in secondary antibody (prepared in TBS, 0.3% triton X-100), washed 2 times in TBS, stained for 3 min with 4□,6-diamidino-2-phenylindole stain (1:10000 in TBS), washed 2 times in TBS, and cover slipped with TBS. For negative controls, the same procedures were performed with omission of the primary antibodies. The antibodies and dilutions used in immunostaining are listed in Table 1.

3.12. Statistical Analysis

Analysis of variance for plasmin activity was performed using SPSS (version14, SPSS Inc., Chicago, IL) software.

4. RESULTS

Fibrin–C5.18 hydrogels were completely degraded within 7 days in the absence of protease inhibitors. Analysis of conditioned medium for fibrin degradation products (FDPs) using SDS-PAGE revealed that FDP started low then increased dramatically at days 4 and 5, started to decrease by day 6, and vanished by day 7 after complete degradation of cross-linked fibrin (data shown in appendix I). Analysis of conditioned medium using gelatin zymography revealed proteolytic bands of MMP-9 at 91, 86, and 80 kDa (latent and the 2 active forms, respectively) and MMP-2 at 68, 62, and 57 kDa (latent and the 2 active forms, respectively). Gelatin zymograms for purified plasmin showed bands at 74, 69, and 48 kDa. Casein zymograms revealed the proteolytic band of MMP-3 at 50 kDa and latent and active forms of MMP-9 at 91 and 80 kDa respectively, in addition to the latent form of MMP-2 at 68 kDa. The use of bovine fibrinogen as a substrate for zymography revealed the secretion of the active form of MMP-3 at 50 kDa and the latent and active forms of MMP-9 at 91 and 80 kDa, respectively, in addition to the latent and active forms of MMP-2 at 68 and 57 kDs, respectively (data shown in appendix I). Metal ion chelators such as EDTA (30 mM) and *o*-phenanthrolene (20 mM) inhibit the bands identified in gelatin zymograms, but the serine protease inhibitor phenylmethylsulfonyl fluoride (10 mM) and a cysteine protease inhibitor 10 μM E-64 do not (data not shown). The identities of the MMP-2, -3, and -9 bands were consistent with quantitative RT-PCR profile and western blotting.

C5.18-mediated breakdown of cross-linked fibrin (day 4) resulted in FDPs at 99, 93, 87, 54, and 39 kDa. Treatment of cross-linked fibrin with purified human plasmin (4 μg/mL)

resulted in prominent FDPs at 83 and 39 kDa and barely visible bands at 93 and 54 kDa, whereas treatment with a mixture of purified MMPs resulted in FDPs at 99, 93, and 54 kDa. Only treatment with a mixture of plasmin and MMPs resulted in FDPs similar to those of day 4 conditioned medium (Fig. 1).

The secretion of different MMPs from C5.18 cells during the breakdown of fibrin hydrogels was subsequently investigated using western blotting of conditioned medium. Specific antibodies revealed the existence of immunoreactive bands at 68 and 57 kDa (latent and active forms of MMP-2), as well as a single immunoreactive band at 50 kDa, which represents MMP-3 active form (data shown in appendix I).

Plasmin activity was detected in conditioned media from fibrin–C5.18 hydrogels using S-2251, a specific chromogenic substrate (Fig. 1). The mean value of plasmin activity in conditioned medium on days 1, 2, and 5 is significantly higher than at day 0 (day 0: the conditioned medium collected directly after the preparation of fibrin-C5.18 hydrogels), whereas no plasmin activity was detected in confluent cell conditioned medium. These results suggest that encapsulation of C5.18 cells in fibrin hydrogels stimulates the synthesis and secretion of plasminogen. MMP-2, -3, and -9 and plasminogen gene expression was evaluated using specific primers for RT-PCR, revealing the expression of these genes in C5.18 cells after encapsulation in fibrin hydrogels. MMP-9 and plasminogen are upregulated in comparison to confluent cells (data shown in appendix I).

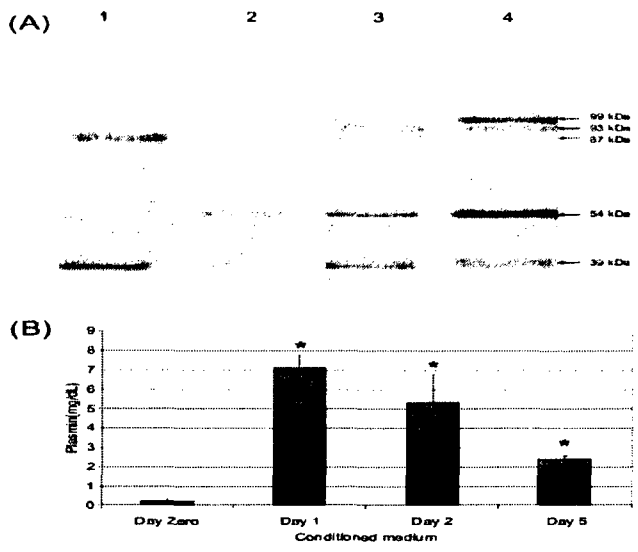

FIG. 1. (A) Comparison of fibrin degradation products (FDPs) obtained after treatment of cross-linked fibrin with purified proteases. Plasmin 4 μg/mL (lane 1), matrix metalloproteinases (MMP) mixture (lane 2), mixture of plasmin and MMPs (lane 3), FDPs obtained from conditioned medium of fibrin–C5.18 hydrogels at day 4 (lane 4). **(B)** Plasmin activity in conditioned medium from fibrin–C5.18 hydrogels. Plasmin activity was detected using a synthetic chromogenic substrate S-2251 at days 0, 1, 2, and 5 of incubation. ($F = 67.39$, $p<0.001$).

Sequencing of the plasminogen amplicon confirmed its identity. Further analysis of MMP-2, -3, and -9 gene expression using real-time PCR showed that MMP-9 gene expression was dramatically upregulated at day 1 and then decreased to minimum expression after complete breakdown of fibrin hydrogels. On the other hand, MMP-2 gene expression was low at day 1 and then started to increase, reaching its maximum expression by day 7. In contrast, the expression of MMP-3 fluctuated throughout 7 days of incubation. Taken together, these results indicate that there is differential expression of MMP-2, -3, and -9 by encapsulated C5.18 cells and that MMP-9 upregulation precedes gel breakdown, whereas MMP-2 expression is upregulated after gel breakdown is complete (Fig. 2).

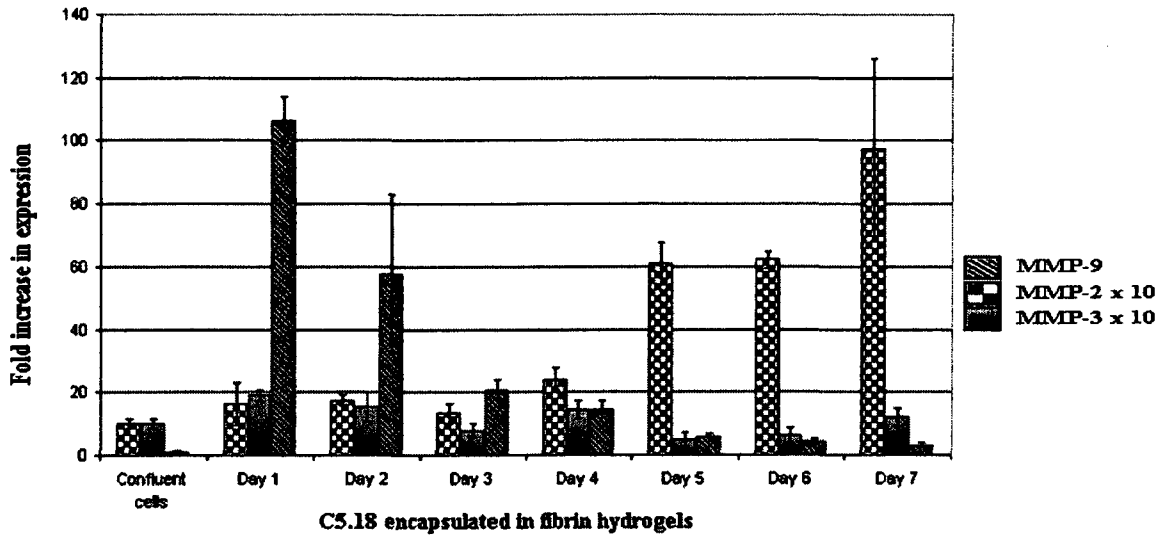

FIG. 2. Analysis of matrix metalloproteinases (MMP) gene expression by real-time reverse transcriptase polymerase chain reaction (PCR). The histogram shows real-time PCR results for MMP-2, MMP-3, and MMP-9 by C5.18 cells before and after encapsulation in fibrin hydrogels.

Cryosectioned fibrin–C5.18 hydrogels were histologically stained using hematoxylin and eosin and Alcian blue. Histology results showed that aprotinin (plasmin inhibitor), galardin (general MMP inhibitor), or the combination of both was capable of preventing fibrin hydrogel breakdown and stabilized the gels for more than 5 weeks, although only a combination of aprotinin and galardin led to enhanced ECM formation within the hydrogel (Fig. 3). Furthermore, specific immunostaining for ECM components collagen II and aggrecan confirmed that only a combination of aprotinin and galardin was associated with a slight accumulation of ECM after 2 weeks and a strong accumulation after 5 weeks in the interface (Fig. 4). To verify that galardin inhibited only MMPs, fibrin hydrogels were constructed and subjected to degradation by plasmin-containing medium to which different concentrations of galardin were added (5-25 μM). It was found that galardin, even at high concentration, did not inhibit plasmin. Statistical analyses for

Live/Dead staining in monolayer culture revealed that aprotinin (20 μg/mL) and galardin- (25 μM) supplemented chondrogenic medium did not have any significant effect on the percentage of live C5.18 cells (aprotinin 94.6±1.7 and galardin 94.9±2.6 vs control 96.8±0.7)

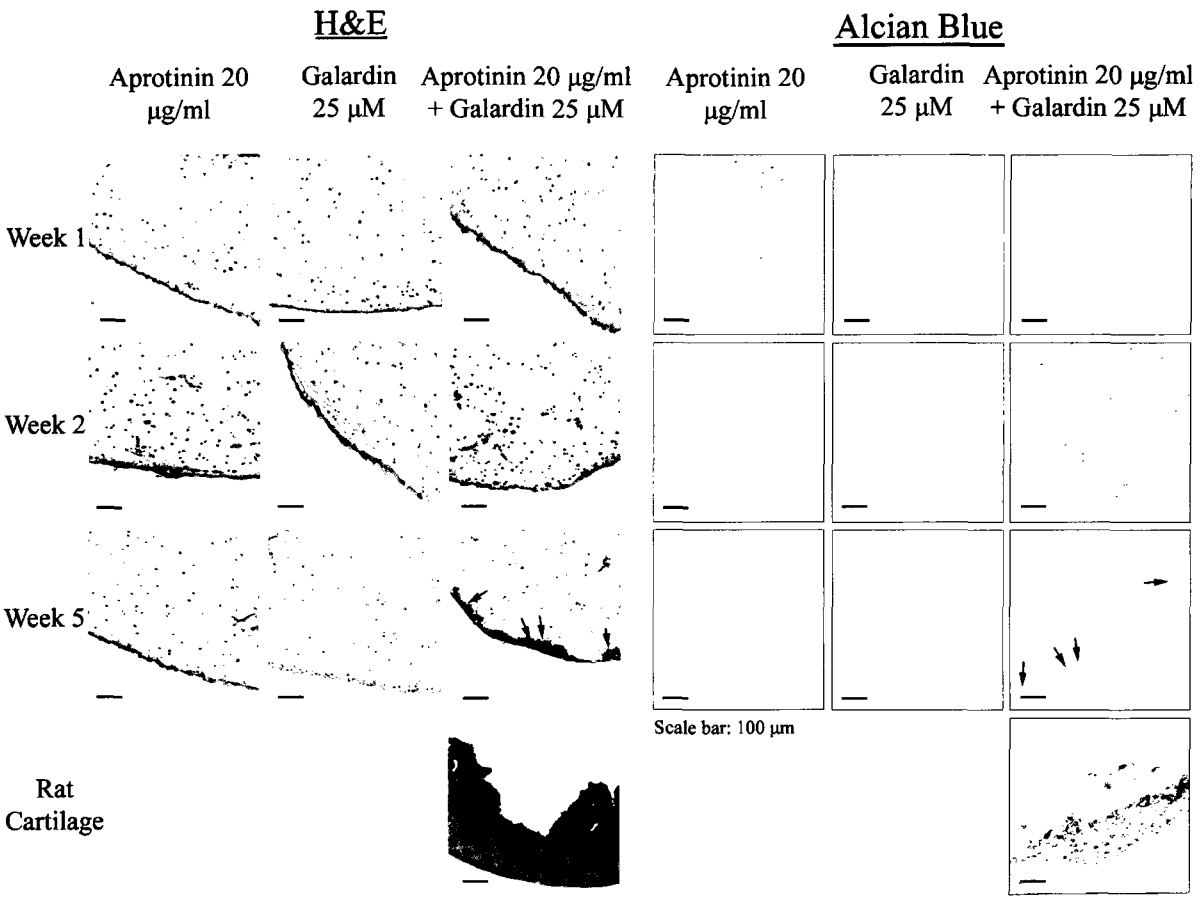

FIG. 3. Hematoxylin and eosin and Alcian blue histological staining. Cryosectioned fibrin C5.18 hydrogels were analyzed after 1, 2, and 5 weeks of culture. Aprotinin, galardin, and a combination of aprotinin and galardin were evaluated for inhibition of hydrogel degradation. The arrows in the interface of hematoxylin and eosin (H&E) and Alcian blue staining show the sites where extracellular matrix formation took place with combined aprotinin and galardin. Color images available online at www.liebertpub.com /ten.

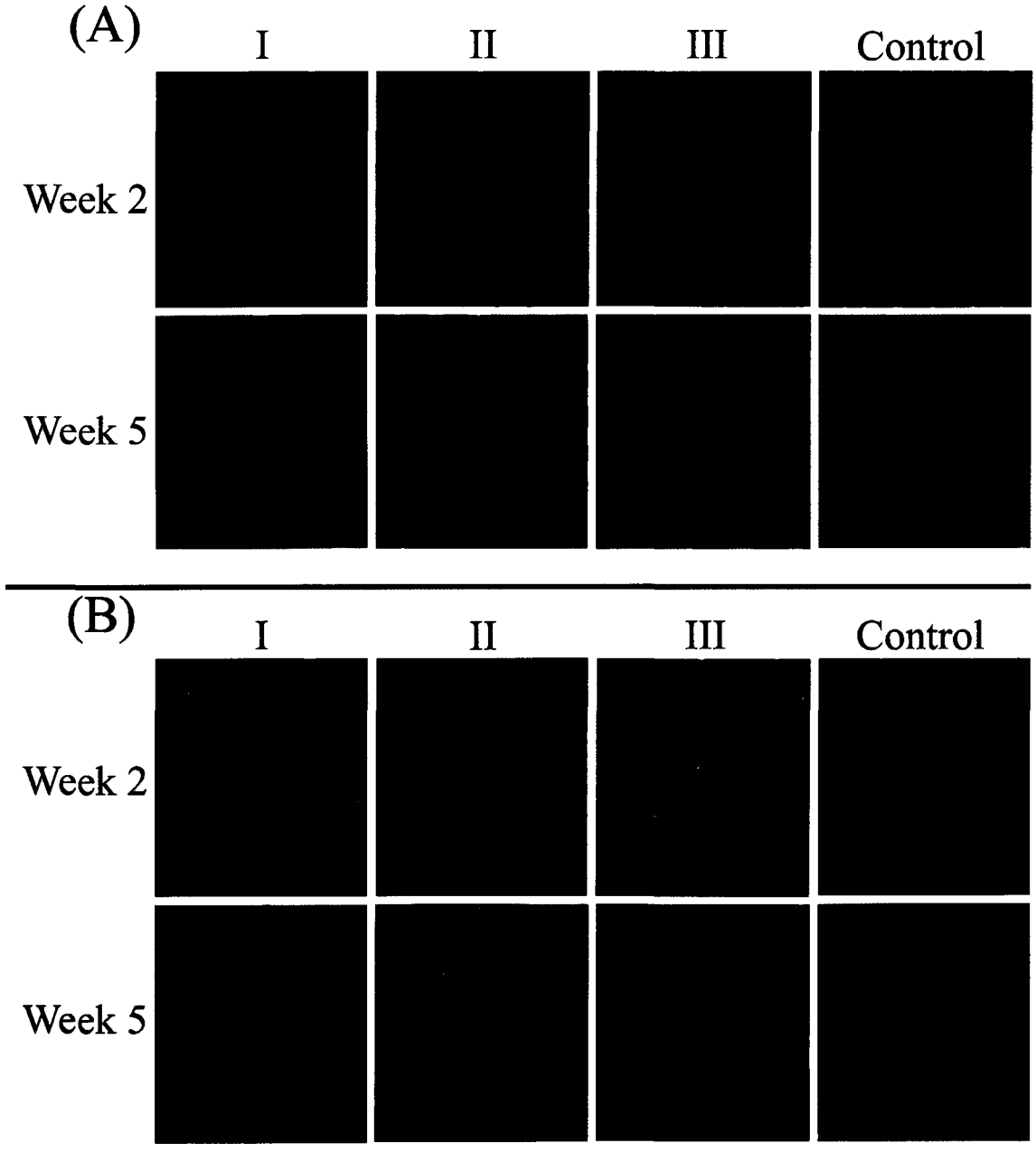

FIG.4. Fibrin–C5.18 hydrogel immunostaining for aggrecan **(A)** and collagen II **(B)**. Hydrogels were stabilized by incubation with protease inhibitors for 2 and 5 weeks using 20 μg/mL aprotinin (I), 25 μM galardin (II), and 20 μg/mL aprotinin plus 25 μM galardin (III). Control is sections stained with omission of primary antibodies. Color images available online at www .liebertpub.com /ten.

5. DISCUSSION

During studies to investigate C5.18 as a model cell line for tissue engineering of articular cartilage, we observed that fibrin–C5.18 hydrogels degraded rapidly within 7 days. It is important to point out that, although fibrinogen may contain trace amounts of plasmin and thrombin, these proteases, if present, did not contribute significantly to fibrin degradation in our system, as indicated by the fact that fibrin degradation is greatly enhanced in the presence of C5.18 cells. Sterile gels prepared without cells were stable for up to 3 weeks. Fibrin hydrogels are degraded by incubation with other cell lines such as endothelial and smooth muscle cells.[5] Furthermore; they can be degraded by myofibroblasts,[4] corneal stem cells,[23] and nasal septum chondrocytes.[24] The data presented in this article clearly show that C5.18, a chondroprogenitor clonal cell line that models some features of cartilage regeneration, is capable of secreting gelatinases (MMP-2 and MMP-9), MMP-3, and plasminogen. We hypothesized that MMPs, the plasminogen/plasmin system, or both together were responsible for the observed rapid degradation of fibrin–C5.18 hydrogels. These results indicate that C5.18 cells demonstrate some chondrocyte-like properties during chondrogenic differentiation, particularly the secretion of proteases such as MMPs. Previous literature has demonstrated that chondrocytes can normally secrete a wide variety of MMPs, such as MMP-1, -2, -3, and -9.[25] Previous studies have also shown that chondrocytes can secrete plasminogen activators under physiological conditions, suggesting a possible mechanism for activation of plasminogen.[26] Although there is no direct evidence for the secretion of plasminogen from chondrocytes, plasminogen can be produced extraheptically.[27,28] In this study, plasminogen secretion from C5.18 cells was confirmed in the conditioned medium using synthetic chromogenic substrate, and plasminogen gene expression was assessed using RT-

PCR and sequencing of the resultant amplicon. MMP-2 and -9 gene expression is upregulated in encapsulated cells in comparison to confluent cells. One possible reason for this upregulation is interaction with cross-linked fibrin itself after encapsulation of C5.18 cells. Fibrin can increase expression of MMPs, particularly gelatinases such as MMP-2 and MMP-9, in addition to its role in the upregulation of plasminogen activator (u-PA and t-PA), whose activation of plasmin promotes activation of MMPs.[29] In addition, t-PA-induced plasminogen activation is accelerated in the presence of fibrin.[30]

It is believed that the imbalance between proteases such as MMPs and plasmin and their inhibitors are the main cause of fibrin hydrogel breakdown in tissue-engineered constructs.[31,32] In our study, treatment of cell-free cross-linked fibrin by MMPs or purified plasmin alone did not produce FDPs resembling those produced by incubation with C5.18 cells. Only treatment with a mixture of plasmin and MMPs produce a FDP pattern like that produced by C5.18 cells. Therefore, we hypothesize that MMPs and plasmin collaborate together in the breakdown of fibrin-C5.18 hydrogels. *In vivo*, plasmin catalyzes fibrinolysis,[33] although other studies have suggested that MMPs also participate in the removal of the fibrin clot.[34,35]

Several strategies have been used to prolong the life span of fibrin hydrogel for tissue engineering applications through controlling the calcium^{2+} concentration and pH,[36] hybridizing fibrin with a synthetic molecule such as polyethylene glycol,[37] modifying the form of fibrin,[38] and using low cell density.[39] In this study, treatment of fibrin-C5.18 hydrogels with aprotinin, galardin, or both together prevented fibrin hydrogel breakdown. Furthermore, fibrin degradation in the control group (without inhibitor) was almost complete by day 4 and 5, whereas plasmin activity was high in the first day then started to decrease, which may be due to decreased expression of plasminogen activators or increased

expression of plasminogen activator inhibitors, plasmin inhibitors or both.[40] These results indicated that MMPs are the main proteases responsible for fibrin hydrogels breakdown and that plasmin indirectly participated in the degradation of fibrin hydrogels by activating proMMPs. To exclude the possibility that galardin may inhibit plasmin at high concentration, the effect of galardin at different concentrations on plasmin-treated cross-linked fibrin was evaluated. Galardin was unable to inhibit plasmin-mediated fibrin gel breakdown. This result was consistent with results from a previous study demonstrating that galardin has no effect on the activities of plasminogen activator or plasmin.[13] Furthermore, aprotinin cannot inhibit MMPs at concentrations as high as 200 μg/mL.[41]

Application of a combination of aprotinin and galardin to the fibrin-C5.18 hydrogel was able to stabilize the structure of ECM, as evidenced by the accumulation of collagen II and aggrecan (2 components of ECM), whereas the use of aprotinin or galardin alone fails to stabilize these components of ECM. One possible explanation is that membrane-type MMP mediates ECM degradation. A recent study indicated that membrane-type 1 - MMP can degrade many components of ECM, including collagen, Furthermore, it can lead to the activation of membrane-bound proMMP-2, suggesting a mechanism for activation of MMPs other than plasmin.[10] Upon inhibition of plasmin by aprotinin, membrane-type MMPs activate MMPs. Membrane-type MMPs in parallel with MMPs then degrade ECM. Galardin-mediated inhibition of MMPs does not prevent plasmin-mediated ECM degradation. These results suggest that plasminogen/plasmin or MMPs systems are capable of degrading ECM and that stabilization of ECM requires the inhibition of both systems simultaneously. Previous studies indicated that ECM degradation involves a proteolytic enzyme cascade. Plasminogen activators, which convert plasminogen to plasmin, which in turn results in the activation of MMPs (directly or indirectly) initiates this cascade. Plasmin

and active MMPs then carry out the degradation of ECM.[42] Further studies must be done to investigate the effects of the addition of plasmin and MMP inhibitors on ECM remodelling of the tissue-engineered construct.

6. CONCLUSIONS

MMPs and plasminogen secreted by cells after encapsulation mediate fibrin–C5.18 hydrogel degradation. Inhibition of MMPs or plasmin by galardin or aprotinin, respectively, greatly inhibited fibrin degradation, whereas inhibition of both together was necessary for enhanced ECM accumulation.

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8. REFERENCES

1. Risbud, M.V., and Sittinger, M. Tissue engineering: advances in in-vitro cartilage generation (review). Trends Biotechnol. **20**, 351, 2002.

2. Oshima, Y., Watanabe, N., Matsuda, K., Takai, S., Kawata, M., and Kubo, T. Fate of transplanted bone-marrow-derived mesenchymal cells during osteochondral repair using transgenic rats to stimulate autologous transplantation. Osteoarthr.Cartil. **12**, 811, 2004.

3. Nehrer, S., Spector, M., and Minas, T. Histological analysis of tissue after failed cartilage repair procedures. Clin. Orthop.Relat. Res. **365**, 149, 1999.

4. Jockenhoevel, S., Zund, G., Hoerstrup, S.P., Chalabi, K., Sachweh, J.S., Demircan, L., Messmer, B.J., and Turina, M. Fibrin gel-advantages of a new scaffold in cardiovascular tissue engineering. Eur. J. Cardiothorac. Surg. **19**, 424,2001.

5. Swartz, D.D., Russel, J.A., and Andreadis, S.T. Engineering of fibrin-based functional and implantable small-diameter blood vessels. Am. J. Physiol. Heart. Circ. Physiol. **288**, H1451, 2005.

6. Fussenegger, M., Meinhart, J., Hobling, W., Kullich, W., Funk, S., and Bernatzky, G. Stabilized autologous fibrin chondrocyte constructs for cartilage repair *in vivo*. Ann. Plast. Surg. **51**, 493, 2003.

7. Muhs, B.E., Gagne, P., Plitas, G., Shaw, J.P., and Shamamian, P. Experimental hind limb ischemia leads to neutrophil-mediated increase in gastrocnemius MMP-2 and -9 activity: a potential mechanism for ischemia induced MMP activation. J. Surg. Res. **117**, 249, 2004.

8. Cawston, T.E. Metalloproteinase inhibitors and the prevention of connective tissue breakdown (review). Pharmacol.Ther. **70**, 163, 1996.

9. Massova, I., Kotra, L.P., Fridman, R., and Mobashery, S. Matrix metalloproteinase: structures, evolution, and diversification. FASEB J. **12**, 1075, 1998.

10. Gingras, D., Page, M., Annabi, I.B., and Beliveau, R. Rapid activation of matrix metalloproteinase-2 by glioma cells occurs through a post-translational MT1-MMP-dependent mechanism. Biochem. Biophys. Acta. **1497**, 341, 2000.

11. Majka, S., McGuire, P., Colombo, S., and Das, A. The balance between proteinases and inhibitors in a murine model of proliferative retinopathy. Invest. Ophthalmol. Vis. Sci. **42**, 210, 2001.

12. Gijbels, K., Galardy, R.E., and Steinman, L. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteinases. J. Clin. Invest. **94**, 2177, 1994.

13. Hao, J.L., Nagano, T., Nakamura, M., Kumagai, N., Mishima, H., and Nishida, T. Galardin inhibits collagen degradation by rabbit keratocytes by inhibiting the activation of pro-matrix metalloproteinases. Exp. Eye Res. **68**, 565, 1999.

14. Collen, D. Ham-Wasserman lecture: role of the plasminogen system in fibrin-homeostasis and tissue remodeling (Review). Hematology Am. Soc. Hematol. Educ. Program 1, 2001.

15. Morgan, H., and Hill, P.A. Human breast cancer cell-mediated bone collagen degradation requires plasminogen activation and matrix metalloproteinase activity. Cancer Cell Int. **5**, 1, 2005.

16. Kang, H.M., Kalnoski, M.H., Frederick, M., and Chandler, W.L. The kinetics of plasmin inhibition by aprotinin *in vivo*. Thromb. Res. **115**, 327, 2005.

17. Grigoriadis, A.E., Heersche, J.N., and Aubin, J.E. Analysis of chondroprogenitor frequency and cartilage differentiation in a novel family of clonal chondrogenic rat cell lines. Differentiation **60**, 299, 1996.

18. Waas, E.T., Lomme. R.M, DeGroot, J., Wobbes, T., and Hendriks, T. Tissue levels of active matrix metalloproteinase- 2 and -9 in colorectal cancer. Br. J. Cancer **86**, 1876, 2002.

19. Lantz, M.S., and Ciborowski, P. Zymographic techniques for detection and characterization of microbial protease. Meth. Enzymol. **235**, 563, 1994.

20. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680, 1970.

21. Towbin, H., Staehelen, T., and Gordon, J. Electophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad.Sci. U. S. A. **76**, 4350, 1979.

22. Byun, Y., and Yang, V.C. Delivery system for targeted thrombolysis without the risk of hemorrhage. ASAIO J. **44**, M638, 1998.

23. Han, B., Schwab, I.R., Madsen, T.K., and Isseroff, R.R. A fibrin- based bioengineered ocular surface with human corneal epithelial stem cells. Cornea **21**, 505, 2002.

24. Meinhart, J., Fussenegger, M., and Hobling, W. Stabilization of fibrin-chondrocyte constructs for cartilage reconstruction. Ann. Plast. Surg. **42**, 673, 1999.

25. Sabatini, M., Lesur, C., Thomas, M., Chomel, A., Anract, P., de Nanteuil, G., and Pastoureau, P. Effect of inhibition of matrix metalloproteinases on cartilage loss *in vitro* and in guinea pig model of osteoarthritis. Arthritis Rheum. **52**, 171, 2005.

26. Yamada, H., Stephens, R.W., Nakagawa, T. and McNicol, D. Detection and partial characterization of a specific plasminogen activator inhibitor in human chondrocyte culture. J. Biochem. (Tokyo) **104**, 960, 1988.

27. Twining, S.S., Wilson, P.M., and Ngamkitidechakul, C. Extrahepatic synthesis of plasminogen in the human cornea is up-regulated by interleukins-1alpha and -1beta. Biochem. J. **339**, 705, 1999.

28. Kasza, A., Kiss, D.L., Gopalan, S., Xu, W., Rydel, R.E., Koj, A., and Kordula, T. Mechanism of plasminogen activator inhibitor-1 regulation by oncostatin M and interleukin-1 in human astrocytes. J. Neurochem. **83**, 696, 2002.

29. Cai, G., Chen, X., Fu, B., and Lu, Y. Activation of gelatinases by fibrin is a PA/plasmin system-dependent in human glomerular endothelial cells. Mol. Cell. Biochem. **277**, 171, 2005.

30. Oliver, J.J., Webb, D., and Newby, D.E. Stimulated tissue plasminogen activator release as a marker of endothelial functions in humans. Arterioscler. Thromb. Vasc. Biol. **25**, 2470, 2005.

31. Dean, D.D., Martel-Pelletier, J., Pelletier, J.P., Howell, D.S., and Woessner, J.F. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. J. Clin. Invest. **84**, 678, 1989.

32. Martel-Pelletier, J., Zafarullah, M., Kodama, S., and Pelletier, J. P. *In vitro* effects of interleukin 1 on the synthesis of metalloproteinases, TIMP, plasminogen activators and inhibitors in human articular cartilage. J. Rheumatol. Suppl. **27**, 80, 1991.

33. Urech, L., Bittermann, A.G., Hubbell, J.A., and Hall, Heike. Mechanical properties, proteolytic degradability and biological modifications affect angiogenic process extension into native and modified fibrin matrices *in vitro*. Biomaterials **26**, 1369, 2005.

34. Hiraoka, N., Allen, E., Apel, I.J., Gyetko, M.R., and Weiss, S.J. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell **95**, 365, 1998.

35. Hiller, O., Lichte, A., Oberpichler, A., Kocourek, A., and Tschesche, H. Matrix metalloproteinases collagenase-3, and membrane type 1- matrix metalloproteinase impair clotting by degradation of fibrinogen and factor XII. J. Biol. Chem. **275**, 33008, 2000.

36. Eyrich, D., Brandl, F., Appel, B., Wiese, H., Maier, G., Wenzel, M., Staudenmaier, R., Goepferich, A., and Blunk, T. Long-term stable fibrin gels for cartilage engineering. Biomaterials **28**, 55, 2007.

37. Dikovsky, D., Bianco-Peled, H., and Seliktar, D. The effect of structural alterations of PEG-fibrinogen hydrogel scaffolds on 3-D cellular morphology and cellular migration. Biomaterials **27**, 1496, 2006.

38. Kassis, I., Zangi, L., Levdansky, L., Samuel, S., Marx, G., and Gorodetsky, R. Isolation of mesenchymal stem cells form GCSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant. **37**, 967, 2006.

39. Passaretti, D., Silvermn, R.P., Huang, W., Kirchhoff, C.H., Ashiku, S., Randolph, M.A., and Yaremchuk, M.J. Cultured chondrocytes produce injectable tissue-engineered cartilage in hydrogel polymer. Tissue Eng. **7**, 805, 2001

40. Ramont, L., Pasco, S., Hornebeck, W., Maquart, F.X., and Monboisse, J.C. Transforming growth factor b1 inhibits tumor growth in a mouse melanoma model by down-regulating the plasminogen activation system. Exp. Cell. Res. **291**, 1, 2003.

41. Ratel, D., Glazier, G., Provencal, M., Boivin, D., Beaulieu, E., Gingras, D., and Beliveau, R. Direct-acting fibrinolytic enzymes in shark cartilage extract: potential therapeutic role in vascular disorders. Thromb. Res. **115**, 143, 2005.

42. Baricos, W.H., Cortez, S.L., El-Dahr, S.S., and Schnaper, H.W. ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. Kidney Int. **47**, 1039, 1995.

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VII. CHAPTER 4. FIBRIN GLUES IN COMBINATION WITH MESENCHYMAL STEM CELLS TO DEVELOP A TISSUE – ENGINEERED CARTILAGE SUBSTITUTE

In chapter 3, I discussed the stabilization of fibrin hydrogels prepared from rat C5.18 cells, commercially purified porcine fibrinogen and thrombin. We have shown that addition of aprotinin (plasmin inhibitor) or galardin (MMPs inhibitor) results in stabilization of fibrin hydrogel, and that only a combination of both inhibitors resulted in enhanced ECM accumulation (Ahmed et al., 2007). Although fibrin gels from commercially produced materials are available in standardized quality, fibrin glue that can be prepared in autologous form has two major advantages: the possibility of viral transmission and prion infection, already minimal with current commercial products, is reduced, and the cost is lower (Ahmed et al 2008). Moreover, bone marrow-derived mesenchymal stem cells (BM-hMSCs) have advantages over chondrocytes such as being harvested in autologous form for tissue engineering and possessing multi-differentiation potential. We hypothesize that fibrin glue produced by the CryoSeal ® FS system from one unit of plasma (Dare et al., 2009), in combination with hMSCs, would move the research closer to clinical trial phases. In order to increase the bioavailability of growth factors and protect them from proteolytic degradation, we assessed the heparin-based delivery system (HBDS) that was originally developed and incorporated into fibrin gels by Sakiyama-Elbert et al., 2000 for these purposes. Different fibrin-hMSCs constructs were maintained in chondrogenic media supplemented with tranexamic acid (plasmin inhibitor), since galardin (MMP inhibitor) alone was shown to fail in stabilizing these constructs. We hypothesized that encapsulation of hMSCs into stabilized fibrin glue, with or without HBDS, will result

in formation of cartilaginous constructs that accumulate cartilage-specific markers to form a cartilage substitute. In the following chapter we present and discuss the chondrogenic differentiation of hMSCs after encapsulation into fibrin glue, rich or poor in platelets, with or without HBDS in the presence of protease inhibitor. The experimental work was done entirely by myself, except for the adipogenic differentiation of hMSCs, which was done by my colleague Roshan K. Sriram who is currently a Ph.D. candidate in Faculty of Medicine, University of Ottawa. The paper was written entirely by myself.

Manuscript 4

Fibrin Glues in Combination with Mesenchymal Stem Cells to Develop a Tissue-Engineered Cartilage Substitute

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1. ABSTRACT

Damage of cartilage due to traumatic or pathological conditions results in disability and severe pain. Regenerative medicine, using tissue engineering-based constructs to enhance cartilage repair by mobilizing chondrogenic cells, is a promising approach for restoration of structure and function. Fresh fibrin (FG) and platelet-rich fibrin (PR-FG) glues produced by the CryoSeal® FS System, in combination with human bone marrow-derived mesenchymal stem cells (BM-hMSCs), were evaluated in this study. We additionally tested the incorporation of heparin-based delivery system (HBDS) into these scaffolds to immobilize endogenous growth factors as well as exogenous TGF-β_2. Strongly CD90+ and CD105+ hMSCs were encapsulated into FG and PR-FG with and without HBDS. Encapsulation of hMSCs in PR-FG led to increased expression of collagen II gene at 2.5 weeks, however, no difference was observed between FG and PR-FG at 5 weeks. The incorporation of HBDS prevented the enhancement of collagen II gene expression. hMSCs in FG initially displayed enhanced aggrecan gene expression and increased accumulation of Alcian blue-positive extracellular matrix (ECM); incorporation of HBDS into these glues did not improve aggrecan gene expression and ECM accumulation. The most significant effect on cartilage marker gene expression and accumulation was observed after encapsulation of hMSCs in FG. We conclude that fibrin glue is more promising than platelet-rich fibrin glue as a scaffold for chondrogenic differentiation of hMSCs; however, immobilization of growth factors inside these fibrin scaffolds with the HBDS system has a negative impact on this process.

Running title: Fresh Fibrin Glue Matrices for Cartilage Tissue Engineering

2. INTRODUCTION

Due to its limited capacity to repair and regenerate, damage to articular cartilage by osteoarthritis or trauma usually heals through scar formation and leads to disability and poorer quality of life especially, in elderly people.[1,2] The major criteria for successful cartilage repair are reduction of pain, improvement of symptoms and maintenance of long-term functionality. Although current methods such as microfracture, osteoarticular autograft transfer system, mosaicplasty, and autologous chondrocyte implantation are beneficial, they have significant limitations.[3] Alternatively, partial and total knee arthroplasty is a very effective in relieving the pain and disability, however they have significant failure rate.[4,5] The successful repair of articular cartilage is therefore a significant challenge for the emerging field of regenerative medicine.

Tissue engineering of articular cartilage requires an appropriate three dimensional (3D) scaffolding material in which a suitable cell source can proliferate and secret specific extracellular matrix (ECM) macromolecules to participate in the repair process.[6] We have focused our research on fibrin matrix, as it possesses some characteristics which make it the scaffold of choice in articular cartilage tissue engineering; for example, it can be isolated autologously from patients and fabricated into a hydrogel scaffold.[7] However, rapid degradation and low mechanical stiffness are major disadvantages.[8,9] We and others have shown that the rapid degradation can be controlled by the addition of protease inhibitors such as aprotonin, galardin, and tranexamic acid to the culture media[9,10], while the mechanical properties of the fibrin hydrogel can be enhanced by further cross-linking using genipin, a natural cross-linker.[11] Fibrin glue has been evaluated in a variety of tissue engineering applications. It can be produced either from allogeneic pooled plasma such as Tissucol/Tisseel® or from autologous plasma through cryoprecipitation technique using

Cryoseal ®-FS system.[8] Product properties of fibrin glue produced by Cryoseal ®-FS in comparison to commercially available Tissucol are shown in table 1. [12] A recent *in vitro* study showed that encapsulation of chondrocytes in FG produced by Cryoseal ®-FS promoted the accumulation of cartilage specific markers, suggesting that it is a promising approach to develop tissue-engineered cartilage replacement. [13]

		FG (Cryoseal ®-FS)	FG (Tissucol®)
Cryoprecipitate	Factor XIII (U/mL)	1.54	10-50
	Fibronectin (mg/mL)	5.4	2-9
	Fibrinogen (mg/mL)	22.1	70-110
Thrombin solution	$CaCl_2$ (µMol/mL)	0.45	40
	Thrombin (U/mL)	38	4 or 500

Table 1. Biochemical composition of fibrin glue produced by (Cryoseal ®-FS) system versus Tissucol®.[12]

Finding the suitable cell source is an absolute requirement for successful tissue engineering applications. The most widely used cell sources for articular cartilage tissue engineering are chondrocytes and mesenchymal stem/stromal cells (MSCs).[8,9,11] However, MSCs have advantages over chondrocytes, since they can be obtained in autologous form in a minimally invasive procedure and can be utilized for creating complex constructs such as cartilage-bone interface.[8] MSCs are characterized by having specific phenotypical and structural properties, for instance adherence to plastic and fibroblast-like morphology. In addition they stain positive for CD29, CD73, CD90, CD105 and CD166, while staining negative for CD14, CD34 and CD45, as indicated by flow cytometry.[14] MSCs in combination with a wide range of scaffolding materials including agarose[15], alginate[16], chitosan[17], hyaluronate[18], collagen[19], gelatin[20], PLGA[21] and PEG[22] have been evaluated during development of tissue engineering-based articular cartilage substitute. In addition, MSCs have been used to develop scaffold-free tissue-engineered constructs (TEC)

to promote cartilage repair. [23,24] Although fibrin hydrogels were used widely as a scaffolding material for development of tissue-enginereed cartilage replacement[8], in this study, we are evaluating different fibrin glues produced by cryoseal®-FS system, since they can be harvested in autologous form from patient plasma.

The mechanical properties of cartilage are largely dependent on the composition and structure of its extracellular matrix (ECM), which is mainly composed of type II collagen and proteoglycans. Therefore, *in vitro* culture conditions that promote ECM accumulation and native-like arrangements is required for the production of functional cartilage constructs.[25] Sakiyama-Elbert and Hubbell recently developed the heparin-based delivery system (HBDS) to provide localized release of heparin-binding growth factors in a biomimetic manner, so that growth factor release takes place through a cell mediated mechanism.[26] The HBDS is composed of heparin, heparin-binding growth factors, and a bifunctional peptide (linker) which contains a heparin-binding sequence linked to transglutaminase substrate sequence. During fibrin hydrogel formation, the bifunctional peptide becomes crosslinked to fibrin through the transglutaminase substrate sequence, while the heparin binding domain immobilizes heparin, which in turn binds many growth factors.[8] The incorporation of HBDS into fibrin and the release mechanism of the different growth factor from fibrin have been studied extensively. For example, HBDS promotes central and peripheral nerve regeneration in chicken and rat models, and represents a feasible method to enhance nerve generation in dorsal root ganglions (DRGs).[26-29]

The objective of this study was to evaluate fresh fibrin glue (FG) and platelet-rich fibrin glue (PR-FG) in combination with bone marrow-derived mesenchymal stem cells (BM-hMSCs) for articular cartilage tissue engineering. The effect of incorporating HBDS into these matrices on the accumulation of ECM components was also investigated.

3. MATERIALS AND METHODS

Unless otherwise specified, all materials were obtained from Sigma-Aldrich (St. Louis, MO). The HBDS bifunctional peptide (K(βA)FAKLAARLYRKANQEQVSP) was purchased from BIO S&T Inc., Montreal, QC.

3.1. Plasma collection

Human plasma (250 ml) was collected from healthy donors (n=2) by apheresis using Trima Accel Version 5 automated blood collection system (Gambro BCT, Inc., Lakewood, CO) after signing informed consent. Two types of plasma were prepared, platelet-rich and platelet-poor plasma according to the setting of the collection system, while the other blood components were returned to the circulation.

3.2. Fibrin glue production

Fibrin glue is composed of two components, cryoprecipitate and thrombin which were both obtained either from platelet- rich or platelet-poor plasma using the CryoSeal® FS System and the Thrombin Processing Device™, respectively (Thermogenesis, Rancho Cordova, CA). The thrombin component is obtained after mixing 10 ml of plasma with 4 mL of thrombin reagent composed of 7.2 mM CaCl2 and 10% ethanol in the Thrombin Processing Device™ reaction chamber, followed by an incubation for 50 min, then agitation and a second incubation for 10 min. Thrombin is then harvested from the chamber, aliquoted, and frozen at -80°C.[13,30] The remaining volume of plasma is transferred to the processing bag and frozen to -27°C within 7 min, then slowly warmed and gently rocked for 20 min until the temperature reaches 2°C. The concentrated cryoprecipitate settles in the pointed end of the processing bag after being placed on a slant for 5 min. Two cycles of freeze–thaw result in enhanced cryoprecipitate recovery. The cryoprecipitate was aliquoted and frozen at -80°C.[13,31]

3.3. MSCs expansion and characterization

Human MSCs (hMSCs) obtained from the Canadian Blood Services (CBS, Ottawa, ON) were expanded in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Thermo Scientific Hyclone, South Logan, UT),[32] while hMSCs obtained from Tulane Centre for Gene Therapy (Tulane University Health Sciences Center, New Orleans, LA) were expanded in complete culture media (CCM) composed of alpha minimum essential medium (αMEM; Invitrogen, Carlsbad, CA) media supplemented with 16.5 % FBS, 100 U/ml Penicillin, and 100 μg/ml streptomycin, according to the manufacturer's protocol. The two cell sources were used for flow cytometry, Alcian blue staining, hydroxyproline quantification, and glycosaminoglycans (GAGs) measurement experiments, while only Tulane Centre hMSCs were used for the rest of experiments. Chondrogenic, adipogenic and osteogenic differentiation of hMSCs was also carried out according to the manufacturer's protocol, where 10^5 cells were plated in 6 well plates and cultured in the expansion media until 70%-80% confluency. After reaching the required confluency, CCM was replaced with the appropriate differentiation media and kept for 3 weeks with media changed every 3-4 days. Osteogenic differentiation was induced with media composed of CCM supplemented with 10 nM dexamethasone, 20 mM β-glycerolphosphate, and 50 μM ascorbic acid-2- phosphate, while, adipogenic differentiation was induced with DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μM insulin, 1 μM dexamethasnone, 0.5 mM isobutylmethylxanthine, and 200 μM indomethacin.[33] Chondrogenic differentiation was induced by DMEM supplemented with 10^{-7} M dexamethasone, 50 μg/mL ascorbic acid-2-phosphate, 100 μg/mL sodium pyruvate, 40 μg/mL L-proline, 1% insulin-transferrin selenous acid-plus premix (BD Bioscience Pharmingen, Mississauga, ON, Canada), 100

U/mL penicillin, 100 μg/mL streptomycin, 10 ng/mL TGF-βs (TGF-β_2 for 3D culture and TGF- β_3 for monolayer culture, R&D Systems, Minneapolis, MN).[1] BM-hMSCs were characterized by flow cytometry (Beckman Coulter Epics XL, Beckman Coulter, Mississauga, ON) using fluorescent-conjugated antibodies against CD34 (CD34-FITC, Miltenyi Biotec Inc, Auburn, CA), CD45 (CD45-FITC, Beckman Coulter), CD90 and CD105 (CD90-PE and CD105-PE, AbD Serotec, Raleigh, NC).[34]

3.4. Fibrin gel formulations

FG and PR-FG prepared as described earlier were used for gel formation. To prepare gels in a 12 transwell plate (~1.5mm thickness), cryoprecipitate (85 μL), cells (25 μL of confluent hMSCS, to a final of 10^7 live cells/mL), and linker peptide (5 μL, to a final concentration of 250 μM for gels containing HBDS)[26] were mixed, and polymerization of fibrinogen completed within a few minutes after adding 85 μL of thrombin (from same unit as cryoprecipitate) to make a final volume of 200 μL. The original protocol of incorporating HBDS into fibrin hydrogels involves addition of heparin to the other constituents before the coagulation process.[26,27] However, we found that FG and PR-FG had significant levels of antithrombin III and that coagulation could not be achieved in the presence of heparin; therefore, heparin was incorporated into fibrin hydrogels after complete coagulation, where 200 μL of 250 μM heparin solution was added to the top of gels containing the bifunctional domain. After incubation (1 hour, 37°C), gels were washed 2 times using DMEM media (each 15 min) to remove any unbound linker and heparin (FIG.1). The different gels were then cultured in transwell plates with the aforementioned chondrogenic media which was supplemented with the protease inhibitor, tranexamic acid (final concentration 1.5 mg/mL) and maintained in 37 °C, 5% CO_2 incubator up to 5 weeks. The different hydrogel systems are illustrated in table 2. In the absence of protease

inhibitors, fibrin gels completely degraded over 10–14 days in culture; however, the gels were fully stabilized with tranexamic acid addition to the medium.[10] TGF- β_2 and TGF- β_3 have the same potency to induce chondrogenesis of hMSCs.[35] However, TGF-β_2 was used for the 3D culture, since heparin has no affinity for TGF- β_3.[36] At 0 day, 2.5 weeks, and 5 weeks one gel from each group was cut into quarters and processed for cryosectioning, papain digestion, and RNA isolation.

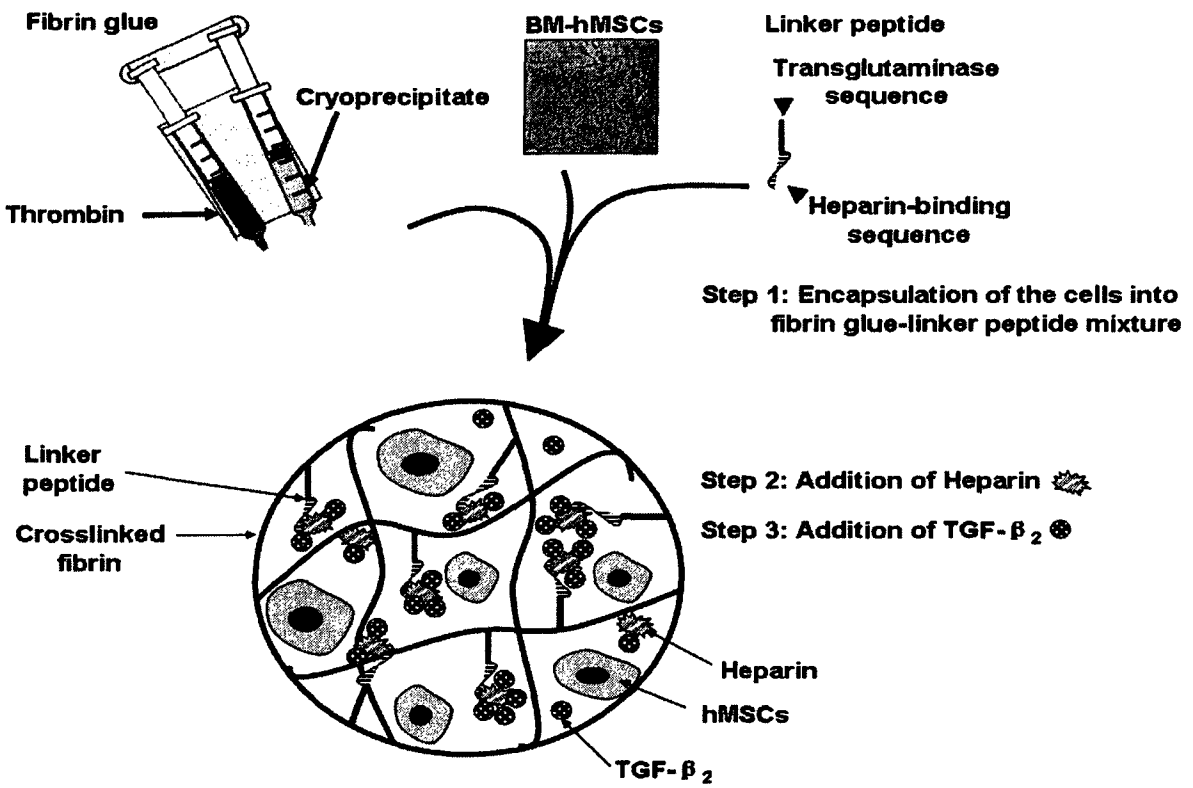

FIG.1. Schematic depiction of the incorporation of HBDS into fibrin glue during tissue engineering of articular cartilage.

Table 2. The different formulations of fibrin glue used in this study.

Fibrin Formulation	Abbreviation
Fibrin glue-hMSCs-hydrogel	FG hydrogel
Platelet-rich fibrin glue-hMSCs hydrogel	PR-FG hydrogel
Fibrin glue-hMSCs hydrogel containing heparin-binding delivery system	FG-HBDS hydrogel
Platelet-rich fibrin glue-hMSCs hydrogel containing heparin-binding delivery system	PR-FG-HBDS hydrogel

3.5. Live/dead staining

Fibrin gels (500 μm thickness prepared as described above, in a 48 well plate) were stained with Syto® 10 green and ethidium homodimer-1 red (Invitrogen) after 1 week of culture. After staining for 30 min, gels were washed with PBS, then flattened by compression between two glass slides to allow for enhanced photography under UV light using a Nikon Eclipse TE2000-E microscope ((Nikon Instruments Inc., Melville, NY). The numbers of dead and live cells were counted using the Nikon microscope software (NIS-Elements™ AR3.10) to calculate the percent viability (live cells/total cell number). At least 4 fields were counted for each gel type.

3.6. Real time-PCR

Human-specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), collagen IIa, and aggrecan I were obtained from SuperArray (Bioscience Corporation, Hornby, ON) and used for Real-Time RT-PCR. RNA was extracted from fibrin-encapsulated hMSCs using the RNeasy Fibrous Tissue Kit (Qiagen, Valencia, CA) and following the manufacturer's protocol. First-strand cDNA was synthesized with 0.2 μg RNA using 200 IU RevertAid™ H Minus M-MuLV Reverse Transcriptase (Fermentas, Burlington, ON) as per manufacturer's instructions. PCR amplification and melt curve analysis were performed using a BioRad iCycler thermocycler as previously described.[9,13]

The results were analyzed using the comparative C_T method. Expression levels for hMSCs after encapsulation into the different fibrin glue preparations at zero time point were used as a baseline for each gene, which was furthermore normalized to GAPDH housekeeping expression. Baseline expression levels were therefore assigned a value of 1.0.

3.7. Histology

Different fibrin hydrogel formulations were fixed in 4% paraformaldehyde overnight, maintained in 30 % sucrose for 24 hours and then frozen in 1:1- 30 % sucrose: OCT for storage at -80 °C. The tissue blocks were cut in 10 μm sections using a Shandon cryotome FSE (Thermo Electron Corporation). Sections were stained with H&E or Alcian blue using standard protocols. For differentiation assays, monolayer cultures were fixed in 4% paraformaldehyde for 1 hour. The chondrogenic and adipogenic differentiation wells were washed with PBS, while the osteogenic differentiation wells were washed with deionized water. The adipogenic differentiation wells were stained with Oil red-O, the chondrogenic differentiation wells were stained with Alcian blue and the osteogenic differentiation wells were stained with Alizarin red-S using standard protocols.

3.8. Immunohistochemistry

After frozen sections were brought to room temperature, excess water was removed and slides are incubated in 50 mM NH_4Cl prepared in TBS (100 mM Tris HCl, 150 mM NaCl, pH 7.7) for 30 min to minimize tissue-associated autofluorescence followed by two washing steps in TBS (each 3 min). The washed sections were digested in 2 % hyaluronidase in TBS for 30 minutes followed by three washing steps in TBS each 3 minutes. The slides were placed in blocking buffer (4% Fetal bovine serum, 1% Bovine serum albumin, 0.3% triton X-100) for 30 minutes, washed again three times in TBS and maintained in the primary antibody for collagen II overnight (AB2036, 1:80 prepared in blocking buffer; Millipore, Etobicoke, ON). The slides were then washed three times in TBS and incubated with secondary antibody (PA42004, 1:500 prepared in TBS, 0.3% triton X-100; GE Healthcare, Baie d'Urfe, QC). After incubation with secondary antibody for 1 hour at room temperature, sections were washed two times in TBS, stained three minutes

with DAPI stain (1:10000 in TBS), then washed two times in TBS and cover slipped with TBS. For negative controls, the same procedures were performed with omission of the primary antibody.

3.9. Biochemical assays

Gels were digested with papain (Sigma; 40 μg/mL in 20 mM ammonium acetate, 1mM EDTA and 2mM dithiothreitol) for 48 h at 65 °C. After complete digestion, the digest were assayed for total glycosaminoglycan (GAG) with dimethylmethylene blue using the Blyscan™ sulfate GAG assay (Bicolor Ltd., Carrickfergus, UK). Aliquots of the digest were hydrolyzed in 6N HCl at 110 °C for 18 h followed by quantification of hydroxyproline content using chloramine-T/Ehrlich's reagent assay.[37] Collagen content was calculated assuming that hydroxyproline represents 14% of total collagen.[38] Total GAG and hydroxyproline were normalized to DNA, which was determined using the Hoechst dye 33258 assay (Invitrogen).[39]

3.10. Statistical Analysis

Analysis of variance (ANOVA, one way) for gene expression, live percentage, GAGs, and total collagen data was performed using SPSS (Ver.10) software. Bonferroni post hoc tests were used for further analysis. The independent sample *t*-test was used to compare 2.5 versus 5 weeks values. Results were expressed as mean ± SE. Results were considered significant when ANOVA was confirmed by Bonferroni post hoc test at $p<0.05$. Graphs were designed using Microsoft Excel.

4. RESULTS

4.1. BM-hMSCs characterization

Flow cytometric results indicated that hMSCs obtained from Tulane Centre were 74% positive for both CD90 and CD105 (FIG.2A,B), while hMSCs obtained from CBS

were 79% positive for CD90 and 71 % positive for CD105. The two sources were almost negative for the hematopoietic cells marker CD34 and the common leukocyte antigen CD45 (FIG.2 A,B). The cells had the potential to differentiate into the different mesodermal lineages including adipocytes, as indicated by Oil red-O staining (FIG.2C), osteocytes as indicated by Alizarin red-S staining (FIG.2D) and chondrocytes as indicated by Alcian blue staining (FIG.2 E).

4.2. Cytotoxicity

Live/dead staining of cells encapsulated in the different fibrin preparations showed 79.8±3.3% viability for FG-hMSCs hydrogels (FIG.2 F) and 76.5 ±3% viability for PRFG-hMSCs-hydrogels (FIG.2 G), while the viability was 90.6 ± 3.1% for FG-hMSCs-HBDS hydrogels (FIG.2 H) and 92.6 ± 4.9% in PRFG-hMSCs-HBDS hydrogels (FIG.2 I) Although, ANOVA showed significant difference between the different fibrin glue preparations (P=0.027, F=4.2), this difference was not significant as indicated by Bonferroni post hoc test.

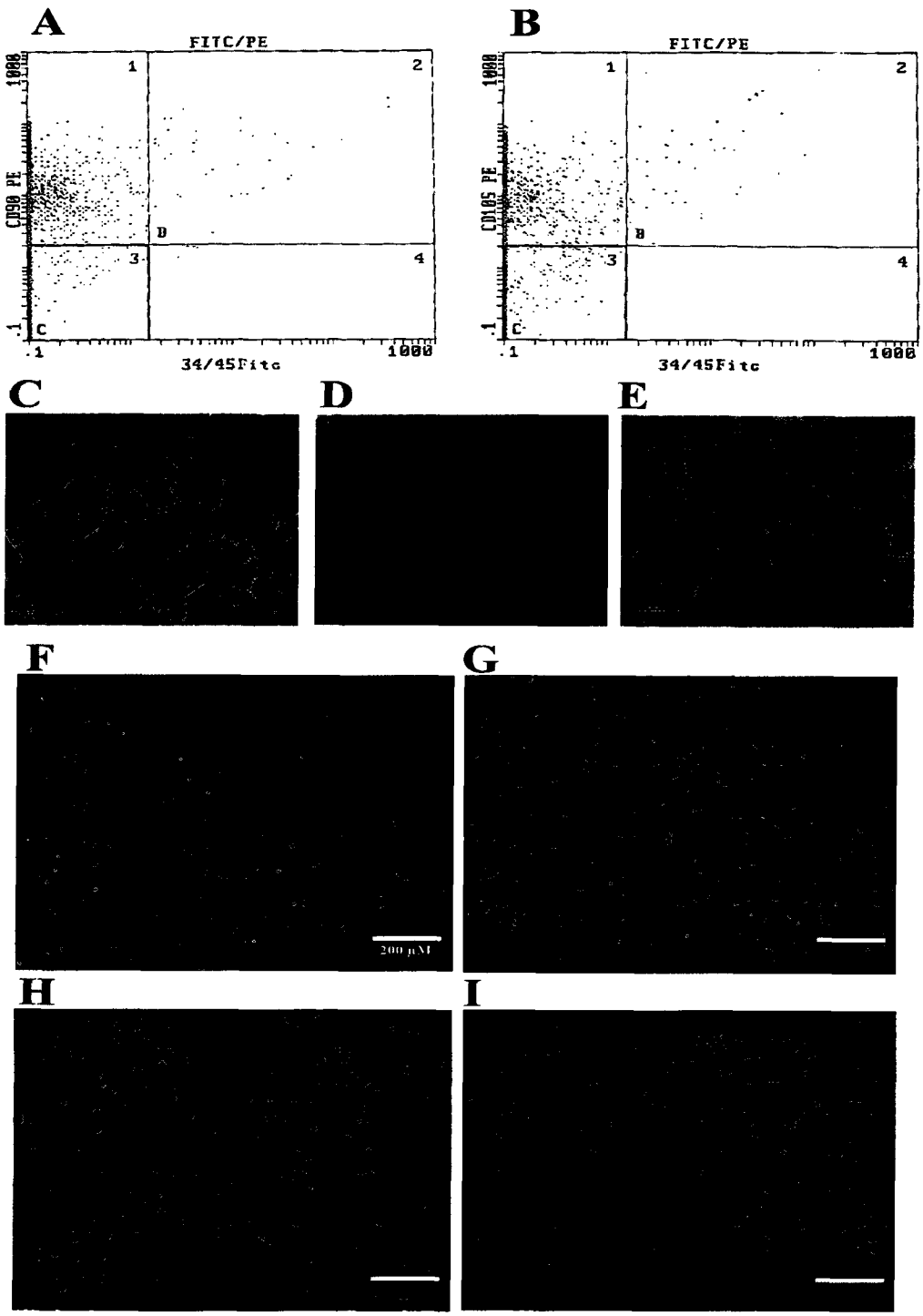

FIG.2. Characterization of hMSCs by flow cytometry (A, B); multi-differentiation potential of hMSCs: adipogenic differentiation revealed by Oil red-O stain (C), osteogenic differentiation revealed by Alizarin red-S stain (D) and chondrogenic differentiation revealed by Alcian blue stain (E); Live/dead stain of hMSCs after encapsulation into fibrin glue (F), platelet-rich fibrin glue (G), fibrin glue-HBDS (H), and platelet-rich fibrin glue-HBDS (I).(Scale Bar = 200 μM)

4.3. Gene expression analysis

Collagen II and aggrecan are major components of the articular cartilage ECM. Encapsulation of hMSCs in FG and PR-FG resulted in 3- and 9-fold increases in collagen II gene expression, respectively, at 2.5 weeks which then increased to 21- and 28-fold increases at 5 weeks (FIG.3A). Collagen II gene expression level was significantly higher in PR-FG compared to FG after encapsulation of hMSCs at 2.5 weeks ($P<0.01$, $F= 14.2$), however, no significant difference was observed between the two groups at 5 weeks. Incorporation of HBDS into PR-FG significantly reduced the enhancement of collagen II expression at 2.5 weeks ($P<0.01$). There was 2-fold (FG-HBDS) and no increase (PR-FG-HBDS) at 2.5 weeks, which then increased to 4- and 5-fold at 5 weeks, respectively. At the same time, there was 17-fold increase in aggrecan expression during encapsulation in fibrin glue at 2.5 weeks, which diminished to 2-fold by 5 weeks; however, in PR-FG there was no change at 2.5 weeks and only 2-fold increase after 5 weeks. Aggrecan gene expression level was significantly higher in FG compared to PR-FG after encapsulation of hMSCs at 2.5 weeks ($P<0.05$, $F= 7.2$), however, no significant difference was observed between the two groups at 5 weeks. The large increase in aggrecan gene expression in FG at 2.5 weeks was completely prevented after incorporation of HBDS ($P<0.05$). There was no effect on aggrecan gene expression after incorporating HBDS in FG at either time point, and also no significant increases were observed in PR-FG at either time point after incorporation of HBDS (FIG.3 B).

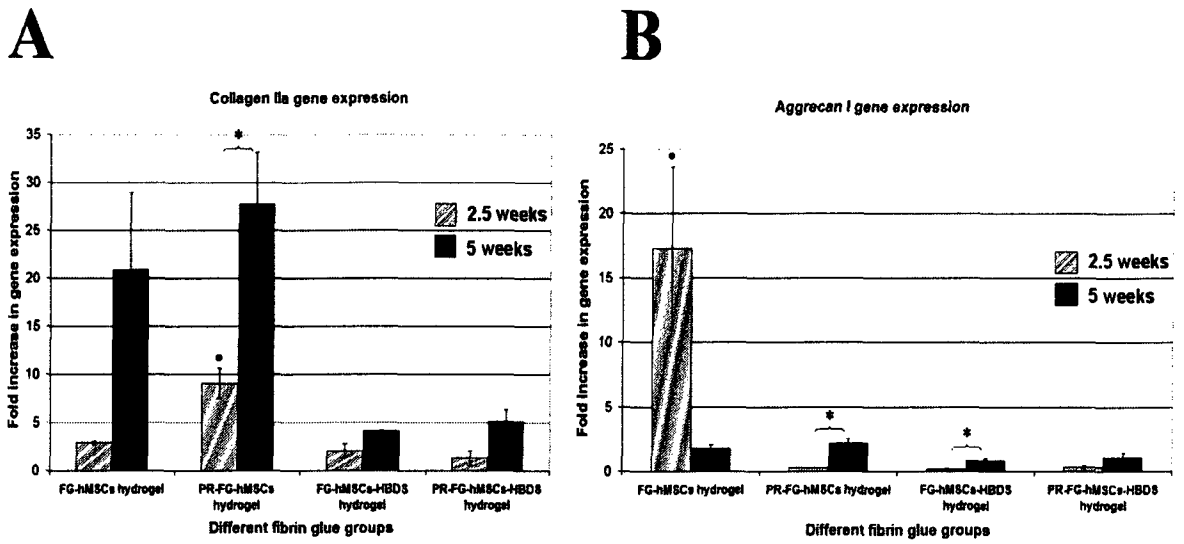

FIG.3. Gene expression analysis of collagen IIa **(A)** and aggrecan I **(B)** after encapsulation of hMSCs in the different types of fibrin glue (n=3) for 2.5 and 5 weeks. The level of expression of collagen II gene after encapsulation of hMSCs into PR-FG was significantly higher than other groups at 2.5 weeks ($P<0.01$, $F= 14.2$), and then only HBDS-containing FG at 5 weeks ($P<0.05$, $F=5.5$) as indicated by ANOVA followed by Bonferroni post-hoc test. However, no significant difference was observed after encapsulation of hMSCs into FG compared to PR-FG at 5 weeks. The level of expression of aggrecan gene was significantly higher after encapsulation of hMSCs in FG compared to the other groups. (*: indicates significant results for a particular fibrin gel preparation at 2.5 weeks versus 5 weeks after analysis using independent sample *t*-test; •: indicates a statistically significant difference of a particular fibrin gel group compared to the other groups at that time point after analysis using ANOVA and Bonferroni post-hoc test).

4.4. ECM production:

4.4.1. Histology

All the gels were prepared as disc-shaped constructs 10 mm diameter and 2 mm thickness. The different fibrin gels preparations were opaque beige, and opacity generally increased over time in culture. H&E staining revealed that FG and PR-FG hydrogels were dominated by chondrocyte-like cells at 2.5 and 5 weeks, while in the remaining groups the differentiated cells showed mixed populations of fibroblast-like and chondrocyte-like cells that were roughly equal in proportion at the same time points. In general, the percentage of

chondrocyte-like cells was higher at 5 weeks than 2.5 weeks in the different groups (FIG.4). Encapsulation of hMSCs into FG and PR-FG led to enhanced accumulation of ECM at 2.5 weeks as indicated by Alcian blue staining of GAGs. This accumulation was further enhanced at 5 weeks, while incorporation of HBDS into these glues resulted in lower observed accumulation of the same ECM components at the same time points. The staining pattern was patchy in both FG and PR-FG, while it was evenly distributed in the HBDS-containing hydrogels (FIG.4). The staining pattern was enhanced at the gel edge, likely due to efficient gas and nutrient exchange compared to the gel interior (FIG.4).

4.4.2. Biochemical assays

A hydroxyproline assay was used to quantify total collagen accumulation in the different fibrin hydrogels (FIG.5 A). Encapsulation of hMSCs into HBDS-containing PR-FG resulted in significantly higher accumulation of total collagen ($P<0.001$, $F=19.3$) at 2.5 weeks (15.2±1.9 μg total collagen/ μg DNA) compared to FG hydrogels (8.8±0.3), PR-FG hydrogels (7.3±0.5), and FG-HBDS hydrogels (7±0.6). However no significant difference was noted amongst the different fibrin preparations at 5 weeks (FG hydrogels: 11.4±0.9, PR-FG hydrogels: 12.4±2.4, FG-HBDS hydrogels: 11.2±1, and PR-FG-HBDS hydrogels: 8.1±0.2 total collagen/ μg DNA) as indicated by ANOVA and Bonferroni as a post-hoc test. Levels of total collagen were significantly higher at 5 weeks compared to 2.5 weeks for FG hydrogels with or without HBDS ($P<0.05$); however, no significant difference was observed between 2.5 and 5 weeks for PR-FG hydrogels (FIG.5 A).

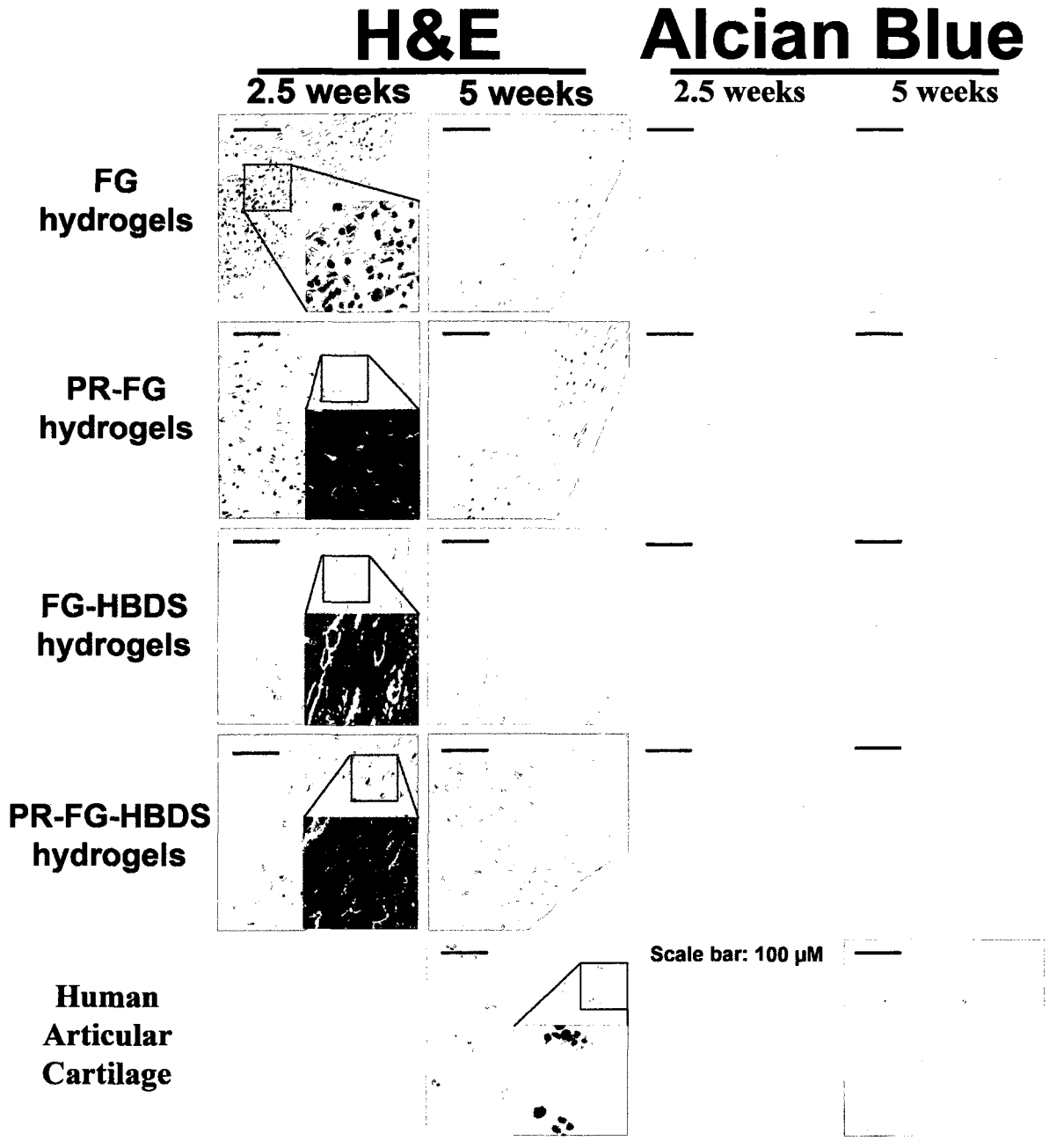

FIG.4. H&E and Alcian blue staining to compare the morphology of hMSCs and GAG production respectively, after encapsulation into the different groups of fibrin glue for 2.5 and 5 weeks. The insert shows a higher magnification of the selected region (2.5X).

The dimethylmethylene blue assay was used to quantify GAGs in the different gel groups (FIG.5 B). A significant difference in total GAGs was detected with ANOVA and Bonferroni as a post-hoc test ($P<0.001$, $F=514$) for HBDS-containing hydrogels at 2.5 weeks (1±0.03 and 1.8±0.05 for FG-HBDS and PR-FG-HBDS hydrogels, respectively) compared to hydrogels lacking HBDS (0.2±0.02 and 0.17±0.03 μg GAGs/μg DNA for FG hydrogels and PR-FG hydrogels, respectively). At 5 weeks FG-HBDS (0.4±0.01 μg GAGs/μg DNA) and PR-FG-HBDS (0.5±0.01) hydrogels showed significant increase in total GAGs compared to FG hydrogels (0.2 ± 0.05) and PR-FG hydrogels (0.3±0.02), respectively as indicated by statistical analysis ($P<0.01$, $F= 9.4$). However, the increase in GAGs in the HBDS-containing gels are mainly due the heparin component of the HBDS, since dimethylmethylene blue binds preferentially to sulfated GAGs including heparin,[40] as indicated at day zero (FIG.5B) This increase in GAGs due to heparin retention that is observed in the different HBDS-containing fibrin glues cannot be visualized histologically by Alcian blue staining, since Alcian blue (pH 2.5) stains weakly acidic sulfated and carboxylated acid GAGs, while heparin is a strongly sulfated GAGs.[41]

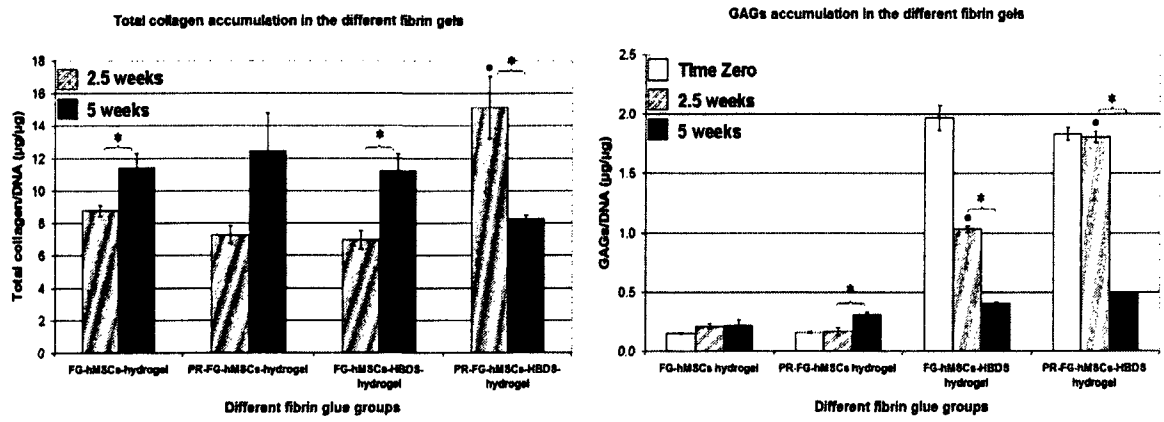

FIG.5. Total collagen accumulation **(A)** and GAG accumulation **(B)** in the different fibrin glue groups after encapsulation of hMSCs for 2.5 and 5 weeks. The level of total collagen was significantly higher in PR-FG-HBDS group compared to other groups at 2.5 weeks, however, no significant difference was observed amongst the different groups at 5 weeks. Total GAGs level was significantly higher in fibrin glue preparations having HBDS compared to preparations lacking HBDS. The levels of GAGs were significantly higher at 5 weeks compared to 2.5 weeks for PR-FG hydrogel (n=6), and the reverse for the different fibrin glues after incorporation of HBDS (n=3). However no significant difference was observed between 2.5 versus 5 weeks for FG hydrogels (n=6). (*: indicates significant results for a particular fibrin gel preparation at 2.5 weeks versus 5 weeks after analysis using independent sample t-test; •: indicates a statistically significant difference of a particular fibrin gel group at one time point compared to the other groups at that time point after analysis using ANOVA and Bonferroni post-hoc test) .

4.4.3. Immunofluorescence

Positive staining for collagen II was observed in immunostained FG, PR-FG, FG-HBDS, and PR-FG-HBDS sections after 2.5 weeks compared to negative control and constructs at time zero (*i.e.* hydrogels collected directly after gelation). Further improvement in staining was observed at 5 weeks in the different constructs. At 5 weeks FG promoted the best collagen II accumulation followed by PR-FG and FG-HBDS hydrogels (FIG.6).

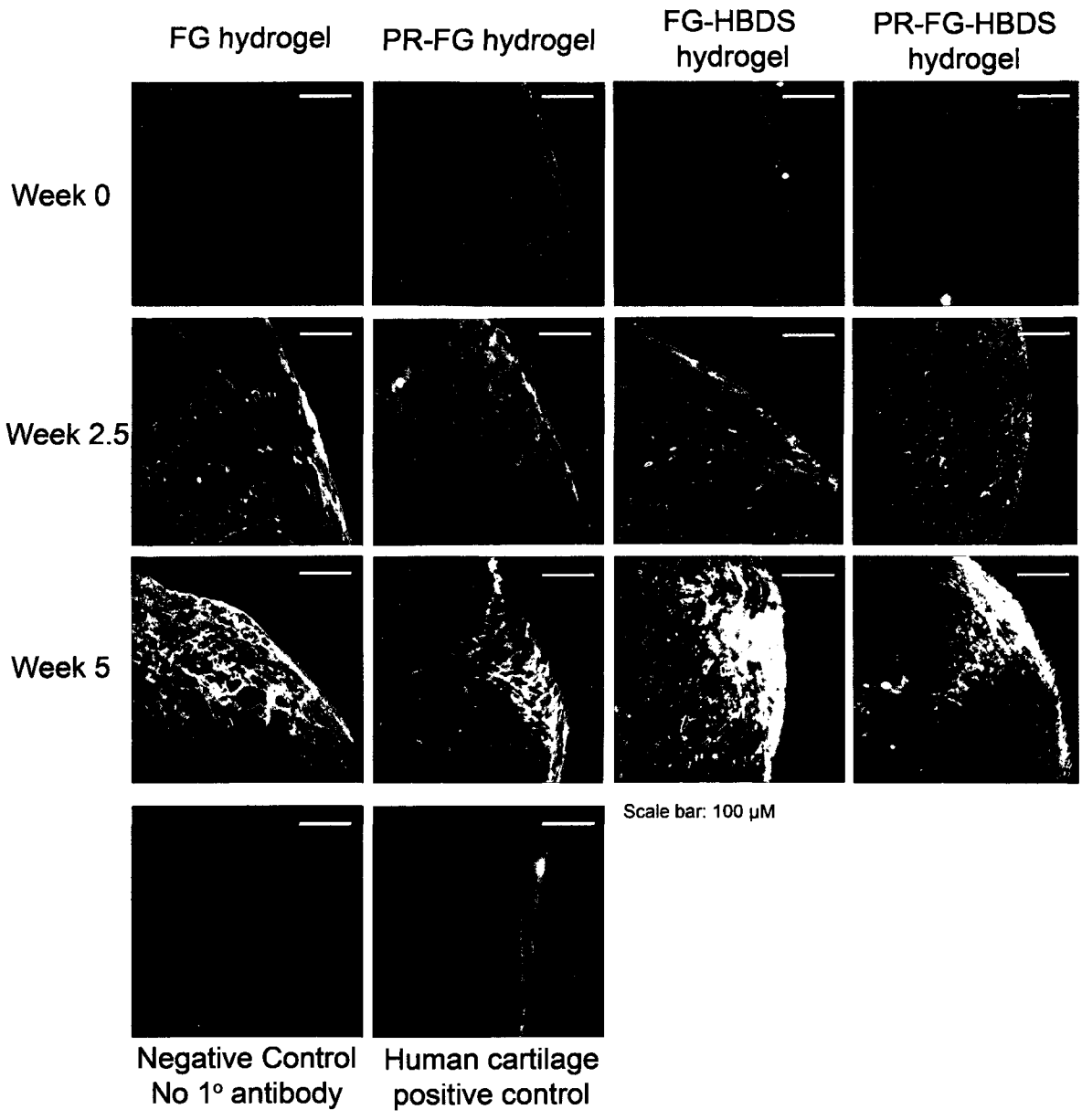

FIG.6. Immunostaining showing the degree of accumulation of collagen II in the different types of fibrin glue after encapsulation of hMSCs for 2.5 and 5 weeks.

5. DISCUSSION

Fibrin glue can be prepared for autologous use from a single unit of plasma, and has been used as a helper tool in many surgical procedures. In addition to its capability to promote hemostasis, judicious use of fibrin glue accelerates wound healing, reduces blood loss and protects against bacterial infection.[8] Our previous study revealed that fresh fibrin

glue obtained from the CryoSeal® FS system, in combination with chondrocytes, is a promising approach towards development of tissue engineering-based cartilage replacement as indicated by collagen II accumulation, especially under hypoxic culture conditions.[13] The primary goal of this study was to evaluate FG and PR-FG matrices from precursors produced with the CryoSeal® FS system, in combination with BM-hMSCs, for development of tissue-engineered cartilage replacement. Chondrocytes and MSCs are the most widely used cell sources for tissue engineering-based approaches to cartilage repair.[8,42] Chondrocytes are relevant to this application, however, the long culturing time required before implantation leads to formation of cartilaginous repair tissue that is of fibrous nature.[2] In addition, chondrocytes implanted into experimental osteochondral lesions filled the defect with new cartilage ECM, but failed to integrate with the subchondral plate, because the chondrocytes did not mature to become hypertrophic chondrocytes (precursors of subchondral bone).[43] Proper integration with subchondral plate is only observed when MSCs were used.[42] MSCs can also be expanded many times without affecting the nature of the tissue formed after implantation.[3] Since osteochondral lesions involve both subchondral bone and cartilage, an ideal cell source for tissue engineering-based repair strategies should be able to regenerate these two very distinct skeletal tissues.[43] MSCs-based approaches would be suitable for constructs at the cartilage-bone interface[44]and to develop ligament[45] and meniscus substitutes[46]. Our results indicated that BM-hMSCs strongly expressing CD90 and CD105 are capable of differentiating into a number of mesodermal lineages including the chondrocytes and osteocytes required for the cartilage-bone interface, in agreement with other studies. [47]

Fibrin glue has been used widely during development of articular cartilage repair strategies.[1,8,13,48-50] However, implantation of fibrin gels prepared from animal source or

allogeneic human pooled plasma can be associated with multiple risks[8,13], although this is now greatly reduced in the current products. To circumvent these potential risks, in this study we have used fibrin derived from single units of plasma, since it can be autologous and can be prepared rich in platelets which release a wide variety of growth factors upon activation by thrombin.[51] We evaluated collagen II and aggrecan expression and accumulation in our constructs, since they are considered to be hyaline cartilage markers and are mainly responsible for the biomechanical properties of native cartilage.[25] The level of expression of collagen II after encapsulation of hMSCs was significantly higher in PR-FG compared to FG at 2.5 weeks. However, no significant difference was observed between the two groups at 5 weeks. These results suggest that PR-FG has a superior effect on collagen II gene expression and ECM accumulation compared to FG; however, no significant difference was observed between the two groups in the long term. In addition, the enhanced collagen II gene expression after encapsulation of hMSCs into FG and PR-FG are consistent with the accumulation of collagen II as indicated by collagen II immunostaining. After encapsulation of hMSCs and induction of chondrogenesis, a majority of chondrocyte-like cells was observed in FG and PRFG by H&E staining. Our results indicated that constructs having more chondrocyte-like cells (i.e. FG and PR-FG) showed enhanced collagen II accumulation (a hyaline cartilage marker) than constructs containing predominantly fibroblast-like cells. These findings are consistent with a previous study which revealed that induction of chondrogenesis in hMSC micromass culture led to formation of cartilage-like zones where the outer zone has elongated cells (fibroblast-like) with prominent collagen I accumulation while the deep zone has spherical cells (chondrocyte-like) with prominent collagen II accumulation.[52] Aggrecan gene expression after encapsulation of hMSCs into FG was 17-fold higher at 2.5 weeks then

decreased at 5 weeks, while for PR-FG the expression was unchanged at 2.5 weeks and only two-fold at 5 weeks. These findings are corroborated by the intense Alcian blue staining pattern in FG at 2.5 weeks which increased slightly at 5 weeks; the staining pattern for PR-FG was stronger at 5 weeks compared to 2.5 weeks. The temporal mismatch is not unusual, since gene expression is quantified at a single time point, but accumulation of GAGs is a gradual process during the entire incubation period. In general, the Alcian blue staining intensity was greater in FG compared to PR-FG. We hypothesize that the difference between FG and PR-FG is due to the effect of a variety growth factors in the PR-FG hydrogels. A wide range of growth factors are released from platelets upon activation by thrombin during formation of PR-FG-based tissue engineering constructs, including platelet-derived epidermal growth factors (PD-EGF), platelet-derived growth factor A+B (PDGF-AA, AB, BB), TGF-β_1, TGF- β_2, insulin like growth factor 1, 2 (IGF 1,2), vascular endothelial growth factor (VEGF) and basic fibroblast growth factors (FGF-2).[53,54] In addition to these bioactive factors, exogenous TGF- β_2 as inducer of chondrogenesis was added to the culture media. The TGF-β superfamily is a potent inducer of chondrogenesis, while PDGF, IGF, and FGF mediate chondrocytic physiology rather than promoting chondrogenesis of MSCs.[35,55,56] VEGF is a potent inducer of endothelial differentiation.[55] Growth factors can either stimulate or inhibit cellular processes such as division, migration, differentiation and gene expression, depending on the cells involved. For example, TGF-βs stimulate proliferation and migration of fibroblasts and promote differentiation of chondrocytes, while inhibiting proliferation of keratinocytes.[56,57] Therefore, after encapsulation of hMSCs, the combination of growth factors in PR-FG would have a complex effect on their cellular activity and patterns of gene expression, leading to altered accumulation of cartilage-specific markers.

A wide range of growth factors bind heparin with high affinity.[8] This binding helps to sequester growth factors in the ECM, serves to localize growth factor activity, protects growth factors from proteolytic degradation, and in some cases enhances binding to cell surface receptors.[26] The HBDS was designed to act as a reservoir of growth factors to enhance their bioavailability and control the release of heparin-binding growth factors through cell mediated proteolytic activity rather than by simple diffusion.[28,58] Incorporation of HBDS into FG and PR-FG did not modify cell survival, since there was no significant difference in viability of encapsulated hMSCs after 1 week. However, adding HBDS to FG and PR-FG scaffolds resulted in decreased expression of chondrocytic markers (*i.e.* collagen II and aggrecan), as well as weaker patterns of Alcian blue staining and collagen II immunostaining. In the long term, there was no difference between the accumulation of total collagen in fibrin glues lacking or containing HBDS. However, enhanced collagen I is usually associated with the fibroblast-like cells which prevail in HBDS-containing fibrin glue preparations, in contrast to the chondrocyte-like cells observed in the FG and PR-FG preparations lacking HBDS.[52] The adverse effect of incorporating HBDS into fibrin glues on the expression and accumulation of cartilage-specific markers might be due to one of the following possible reasons. First, since the bound growth factor must be released by a cell-mediated proteolytic activity, tranexamic acid added to the media to stabilize fibrin glue for extended time might interfere with this activity. When used for central and peripheral nerve generation, HBDS was incorporated into plasminogen-free fibrinogen. In addition, most of the nerve regeneration experiments were carried out in a period of less than one week.[26-28] However, for cartilage regeneration, extended times are required to obtain satisfactory cartilaginous tissue formation. In a related study, the degree of neurite extension within three-dimensional

matrices of fibrin was diminished in a concentration-dependent manner by agents that inhibited plasmin such as aprotinin.[59] Second, it has been shown that ECM components act not only as activators or protectors of growth factors, but also can inhibit bound growth factor activities. For example, the N-terminal region of betaglycan (chondroitin sulfate/heparan sulfate proteoglycan) [60] binds to TGF-β_2 and acts as a potent inhibitor of its activity.[57] Finally, as indicated by the quantification of GAGs in the different fibrin glue preparations, FG-HBDS and PR-FG-HBDS hydrogels showed approximately ten-fold increase in GAGs concentration compared to HBDS-free fibrin glues at zero time before cell-mediated accumulation of any GAGs. This apparent background is due to the incorporated heparin. In our study heparin will bind to the heparin binding domain of the linker peptide. However, it has been shown that heparin can bind to the E-domain of fibrin without any linker.[61] Theoretic and experimental studies have demonstrated that at a constant growth factor concentration, increased heparin concentration will increase the overall growth factor binding and subsequently most of the growth factor will be in the bound state. [58]

In conclusion, fresh human fibrin and platelet-rich fibrin glues produced with the CryoSeal® FS system are potential matrices for articular cartilage tissue engineering. Fibrin glue is particularly appealing, since encapsulated hMSCs exhibited greatly enhanced collagen II gene expression as well as cartilage-like ECM accumulation. Incorporation of HBDS into these matrices had a negative impact on cartilage-specific markers. Further experiments should be conducted first to enhance the final mechanical properties of fibrin glue-based cartilage repair strategies; and second, to find the best growth factor combination and physical stimulant that will lead to optimum expression and accumulation of the hyaline-specific chondrocytic markers. These studies should be followed by *in vivo*

testing to evaluate the characteristics of fibrin glue-based constructs for articular cartilage repair.

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7. REFERENCES

1. Peretti, G.M., Xu, J.W., Bonassar, L.J., Kirchhoff, C.H., Yaremchuk, M.J., Randolph, M.A. Review of injectable cartilage engineering using fibrin gel in mice and swine models. Tissue Eng.**12**:1151, 2006.

2. Temenoff, J.S., Mikos, A.G. Review: tissue engineering for regeneration of articular cartilage. Biomaterials.**21**:431, 2000.

3. Kessler, M.W., Ackerman, G., Dines, J.S., Grande, D. Emerging technologies and fourth generation issues in cartilage repair. Sports Med Arthrosc.**16**:246, 2008.

4. Mulhall, K.J., Ghomrawi, H.M., Scully, S., Callaghan, J.J., Saleh, K.J. Current etiologies and modes of failure in total knee arthroplasty revision. Clin Orthop Relat Res.**446**:45, 2006.

5. Berger, R.A., Meneghini, R.M., Jacobs, J.J., Sheinkop, M.B., Della Valle, C.J., Rosenberg, A.G., Galante, J.O. Results of unicompartmental knee arthroplasty at a minimum of ten years of follow-up. J Bone Joint Surg Am.**87**:999, 2005.

6. Kisiday, J., Jin, M., Kurz, B., Hung, H., Semino, C., Zhang, S., Grodzinsky, A.J. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. Proc Natl Acad Sci U S A.**99**:9996, 2002.

7. Jockenhoevel, S., Zund, G., Hoerstrup, S.P., Chalabi, K., Sachweh, J.S., Demircan, L., Messmer, B.J., Turina, M. Fibrin gel -- advantages of a new scaffold in cardiovascular tissue engineering. Eur J Cardiothorac Surg.**19**:424, 2001.

8. Ahmed, T.A., Dare, E.V., Hincke, M. Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng Part B Rev.**14**:199, 2008.

9. Ahmed, T.A., Griffith, M., Hincke, M. Characterization and inhibition of fibrin hydrogel-degrading enzymes during development of tissue engineering scaffolds. Tissue Eng.**13**:1469, 2007.

10. Meinhart, J., Fussenegger, M., Hobling, W. Stabilization of fibrin-chondrocyte constructs for cartilage reconstruction. Ann Plast Surg.**42**:673, 1999.

11. Dare, E.V., Griffith, M., Poitras, P., Kaupp, J.A., Waldman, S.D., Carlsson, D.J., Dervin, G., Mayoux, C., Hincke, M.T. Genipin Cross-Linked Fibrin Hydrogels for in vitro Human Articular Cartilage Tissue-Engineered Regeneration. Cells Tissues Organs. 2009.

12. Buchta, C., Dettke, M., Funovics, P.T., Hocker, P., Knobl, P., Macher, M., Quehenberger, P., Treitl, C., Worel, N. Fibrin sealant produced by the CryoSeal FS System: product chemistry, material properties and possible preparation in the autologous preoperative setting. Vox Sang.**86**:257, 2004.

13. Dare, E.V., Griffith, M., Poitras, P., Wang, T., Dervin, G.F., Giulivi, A., Hincke, M.T. Fibrin sealants from fresh or fresh/frozen plasma as scaffolds for in vitro articular cartilage regeneration. Tissue Eng Part A.**15**:2285, 2009.

14. Le Blanc, K., Ringden, O. Immunomodulation by mesenchymal stem cells and clinical experience. J Intern Med.**262**:509, 2007.

15. Vinardell, T., Thorpe, S.D., Buckley, C.T., Kelly, D.J. Chondrogenesis and integration of mesenchymal stem cells within an in vitro cartilage defect repair model. Ann Biomed Eng.**37**:2556, 2009.

16. Xu, J., Wang, W., Ludeman, M., Cheng, K., Hayami, T., Lotz, J.C., Kapila, S. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. Tissue Eng Part A.**14**:667, 2008.

17. Cho, J.H., Kim, S.H., Park, K.D., Jung, M.C., Yang, W.I., Han, S.W., Noh, J.Y., Lee, J.W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. Biomaterials.**25**:5743, 2004.

18. Kayakabe, M., Tsutsumi, S., Watanabe, H., Kato, Y., Takagishi, K. Transplantation of autologous rabbit BM-derived mesenchymal stromal cells embedded in hyaluronic acid gel sponge into osteochondral defects of the knee. Cytotherapy.**8**:343, 2006.

19. Dorotka, R., Windberger, U., Macfelda, K., Bindreiter, U., Toma, C., Nehrer, S. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials.**26**:3617, 2005.

20. Ponticiello, M.S., Schinagl, R.M., Kadiyala, S., Barry, F.P. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. J Biomed Mater Res.**52**:246, 2000.

21. Han, S.H., Kim, Y.H., Park, M.S., Kim, I.A., Shin, J.W., Yang, W.I., Jee, K.S., Park, K.D., Ryu, G.H., Lee, J.W. Histological and biomechanical properties of regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold in vivo. J Biomed Mater Res A.**87**:850, 2008.

22. Buxton, A.N., Zhu, J., Marchant, R., West, J.L., Yoo, J.U., Johnstone, B. Design and characterization of poly(ethylene glycol) photopolymerizable semi-interpenetrating networks for chondrogenesis of human mesenchymal stem cells. Tissue Eng.**13**:2549, 2007.

23. Ando, W., Tateishi, K., Katakai, D., Hart, D.A., Higuchi, C., Nakata, K., Hashimoto, J., Fujie, H., Shino, K., Yoshikawa, H., Nakamura, N. In vitro generation of a scaffold-free tissue-engineered construct (TEC) derived from human synovial mesenchymal stem cells: biological and mechanical properties and further chondrogenic potential. Tissue Eng Part A.**14**:2041, 2008.

24. Katakai, D., Imura, M., Ando, W., Tateishi, K., Yoshikawa, H., Nakamura, N., Fujie, H. Compressive properties of cartilage-like tissues repaired in vivo with scaffold-free, tissue engineered constructs. Clin Biomech (Bristol, Avon).**24**:110, 2009.

25. Bueno, E.M., Bilgen, B., Barabino, G.A. Hydrodynamic parameters modulate biochemical, histological, and mechanical properties of engineered cartilage. Tissue Eng Part A.**15**:773, 2009.

26. Sakiyama-Elbert, S.E., Hubbell, J.A. Development of fibrin derivatives for controlled release of heparin-binding growth factors. J Control Release.**65**:389, 2000.

27. Sakiyama-Elbert, S.E., Hubbell, J.A. Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. J Control Release.**69**:149, 2000.

28. Taylor, S.J., McDonald, J.W., 3rd, Sakiyama-Elbert, S.E. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J Control Release.**98**:281, 2004.

29. Lee, A.C., Yu, V.M., Lowe, J.B., 3rd, Brenner, M.J., Hunter, D.A., Mackinnon, S.E., Sakiyama-Elbert, S.E. Controlled release of nerve growth factor enhances sciatic nerve regeneration. Exp Neurol.**184**:295, 2003.

30. Rock, G., Neurath, D., Semple, E., Harvey, M., Freedman, M. Preparation and characterization of human thrombin for use in a fibrin glue. Transfus Med.**17**:187, 2007.

31. Rock, G., Berger, R., Lange, J., Tokessy, M., Palmer, D.S., Giulivi, A. A novel, automated method of temperature cycling to produce cryoprecipitate. Transfusion.**41**:232, 2001.

32. Wilkins, A., Kemp, K., Ginty, M., Hares, K., Mallam, E., Scolding, N. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. Stem Cell Res. 2009.

33. Zuk, P.A., Zhu, M., Ashjian, P., De Ugarte, D.A., Huang, J.I., Mizuno, H., Alfonso, Z.C., Fraser, J.K., Benhaim, P., Hedrick, M.H. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell.**13**:4279, 2002.

34. Mehlhorn, A.T., Niemeyer, P., Kaiser, S., Finkenzeller, G., Stark, G.B., Sudkamp, N.P., Schmal, H. Differential expression pattern of extracellular matrix molecules during chondrogenesis of mesenchymal stem cells from bone marrow and adipose tissue. Tissue Eng.**12**:2853, 2006.

35. Barry, F., Boynton, R.E., Liu, B., Murphy, J.M. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Exp Cell Res.**268**:189, 2001.

36. Rider, C.C. Heparin/heparan sulphate binding in the TGF-beta cytokine superfamily. Biochem Soc Trans.**34**:458, 2006.

37. Woessner, J.F., Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys.**93**:440, 1961.

38. Colgrave, M.L., Allingham, P.G., Jones, A. Hydroxyproline quantification for the estimation of collagen in tissue using multiple reaction monitoring mass spectrometry. J Chromatogr A.**1212**:150, 2008.

39. Kim, Y.J., Sah, R.L., Doong, J.Y., Grodzinsky, A.J. Fluorometric assay of DNA in cartilage explants using Hoechst 33258. Anal Biochem.**174**:168, 1988.

40. Barbosa, I., Garcia, S., Barbier-Chassefiere, V., Caruelle, J.P., Martelly, I., Papy-Garcia, D. Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies. Glycobiology.**13**:647, 2003.

41. Lev, R., Spicer, S.S. Specific Staining of Sulphate Groups with Alcian Blue at Low Ph. J Histochem Cytochem.**12**:309, 1964.

42. Yan, H., Yu, C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy.**23**:178, 2007.

43. Caplan, A.I., Elyaderani, M., Mochizuki, Y., Wakitani, S., Goldberg, V.M. Principles of cartilage repair and regeneration. Clin Orthop Relat Res.254, 1997.

44. Heymer, A., Bradica, G., Eulert, J., Noth, U. Multiphasic collagen fibre-PLA composites seeded with human mesenchymal stem cells for osteochondral defect repair: an in vitro study. J Tissue Eng Regen Med.**3**:389, 2009.

45. Fan, H., Liu, H., Toh, S.L., Goh, J.C. Anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold in large animal model. Biomaterials.**30**:4967, 2009.

46. Angele, P., Johnstone, B., Kujat, R., Zellner, J., Nerlich, M., Goldberg, V., Yoo, J. Stem cell based tissue engineering for meniscus repair. J Biomed Mater Res A.**85**:445, 2008.

47. Zheng, Z.H., Li, X.Y., Ding, J., Jia, J.F., Zhu, P. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. Rheumatology (Oxford).**47**:22, 2008.

48. Vinatier, C., Gauthier, O., Masson, M., Malard, O., Moreau, A., Fellah, B.H., Bilban, M., Spaethe, R., Daculsi, G., Guicheux, J. Nasal chondrocytes and fibrin sealant for cartilage tissue engineering. J Biomed Mater Res A.**89**:176, 2009.

49. Chou, C.H., Cheng, W.T., Kuo, T.F., Sun, J.S., Lin, F.H., Tsai, J.C. Fibrin glue mixed with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular cartilage tissue engineering: the results of real-time polymerase chain reaction. J Biomed Mater Res A.**82**:757, 2007.

50. Chang, J., Rasamny, J.J., Park, S.S. Injectable tissue-engineered cartilage using a fibrin sealant. Arch Facial Plast Surg.**9**:161, 2007.

51. Jung, R.E., Schmoekel, H.G., Zwahlen, R., Kokovic, V., Hammerle, C.H., Weber, F.E. Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morphogenetic protein-2. Clin Oral Implants Res.**16**:676, 2005.

52. Ichinose, S., Tagami, M., Muneta, T., Sekiya, I. Morphological examination during in vitro cartilage formation by human mesenchymal stem cells. Cell Tissue Res.**322**:217, 2005.

53. Simon, B.I., Zatcoff, A.L., Kong, J.J., O'Connell, S.M. Clinical and Histological Comparison of Extraction Socket Healing Following the Use of Autologous Platelet-Rich Fibrin Matrix (PRFM) to Ridge Preservation Procedures Employing Demineralized Freeze Dried Bone Allograft Material and Membrane. Open Dent J.**3**:92, 2009.

54. Gassling, V.L., Acil, Y., Springer, I.N., Hubert, N., Wiltfang, J. Platelet-rich plasma and platelet-rich fibrin in human cell culture. Oral Surg Oral Med Oral Pathol Oral Radiol Endod.**108**:48, 2009.

55. Liu, Z.J., Zhuge, Y., Velazquez, O.C. Trafficking and differentiation of mesenchymal stem cells. J Cell Biochem.**106**:984, 2009.

56. Kuo, C.K., Li, W.J., Mauck, R.L., Tuan, R.S. Cartilage tissue engineering: its potential and uses. Curr Opin Rheumatol.**18**:64, 2006.

57. Jin'gen, B., Jian, Y., Dunwan, Z., Junjie, L., Kangde, Y. The multiple interactions between growth factors and microenvironment in vivo. Chinese Sci Bull.**51**:761, 2006.

58. Han, D., Liu, W., Ao, Q., Wang, G. Optimal delivery systems for bone morphogenetic proteins in orthopedic applications should model initial tissue repair structures by using a heparin-incorporated fibrin-fibronectin matrix. Med Hypotheses.**71**:374, 2008.

59. Herbert, C.B., Bittner, G.D., Hubbell, J.A. Effects of fibinolysis on neurite growth from dorsal root ganglia cultured in two- and three-dimensional fibrin gels. J Comp Neurol.**365**:380, 1996.

60. Eickelberg, O., Centrella, M., Reiss, M., Kashgarian, M., Wells, R.G. Betaglycan inhibits TGF-beta signaling by preventing type I-type II receptor complex formation. Glycosaminoglycan modifications alter betaglycan function. J Biol Chem.**277**:823, 2002.

61. Yakovlev, S., Gorlatov, S., Ingham, K., Medved, L. Interaction of fibrin(ogen) with heparin: further characterization and localization of the heparin-binding site. Biochemistry.**42**:7709, 2003.

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VIII. CHAPTER 5. GENERAL DISCUSSION

1. Significant Scientific Contributions

As discussed in chapter 1, articular cartilage has poor ability for self-repair and regeneration. Even under optimal conditions, when cartilage injury extends to the subchondral bone to promote the migration of progenitor/stem cells that aid in the healing process, fibrocartilage is formed (McCormick et al., 2008; Peretti et al., 2006; Beris et al., 2005; Temenoff et al., 2000). Fibrocartilage possesses substandard biomechanical properties compared to hyaline cartilage (McCormick et al., 2008; Zehbe et al., 2005; Aigner et al. 2003). Several repair approaches were established in the last century, primarily to reduce pain including conservative treatments (Rosneck et al., 2007; Detterline et al, 2005), along with nonreparative procedures (debridement: Li et al., 2008; Laupattarakasem et al., 2008, chondral shaving: Spahn et al., 2006; Burkart et al., 2001, and knee lavage: Fu el., 2009; Lutzner et al., 2009). Other strategies were established to aid in the repair process and functional restoration after various cartilage injuries; these include marrow stimulation techniques (Dorotka et al., 2005), high tibial osteotomy (Mina et al.,2008; Tang et al., 2005; Wright et al., 2005) and knee replacement (Miller et al., 2005; Bert et al., 2005). However, none of these techniques are able to entirely restore articular cartilage function or structure. A number of transplantation strategies have been established over the last two decades to help restore the function and structure of injured cartilage, either through ostochondral grafting (autologous and allogeneic, McCormick et al., 2008; Kessler et al., 2008; Recht et al., 2003) or chondrocyte implantation (autologous, McNickle 2009, 2008; Iwasa et al., 2008; Hettrich et al., 2008); however, most of these strategies

require two surgeries and are associated with donor site morbidity along with numerous technical difficulties.

Tissue engineering-based substitutes are considered to be the future of regenerative medicine as they mimic the 3D architecture of native cartilage and eliminate some of the drawbacks associated with the routinely performed transplantation procedures. A wide range of materials have been developed for tissue engineering applications, including carbohydrate-based natural scaffolds (Lee et al., 2009), protein-based natural scaffolds, and synthetic scaffolds (Iwasa et al., 2009; Kerker et al., 2008). Amongst the protein-based matrices, fibrin is a promising scaffold that can be utilized for a wide range of tissue engineering applications, including articular cartilage, as discussed in detail in chapters 1 and 2. Generally, scaffolds/carrier matrices utilized for tissue engineering of articular cartilage should follow specific criteria: first, they should mimic the effect of native cartilage ECM on cell proliferation, differentiation and cell-to-cell interaction; second, they should be biocompatible and promote uniform cell distribution; third, they should have suitable life span *in vivo* and/or *in vitro* until being replaced gradually by cartilage-like ECM secreted from the cells (i.e. remodelling) with subsequent formation of adequate cartilaginous tissue; fourth, they should promote optimum integration with the surrounding native cartilage while maintaining the cells at the lesion site; and finally, they should have sufficient mechanical properties to withstand *in vivo* forces (Iwasa et al., 2009; Kerker et al., 2008; Beris et al., 2005). The nature of the scaffolding material and its porosity has tremendous effects on the proliferation and phenotype of chondrocytes (Lien et al., 2009). Of all the currently used scaffolding materials, fibrin combines many of these features (Ahmed et al., 2008; Rosso et al., 2005; Swartz et al., 2005; Jockenhoevel et al., 2001). In addition, fibrin is the only scaffold that can be easily prepared in autologous form to

circumvent foreign body reaction and disease transmission. However, fibrin matrix has two major disadvantages: rapid degradation before formation of satisfactory cartilaginous structures, and poor mechanical properties (Mol et al., 2005; Jockenhoevel et al., 2001).

In chapter 3 we addressed the first disadvantage of fibrin scaffold during tissue engineering of articular cartilage, which is the observed rapid degradation that is mediated by enzymes secreted from the cells after encapsulation. Our novel work with the chondrogenic C5.18 cell line demonstrated that secreted members of matrix metalloproteinase family (MMPs), including MMP-2, MMP-3, and MMP-9, in addition to plasminogen, are responsible for the accelerated degradation of fibrin hydrogels. We are also the first to report that either the plasmin inhibitor, aprotinin, or the MMPs inhibitor, galardin, are capable of stabilizing fibrin hydrogel for extended times, while only a combination of both inhibitors promotes enhanced accumulation of the cartilage ECM-specific markers aggrecan and collagen II. Alternatively, other approaches can prolong the life span of fibrin hydrogels including optimizing the pH, Ca^{2+} and fibrinogen concentrations simultaneously (Eyrich et al., 2007), altering the properties of fibrin gel by denaturation, increased cross-linking, or increased fibrin density through formation of fibrin microbeads (Rivkin et al., 2007; Kassis et al., 2006), or decreasing the cell density (Passaretti et al., 2001). In comparison to these approaches, adding protease inhibitors is a highly feasible approach for development of *in vivo* tissue-engineered cartilage substitute, since the protease inhibitor aprotinin can be immobilized into the fibrin hydrogel to promote fibrin stability *in vivo* (Smith et al., 2007). In addition, chondrogenic cells such as chondrocytes can be transduced to express tissue inhibitors of matrix metalloproteinases (TIMPs) including TIMP-1 with subsequent reduced MMPs activity and diminished collagen II degradation in tissue-engineered cartilage constructs (Kafienah et al., 2003).

Enhancing the mechanical properties of fibrin-based constructs has been achieved through cross-linking by genipin, a natural product cross-linker, which was demonstrated by our research group in 2009 (Dare et al., 2009).

In chapter 4, we addressed the encapsulation of bone marrow-derived mesenchymal/stromal cells into fibrin glues, since the major difficulty of engineering cartilage is to obtain sufficient number of cells for generating the cartilage tissue (Peretti et al., 2006). MSCs have two major advantages in that they can be expanded many times without affecting their capacity to form cartilaginous tissue after implantation (Kessler et al., 2008), and that they possess multi-differentiation potential which facilitates the utilization of the same cell source to create different tissue structures including cartilage-bone interface (Heymer et al., 2009), meniscus (Angele et al., 2008), and ligament (Fan et al., 2009). In addition, MSCs can be obtained from many sources including adipose tissue, blood, bone marrow, dermis, muscle periosteum, synovial membrane and trabecular bone (Tuan et al., 2003), usually by a minimally invasive procedure. A number of fibrin-based products are currently in different phases of human clinical trials including Tissucol® in combination with autologous chondrocytes, fibrin glue with allogeneic minced cartilage (DeNovo NT graft) or allogeneic chondrocytes (DeNovo ET graft) and, finally, photo-polymerized poly(ethylene glycol)-modified fibrin, known as Gelrin C (Clair et al 2009; Cascio et al., 2008; Kerker et al., 2008; Kon et al., 2008; Hettrich et al., 2008; Iwasa et al., 2008; McCormick et al., 2008; McNickle et al., 2008). Tissucol® is commercial non-autologous fibrin product, while DeNovo NT and DeNovo ET utilize allogeneic cell sources. Gelrin C is an adjuvant to the microfracture technique, and acts to promote formation of fibrocartilage mediated by migrating stem cells. Our approach is the first to combine fibrin glue produced by the CryoSeal® FS system with MSCs that can be both

obtained in autologous form and represent the first move to develop an autologous fibrin-based tissue-engineered cartilage substitute for clinical application in human. Enhanced expression of the cartilage-specific marker, collagen II, was observed after encapsulation of hMSCs into fibrin glue and induction of chondrogenesis, confirming the chondrogenic potential of these cells. However, when the heparin-based delivery system was incorporated into fibrin glues to enhance the bioavailability and to protect growth factors from proteolytic degradation, considerable reduction in the expression of cartilage-specific markers was observed. This was possibly due to interference with cell-mediated proteolytic release of the growth factors by the plasmin protease inhibitor added to the culture media (Herbert et al., 1996). This is a critical finding for the future development of the project, as it reveals that immobilizing growth factors inside fibrin glue does not necessarily result in potentiation of the chondrogenic effect of bioactive factors. Although fibrin gels have been used clinically for tissue-engineered skin and adipose tissue substitutes, in addition to its use as a helper tool in many surgical settings, its clinical utilization for the treatment of human articular cartilage defects is still limited. In the following section, we will discuss some strategies to advance the utilization of fibrin-based tissue-engineered cartilage substitutes further towards clinical applications.

2. Approaches for future consideration

2. 1. Optimizing the mechanical properties

In order to proceed with application of fibrin for human clinical trials, the final mechanical properties of a fibrin-based construct should be sufficient to withstand intra joint physiological forces until proper formation of cartilage-specific ECM. Mechanical properties can be enhanced as mentioned earlier by cross-linking (Dare et al., 2009); however, the mechanical properties still need to be optimized to match those of native

cartilage, since the compressive modulus of adult human articular cartilage is in the range 4.4 - 27 MPa (Shepherd et al., 1997). Combining fibrin with other scaffolding materials that have good mechanical properties, such as polyethylene glycol (PEG), is a relevant approach (Peled et al., 2007). PEG is a biodegradable synthetic polymer and approved by FDA for medicinal purposes (Barker et al., 2001). Photocrosslinkable derivatives of PEG, such as poly(ethylene glycol) dimethacrylate (PEGDM) and poly(ethylene glycol) diacrylate (PEGDA), facilitate *in vivo* applications, since they can be injected in a liquid form that gelates *in situ* with UV light (Buxton et al., 2007; Nguyen et al., 2002). The mechanical properties of PEG - based scaffolds are dependent on mesh size, which in turn depends on the PEG molecular weight, concentration or cross-linking density (Bryant et al., 2004; Temenoff et al., 2002). Controlling the mesh size helps also to fabricate multilayered PEG-based constructs that resemble the zonal pattern of articular cartilage (Temenoff et al., 2002). Therefore combining autologous fibrin glue with these derivatives of PEG is a good strategy to develop constructs that combine most of the required features for successful tissue engineering of articular cartilage. In support of this idea, Gelrin C, a commercial product based on a PEGylated derivative of fibrin, is now in preclinical phases as an adjuvant to microfracture. However, its utilization could be extended further to restore different elements of damaged cartilage. The proposed utilization of fibrin in combination with PEG for cartilage clinical applications is illustrated in FIG.1

2.2. Optimizing the accumulation of cartilage-specific markers

The mechanical properties of native articular cartilage are attributed to its ECM components mainly type II collagen and proteoglycans such as aggrecan. Therefore, optimizing the accumulation of these markers *in vitro* and *in vivo* to promote native-like structures would be an important requirement during development of the tissue

engineering-based cartilage substitute (Bueno et al., 2009). Bioactive factors regulate the expression and accumulation of cartilage-specific markers. However, better understanding of signalling involved in chondrogenesis is necessary. Growth factors should induce proliferation, differentiation, and maturation, while maintaining chemotactic activity and optimum ECM production by the cell source (Ahmed et al., 2008; Leo et al., 2006). The TGF-β superfamily is a group of growth factors that are known to induce chondrogenesis; other growth factors promote chondrocyte physiology rather than inducing chondrogenesis; for instance, FGF and IGF promote cell proliferation and in combination with TGF-βs increase neo-cartilage formation (Liu et al., 2009; Kuo et al., 2006; Barry et al., 2001). Therefore, for the development of tissue-engineered cartilage constructs, a combination of these distinct groups of bioactive factors should be selected. This approach has been utilized by other research groups, where a combination of IGF-1 either with TGF-β_3 (Indrawattana et al., 2004), TGF-β_2 (Yasuda et al., 2006), or TGF-β_1 (Xiang et al., 2007) resulted in improved chondrogenesis in 3D cultures. This approach is particularly helpful for the development of *in vitro* tissue-engineered cartilage constructs. However, this concept can be adapted for *in vivo* application through delivery of genetic information to the chondrogenic cell source. Implantation of MSCs that overexpress BMP-7(Grande et al., 2003) or TGF-β_1 (Guo et al., 2006), along with a supporting matrix, into the chondral defect results in satisfactory hyaline-like tissue formation. Similar results can be obtained when chondrocytes overexpressing both TGF-β_1 and PTHrP are implanted into a chondral defect (Goomer et al., 2001). Alternatively, genetic manipulation of MSCs to overexpress SOX-9 (high-mobility-group domain transcription factor; a key regulator of collagen II and aggrecan gene expression) leads to formation of cartilaginous tissue both *in vivo* and *in vitro* (Babister et al., 2008). Therefore, encapsulation of MSCs that overexpress SOX-9,

along with TGF-β3 and IGF-1, in fibrin glue/photo-crosslinkable PEG hybrid, would be a good approach for development of an *in vivo* implantation system. For genetic transfer, viral vectors should be utilized, since they have higher transfection efficiency and exhibit long duration expression of the delivered gene (Kim et al., 2006; Saraf et al., 2006). The proposed utilization of genetically-modified hMSCs in combination with Fibrin/PEG hybrid for cartilage clinical applications is illustrated in FIG.1. Finally, applying low intensity ultrasound is efficient way to promote the maturation of tissue-engineered cartilage constructs *in vitro* before implantation (Lee et al., 2007; Ebisawa et al., 2004).

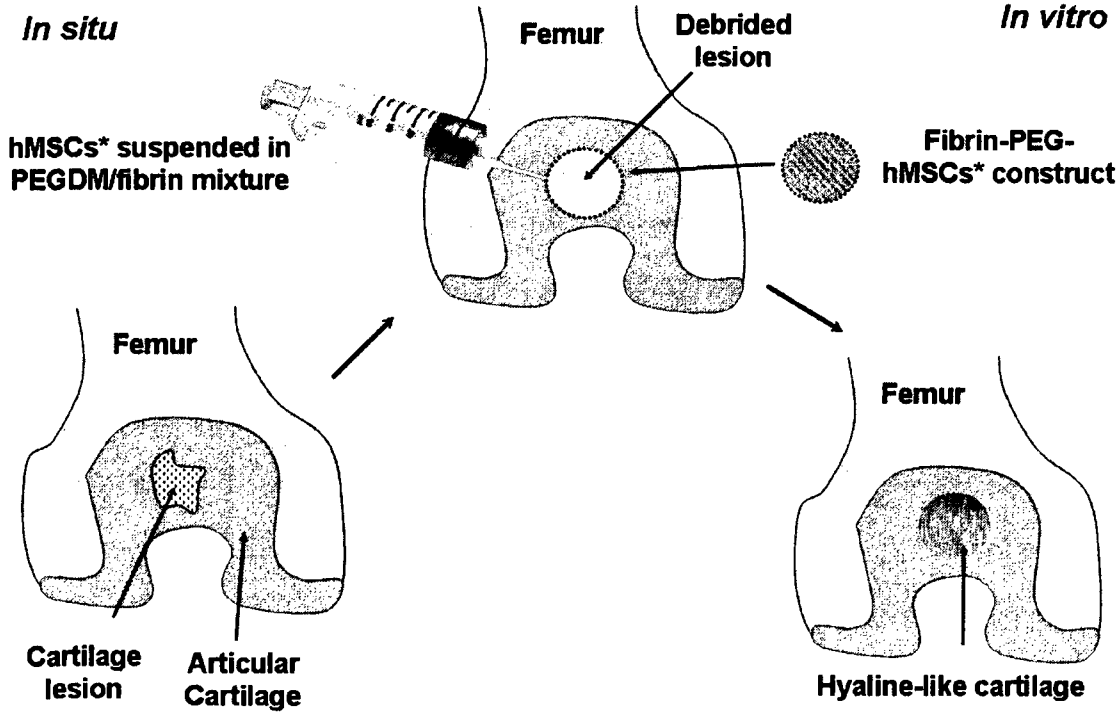

FIG.1. Anticipated strategy for implantation of fibrin-based cartilage constructs. Normal or genetically-modified hMSCs are cultured *in vitro* after encapsulation in a fibrin/photo-crosslinkable PEG copolymer under the effect of growth factor and physical stimulants, followed by *in vivo* implantation. Alternatively, normal or genetically-modified hMSCs can be suspended in the fibrin/photo-crosslinkable PEG mixture for *in situ* gelation to promote the regeneration process. (hMSCs*: Normal or genetically modified cells).

2.3. Selecting an appropriate animal model for experimental testing

Several considerations should be followed to choose the appropriate animal model for experimental work involving osteochondral defects, including, age and species of the model, the nature of the required osteochondral defect and the duration of the experiment. A wide range of experimental animals can be utilized for experimental testing, including rats, rabbits, pigs, goats, dogs, and horses. Rabbits are the most utilized animal model, since their knee joint gross morphology is similar to human, including bone, cartilage and tendon anatomy. Horses are a model of choice to achieve a cartilage defect having the same dimensions as those of human. However, using horses is not cost effective, particularly when large numbers of animals are required (Rudert et al., 2002). Again, rabbits should be considered for reasons of cost effectiveness. Experimental work should be done in rabbits older than 6 months (age of skeletal maturity), since wound healing is of superior quality and take places faster in young compared to old animals (Wei et al., 1997). Experimentally created defects should be condylar and of reproducible dimensions, along with having a critical size to resist substantial loads without causing condylar fracture. Finally, a follow-up of 6 months is sufficient for the first evaluation of osteochondral defects created in rabbit model (Rudert et al., 2002). Goats are a second good candidate as an osteochondral model before clinical trials in human, since they have large joint size, good cartilage thickness (~1 mm). In addition, their availability and ease of handling facilitate the experimental procedures (Niederauer et al., 2000).

IX. CONCLUSIONS

A number of objectives of this project have been addressed during the course of this thesis research. One was to characterize and identify the enzymes responsible for fibrin hydrogel breakdown after encapsulation of the chondroprogenitor clonal cell line RCJ 3.1 C5.18 (C5.18). Another goal of the project was to evaluate bone-marrow derived mesenchymal stem cells (BM-hMSCs) in combination with different fibrin glues produced by the CryoSeal®-FS system (containing or lacking heparin-based delivery system (HBDS)), to develop stem cells-based tissue-engineered cartilage constructs *in vitro*. The results discussed in chapter 3 and 4 showed that the above objectives have been achieved. Plasmin and matrix metalloproteinases (MMPs) that are secreted by C5.18 cells after encapsulation led to rapid degradation of fibrin hydrogel before formation of satisfactory cartilaginous tissue. Stabilization of plasmin by aprotinin or MMPs by galardin is necessary to control gel degradation, while only a combination of both inhibitors resulted in improved accumulation of cartilage-specific markers. BM-hMSCs are a potential chondrogenic cell source that can promote the development of cartilaginous tissue and expression of cartilage specific markers, and in combination with supportive 3D fibrin glue advance the project closer to clinical phase trials. However, enhancing the bioavailability and protection of growth factors via immobilization with the HBDS did not promote enhanced expression of cartilage specific markers. The project represents a significant contribution to regenerative medicine for repair of cartilage; the project continuation should include optimizing conditions to enhance mechanical properties of fibrin scaffold and to maximize ECM accumulation, along with evaluation of the resulting constructs in orthopaedic animal models.

X. REFERENCES

Abe M., Takahashi M., Tokura S., Tamura H., and Nagano A. 2004. Cartilage-scaffold composites produced by bioresorbable beta-chitin sponge with cultured rabbit chondrocytes. *Tissue Eng.* 10(3-4), 585-94.

Ahmed T.A., and Hincke M.T. 2010. Strategies for Articular Cartilage Lesion Repair and Functional Restoration. *Tissue Eng Part B Rev.* (Epub ahead of print). Review.

Ahmed T.A., Dare E.V., and Hincke M. 2008. Fibrin: A Versatile Scaffold for Tissue Engineering Applications. *Tissue Eng Part B Rev.* 14(2):199-215. Review.

Ahmed T.A., Griffith M., and Hincke M. 2007. Characterization and inhibition of fibrin hydrogel-degrading enzymes during development of tissue engineering scaffolds. *Tissue Eng.* 13(7), 1469-77.

Aigner T., and Stove J. 2003. Collagens--major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. *Adv Drug Deliv Rev.* 55(12), 1569-93.

Alaminos M., Del Carmen Sanchez-Quevedo M., Munoz-Avila J.I., Serrano D., Medialdea S., Carreras I., and Campos A. 2006. Construction of a complete rabbit cornea substitute using a fibrin-agarose scaffold. *Invest Ophthalmol Vis Sci.* 47(8), 3311-7.

Allison D.D., and Grande-Allen K.J. 2006. Review. Hyaluronan: a powerful tissue engineering tool. *Tissue Eng.* 12(8), 2131-40.

Almond A. 2007. Hyaluronan. *Cell Mol Life Sci.* 64(13), 1591-6.

Almqvist K.F., Wang L., Wang J., Baeten D., Cornelissen M., Verdonk R., Veys E.M., and Verbruggen G. 2001. Culture of chondrocytes in alginate surrounded by fibrin gel:

characteristics of the cells over a period of eight weeks. *Ann Rheum Dis.* 60(8), 781-90.

Alsberg E., Feinstein E., Joy M.P., Prentiss M., and Ingber D.E. 2006. Magnetically-guided self-assembly of fibrin matrices with ordered nano-scale structure for tissue engineering. *Tissue Eng.* 12(11), 3247-56.

Altman R.D., and Gray R. 1983. Diagnostic and therapeutic uses of the arthroscope in rheumatoid arthritis and osteoarthritis. *Am J Med.* 75(4B), 50-5.

Altmeppen J., Hansen E., Bonnlander G.L., Horch R.E., and Jeschke M.G. 2004. Composition and characteristics of an autologous thrombocyte gel. *J Surg Res.* 117(2), 202-7.

Ameer G.A., Mahmood T.A., and Langer R. 2002. A biodegradable composite scaffold for cell transplantation. *J Orthop Res.* 20(1), 16-9.

Anderson A.F., Richards D.B., Pagnani M.J., and Hovis W.D. 1997. Antegrade drilling for osteochondritis dissecans of the knee. *Arthroscopy.* 13(3), 319-24.

Ando W., Tateishi K., Katakai D., Hart D.A., Higuchi C., Nakata K., Hashimoto J., Fujie H., Shino K., Yoshikawa H., and Nakamura N. 2008. *In vitro* generation of a scaffold-free tissue-engineered construct (TEC) derived from human synovial mesenchymal stem cells: biological and mechanical properties and further chondrogenic potential. *Tissue Eng Part A.* 14(12), 2041-9.

Angele P., Johnstone B., Kujat R., Zellner J., Nerlich M., Goldberg V., and Yoo J. 2008. Stem cell based tissue engineering for meniscus repair. *J Biomed Mater Res A.* 85(2), 445-55.

Antoniou J., Hadjipavlou A., Enker P., and Antoniou A. 1996. Unicompartmental knee arthroplasty with patelloplasty. *Int Orthop.* 20(2), 94-9.

Arkudas A., Tjiawi J., Bleiziffer O., Grabinger L., Polykandriotis E., Beier J.P., Sturzl M., Horch R.E., and Kneser U. 2007. Fibrin gel-immobilized VEGF and bFGF efficiently stimulate angiogenesis in the AV loop model. *Mol Med.* 13(9-10), 480-7.

Aubin P.P., Cheah H.K., Davis A.M., and Gross A.E. 2001. Long-term followup of fresh femoral osteochondral allografts for posttraumatic knee defects. *Clin Orthop Relat Res*(391 Suppl), S318-27.

Babister J.C., Tare R.S., Green D.W., Inglis S., Mann S., and Oreffo R.O. 2008. Genetic manipulation of human mesenchymal progenitors to promote chondrogenesis using "bead-in-bead" polysaccharide capsules. *Biomaterials.* 29(1), 58-65.

Badley E.M., and Wang P.P. 1998. Arthritis and the aging population: projections of arthritis prevalence in Canada 1991 to 2031. *J Rheumatol.* 25(1), 138-44.

Balestrini J.L., and Billiar K.L. 2006. Equibiaxial cyclic stretch stimulates fibroblasts to rapidly remodel fibrin. *J Biomech.* 39(16), 2983-90.

Banu N., and Tsuchiya T. 2007. Markedly different effects of hyaluronic acid and chondroitin sulfate-A on the differentiation of human articular chondrocytes in micromass and 3-D honeycomb rotation cultures. *J Biomed Mater Res A.* 80(2), 257-67.

Barbosa I., Garcia S., Barbier-Chassefiere V., Caruelle J.P., Martelly I., and Papy-Garcia D. 2003. Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies. *Glycobiology.* 13(9), 647-53.

Baricos W.H., Cortez S.L., el-Dahr S.S., and Schnaper H.W. 1995. ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. *Kidney Int.* 47(4), 1039-47.

Barker T.H., Fuller G.M., Klinger M.M., Feldman D.S., and Hagood J.S. 2001. Modification of fibrinogen with poly(ethylene glycol) and its effects on fibrin clot characteristics. *J Biomed Mater Res.* 56(4), 529-35.

Barry F., Boynton R.E., Liu B., and Murphy J.M. 2001. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res.* 268(2), 189-200.

Bastiaansen-Jenniskens Y.M., Koevoet W., de Bart A.C., van der Linden J.C., Zuurmond A.M., Weinans H., Verhaar J.A., van Osch G.J., and Degroot J. 2008. Contribution of collagen network features to functional properties of engineered cartilage. *Osteoarthritis Cartilage.* 16(3), 359-66.

Bedran-Russo A.K., Pereira P.N., Duarte W.R., Drummond J.L., and Yamauchi M. 2007. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. *J Biomed Mater Res B Appl Biomater.* 80(1), 268-72.

Berger R.A., Meneghini R.M., Jacobs J.J., Sheinkop M.B., Della Valle C.J., Rosenberg A.G., and Galante J.O. 2005. Results of unicompartmental knee arthroplasty at a minimum of ten years of follow-up. *J Bone Joint Surg Am.* 87(5), 999-1006.

Beris A.E., Lykissas M.G., Papageorgiou C.D., and Georgoulis A.D. 2005. Advances in articular cartilage repair. *Injury.* 36 Suppl 4, S14-23.

Bert J.M. 2005. Unicompartmental knee replacement. *Orthop Clin North Am.* 36(4), 513-22.

Bert J.M. 2007. Unicompartmental arthroplasty for unicompartmental knee arthritis. *Tech Knee Surg.* 7(1), 51-60.

Bhosale A.M., and Richardson J.B. 2008. Articular cartilage: structure, injuries and review of management. *British Medical Bulletin.* 87(1), 77-95.

Birla R.K., Borschel G.H., Dennis R.G., and Brown D.L. 2005. Myocardial engineering in vivo: formation and characterization of contractile, vascularized three-dimensional cardiac tissue. *Tissue Eng.* 11(5-6), 803-13.

Blackburn T.A., and Craig E. 1980. Knee anatomy: a brief review. *Phys Ther.* 60(12), 1556-60.

Borges J., Mueller M.C., Padron N.T., Tegtmeier F., Lang E.M., and Stark G.B. 2003. Engineered adipose tissue supplied by functional microvessels. *Tissue Eng.* 9(6), 1263-70.

Bruns H., Kneser U., Holzhuter S., Roth B., Kluth J., Kaufmann P.M., Kluth D., and Fiegel H.C. 2005. Injectable liver: a novel approach using fibrin gel as a matrix for culture and intrahepatic transplantation of hepatocytes. *Tissue Eng.* 11(11-12), 1718-26.

Bryant S.J., Anseth K.S., Lee D.A., and Bader D.L. 2004a. Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain. *J Orthop Res.* 22(5), 1143-9.

Bryant S.J., Arthur J.A., and Anseth K.S. 2005. Incorporation of tissue-specific molecules alters chondrocyte metabolism and gene expression in photocrosslinked hydrogels. *Acta Biomater.* 1(2), 243-52.

Bryant S.J., Bender R.J., Durand K.L., and Anseth K.S. 2004b. Encapsulating chondrocytes in degrading PEG hydrogels with high modulus: engineering gel structural changes to facilitate cartilaginous tissue production. *Biotechnol Bioeng.* 86(7), 747-55.

Bryant S.J., Chowdhury T.T., Lee D.A., Bader D.L., and Anseth K.S. 2004c. Crosslinking density influences chondrocyte metabolism in dynamically loaded photocrosslinked poly(ethylene glycol) hydrogels. *Ann Biomed Eng.* 32(3), 407-17.

Bryant S.J., Durand K.L., and Anseth K.S. 2003. Manipulations in hydrogel chemistry control photoencapsulated chondrocyte behavior and their extracellular matrix production. *J Biomed Mater Res A*. 67(4), 1430-6.

Buchta C., Dettke M., Funovics P.T., Hocker P., Knobl P., Macher M., Quehenberger P., Treitl C., and Worel N. 2004. Fibrin sealant produced by the CryoSeal FS System: product chemistry, material properties and possible preparation in the autologous preoperative setting. *Vox Sang*. 86(4), 257-62.

Buchta C., Hedrich H.C., Macher M., Hocker P., and Redl H. 2005. Biochemical characterization of autologous fibrin sealants produced by CryoSeal and Vivostat in comparison to the homologous fibrin sealant product Tissucol/Tisseel. *Biomaterials*. 26(31), 6233-41.

Bueno E.M., Bilgen B., and Barabino G.A. 2009. Hydrodynamic parameters modulate biochemical, histological, and mechanical properties of engineered cartilage. *Tissue Eng Part A*. 15(4), 773-85.

Buly R.L., and Sculco T.P. 1995. Recent advances in total knee replacement surgery. *Curr Opin Rheumatol*. 7(2), 107-13.

Buma P., Pieper J.S., van Tienen T., van Susante J.L., van der Kraan P.M., Veerkamp J.H., van den Berg W.B., Veth R.P., and van Kuppevelt T.H. 2003. Cross-linked type I and type II collagenous matrices for the repair of full-thickness articular cartilage defects--a study in rabbits. *Biomaterials*. 24(19), 3255-63.

Burkart A.C., Schoettle P.B., and Imhoff A.B. 2001. [Surgical therapeutic possibilities of cartilage damage]. *Unfallchirurg*. 104(9), 798-807.

Burnouf T., and Radosevich M. 2001. Affinity chromatography in the industrial purification of plasma proteins for therapeutic use. *J Biochem Biophys Methods.* 49(1-3), 575-86.

Buxton A.N., Zhu J., Marchant R., West J.L., Yoo J.U., and Johnstone B. 2007. Design and characterization of poly(ethylene glycol) photopolymerizable semi-interpenetrating networks for chondrogenesis of human mesenchymal stem cells. *Tissue Eng.* 13(10), 2549-60.

Byun Y., and Yang V.C. 1998. Delivery system for targeted thrombolysis without the risk of hemorrhage. *Asaio J.* 44(5), M638-41.

Cai G., Chen X., Fu B., and Lu Y. 2005. Activation of gelatinases by fibrin is PA/plasmin system-dependent in human glomerular endothelial cells. *Mol Cell Biochem.* 277(1-2), 171-9.

Caplan A.I., Elyaderani M., Mochizuki Y., Wakitani S., and Goldberg V.M. 1997. Principles of cartilage repair and regeneration. *Clin Orthop Relat Res*(342), 254-69.

Carless P.A., Anthony D.M., and Henry D.A. 2002. Systematic review of the use of fibrin sealant to minimize perioperative allogeneic blood transfusion. *Br J Surg.* 89(6), 695-703.

Cascio B.M., and Sharma B. 2008. The future of cartilage repair. *Oper Tech Sports Med.* 16, 221-4.

Cawston T.E. 1996. Metalloproteinase inhibitors and the prevention of connective tissue breakdown. *Pharmacol Ther.* 70(3), 163-82.

Cen L., Liu W., Cui L., Zhang W., and Cao Y. 2008. Collagen tissue engineering: development of novel biomaterials and applications. *Pediatr Res.* 63(5), 492-6.

Chajra H., Rousseau C.F., Cortial D., Ronziere M.C., Herbage D., Mallein-Gerin F., and Freyria A.M. 2008. Collagen-based biomaterials and cartilage engineering. Application to osteochondral defects. *Biomed Mater Eng.* 18(1 Suppl), S33-45.

Chang C.H., Kuo T.F., Lin C.C., Chou C.H., Chen K.H., Lin F.H., and Liu H.C. 2006. Tissue engineering-based cartilage repair with allogenous chondrocytes and gelatin-chondroitin-hyaluronan tri-copolymer scaffold: A porcine model assessed at 18, 24, and 36 weeks. *Biomaterials.* 27(9), 1876-88.

Chang C.H., Lin F.H., Lin C.C., Chou C.H., and Liu H.C. 2004. Cartilage tissue engineering on the surface of a novel gelatin-calcium-phosphate biphasic scaffold in a double-chamber bioreactor. *J Biomed Mater Res B Appl Biomater.* 71(2), 313-21.

Chang C.H., Liu H.C., Lin C.C., Chou C.H., and Lin F.H. 2003. Gelatin-chondroitin-hyaluronan tri-copolymer scaffold for cartilage tissue engineering. *Biomaterials.* 24(26), 4853-8.

Chang F., Ishii T., Yanai T., Mishima H., Akaogi H., Ogawa T., and Ochiai N. 2008. Repair of large full-thickness articular cartilage defects by transplantation of autologous uncultured bone-marrow-derived mononuclear cells. *J Orthop Res.* 26(1), 18-26.

Chang J., Rasamny J.J., and Park S.S. 2007. Injectable tissue-engineered cartilage using a fibrin sealant. *Arch Facial Plast Surg.* 9(3), 161-6.

Chen G., Sato T., Ushida T., Hirochika R., Shirasaki Y., Ochiai N., and Tateishi T. 2003. The use of a novel PLGA fiber/collagen composite web as a scaffold for engineering of articular cartilage tissue with adjustable thickness. *J Biomed Mater Res A.* 67(4), 1170-80.

Chen G., Sato T., Ushida T., Ochiai N., and Tateishi T. 2004. Tissue engineering of cartilage using a hybrid scaffold of synthetic polymer and collagen. *Tissue Eng.* 10(3-4), 323-30.

Chen H.C., Sung L.Y., Lo W.H., Chuang C.K., Wang Y.H., Lin J.L., and Hu Y.C. 2008. Combination of baculovirus-expressed BMP-2 and rotating-shaft bioreactor culture synergistically enhances cartilage formation. *Gene Ther.* 15(4), 309-17.

Chen Y.L., Lee H.P., Chan H.Y., Sung L.Y., Chen H.C., and Hu Y.C. 2007. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. *Biomaterials.* 28(14), 2294-305.

Cheng H., Cao Y., and Olson L. 1996. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. *Science.* 273(5274), 510-3.

Chia S.M., Leong K.W., Li J., Xu X., Zeng K., Er P.N., Gao S., and Yu H. 2000. Hepatocyte encapsulation for enhanced cellular functions. *Tissue Eng.* 6(5), 481-95.

Cho J.H., Kim S.H., Park K.D., Jung M.C., Yang W.I., Han S.W., Noh J.Y., and Lee J.W. 2004. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. *Biomaterials.* 25(26), 5743-51.

Cho S.W., Kim I., Kim S.H., Rhie J.W., Choi C.Y., and Kim B.S. 2006. Enhancement of adipose tissue formation by implantation of adipogenic-differentiated preadipocytes. *Biochem Biophys Res Commun.* 345(2), 588-94.

Cho S.W., Kim S.S., Rhie J.W., Cho H.M., Choi C.Y., and Kim B.S. 2005. Engineering of volume-stable adipose tissues. *Biomaterials.* 26(17), 3577-85.

Choi B.H., Woo J.I., Min B.H., and Park S.R. 2006. Low-intensity ultrasound stimulates the viability and matrix gene expression of human articular chondrocytes in alginate bead culture. *J Biomed Mater Res A*. 79(4), 858-64.

Chong A.K., Ang A.D., Goh J.C., Hui J.H., Lim A.Y., Lee E.H., and Lim B.H. 2007. Bone marrow-derived mesenchymal stem cells influence early tendon-healing in a rabbit achilles tendon model. *J Bone Joint Surg Am*. 89(1), 74-81.

Chou C.H., Cheng W.T., Kuo T.F., Sun J.S., Lin F.H., and Tsai J.C. 2007. Fibrin glue mixed with gelatin/hyaluronic acid/chondroitin-6-sulfate tri-copolymer for articular cartilage tissue engineering: the results of real-time polymerase chain reaction. *J Biomed Mater Res A*. 82(3), 757-67.

Chowdhury T.T., Bader D.L., Shelton J.C., and Lee D.A. 2003. Temporal regulation of chondrocyte metabolism in agarose constructs subjected to dynamic compression. *Arch Biochem Biophys*. 417(1), 105-11.

Chu C.R., Coutts R.D., Yoshioka M., Harwood F.L., Monosov A.Z., and Amiel D. 1995. Articular cartilage repair using allogeneic perichondrocyte-seeded biodegradable porous polylactic acid (PLA): a tissue-engineering study. *J Biomed Mater Res*. 29(9), 1147-54.

Chung C., Erickson I.E., Mauck R.L., and Burdick J.A. 2008. Differential behavior of auricular and articular chondrocytes in hyaluronic acid hydrogels. *Tissue Eng Part A*. 14(7), 1121-31.

Chung Y.I., Ahn K.M., Jeon S.H., Lee S.Y., Lee J.H., and Tae G. 2007. Enhanced bone regeneration with BMP-2 loaded functional nanoparticle-hydrogel complex. *J Control Release*. 121(1-2), 91-9.

Clair B.L., Johnson A.R., and Howard T. 2009. Cartilage repair: current and emerging options in treatment. *Foot Ankle Spec.* 2(4), 179-88.

Clark R.A. 2003. Fibrin is a many splendored thing. *J Invest Dermatol.* 121(5), xxi-xxii.

Clarke H.D., and Scott W.N. 2003. The role of debridement: through small portals. *J Arthroplasty.* 18(3 Suppl 1), 10-3.

Colgrave M.L., Allingham P.G., and Jones A. 2008. Hydroxyproline quantification for the estimation of collagen in tissue using multiple reaction monitoring mass spectrometry. *J Chromatogr A.* 1212(1-2), 150-3.

Collen D. 2001. Ham-Wasserman lecture: role of the plasminogen system in fibrin-homeostasis and tissue remodeling. *Hematology Am Soc Hematol Educ Program*, 1-9.

Connelly J.T., Vanderploeg E.J., and Levenston M.E. 2004. The influence of cyclic tension amplitude on chondrocyte matrix synthesis: experimental and finite element analyses. *Biorheology.* 41(3-4), 377-87.

Cummings C.L., Gawlitta D., Nerem R.M., and Stegemann J.P. 2004. Properties of engineered vascular constructs made from collagen, fibrin, and collagen-fibrin mixtures. *Biomaterials.* 25(17), 3699-706.

Currie L.J., Sharpe J.R., and Martin R. 2001. The use of fibrin glue in skin grafts and tissue-engineered skin replacements: a review. *Plast Reconstr Surg.* 108(6), 1713-26.

Dare E.V., Griffith M., Poitras P., Kaupp J.A., Waldman S.D., Carlsson D.J., Dervin G., Mayoux C., and Hincke M.T. 2009a. Genipin cross-linked fibrin hydrogels for in vitro human articular cartilage tissue-engineered regeneration. *Cells Tissues Organs.* 190(6), 313-25.

Dare E.V., Griffith M., Poitras P., Wang T., Dervin G.F., Giulivi A., and Hincke M.T. 2009b. Fibrin sealants from fresh or fresh/frozen plasma as scaffolds for in vitro articular cartilage regeneration. *Tissue Eng Part A*. 15(8), 2285-97.

Dare E.V., Vascotto S.G., Carlsson D., Hincke M.T., and Griffith M. 2007. Differentiation of a fibrin gel encapsulated chondrogenic cell line. *Int J Artif Organs*. 30(7), 619-27.

David T.S., and Shields C.L. 2004. Radiofrequency and articular cartilage. *Tech Knee Surg*. 3(3), 193-7.

Day B. 2005. The indications for arthroscopic debridement for osteoarthritis of the knee. *Orthop Clin North Am*. 36(4), 413-7.

Dean D.D., Martel-Pelletier J., Pelletier J.P., Howell D.S., and Woessner J.F., Jr. 1989. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. *J Clin Invest*. 84(2), 678-85.

Demetriou A.A., Whiting J.F., Feldman D., Levenson S.M., Chowdhury N.R., Moscioni A.D., Kram M., and Chowdhury J.R. 1986. Replacement of liver function in rats by transplantation of microcarrier-attached hepatocytes. *Science*. 233(4769), 1190-2.

Dempfle C.E., and Heene D.L. 1987. Purification of human plasma fibrinogen by chromatography on protamine-agarose. *Thromb Res*. 46(1), 19-27.

Detterline A.J., Goldberg S., Bach B.R., Jr., and Cole B.J. 2005. Treatment options for articular cartilage defects of the knee. *Orthop Nurs*. 24(5), 361-6; quiz 367-8.

Di Martino A., Sittinger M., and Risbud M.V. 2005. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials*. 26(30), 5983-90.

Dietrich F., and Lelkes P.I. 2006. Fine-tuning of a three-dimensional microcarrier-based angiogenesis assay for the analysis of endothelial-mesenchymal cell co-cultures in fibrin and collagen gels. *Angiogenesis*. 9(3), 111-25.

Dikovsky D., Bianco-Peled H., and Seliktar D. 2006. The effect of structural alterations of PEG-fibrinogen hydrogel scaffolds on 3-D cellular morphology and cellular migration. *Biomaterials*. 27(8), 1496-506.

Donati I., Stredanska S., Silvestrini G., Vetere A., Marcon P., Marsich E., Mozetic P., Gamini A., Paoletti S., and Vittur F. 2005. The aggregation of pig articular chondrocyte and synthesis of extracellular matrix by a lactose-modified chitosan. *Biomaterials*. 26(9), 987-98.

Dorotka R., Bindreiter U., Macfelda K., Windberger U., and Nehrer S. 2005a. Marrow stimulation and chondrocyte transplantation using a collagen matrix for cartilage repair. *Osteoarthritis Cartilage*. 13(8), 655-64.

Dorotka R., Windberger U., Macfelda K., Bindreiter U., Toma C., and Nehrer S. 2005b. Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. *Biomaterials*. 26(17), 3617-29.

Dragoo J.L., Carlson G., McCormick F., Khan-Farooqi H., Zhu M., Zuk P.A., and Benhaim P. 2007. Healing full-thickness cartilage defects using adipose-derived stem cells. *Tissue Eng*. 13(7), 1615-21.

Dragoo J.L., Samimi B., Zhu M., Hame S.L., Thomas B.J., Lieberman J.R., Hedrick M.H., and Benhaim P. 2003. Tissue-engineered cartilage and bone using stem cells from human infrapatellar fat pads. *J Bone Joint Surg Br*. 85(5), 740-7.

Dresdale A., Rose E.A., Jeevanandam V., Reemtsma K., Bowman F.O., and Malm J.R. 1985. Preparation of fibrin glue from single-donor fresh-frozen plasma. *Surgery.* 97(6), 750-5.

Duchesne B., Tahi H., and Galand A. 2001. Use of human fibrin glue and amniotic membrane transplant in corneal perforation. *Cornea.* 20(2), 230-2.

Ebisawa K., Hata K., Okada K., Kimata K., Ueda M., Torii S., and Watanabe H. 2004. Ultrasound enhances transforming growth factor beta-mediated chondrocyte differentiation of human mesenchymal stem cells. *Tissue Eng.* 10(5-6), 921-9.

Eickelberg O., Centrella M., Reiss M., Kashgarian M., and Wells R.G. 2002. Betaglycan inhibits TGF-beta signaling by preventing type I-type II receptor complex formation. Glycosaminoglycan modifications alter betaglycan function. *J Biol Chem.* 277(1), 823-9.

Elisseeff J., McIntosh W., Anseth K., Riley S., Ragan P., and Langer R. 2000. Photoencapsulation of chondrocytes in poly(ethylene oxide)-based semi-interpenetrating networks. *J Biomed Mater Res.* 51(2), 164-71.

Erggelet C., Kreuz P.C., Mrosek E.H., Schagemann J.C., Lahm A., Ducommun P.P., and Ossendorf C. 2009. Autologous chondrocyte implantation versus ACI using 3D-bioresorbable graft for the treatment of large full-thickness cartilage lesions of the knee. *Arch Orthop Trauma Surg.* [Epub ahead of print]

Erzurum V.Z., Bian J.F., Husak V.A., Ellinger J., Xue L., Burgess W.H., and Greisler H.P. 2003. R136K fibroblast growth factor-1 mutant induces heparin-independent migration of endothelial cells through fibrin glue. *J Vasc Surg.* 37(5), 1075-81.

Evatt B., Austin H., Leon G., Ruiz-Saez A., and de Bosch N. 2000. Hemophilia treatment. Predicting the long-term risk of HIV exposure by cryoprecipitate. *Haemophilia.* 6 Suppl 1, 128-32.

Eyrich D., Brandl F., Appel B., Wiese H., Maier G., Wenzel M., Staudenmaier R., Goepferich A., and Blunk T. 2007a. Long-term stable fibrin gels for cartilage engineering. *Biomaterials.* 28(1), 55-65.

Eyrich D., Wiese H., Maier G., Skodacek D., Appel B., Sarhan H., Tessmar J., Staudenmaier R., Wenzel M.M., Goepferich A., and Blunk T. 2007b. In vitro and in vivo cartilage engineering using a combination of chondrocyte-seeded long-term stable fibrin gels and polycaprolactone-based polyurethane scaffolds. *Tissue Eng.* 13(9), 2207-18.

Falanga V., Iwamoto S., Chartier M., Yufit T., Butmarc J., Kouttab N., Shrayer D., and Carson P. 2007. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. *Tissue Eng.* 13(6), 1299-312.

Fan H., Liu H., Toh S.L., and Goh J.C. 2009. Anterior cruciate ligament regeneration using mesenchymal stem cells and silk scaffold in large animal model. *Biomaterials.* 30(28), 4967-77.

Ferruzzi A., Buda R., Faldini C., Vannini F., Di Caprio F., Luciani D., and Giannini S. 2008. Autologous chondrocyte implantation in the knee joint: open compared with arthroscopic technique. Comparison at a minimum follow-up of five years. *J Bone Joint Surg Am.* 90 Suppl 4, 90-101.

Ficat R.P., Ficat C., Gedeon P., and Toussaint J.B. 1979. Spongialization: a new treatment for diseased patellae. *Clin Orthop Relat Res*(144), 74-83.

Fithian D.C. 2001. A historical perspective of anterior knee pain. *Sports Medicine and Arthroscopy Review*. 9(4), 273-281.

Flanagan T.C., Cornelissen C., Koch S., Tschoeke B., Sachweh J.S., Schmitz-Rode T., and Jockenhoevel S. 2007. The in vitro development of autologous fibrin-based tissue-engineered heart valves through optimised dynamic conditioning. *Biomaterials*. 28(23), 3388-97.

Follmar K.E., Prichard H.L., DeCroos F.C., Wang H.T., Levin L.S., Klitzman B., Olbrich K.C., and Erdmann D. 2007. Combined bone allograft and adipose-derived stem cell autograft in a rabbit model. *Ann Plast Surg*. 58(5), 561-5.

Fortier L.A., Mohammed H.O., Lust G., and Nixon A.J. 2002. Insulin-like growth factor-I enhances cell-based repair of articular cartilage. *J Bone Joint Surg Br*. 84(2), 276-88.

Freyria A.M., Cortial D., Ronziere M.C., Guerret S., and Herbage D. 2004. Influence of medium composition, static and stirred conditions on the proliferation of and matrix protein expression of bovine articular chondrocytes cultured in a 3-D collagen scaffold. *Biomaterials*. 25(4), 687-97.

Freyria A.M., Ronziere M.C., Cortial D., Galois L., Hartmann D., Herbage D., and Mallein-Gerin F. 2009. Comparative phenotypic analysis of articular chondrocytes cultured within type I or type II collagen scaffolds. *Tissue Eng Part A*. 15(6), 1233-45.

Fu X., Lin L., Zhang J., and Yu C. 2009. Assessment of the efficacy of joint lavage in rabbits with osteoarthritis of the knee. *J Orthop Res*. 27(1), 91-6.

Fujisato T., Sajiki T., Liu Q., and Ikada Y. 1996. Effect of basic fibroblast growth factor on cartilage regeneration in chondrocyte-seeded collagen sponge scaffold. *Biomaterials*. 17(2), 155-62.

Fussenegger M., Meinhart J., Hobling W., Kullich W., Funk S., and Bernatzky G. 2003. Stabilized autologous fibrin-chondrocyte constructs for cartilage repair in vivo. *Ann Plast Surg*. 51(5), 493-8.

Gaissmaier C., Fritz J., Krackhardt T., Flesch I., Aicher W.K., and Ashammakhi N. 2005. Effect of human platelet supernatant on proliferation and matrix synthesis of human articular chondrocytes in monolayer and three-dimensional alginate cultures. *Biomaterials*. 26(14), 1953-60.

Galois L., Freyria A.M., Grossin L., Hubert P., Mainard D., Herbage D., Stoltz J.F., Netter P., Dellacherie E., and Payan E. 2004. Cartilage repair: surgical techniques and tissue engineering using polysaccharide- and collagen-based biomaterials. *Biorheology*. 41(3-4), 433-43.

Gao J., Dennis J.E., Solchaga L.A., Goldberg V.M., and Caplan A.I. 2002. Repair of osteochondral defect with tissue-engineered two-phase composite material of injectable calcium phosphate and hyaluronan sponge. *Tissue Eng*. 8(5), 827-37.

Gassling V.L., Acil Y., Springer I.N., Hubert N., and Wiltfang J. 2009. Platelet-rich plasma and platelet-rich fibrin in human cell culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 108(1), 48-55.

Geer D.J., Swartz D.D., and Andreadis S.T. 2002. Fibrin promotes migration in a three-dimensional in vitro model of wound regeneration. *Tissue Eng*. 8(5), 787-98.

Gelse K., von der Mark K., Aigner T., Park J., and Schneider H. 2003. Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. *Arthritis Rheum.* 48(2), 430-41.

Gerard C., Catuogno C., Amargier-Huin C., Grossin L., Hubert P., Gillet P., Netter P., Dellacherie E., and Payan E. 2005. The effect of alginate, hyaluronate and hyaluronate derivatives biomaterials on synthesis of non-articular chondrocyte extracellular matrix. *J Mater Sci Mater Med.* 16(6), 541-51.

Gijbels K., Galardy R.E., and Steinman L. 1994. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. *J Clin Invest.* 94(6), 2177-82.

Gingras D., Page M., Annabi B., and Beliveau R. 2000. Rapid activation of matrix metalloproteinase-2 by glioma cells occurs through a posttranslational MT1-MMP-dependent mechanism. *Biochim Biophys Acta.* 1497(3), 341-50.

Glowacki J., and Mizuno S. 2008. Collagen scaffolds for tissue engineering. *Biopolymers.* 89(5), 338-44.

Gong Y., Zhou Q., Gao C., and Shen J. 2007. In vitro and in vivo degradability and cytocompatibility of poly(l-lactic acid) scaffold fabricated by a gelatin particle leaching method. *Acta Biomater.* 3(4), 531-40.

Gonzalez Della Valle A., Leali A., and Haas S. 2007. Etiology and surgical interventions for stiff total knee replacements. *Hss J.* 3(2), 182-9.

Goodstone N.J., Cartwright A., and Ashton B. 2004. Effects of high molecular weight hyaluronan on chondrocytes cultured within a resorbable gelatin sponge. *Tissue Eng.* 10(3-4), 621-31.

Goomer R.S., Deftos L.J., Terkeltaub R., Maris T., Lee M.C., Harwood F.L., and Amiel D. 2001. High-efficiency non-viral transfection of primary chondrocytes and perichondrial cells for ex-vivo gene therapy to repair articular cartilage defects. *Osteoarthritis Cartilage*. 9(3), 248-56.

Gorodetsky R., Clark R.A., An J., Gailit J., Levdansky L., Vexler A., Berman E., and Marx G. 1999. Fibrin microbeads (FMB) as biodegradable carriers for culturing cells and for accelerating wound healing. *J Invest Dermatol*. 112(6), 866-72.

Grande D.A., Mason J., Light E., and Dines D. 2003. Stem cells as platforms for delivery of genes to enhance cartilage repair. *J Bone Joint Surg Am*. 85-A Suppl 2, 111-6.

Grant I., Warwick K., Marshall J., Green C., and Martin R. 2002. The co-application of sprayed cultured autologous keratinocytes and autologous fibrin sealant in a porcine wound model. *Br J Plast Surg*. 55(3), 219-27.

Greisler H.P., Gosselin C., Ren D., Kang S.S., and Kim D.U. 1996. Biointeractive polymers and tissue engineered blood vessels. *Biomaterials*. 17(3), 329-36.

Grelsamer R.P., and Stein D.A. 2006. Patellofemoral arthritis. *J Bone Joint Surg Am*. 88(8), 1849-60.

Grigolo B., Roseti L., Fiorini M., Fini M., Giavaresi G., Aldini N.N., Giardino R., and Facchini A. 2001. Transplantation of chondrocytes seeded on a hyaluronan derivative (hyaff-11) into cartilage defects in rabbits. *Biomaterials*. 22(17), 2417-24.

Grigoriadis A.E., Heersche J.N., and Aubin J.E. 1996. Analysis of chondroprogenitor frequency and cartilage differentiation in a novel family of clonal chondrogenic rat cell lines. *Differentiation*. 60(5), 299-307.

Groger A., Klaring S., Merten H.A., Holste J., Kaps C., and Sittinger M. 2003. Tissue engineering of bone for mandibular augmentation in immunocompetent minipigs: preliminary study. *Scand J Plast Reconstr Surg Hand Surg.* 37(3), 129-33.

Gruber H.E., Leslie K., Ingram J., Norton H.J., and Hanley E.N. 2004. Cell-based tissue engineering for the intervertebral disc: in vitro studies of human disc cell gene expression and matrix production within selected cell carriers. *Spine J.* 4(1), 44-55.

Grunder T., Gaissmaier C., Fritz J., Stoop R., Hortschansky P., Mollenhauer J., and Aicher W.K. 2004. Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. *Osteoarthritis Cartilage.* 12(7), 559-67.

Gudas R., Stankevicius E., Monastyreckiene E., Pranys D., and Kalesinskas R.J. 2006. Osteochondral autologous transplantation versus microfracture for the treatment of articular cartilage defects in the knee joint in athletes. *Knee Surg Sports Traumatol Arthrosc.* 14(9), 834-42.

Guo X., Zheng Q., Yang S., Shao Z., Yuan Q., Pan Z., Tang S., Liu K., and Quan D. 2006. Repair of full-thickness articular cartilage defects by cultured mesenchymal stem cells transfected with the transforming growth factor beta1 gene. *Biomed Mater.* 1(4), 206-15.

Gurevich O., Vexler A., Marx G., Prigozhina T., Levdansky L., Slavin S., Shimeliovich I., and Gorodetsky R. 2002. Fibrin microbeads for isolating and growing bone marrow-derived progenitor cells capable of forming bone tissue. *Tissue Eng.* 8(4), 661-72.

Hafemann B., Hettich R., Ensslen S., Kowol B., Zuhlke A., Ebert R., Konigs M., and Kirkpatrick C.J. 1994. Treatment of skin defects using suspensions of in vitro cultured keratinocytes. *Burns*. 20(2), 168-72.

Han B., Schwab I.R., Madsen T.K., and Isseroff R.R. 2002. A fibrin-based bioengineered ocular surface with human corneal epithelial stem cells. *Cornea*. 21(5), 505-10.

Han D., Liu W., Ao Q., and Wang G. 2008a. Optimal delivery systems for bone morphogenetic proteins in orthopedic applications should model initial tissue repair structures by using a heparin-incorporated fibrin-fibronectin matrix. *Med Hypotheses*. 71(3), 374-8.

Han S.H., Kim Y.H., Park M.S., Kim I.A., Shin J.W., Yang W.I., Jee K.S., Park K.D., Ryu G.H., and Lee J.W. 2008b. Histological and biomechanical properties of regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold in vivo. *J Biomed Mater Res A*. 87(4), 850-61.

Handl M., Trc T., Hanus M., Stastny E., Fricova-Poulova M., Neuwirth J., Adler J., Havranova D., and Varga F. 2006. [Therapy of severe chondral defects of the patella by autologous chondrocyte implantation]. *Acta Chir Orthop Traumatol Cech*. 73(6), 373-9.

Hangody L., Vasarhelyi G., Hangody L.R., Sukosd Z., Tibay G., Bartha L., and Bodo G. 2008. Autologous osteochondral grafting--technique and long-term results. *Injury*. 39 Suppl 1, S32-9.

Hankemeier S., van Griensven M., Ezechieli M., Barkhausen T., Austin M., Jagodzinski M., Meller R., Bosch U., Krettek C., and Zeichen J. 2007. Tissue engineering of tendons and ligaments by human bone marrow stromal cells in a liquid fibrin matrix

in immunodeficient rats: results of a histologic study. *Arch Orthop Trauma Surg.* 127(9), 815-21.

Hao J., Yao Y., Varshney R.R., Wang L., Prakash C., Li H., and Wang D.A. 2008. Gene transfer and living release of transforming growth factor-beta3 for cartilage tissue engineering applications. *Tissue Eng Part C Methods.* 14(4), 273-80.

Hao J.L., Nagano T., Nakamura M., Kumagai N., Mishima H., and Nishida T. 1999. Galardin inhibits collagen degradation by rabbit keratocytes by inhibiting the activation of pro-matrix metalloproteinases. *Exp Eye Res.* 68(5), 565-72.

Hecker L., Baar K., Dennis R.G., and Bitar K.N. 2005. Development of a three-dimensional physiological model of the internal anal sphincter bioengineered in vitro from isolated smooth muscle cells. *Am J Physiol Gastrointest Liver Physiol.* 289(2), G188-96.

Hempfling H. 2007. Intra-articular hyaluronic acid after knee arthroscopy: a two-year study. *Knee Surg Sports Traumatol Arthrosc.* 15(5), 537-46.

Hennig T., Lorenz H., Thiel A., Goetzke K., Dickhut A., Geiger F., and Richter W. 2007. Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. *J Cell Physiol.* 211(3), 682-91.

Henning C.E., Lynch M.A., Yearout K.M., Vequist S.W., Stallbaumer R.J., and Decker K.A. 1990. Arthroscopic meniscal repair using an exogenous fibrin clot. *Clin Orthop Relat Res*(252), 64-72.

Herbert C.B., Bittner G.D., and Hubbell J.A. 1996. Effects of fibinolysis on neurite growth from dorsal root ganglia cultured in two- and three-dimensional fibrin gels. *J Comp Neurol.* 365(3), 380-91.

Hettrich C.M., Crawford D., and Rodeo S.A. 2008. Cartilage repair: third-generation cell-based technologies--basic science, surgical techniques, clinical outcomes. *Sports Med Arthrosc.* 16(4), 230-5.

Heymer A., Bradica G., Eulert J., and Noth U. 2009. Multiphasic collagen fibre-PLA composites seeded with human mesenchymal stem cells for osteochondral defect repair: an in vitro study. *J Tissue Eng Regen Med.* 3(5), 389-97.

Heywood H.K., Bader D.L., and Lee D.A. 2006. Glucose concentration and medium volume influence cell viability and glycosaminoglycan synthesis in chondrocyte-seeded alginate constructs. *Tissue Eng.* 12(12), 3487-96.

Heywood H.K., Sembi P.K., Lee D.A., and Bader D.L. 2004. Cellular utilization determines viability and matrix distribution profiles in chondrocyte-seeded alginate constructs. *Tissue Eng.* 10(9-10), 1467-79.

Higa K., Shimmura S., Kato N., Kawakita T., Miyashita H., Itabashi Y., Fukuda K., Shimazaki J., and Tsubota K. 2007. Proliferation and differentiation of transplantable rabbit epithelial sheets engineered with or without an amniotic membrane carrier. *Invest Ophthalmol Vis Sci.* 48(2), 597-604.

Hiller O., Lichte A., Oberpichler A., Kocourek A., and Tschesche H. 2000. Matrix metalloproteinases collagenase-2, macrophage elastase, collagenase-3, and membrane type 1-matrix metalloproteinase impair clotting by degradation of fibrinogen and factor XII. *J Biol Chem.* 275(42), 33008-13.

Hiraoka N., Allen E., Apel I.J., Gyetko M.R., and Weiss S.J. 1998. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell.* 95(3), 365-77.

Hoemann C.D., Sun J., Legare A., McKee M.D., and Buschmann M.D. 2005. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. *Osteoarthritis Cartilage*. 13(4), 318-29.

Hojo M., Inokuchi S., Kidokoro M., Fukuyama N., Tanaka E., Tsuji C., Miyasaka M., Tanino R., and Nakazawa H. 2003. Induction of vascular endothelial growth factor by fibrin as a dermal substrate for cultured skin substitute. *Plast Reconstr Surg.* 111(5), 1638-45.

Holland T.A., Bodde E.W., Baggett L.S., Tabata Y., Mikos A.G., and Jansen J.A. 2005. Osteochondral repair in the rabbit model utilizing bilayered, degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds. *J Biomed Mater Res A.* 75(1), 156-67.

Horan J.T., and Francis C.W. 2001. Fibrin degradation products, fibrin monomer and soluble fibrin in disseminated intravascular coagulation. *Semin Thromb Hemost.* 27(6), 657-66.

Horch R.E., Kopp J., Kneser U., Beier J., and Bach A.D. 2005. Tissue engineering of cultured skin substitutes. *J Cell Mol Med.* 9(3), 592-608.

Hoshikawa A., Nakayama Y., Matsuda T., Oda H., Nakamura K., and Mabuchi K. 2006. Encapsulation of chondrocytes in photopolymerizable styrenated gelatin for cartilage tissue engineering. *Tissue Eng.* 12(8), 2333-41.

Hsu S.H., Chang S.H., Yen H.J., Whu S.W., Tsai C.L., and Chen D.C. 2006. Evaluation of biodegradable polyesters modified by type II collagen and Arg-Gly-Asp as tissue engineering scaffolding materials for cartilage regeneration. *Artif Organs.* 30(1), 42-55.

Hsu S.H., Whu S.W., Hsieh S.C., Tsai C.L., Chen D.C., and Tan T.S. 2004. Evaluation of chitosan-alginate-hyaluronate complexes modified by an RGD-containing protein as tissue-engineering scaffolds for cartilage regeneration. *Artif Organs*. 28(8), 693-703.

Huang Y.C., Dennis R.G., Larkin L., and Baar K. 2005. Rapid formation of functional muscle in vitro using fibrin gels. *J Appl Physiol*. 98(2), 706-13.

Hunter C.J., Imler S.M., Malaviya P., Nerem R.M., and Levenston M.E. 2002. Mechanical compression alters gene expression and extracellular matrix synthesis by chondrocytes cultured in collagen I gels. *Biomaterials*. 23(4), 1249-59.

Hunter C.J., Mouw J.K., and Levenston M.E. 2004. Dynamic compression of chondrocyte-seeded fibrin gels: effects on matrix accumulation and mechanical stiffness. *Osteoarthritis Cartilage*. 12(2), 117-30.

Hunter N., and Houston F. 2002. Can prion diseases be transmitted between individuals via blood transfusion: evidence from sheep experiments. *Dev Biol (Basel)*. 108, 93-8.

Hunziker E.B. 2002. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage*. 10(6), 432-63.

Hunziker E.B., and Quinn T.M. 2003. Surgical removal of articular cartilage leads to loss of chondrocytes from cartilage bordering the wound edge. *J Bone Joint Surg Am*. 85-A Suppl 2, 85-92.

Ibusuki S., Fujii Y., Iwamoto Y., and Matsuda T. 2003. Tissue-engineered cartilage using an injectable and in situ gelable thermoresponsive gelatin: fabrication and in vitro performance. *Tissue Eng*. 9(2), 371-84.

Ibusuki S., Iwamoto Y., and Matsuda T. 2003. System-engineered cartilage using poly(N-isopropylacrylamide)-grafted gelatin as in situ-formable scaffold: in vivo performance. *Tissue Eng.* 9(6), 1133-42.

Ichinose S., Tagami M., Muneta T., and Sekiya I. 2005. Morphological examination during in vitro cartilage formation by human mesenchymal stem cells. *Cell Tissue Res.* 322(2), 217-26.

Ifkovits J.L., and Burdick J.A. 2007. Review: photopolymerizable and degradable biomaterials for tissue engineering applications. *Tissue Eng.* 13(10), 2369-85.

Im G.I., Jung N.H., and Tae S.K. 2006. Chondrogenic differentiation of mesenchymal stem cells isolated from patients in late adulthood: the optimal conditions of growth factors. *Tissue Eng.* 12(3), 527-36.

Im G.I., Shin Y.W., and Lee K.B. 2005. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthritis Cartilage.* 13(10), 845-53.

Indrawattana N., Chen G., Tadokoro M., Shann L.H., Ohgushi H., Tateishi T., Tanaka J., and Bunyaratvej A. 2004. Growth factor combination for chondrogenic induction from human mesenchymal stem cell. *Biochem Biophys Res Commun.* 320(3), 914-9.

Isogai N., Kusuhara H., Ikada Y., Ohtani H., Jacquet R., Hillyer J., Lowder E., and Landis W.J. 2006. Comparison of different chondrocytes for use in tissue engineering of cartilage model structures. *Tissue Eng.* 12(4), 691-703.

Ito K., Yamada Y., Naiki T., and Ueda M. 2006. Simultaneous implant placement and bone regeneration around dental implants using tissue-engineered bone with fibrin glue, mesenchymal stem cells and platelet-rich plasma. *Clin Oral Implants Res.* 17(5), 579-86.

Iwasa J., Engebretsen L., Shima Y., and Ochi M. 2009. Clinical application of scaffolds for cartilage tissue engineering. *Knee Surg Sports Traumatol Arthrosc.* 17(6), 561-77.

Iwasaki N., Yamane S.T., Majima T., Kasahara Y., Minami A., Harada K., Nonaka S., Maekawa N., Tamura H., Tokura S., Shiono M., Monde K., and Nishimura S. 2004. Feasibility of polysaccharide hybrid materials for scaffolds in cartilage tissue engineering: evaluation of chondrocyte adhesion to polyion complex fibers prepared from alginate and chitosan. *Biomacromolecules.* 5(3), 828-33.

Iwaya K., Mizoi K., Tessler A., and Itoh Y. 1999. Neurotrophic agents in fibrin glue mediate adult dorsal root regeneration into spinal cord. *Neurosurgery.* 44(3), 589-95; discussion 595-6.

Izuta Y., Ochi M., Adachi N., Deie M., Yamasaki T., and Shinomiya R. 2005. Meniscal repair using bone marrow-derived mesenchymal stem cells: experimental study using green fluorescent protein transgenic rats. *Knee.* 12(3), 217-23.

Jackson M.R. 2001. Fibrin sealants in surgical practice: An overview. *Am J Surg.* 182(2 Suppl), 1S-7S.

Jackson R.W., and Dieterichs C. 2003. The results of arthroscopic lavage and debridement of osteoarthritic knees based on the severity of degeneration: a 4- to 6-year symptomatic follow-up. *Arthroscopy.* 19(1), 13-20.

Jian-Wei X., Randolph M.A., Peretti G.M., Nazzal J.A., Roses R.E., Morse K.R., and Yaremchuk M.J. 2005. Producing a flexible tissue-engineered cartilage framework using expanded polytetrafluoroethylene membrane as a pseudoperichondrium. *Plast Reconstr Surg.* 116(2), 577-89.

Jin'gen B., Jian Y., Dunwan Z., Junjie L., and Kangde Y. 2006. The multiple interactions between growth factors and microenvironment in vivo. *Chinese Sci Bull.* 51(7), 761-9.

Jobanputra P., Parry D., Fry-Smith A., and Burls A. 2001. Effectiveness of autologous chondrocyte transplantation for hyaline cartilage defects in knees: a rapid and systematic review. *Health Technol Assess.* 5(11), 1-57.

Jockenhoevel S., Chalabi K., Sachweh J.S., Groesdonk H.V., Demircan L., Grossmann M., Zund G., and Messmer B.J. 2001a. Tissue engineering: complete autologous valve conduit--a new moulding technique. *Thorac Cardiovasc Surg.* 49(5), 287-90.

Jockenhoevel S., Zund G., Hoerstrup S.P., Chalabi K., Sachweh J.S., Demircan L., Messmer B.J., and Turina M. 2001b. Fibrin gel -- advantages of a new scaffold in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 19(4), 424-30.

Johnsen S., Ermuth T., Tanczos E., Bannasch H., Horch R.E., Zschocke I., Peschen M., Schopf E., Vanscheidt W., and Augustin M. 2005. Treatment of therapy-refractive ulcera cruris of various origins with autologous keratinocytes in fibrin sealant. *Vasa.* 34(1), 25-9.

Johnson L.L. 1986. Arthroscopic abrasion arthroplasty historical and pathologic perspective: present status. *Arthroscopy.* 2(1), 54-69.

Johnson L.L. 2001. Arthroscopic abrasion arthroplasty: a review. *Clin Orthop Relat Res*(391 Suppl), S306-17.

Johnson T.S., Xu J.W., Zaporojan V.V., Mesa J.M., Weinand C., Randolph M.A., Bonassar L.J., Winograd J.M., and Yaremchuk M.J. 2004. Integrative repair of cartilage with articular and nonarticular chondrocytes. *Tissue Eng.* 10(9-10), 1308-15.

Jubel A., Andermahr J., Schiffer G., Fischer J., Rehm K.E., Stoddart M.J., and Häuselmann H.J. 2008. Transplantation of de novo scaffold-free cartilage implants into sheep knee chondral defects. *Am J Sports Med.* 36(8):1555-64.

Jung R.E., Schmoekel H.G., Zwahlen R., Kokovic V., Hammerle C.H., and Weber F.E. 2005. Platelet-rich plasma and fibrin as delivery systems for recombinant human bone morphogenetic protein-2. *Clin Oral Implants Res.* 16(6), 676-82.

Kafienah W., Al-Fayez F., Hollander A.P., and Barker M.D. 2003. Inhibition of cartilage degradation: a combined tissue engineering and gene therapy approach. *Arthritis Rheum.* 48(3), 709-18.

Kafienah W., Mistry S., Dickinson S.C., Sims T.J., Learmonth I., and Hollander A.P. 2007a. Three-dimensional cartilage tissue engineering using adult stem cells from osteoarthritis patients. *Arthritis Rheum.* 56(1), 177-87.

Kafienah W., Mistry S., Perry M.J., Politopoulou G., and Hollander A.P. 2007b. Pharmacological regulation of adult stem cells: chondrogenesis can be induced using a synthetic inhibitor of the retinoic acid receptor. *Stem Cells.* 25(10), 2460-8.

Kaiser H.W., Stark G.B., Kopp J., Balcerkiewicz A., Spilker G., and Kreysel H.W. 1994. Cultured autologous keratinocytes in fibrin glue suspension, exclusively and combined with STS-allograft (preliminary clinical and histological report of a new technique). *Burns.* 20(1), 23-9.

Kanemaru S., Nakamura T., Omori K., Magrufov A., Yamashita M., and Ito J. 2005. Regeneration of mastoid air cells in clinical applications by in situ tissue engineering. *Laryngoscope.* 115(2), 253-8.

Kang H.M., Kalnoski M.H., Frederick M., and Chandler W.L. 2005. The kinetics of plasmin inhibition by aprotinin in vivo. *Thromb Res.* 115(4), 327-40.

Kang S.W., Jeon O., and Kim B.S. 2005. Poly(lactic-co-glycolic acid) microspheres as an injectable scaffold for cartilage tissue engineering. *Tissue Eng.* 11(3-4), 438-47.

Kang S.W., Yoon J.R., Lee J.S., Kim H.J., Lim H.W., Lim H.C., Park J.H., and Kim B.S. 2006. The use of poly(lactic-co-glycolic acid) microspheres as injectable cell carriers for cartilage regeneration in rabbit knees. *J Biomater Sci Polym Ed.* 17(8), 925-39.

Kaps C., Bramlage C., Smolian H., Haisch A., Ungethum U., Burmester G.R., Sittinger M., Gross G., and Haupl T. 2002. Bone morphogenetic proteins promote cartilage differentiation and protect engineered artificial cartilage from fibroblast invasion and destruction. *Arthritis Rheum.* 46(1), 149-62.

Kassis I., Zangi L., Rivkin R., Levdansky L., Samuel S., Marx G., and Gorodetsky R. 2006. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. *Bone Marrow Transplant.* 37(10), 967-76.

Kasza A., Kiss D.L., Gopalan S., Xu W., Rydel R.E., Koj A., and Kordula T. 2002. Mechanism of plasminogen activator inhibitor-1 regulation by oncostatin M and interleukin-1 in human astrocytes. *J Neurochem.* 83(3), 696-703.

Katakai D., Imura M., Ando W., Tateishi K., Yoshikawa H., Nakamura N., and Fujie H. 2009. Compressive properties of cartilage-like tissues repaired in vivo with scaffold-free, tissue engineered constructs. *Clin Biomech (Bristol, Avon).* 24(1), 110-6.

Kawamura M., Sawafuji M., Watanabe M., Horinouchi H., and Kobayashi K. 2002. Frequency of transmission of human parvovirus B19 infection by fibrin sealant used during thoracic surgery. *Ann Thorac Surg.* 73(4), 1098-100.

Kayakabe M., Tsutsumi S., Watanabe H., Kato Y., and Takagishi K. 2006. Transplantation of autologous rabbit BM-derived mesenchymal stromal cells embedded in hyaluronic acid gel sponge into osteochondral defects of the knee. *Cytotherapy.* 8(4), 343-53.

Kerker J.T., Leo A.J., and Sgaglione N.A. 2008. Cartilage repair: synthetics and scaffolds: basic science, surgical techniques, and clinical outcomes. *Sports Med Arthrosc.* 16(4), 208-16.

Kessler M.W., Ackerman G., Dines J.S., and Grande D. 2008. Emerging technologies and fourth generation issues in cartilage repair. *Sports Med Arthrosc.* 16(4), 246-54.

Kessler M.W., and Grande D.A. 2008. Tissue engineering and cartilage. *Organogenesis.* 4(1), 28-32.

Khor H.L., Ng K.W., Htay A.S., Schantz J.T., Teoh S.H., and Hutmacher D.W. 2003. Preliminary study of a polycaprolactone membrane utilized as epidermal substrate. *J Mater Sci Mater Med.* 14(2), 113-20.

Kim H.J., and Im G.I. 2009. Combination of transforming growth factor-beta2 and bone morphogenetic protein 7 enhances chondrogenesis from adipose tissue-derived mesenchymal stem cells. *Tissue Eng Part A.* 15(7), 1543-51.

Kim H.K., Moran M.E., and Salter R.B. 1991. The potential for regeneration of articular cartilage in defects created by chondral shaving and subchondral abrasion. An experimental investigation in rabbits. *J Bone Joint Surg Am.* 73(9), 1301-15.

Kim H.T., Zaffagnini S., Mizuno S., Abelow S., and Safran M.R. 2006. A peek into the possible future of management of articular cartilage injuries: gene therapy and scaffolds for cartilage repair. *J Orthop Sports Phys Ther.* 36(10), 765-73.

Kim H.W., and Han C.D. 2000. An overview of cartilage tissue engineering. *Yonsei Med J.* 41(6), 766-73.

Kim Y.J., Sah R.L., Doong J.Y., and Grodzinsky A.J. 1988. Fluorometric assay of DNA in cartilage explants using Hoechst 33258. *Anal Biochem.* 174(1), 168-76.

Kisiday J., Jin M., Kurz B., Hung H., Semino C., Zhang S., and Grodzinsky A.J. 2002. Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. *Proc Natl Acad Sci U S A.* 99(15), 9996-10001.

Ko C.S., Huang J.P., Huang C.W., and Chu I.M. 2009. Type II collagen-chondroitin sulfate-hyaluronan scaffold cross-linked by genipin for cartilage tissue engineering. *J Biosci Bioeng.* 107(2), 177-82.

Kocheta A., and Toms A. 2004. Isolated chondral injuries of the knee (diagnosis and treatment). *Current Orthopaedics.* 18(2), 154-163.

Kofidis T., Lenz A., Boublik J., Akhyari P., Wachsmann B., Mueller-Stahl K., Hofmann M., and Haverich A. 2003. Pulsatile perfusion and cardiomyocyte viability in a solid three-dimensional matrix. *Biomaterials.* 24(27), 5009-14.

Kon E., Delcogliano M., Filardo G., Montaperto C., and Marcacci M. 2008. Second generation issues in cartilage repair. *Sports Med Arthrosc.* 16(4), 221-9.

Kopp J., Jeschke M.G., Bach A.D., Kneser U., and Horch R.E. 2004. Applied tissue engineering in the closure of severe burns and chronic wounds using cultured human autologous keratinocytes in a natural fibrin matrix. *Cell Tissue Bank.* 5(2), 89-96.

Koster A., Sanger S., Knorig F.J., Kuppe H., Hetzer R., and Loebe M. 2002. Autologous plasma and platelet sequestration at the beginning of cardiopulmonary bypass: a

pilot investigation in five patients undergoing extended vascular surgery in deep hypothermia. *Asaio J.* 48(1), 106-9.

Kreuz P.C., Muller S., Ossendorf C., Kaps C., and Erggelet C. 2009. Treatment of focal degenerative cartilage defects with polymer-based autologous chondrocyte grafts: four-year clinical results. *Arthritis Res Ther.* 11(2), R33.

Kuo C.K., Li W.J., Mauck R.L., and Tuan R.S. 2006. Cartilage tissue engineering: its potential and uses. *Curr Opin Rheumatol.* 18(1), 64-73.

Kuo S.M., Wang Y.J., Weng C.L., Lu H.E., and Chang S.J. 2005. Influence of alginate on type II collagen fibrillogenesis. *J Mater Sci Mater Med.* 16(6), 525-31.

Kuo Y.C., and Lin C.Y. 2006. Effect of genipin-crosslinked chitin-chitosan scaffolds with hydroxyapatite modifications on the cultivation of bovine knee chondrocytes. *Biotechnol Bioeng.* 95(1), 132-44.

Kurth T., Hedbom E., Shintani N., Sugimoto M., Chen F.H., Haspl M., Martinovic S., and Hunziker E.B. 2007. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. *Osteoarthritis Cartilage.* 15(10), 1178-89.

Laemmli U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227(5259), 680-5.

Lahiji A., Sohrabi A., Hungerford D.S., and Frondoza C.G. 2000. Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. *J Biomed Mater Res.* 51(4), 586-95.

Lantz M.S., and Ciborowski P. 1994. Zymographic techniques for detection and characterization of microbial proteases. *Methods Enzymol.* 235, 563-94.

Lanza R.P., Jackson R., Sullivan A., Ringeling J., McGrath C., Kuhtreiber W., and Chick W.L. 1999. Xenotransplantation of cells using biodegradable microcapsules. *Transplantation*. 67(8), 1105-11.

Laupattarakasem W., Laopaiboon M., Laupattarakasem P., and Sumananont C. 2008. Arthroscopic debridement for knee osteoarthritis. *Cochrane Database Syst Rev*(1), CD005118.

Lavik E., and Langer R. 2004. Tissue engineering: current state and perspectives. *Appl Microbiol Biotechnol*. 65(1), 1-8.

Le Blanc K., and Ringdén O. 2007. Immunomodulation by mesenchymal stem cells and clinical experience. *J Intern Med*. 262(5), 509-25.

Le Nihouannen D., Guehennec L.L., Rouillon T., Pilet P., Bilban M., Layrolle P., and Daculsi G. 2006. Micro-architecture of calcium phosphate granules and fibrin glue composites for bone tissue engineering. *Biomaterials*. 27(13), 2716-22.

Lechner S., and Huss R. 2006. Bone engineering: combining smart biomaterials and the application of stem cells. *Artif Organs*. 30(10), 770-4.

Lee A.C., Yu V.M., Lowe J.B., 3rd, Brenner M.J., Hunter D.A., Mackinnon S.E., and Sakiyama-Elbert S.E. 2003a. Controlled release of nerve growth factor enhances sciatic nerve regeneration. *Exp Neurol*. 184(1), 295-303.

Lee C.R., Grad S., Gorna K., Gogolewski S., Goessl A., and Alini M. 2005a. Fibrin-polyurethane composites for articular cartilage tissue engineering: a preliminary analysis. *Tissue Eng*. 11(9-10), 1562-73.

Lee C.R., Grodzinsky A.J., Hsu H.P., and Spector M. 2003b. Effects of a cultured autologous chondrocyte-seeded type II collagen scaffold on the healing of a chondral defect in a canine model. *J Orthop Res*. 21(2), 272-81.

Lee C.R., Grodzinsky A.J., and Spector M. 2003. Biosynthetic response of passaged chondrocytes in a type II collagen scaffold to mechanical compression. *J Biomed Mater Res A.* 64(3), 560-9.

Lee D.A., Reisler T., and Bader D.L. 2003. Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared to conventional monolayer techniques. *Acta Orthop Scand.* 74(1), 6-15.

Lee H.J., Choi B.H., Min B.H., and Park S.R. 2007. Low-intensity ultrasound inhibits apoptosis and enhances viability of human mesenchymal stem cells in three-dimensional alginate culture during chondrogenic differentiation. *Tissue Eng.* 13(5), 1049-57.

Lee H.J., Choi B.H., Min B.H., Son Y.S., and Park S.R. 2006. Low-intensity ultrasound stimulation enhances chondrogenic differentiation in alginate culture of mesenchymal stem cells. *Artif Organs.* 30(9), 707-15.

Lee J.E., Kim K.E., Kwon I.C., Ahn H.J., Lee S.H., Cho H., Kim H.J., Seong S.C., and Lee M.C. 2004. Effects of the controlled-released TGF-beta 1 from chitosan microspheres on chondrocytes cultured in a collagen/chitosan/glycosaminoglycan scaffold. *Biomaterials.* 25(18), 4163-73.

Lee K.Y., Jeong L., Kang Y.O., Lee S.J., and Park W.H. 2009. Electrospinning of polysaccharides for regenerative medicine. *Adv Drug Deliv Rev.* 61(12), 1020-32.

Lee O.K., Coathup M.J., Goodship A.E., and Blunn G.W. 2005b. Use of mesenchymal stem cells to facilitate bone regeneration in normal and chemotherapy-treated rats. *Tissue Eng.* 11(11-12), 1727-35.

Leo A.J., and Grande D.A. 2006. Mesenchymal stem cells in tissue engineering. *Cells Tissues Organs.* 183(3), 112-22.

Lev R., and Spicer S.S. 1964. Specific Staining of Sulphate Groups with Alcian Blue at Low Ph. *J Histochem Cytochem.* 12, 309.

Li X., Shah A., Franklin P., Merolli R., Bradley J., and Busconi B. 2008. Arthroscopic debridement of the osteoarthritic knee combined with hyaluronic acid (Orthovisc(R)) treatment: A case series and review of the literature. *J Orthop Surg.* 3, 43.

Li Z., and Zhang M. 2005. Chitosan-alginate as scaffolding material for cartilage tissue engineering. *J Biomed Mater Res A.* 75(2), 485-93.

Lien S.M., Ko L.Y., and Huang T.J. 2009. Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering. *Acta Biomater.* 5(2), 670-9.

Lind M., Larsen A., Clausen C., Osther K., and Everland H. 2008. Cartilage repair with chondrocytes in fibrin hydrogel and MPEG polylactide scaffold: an in vivo study in goats. *Knee Surg Sports Traumatol Arthrosc.* 16(7), 690-8.

Lindenhayn K., Perka C., Spitzer R., Heilmann H., Pommerening K., Mennicke J., and Sittinger M. 1999. Retention of hyaluronic acid in alginate beads: aspects for in vitro cartilage engineering. *J Biomed Mater Res.* 44(2), 149-55.

Liu J.Y., Swartz D.D., Peng H.F., Gugino S.F., Russell J.A., and Andreadis S.T. 2007. Functional tissue-engineered blood vessels from bone marrow progenitor cells. *Cardiovasc Res.* 75(3), 618-28.

Liu Z.J., Zhuge Y., and Velazquez O.C. 2009. Trafficking and differentiation of mesenchymal stem cells. *J Cell Biochem.* 106(6), 984-91.

Long J.L., and Tranquillo R.T. 2003. Elastic fiber production in cardiovascular tissue-equivalents. *Matrix Biol.* 22(4), 339-50.

Lu J.M., Wang X., Marin-Muller C., Wang H., Lin P.H., Yao Q., and Chen C. 2009. Current advances in research and clinical applications of PLGA-based nanotechnology. *Expert Rev Mol Diagn.* 9(4), 325-41.

Lu L., Zhu X., Pederson L.G., Jabbari E., Currier B., O'Driscoll S., and Yaszemski M. 2005. Effects of dynamic fluid pressure on chondrocytes cultured in biodegradable poly(glycolic acid) fibrous scaffolds. *Tissue Eng.* 11(11-12), 1852-9.

Lutzner J., Kasten P., Gunther K.P., and Kirschner S. 2009. Surgical options for patients with osteoarthritis of the knee. *Nat Rev Rheumatol.* 5(6), 309-16.

Lyon M., Rushton G., and Gallagher J.T. 1997. The interaction of the transforming growth factor-betas with heparin/heparan sulfate is isoform-specific. *J Biol Chem.* 272(29), 18000-6.

Madry H., Padera R., Seidel J., Langer R., Freed L.E., Trippel S.B., and Vunjak-Novakovic G. 2002. Gene transfer of a human insulin-like growth factor I cDNA enhances tissue engineering of cartilage. *Hum Gene Ther.* 13(13), 1621-30.

Mahomed N.N., Barrett J., Katz J.N., Baron J.A., Wright J., and Losina E. 2005. Epidemiology of total knee replacement in the United States Medicare population. *J Bone Joint Surg Am.* 87(6), 1222-8.

Majka S., McGuire P., Colombo S., and Das A. 2001. The balance between proteinases and inhibitors in a murine model of proliferative retinopathy. *Invest Ophthalmol Vis Sci.* 42(1), 210-5.

Malda J., Woodfield T.B., van der Vloodt F., Wilson C., Martens D.E., Tramper J., van Blitterswijk C.A., and Riesle J. 2005. The effect of PEGT/PBT scaffold architecture on the composition of tissue engineered cartilage. *Biomaterials.* 26(1), 63-72.

Malicev E., Radosavljevic D., and Velikonja N.K. 2007. Fibrin gel improved the spatial uniformity and phenotype of human chondrocytes seeded on collagen scaffolds. *Biotechnol Bioeng.* 96(2), 364-70.

Marcacci M., Berruto M., Brocchetta D., Delcogliano A., Ghinelli D., Gobbi A., Kon E., Pederzini L., Rosa D., Sacchetti G.L., Stefani G., and Zanasi S. 2005. Articular cartilage engineering with Hyalograft C: 3-year clinical results. *Clin Orthop Relat Res*(435), 96-105.

Marcacci M., Kon E., Delcogliano M., Filardo G., Busacca M., and Zaffagnini S. 2007. Arthroscopic autologous osteochondral grafting for cartilage defects of the knee: prospective study results at a minimum 7-year follow-up. *Am J Sports Med.* 35(12), 2014-21.

Marlovits S., Striessnig G., Kutscha-Lissberg F., Resinger C., Aldrian S.M., Vecsei V., and Trattnig S. 2005. Early postoperative adherence of matrix-induced autologous chondrocyte implantation for the treatment of full-thickness cartilage defects of the femoral condyle. *Knee Surg Sports Traumatol Arthrosc.* 13(6), 451-7.

Martel-Pelletier J., Zafarullah M., Kodama S., and Pelletier J.P. 1991. In vitro effects of interleukin 1 on the synthesis of metalloproteases, TIMP, plasminogen activators and inhibitors in human articular cartilage. *J Rheumatol Suppl.* 27, 80-4.

Marx G. 2003. Evolution of fibrin glue applicators. *Transfus Med Rev.* 17(4), 287-98.

Marx G., and Mou X. 2002. Characterizing fibrin glue performance as modulated by heparin, aprotinin, and factor XIII. *J Lab Clin Med.* 140(3), 152-60.

Marx G., Mou X., Hotovely-Salomon A., Levdansky L., Gaberman E., Belenky D., and Gorodetsky R. 2008. Heat denaturation of fibrinogen to develop a biomedical matrix. *J Biomed Mater Res B Appl Biomater.* 84(1), 49-57.

Massova I., Kotra L.P., Fridman R., and Mobashery S. 1998. Matrix metalloproteinases: structures, evolution, and diversification. *Faseb J.* 12(12), 1075-95.

Masuda K., Pfister B.E., Sah R.L., and Thonar E.J. 2006. Osteogenic protein-1 promotes the formation of tissue-engineered cartilage using the alginate-recovered-chondrocyte method. *Osteoarthritis Cartilage.* 14(4), 384-91.

Matthias F.R., Hocke G., and Lasch H.G. 1975. Isolation of fibrinogen from plasma by affinity chromatography on insolubilized fibrinogen (FG-ag) and insolubilized fibrinmonomer (FM-ag). *Thromb Res.* 7(6), 861-70.

Mauck R.L., Seyhan S.L., Ateshian G.A., and Hung C.T. 2002. Influence of seeding density and dynamic deformational loading on the developing structure/function relationships of chondrocyte-seeded agarose hydrogels. *Ann Biomed Eng.* 30(8), 1046-56.

Mauck R.L., Soltz M.A., Wang C.C., Wong D.D., Chao P.H., Valhmu W.B., Hung C.T., and Ateshian G.A. 2000. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J Biomech Eng.* 122(3), 252-60.

McCormick F., Yanke A., Provencher M.T., and Cole B.J. 2008. Minced articular cartilage--basic science, surgical technique, and clinical application. *Sports Med Arthrosc.* 16(4), 217-20.

McLaren A.C., Blokker C.P., Fowler P.J., Roth J.N., and Rock M.G. 1991. Arthroscopic debridement of the knee for osteoarthrosis. *Can J Surg.* 34(6), 595-8.

McMahon L.A., Reid A.J., Campbell V.A., and Prendergast P.J. 2008. Regulatory effects of mechanical strain on the chondrogenic differentiation of MSCs in a collagen-

GAG scaffold: experimental and computational analysis. *Ann Biomed Eng.* 36(2), 185-94.

McNickle A.G., L'Heureux D.R., Yanke A.B., and Cole B.J. 2009. Outcomes of autologous chondrocyte implantation in a diverse patient population. *Am J Sports Med.* 37(7), 1344-50.

McNickle A.G., Provencher M.T., and Cole B.J. 2008. Overview of existing cartilage repair technology. *Sports Med Arthrosc.* 16(4), 196-201.

Meek R.M., Masri B.A., and Duncan C.P. 2004. Minimally invasive unicompartmental knee replacement: rationale and correct indications. *Orthop Clin North Am.* 35(2), 191-200.

Mehlhorn A.T., Niemeyer P., Kaiser S., Finkenzeller G., Stark G.B., Sudkamp N.P., and Schmal H. 2006. Differential expression pattern of extracellular matrix molecules during chondrogenesis of mesenchymal stem cells from bone marrow and adipose tissue. *Tissue Eng.* 12(10), 2853-62.

Mehrotra C., Remington P.L., Naimi T.S., Washington W., and Miller R. 2005. Trends in total knee replacement surgeries and implications for public health, 1990-2000. *Public Health Rep.* 120(3), 278-82.

Meinhart J., Fussenegger M., and Hobling W. 1999. Stabilization of fibrin-chondrocyte constructs for cartilage reconstruction. *Ann Plast Surg.* 42(6), 673-8.

Mercier N.R., Costantino H.R., Tracy M.A., and Bonassar L.J. 2005. Poly(lactide-co-glycolide) microspheres as a moldable scaffold for cartilage tissue engineering. *Biomaterials.* 26(14), 1945-52.

Mesa J.M., Zaporojan V., Weinand C., Johnson T.S., Bonassar L., Randolph M.A., Yaremchuk M.J., and Butler P.E. 2006. Tissue engineering cartilage with aged articular chondrocytes in vivo. *Plast Reconstr Surg.* 118(1), 41-9; discussion 50-3.

Miller T.T. 2005. Imaging of knee arthroplasty. *Eur J Radiol.* 54(2), 164-77.

Mina C., Garrett W.E., Jr., Pietrobon R., Glisson R., and Higgins L. 2008. High tibial osteotomy for unloading osteochondral defects in the medial compartment of the knee. *Am J Sports Med.* 36(5), 949-55.

Mironov V., Boland T., Trusk T., Forgacs G., and Markwald R.R. 2003. Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* 21(4), 157-61.

Mitchell N., and Shepard N. 1976. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone Joint Surg Am.* 58(2), 230-3.

Miyanishi K., Trindade M.C., Lindsey D.P., Beaupre G.S., Carter D.R., Goodman S.B., Schurman D.J., and Smith R.L. 2006. Effects of hydrostatic pressure and transforming growth factor-beta 3 on adult human mesenchymal stem cell chondrogenesis in vitro. *Tissue Eng.* 12(6), 1419-28.

Mohri H., and Ohkubo T. 1993. Fibrinogen binds to heparin: the relationship of the binding of other adhesive proteins to heparin. *Arch Biochem Biophys.* 303(1), 27-31.

Mol A., van Lieshout M.I., Dam-de Veen C.G., Neuenschwander S., Hoerstrup S.P., Baaijens F.P., and Bouten C.V. 2005. Fibrin as a cell carrier in cardiovascular tissue engineering applications. *Biomaterials.* 26(16), 3113-21.

Morag G., Kulidjian A., Zalzal P., Shasha N., Gross A.E., and Backstein D. 2006. Total knee replacement in previous recipients of fresh osteochondral allograft transplants. *J Bone Joint Surg Am.* 88(3), 541-6.

Morgan H., and Hill P.A. 2005. Human breast cancer cell-mediated bone collagen degradation requires plasminogen activation and matrix metalloproteinase activity. *Cancer Cell Int.* 5(1), 1.

Mosesson M.W. 2005. Fibrinogen and fibrin structure and functions. *J Thromb Haemost.* 3(8), 1894-904.

Mosesson M.W., Siebenlist K.R., and Meh D.A. 2001. The structure and biological features of fibrinogen and fibrin. *Ann N Y Acad Sci.* 936, 11-30.

Muhs B.E., Gagne P., Plitas G., Shaw J.P., and Shamamian P. 2004. Experimental hindlimb ischemia leads to neutrophil-mediated increases in gastrocnemius MMP-2 and -9 activity: a potential mechanism for ischemia induced MMP activation. *J Surg Res.* 117(2), 249-54.

Mulhall K.J., Ghomrawi H.M., Scully S., Callaghan J.J., and Saleh K.J. 2006. Current etiologies and modes of failure in total knee arthroplasty revision. *Clin Orthop Relat Res.* 446, 45-50.

Munirah S., Kim S.H., Ruszymah B.H., and Khang G. 2008. The use of fibrin and poly(lactic-co-glycolic acid) hybrid scaffold for articular cartilage tissue engineering: an in vivo analysis. *Eur Cell Mater.* 15, 41-52.

Nakamura H., Matsuyama Y., Yoshihara H., Sakai Y., Katayama Y., Nakashima S., Takamatsu J., and Ishiguro N. 2005. The effect of autologous fibrin tissue adhesive on postoperative cerebrospinal fluid leak in spinal cord surgery: a randomized controlled trial. *Spine (Phila Pa 1976).* 30(13), E347-51.

Nakamura K., Koshino T., and Saito T. 1998. Osteogenic response of the rabbit femur to a hydroxyapatite thermal decomposition product-fibrin glue mixture. *Biomaterials.* 19(20), 1901-7.

Nakamura Y., Masuda M., Toshima Y., Asou T., Oe M., Kinoshita K., Kawachi Y., Tanaka J., and Tokunaga K. 1990. Comparative study of cell saver and ultrafiltration nontransfusion in cardiac surgery. *Ann Thorac Surg.* 49(6), 973-8.

Nehrer S., Domayer S., Dorotka R., Schatz K., Bindreiter U., and Kotz R. 2006. Three-year clinical outcome after chondrocyte transplantation using a hyaluronan matrix for cartilage repair. *Eur J Radiol.* 57(1), 3-8.

Nehrer S., and Minas T. 2000. Treatment of articular cartilage defects. *Invest Radiol.* 35(10), 639-46.

Nehrer S., Spector M., and Minas T. 1999. Histologic analysis of tissue after failed cartilage repair procedures. *Clin Orthop Relat Res*(365), 149-62.

Neovius E.B., and Kratz G. 2003. Tissue engineering by cocultivating human elastic chondrocytes and keratinocytes. *Tissue Eng.* 9(2), 365-9.

Nettles D.L., Vail T.P., Morgan M.T., Grinstaff M.W., and Setton L.A. 2004. Photocrosslinkable hyaluronan as a scaffold for articular cartilage repair. *Ann Biomed Eng.* 32(3), 391-7.

Neuman A.R., Weinberg A., Benmeir P., Eldad A., and Wexler M.R. 1991. Spray application of fibrin glue by a simple device. *Plast Reconstr Surg.* 88(5), 922-3.

Neumeister M.W., Wu T., and Chambers C. 2006. Vascularized tissue-engineered ears. *Plast Reconstr Surg.* 117(1), 116-22.

Ng K.W., Saliman J.D., Lin E.Y., Statman L.Y., Kugler L.E., Lo S.B., Ateshian G.A., and Hung C.T. 2007. Culture duration modulates collagen hydrolysate-induced tissue remodeling in chondrocyte-seeded agarose hydrogels. *Ann Biomed Eng.* 35(11), 1914-23.

Ng K.W., Wang C.C., Mauck R.L., Kelly T.A., Chahine N.O., Costa K.D., Ateshian G.A., and Hung C.T. 2005. A layered agarose approach to fabricate depth-dependent inhomogeneity in chondrocyte-seeded constructs. *J Orthop Res.* 23(1), 134-41.

Nguyen K.T., and West J.L. 2002. Photopolymerizable hydrogels for tissue engineering applications. *Biomaterials.* 23(22), 4307-14.

Niederauer G.G., Slivka M.A., Leatherbury N.C., Korvick D.L., Harroff H.H., Ehler W.C., Dunn C.J., and Kieswetter K. 2000. Evaluation of multiphase implants for repair of focal osteochondral defects in goats. *Biomaterials.* 21(24), 2561-74.

Nieponice A., Maul T.M., Cumer J.M., Soletti L., and Vorp D.A. 2007. Mechanical stimulation induces morphological and phenotypic changes in bone marrow-derived progenitor cells within a three-dimensional fibrin matrix. *J Biomed Mater Res A.* 81(3), 523-30.

Nishida K. 2003. Tissue engineering of the cornea. *Cornea.* 22(7 Suppl), S28-34.

O'Neill T., Jinks C., and Ong B.N. 2007. Decision-making regarding total knee replacement surgery: a qualitative meta-synthesis. *BMC Health Serv Res.* 7, 52.

Oliver J.J., Webb D.J., and Newby D.E. 2005. Stimulated tissue plasminogen activator release as a marker of endothelial function in humans. *Arterioscler Thromb Vasc Biol.* 25(12), 2470-9.

Oshima Y., Watanabe N., Matsuda K., Takai S., Kawata M., and Kubo T. 2004. Fate of transplanted bone-marrow-derived mesenchymal cells during osteochondral repair using transgenic rats to simulate autologous transplantation. *Osteoarthritis Cartilage.* 12(10), 811-7.

Oshima Y., Watanabe N., Matsuda K., Takai S., Kawata M., and Kubo T. 2005. Behavior of transplanted bone marrow-derived GFP mesenchymal cells in osteochondral

defect as a simulation of autologous transplantation. *J Histochem Cytochem.* 53(2), 207-16.

Palmer G.D., Steinert A., Pascher A., Gouze E., Gouze J.N., Betz O., Johnstone B., Evans C.H., and Ghivizzani S.C. 2005. Gene-induced chondrogenesis of primary mesenchymal stem cells in vitro. *Mol Ther.* 12(2), 219-28.

Park G.E., Pattison M.A., Park K., and Webster T.J. 2005a. Accelerated chondrocyte functions on NaOH-treated PLGA scaffolds. *Biomaterials.* 26(16), 3075-82.

Park S.H., Park S.R., Chung S.I., Pai K.S., and Min B.H. 2005b. Tissue-engineered cartilage using fibrin/hyaluronan composite gel and its in vivo implantation. *Artif Organs.* 29(10), 838-45.

Park Y., Sugimoto M., Watrin A., Chiquet M., and Hunziker E.B. 2005c. BMP-2 induces the expression of chondrocyte-specific genes in bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. *Osteoarthritis Cartilage.* 13(6), 527-36.

Passaretti D., Silverman R.P., Huang W., Kirchhoff C.H., Ashiku S., Randolph M.A., and Yaremchuk M.J. 2001. Cultured chondrocytes produce injectable tissue-engineered cartilage in hydrogel polymer. *Tissue Eng.* 7(6), 805-15.

Patrick C.W., Jr., Zheng B., Johnston C., and Reece G.P. 2002. Long-term implantation of preadipocyte-seeded PLGA scaffolds. *Tissue Eng.* 8(2), 283-93.

Pavone V., Boettner F., Fickert S., and Sculco T.P. 2001. Total condylar knee arthroplasty: a long-term followup. *Clin Orthop Relat Res*(388), 18-25.

Peled E., Boss J., Bejar J., Zinman C., and Seliktar D. 2007. A novel poly(ethylene glycol)-fibrinogen hydrogel for tibial segmental defect repair in a rat model. *J Biomed Mater Res A.* 80(4), 874-84.

Peretti G.M., Campo-Ruiz V., Gonzalez S., Randolph M.A., Wei Xu J., Morse K.R., Roses R.E., and Yaremchuk M.J. 2006a. Tissue engineered cartilage integration to live and devitalized cartilage: a study by reflectance mode confocal microscopy and standard histology. *Connect Tissue Res.* 47(4), 190-9.

Peretti G.M., Xu J.W., Bonassar L.J., Kirchhoff C.H., Yaremchuk M.J., and Randolph M.A. 2006b. Review of injectable cartilage engineering using fibrin gel in mice and swine models. *Tissue Eng.* 12(5), 1151-68.

Perka C., Schultz O., Lindenhayn K., Spitzer R.S., Muschik M., Sittinger M., and Burmester G.R. 2000a. Joint cartilage repair with transplantation of embryonic chondrocytes embedded in collagen-fibrin matrices. *Clin Exp Rheumatol.* 18(1), 13-22.

Perka C., Schultz O., Spitzer R.S., and Lindenhayn K. 2000b. The influence of transforming growth factor beta1 on mesenchymal cell repair of full-thickness cartilage defects. *J Biomed Mater Res.* 52(3), 543-52.

Perka C., Spitzer R.S., Lindenhayn K., Sittinger M., and Schultz O. 2000c. Matrix-mixed culture: new methodology for chondrocyte culture and preparation of cartilage transplants. *J Biomed Mater Res.* 49(3), 305-11.

Peterson L., Minas T., Brittberg M., Nilsson A., Sjogren-Jansson E., and Lindahl A. 2000. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*(374), 212-34.

Pieper J.S., van der Kraan P.M., Hafmans T., Kamp J., Buma P., van Susante J.L., van den Berg W.B., Veerkamp J.H., and van Kuppevelt T.H. 2002. Crosslinked type II collagen matrices: preparation, characterization, and potential for cartilage engineering. *Biomaterials.* 23(15), 3183-92.

Ponticiello M.S., Schinagl R.M., Kadiyala S., and Barry F.P. 2000. Gelatin-based resorbable sponge as a carrier matrix for human mesenchymal stem cells in cartilage regeneration therapy. *J Biomed Mater Res.* 52(2), 246-55.

Provenzano P.P., Hurschler C., and Vanderby R., Jr. 2001. Microstructural morphology in the transition region between scar and intact residual segments of a healing rat medial collateral ligament. *Connect Tissue Res.* 42(2), 123-33.

Pulliainen O., Vasara A.I., Hyttinen M.M., Tiitu V., Valonen P., Kellomaki M., Jurvelin J.S., Peterson L., Lindahl A., Kiviranta I., and Lammi M.J. 2007. Poly-L-D-lactic acid scaffold in the repair of porcine knee cartilage lesions. *Tissue Eng.* 13(6), 1347-55.

Quinn T.M., Maung A.A., Grodzinsky A.J., Hunziker E.B., and Sandy J.D. 1999. Physical and biological regulation of proteoglycan turnover around chondrocytes in cartilage explants. Implications for tissue degradation and repair. *Ann N Y Acad Sci.* 878, 420-41.

Quinn T.M., Schmid P., Hunziker E.B., and Grodzinsky A.J. 2002. Proteoglycan deposition around chondrocytes in agarose culture: construction of a physical and biological interface for mechanotransduction in cartilage. *Biorheology.* 39(1-2), 27-37.

Radice M., Brun P., Cortivo R., Scapinelli R., Battaliard C., and Abatangelo G. 2000. Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors. *J Biomed Mater Res.* 50(2), 101-9.

Rama P., Bonini S., Lambiase A., Golisano O., Paterna P., De Luca M., and Pellegrini G. 2001. Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency. *Transplantation.* 72(9), 1478-85.

Ramaswamy S., Wang D.A., Fishbein K.W., Elisseeff J.H., and Spencer R.G. 2006. An analysis of the integration between articular cartilage and nondegradable hydrogel using magnetic resonance imaging. *J Biomed Mater Res B Appl Biomater.* 77(1), 144-8.

Ramont L., Pasco S., Hornebeck W., Maquart F.X., and Monboisse J.C. 2003. Transforming growth factor-beta1 inhibits tumor growth in a mouse melanoma model by down-regulating the plasminogen activation system. *Exp Cell Res.* 291(1), 1-10.

Ratel D., Glazier G., Provencal M., Boivin D., Beaulieu E., Gingras D., and Beliveau R. 2005. Direct-acting fibrinolytic enzymes in shark cartilage extract: potential therapeutic role in vascular disorders. *Thromb Res.* 115(1-2), 143-52.

Recht M., White L.M., Winalski C.S., Miniaci A., Minas T., and Parker R.D. 2003. MR imaging of cartilage repair procedures. *Skeletal Radiol.* 32(4), 185-200.

Redwan el R.M. 2009. Animal-derived pharmaceutical proteins. *J Immunoassay Immunochem.* 30(3), 262-90.

Rees J.L., Price A.J., Lynskey T.G., Svard U.C., Dodd C.A., and Murray D.W. 2001. Medial unicompartmental arthroplasty after failed high tibial osteotomy. *J Bone Joint Surg Br.* 83(7), 1034-6.

Richmond J.C. 2008. Surgery for osteoarthritis of the knee. *Rheum Dis Clin North Am.* 34(3), 815-25.

Rider C.C. 2006. Heparin/heparan sulphate binding in the TGF-beta cytokine superfamily. *Biochem Soc Trans.* 34(Pt 3), 458-60.

Ringe J., Kaps C., Burmester G.R., and Sittinger M. 2002. Stem cells for regenerative medicine: advances in the engineering of tissues and organs. *Naturwissenschaften.* 89(8), 338-51.

Risbud M. 2001. Tissue engineering: implications in the treatment of organ and tissue defects. *Biogerontology.* 2(2), 117-25.

Risbud M.V., and Sittinger M. 2002. Tissue engineering: advances in in vitro cartilage generation. *Trends Biotechnol.* 20(8), 351-6.

Rivkin R., Ben-Ari A., Kassis I., Zangi L., Gaberman E., Levdansky L., Marx G., and Gorodetsky R. 2007. High-yield isolation, expansion, and differentiation of murine bone marrow-derived mesenchymal stem cells using fibrin microbeads (FMB). *Cloning Stem Cells.* 9(2), 157-75.

Rock G., Berger R., Lange J., Tokessy M., Palmer D.S., and Giulivi A. 2001. A novel, automated method of temperature cycling to produce cryoprecipitate. *Transfusion.* 41(2), 232-5.

Ronga M., Grassi F.A., and Bulgheroni P. 2004. Arthroscopic autologous chondrocyte implantation for the treatment of a chondral defect in the tibial plateau of the knee. *Arthroscopy.* 20(1), 79-84.

Rorabeck C.H., Bourne R.B., and Nott L. 1988. The cemented kinematic-II and the non-cemented porous-coated anatomic prostheses for total knee replacement. A prospective evaluation. *J Bone Joint Surg Am.* 70(4), 483-90.

Rosneck J., Higuera C.A., Tadross N., Krebs V., and Barsoum W.K. 2007. Managing knee osteoarthritis before and after arthroplasty. *Cleve Clin J Med.* 74(9), 663-71.

Rosowski M., Falb M., Tschirschmann M., and Lauster R. 2006. Initiation of mesenchymal condensation in alginate hollow spheres--a useful model for understanding cartilage repair? *Artif Organs*. 30(10), 775-84.

Rosso F., Marino G., Giordano A., Barbarisi M., Parmeggiani D., and Barbarisi A. 2005. Smart materials as scaffolds for tissue engineering. *J Cell Physiol*. 203(3), 465-70.

Roth E.A., Xu T., Das M., Gregory C., Hickman J.J., and Boland T. 2004. Inkjet printing for high-throughput cell patterning. *Biomaterials*. 25(17), 3707-15.

Rowe S.L., Lee S., and Stegemann J.P. 2007. Influence of thrombin concentration on the mechanical and morphological properties of cell-seeded fibrin hydrogels. *Acta Biomater*. 3(1), 59-67.

Rudert M. 2002. Histological evaluation of osteochondral defects: consideration of animal models with emphasis on the rabbit, experimental setup, follow-up and applied methods. *Cells Tissues Organs*. 171(4), 229-40.

Ruszymah B.H., Lokman B.S., Asma A., Munirah S., Chua K., Mazlyzam A.L., Isa M.R., Fuzina N.H., and Aminuddin B.S. 2007. Pediatric auricular chondrocytes gene expression analysis in monolayer culture and engineered elastic cartilage. *Int J Pediatr Otorhinolaryngol*. 71(8), 1225-34.

Sabatini M., Lesur C., Thomas M., Chomel A., Anract P., de Nanteuil G., and Pastoureau P. 2005. Effect of inhibition of matrix metalloproteinases on cartilage loss in vitro and in a guinea pig model of osteoarthritis. *Arthritis Rheum*. 52(1), 171-80.

Sakiyama-Elbert S.E., and Hubbell J.A. 2000a. Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. *J Control Release*. 69(1), 149-58.

Sakiyama-Elbert S.E., and Hubbell J.A. 2000b. Development of fibrin derivatives for controlled release of heparin-binding growth factors. *J Control Release*. 65(3), 389-402.

Sakiyama S.E., Schense J.C., and Hubbell J.A. 1999. Incorporation of heparin-binding peptides into fibrin gels enhances neurite extension: an example of designer matrices in tissue engineering. *Faseb J.* 13(15), 2214-24.

Sanjana N.E., and Fuller S.B. 2004. A fast flexible ink-jet printing method for patterning dissociated neurons in culture. *J Neurosci Methods*. 136(2), 151-63.

Saraf A., and Mikos A.G. 2006. Gene delivery strategies for cartilage tissue engineering. *Adv Drug Deliv Rev*. 58(4), 592-603.

Sato M., Ishihara M., Ishihara M., Kaneshiro N., Mitani G., Nagai T., Kutsuna T., Asazuma T., Kikuchi M., and Mochida J. 2007. Effects of growth factors on heparin-carrying polystyrene-coated atelocollagen scaffold for articular cartilage tissue engineering. *J Biomed Mater Res B Appl Biomater*. 83(1), 181-8.

Savaiano J.K., and Webster T.J. 2004. Altered responses of chondrocytes to nanophase PLGA/nanophase titania composites. *Biomaterials*. 25(7-8), 1205-13.

Schense J.C., Bloch J., Aebischer P., and Hubbell J.A. 2000. Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. *Nat Biotechnol*. 18(4), 415-9.

Schense J.C., and Hubbell J.A. 1999. Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjug Chem*. 10(1), 75-81.

Schmitt B., Ringe J., Haupl T., Notter M., Manz R., Burmester G.R., Sittinger M., and Kaps C. 2003. BMP2 initiates chondrogenic lineage development of adult human mesenchymal stem cells in high-density culture. *Differentiation*. 71(9-10), 567-77.

Schoen F.J. 1999. Future directions in tissue heart valves: impact of recent insights from biology and pathology. *J Heart Valve Dis.* 8(4), 350-8.

Schulz R.M., Zscharnack M., Hanisch I., Geiling M., Hepp P., and Bader A. 2008. Cartilage tissue engineering by collagen matrix associated bone marrow derived mesenchymal stem cells. *Biomed Mater Eng.* 18(1 Suppl), S55-70.

Sekiya I., Larson B.L., Vuoristo J.T., Reger R.L., and Prockop D.J. 2005. Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. *Cell Tissue Res.* 320(2), 269-76.

Sha'ban M., Kim S.H., Idrus R.B., and Khang G. 2008. Fibrin and poly(lactic-co-glycolic acid) hybrid scaffold promotes early chondrogenesis of articular chondrocytes: an in vitro study. *J Orthop Surg.* 3, 17.

Sharkey P.F., Hozack W.J., Rothman R.H., Shastri S., and Jacoby S.M. 2002. Insall Award paper. Why are total knee arthroplasties failing today? *Clin Orthop Relat Res*(404), 7-13.

Shepherd D.E., and Seedhom B.B. 1997. A technique for measuring the compressive modulus of articular cartilage under physiological loading rates with preliminary results. Proc Inst Mech Eng H. 211(2), 155-65.

Shields K.J., Beckman M.J., Bowlin G.L., and Wayne J.S. 2004. Mechanical properties and cellular proliferation of electrospun collagen type II. *Tissue Eng.* 10(9-10), 1510-7.

Shimony N., Gorodetsky R., Marx G., Gal D., Rivkin R., Ben-Ari A., Landsman A., and Haviv Y.S. 2006. Fibrin microbeads (FMB) as a 3D platform for kidney gene and cell therapy. *Kidney Int.* 69(3), 625-33.

Shin H.J., Lee C.H., Cho I.H., Kim Y.J., Lee Y.J., Kim I.A., Park K.D., Yui N., and Shin J.W. 2006. Electrospun PLGA nanofiber scaffolds for articular cartilage

reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation in vitro. *J Biomater Sci Polym Ed.* 17(1-2), 103-19.

Shireman P.K., Hampton B., Burgess W.H., and Greisler H.P. 1999. Modulation of vascular cell growth kinetics by local cytokine delivery from fibrin glue suspensions. *J Vasc Surg.* 29(5), 852-61; discussion 862.

Siedentop K.H., O'Grady K., Bhattacharyya T.K., and Shah A. 2004. Fibrin tissue adhesive and autologous concha cartilage for reconstruction of the posterior-superior canal wall of the chinchilla middle ear. *Otol Neurotol.* 25(3), 220-2.

Siedler S., and Schuller-Petrovic S. 2002. Wound healing enhancement in leg ulcers: A case report. *Cell Tissue Bank.* 3(1), 25-8.

Silver F.H., Wang M.C., and Pins G.D. 1995. Preparation of fibrin glue: a study of chemical and physical methods. *J Appl Biomater.* 6(3), 175-83.

Silverman R.P., Passaretti D., Huang W., Randolph M.A., and Yaremchuk M.J. 1999. Injectable tissue-engineered cartilage using a fibrin glue polymer. *Plast Reconstr Surg.* 103(7), 1809-18.

Simon B.I., Zatcoff A.L., Kong J.J., and O'Connell S.M. 2009. Clinical and Histological Comparison of Extraction Socket Healing Following the Use of Autologous Platelet-Rich Fibrin Matrix (PRFM) to Ridge Preservation Procedures Employing Demineralized Freeze Dried Bone Allograft Material and Membrane. *Open Dent J.* 3, 92-99.

Sims C.D., Butler P.E., Cao Y.L., Casanova R., Randolph M.A., Black A., Vacanti C.A., and Yaremchuk M.J. 1998. Tissue engineered neocartilage using plasma derived polymer substrates and chondrocytes. *Plast Reconstr Surg.* 101(6), 1580-5.

Siparsky P., Ryzewicz M., Peterson B., and Bartz R. 2007. Arthroscopic treatment of osteoarthritis of the knee: are there any evidence-based indications? *Clin Orthop Relat Res.* 455, 107-12.

Sirieix D., Chemla E., Castier Y., Massonnet-Castel S., Fabiani J.N., and Baron J.F. 1998. Comparative study of different biological glues in an experimental model of surgical bleeding in anesthetized rats: platelet-rich and -poor plasma-based glue with and without aprotinin versus commercial fibrinogen-based glue. *Ann Vasc Surg.* 12(4), 311-6.

Smith J.D., Chen A., Ernst L.A., Waggoner A.S., and Campbell P.G. 2007. Immobilization of aprotinin to fibrinogen as a novel method for controlling degradation of fibrin gels. *Bioconjug Chem.* 18(3), 695-701.

Smith R.C., Spalding T., and Masri B.A. 2009. Trufit polymer scaffolds for the management of cartilage defects of the knee. *Tech Knee Surg.* 8(2), 136-41.

Solchaga L.A., Gao J., Dennis J.E., Awadallah A., Lundberg M., Caplan A.I., and Goldberg V.M. 2002. Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. *Tissue Eng.* 8(2), 333-47.

Song L., Baksh D., and Tuan R.S. 2004. Mesenchymal stem cell-based cartilage tissue engineering: cells, scaffold and biology. *Cytotherapy.* 6(6), 596-601.

Song S.U., Cha Y.D., Han J.U., Oh I.S., Choi K.B., Yi Y., Hyun J.P., Lee H.Y., Chi G.F., Lim C.L., Ganjei J.K., Noh M.J., Kim S.J., Lee D.K., and Lee K.H. 2005. Hyaline cartilage regeneration using mixed human chondrocytes and transforming growth factor-beta1- producing chondrocytes. *Tissue Eng.* 11(9-10), 1516-26.

Soon M.Y., Hassan A., Hui J.H., Goh J.C., and Lee E.H. 2007. An analysis of soft tissue allograft anterior cruciate ligament reconstruction in a rabbit model: a short-term

study of the use of mesenchymal stem cells to enhance tendon osteointegration. *Am J Sports Med.* 35(6), 962-71.

Spahn G., Frober R., and Linss W. 2006. Treatment of chondral defects by hydro jet. Results of a preliminary scanning electron microscopic evaluation. *Arch Orthop Trauma Surg.* 126(4), 223-7.

Spotnitz W.D., Mintz P.D., Avery N., Bithell T.C., Kaul S., and Nolan S.P. 1987. Fibrin glue from stored human plasma. An inexpensive and efficient method for local blood bank preparation. *Am Surg.* 53(8), 460-2.

Spotnitz W.D., and Prabhu R. 2005. Fibrin sealant tissue adhesive--review and update. *J Long Term Eff Med Implants.* 15(3), 245-70.

Stammen J.A., Williams S., Ku D.N., and Guldberg R.E. 2001. Mechanical properties of a novel PVA hydrogel in shear and unconfined compression. *Biomaterials.* 22(8), 799-806.

Stark Y., Suck K., Kasper C., Wieland M., van Griensven M., and Scheper T. 2006. Application of collagen matrices for cartilage tissue engineering. *Exp Toxicol Pathol.* 57(4), 305-11.

Steinert A.F., Noth U., and Tuan R.S. 2008. Concepts in gene therapy for cartilage repair. *Injury.* 39 Suppl 1, S97-113.

Steinwachs M.R., Guggi T., and Kreuz P.C. 2008. Marrow stimulation techniques. *Injury.* 39 Suppl 1, S26-31.

Stevens M.M., Qanadilo H.F., Langer R., and Prasad Shastri V. 2004. A rapid-curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. *Biomaterials.* 25(5), 887-94.

Stevenson L.W., Warner S.L., Steimle A.E., Fonarow G.C., Hamilton M.A., Moriguchi J.D., Kobashigawa J.A., Tillisch J.H., Drinkwater D.C., and Laks H. 1994. The impending crisis awaiting cardiac transplantation. Modeling a solution based on selection. *Circulation*. 89(1), 450-7.

Stuart M.J., and Lubowitz J.H. 2006. What, if any, are the indications for arthroscopic debridement of the osteoarthritic knee? *Arthroscopy*. 22(3), 238-9.

Sun T., Chan M.L., Quek C.H., and Yu H. 2004. Improving mechanical stability and density distribution of hepatocyte microcapsules by fibrin clot and gold nano-particles. *J Biotechnol*. 111(2), 169-77.

Sun X.D., Jeng L., Bolliet C., Olsen B.R., and Spector M. 2009. Non-viral endostatin plasmid transfection of mesenchymal stem cells via collagen scaffolds. *Biomaterials*. 30(6), 1222-31.

Suuronen E.J., Muzakare L., Doillon C.J., Kapila V., Li F., Ruel M., and Griffith M. 2006. Promotion of angiogenesis in tissue engineering: developing multicellular matrices with multiple capacities. *Int J Artif Organs*. 29(12), 1148-57.

Swartz D.D., Russell J.A., and Andreadis S.T. 2005. Engineering of fibrin-based functional and implantable small-diameter blood vessels. *Am J Physiol Heart Circ Physiol*. 288(3), H1451-60.

Swenson T.M. 2007. The use of exogenous fibrin clot to supplement meniscal surgery techniques. *Orthopedics*. 30(9), 718-23.

Taguchi T., Xu L., Kobayashi H., Taniguchi A., Kataoka K., and Tanaka J. 2005. Encapsulation of chondrocytes in injectable alkali-treated collagen gels prepared using poly(ethylene glycol)-based 4-armed star polymer. *Biomaterials*. 26(11), 1247-52.

Takagi M., Umetsu Y., Fujiwara M., and Wakitani S. 2007. High inoculation cell density could accelerate the differentiation of human bone marrow mesenchymal stem cells to chondrocyte cells. *J Biosci Bioeng.* 103(1), 98-100.

Tamai N., Myoui A., Hirao M., Kaito T., Ochi T., Tanaka J., Takaoka K., and Yoshikawa H. 2005. A new biotechnology for articular cartilage repair: subchondral implantation of a composite of interconnected porous hydroxyapatite, synthetic polymer (PLA-PEG), and bone morphogenetic protein-2 (rhBMP-2). *Osteoarthritis Cartilage.* 13(5), 405-17.

Tanaka N., Sakahashi H., Sato E., Hirose K., and Ishii S. 2002. Effects of needle-arthroscopic lavage with different volumes of fluid on knee synovitis in rheumatoid arthritis. *Clin Rheumatol.* 21(1), 4-9.

Tang W.C., and Henderson I.J. 2005. High tibial osteotomy: long term survival analysis and patients' perspective. *Knee.* 12(6), 410-3.

Taylor J.P., Hardy J., and Fischbeck K.H. 2002. Toxic proteins in neurodegenerative disease. *Science.* 296(5575), 1991-5.

Taylor S.J., McDonald J.W., 3rd, and Sakiyama-Elbert S.E. 2004. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. *J Control Release.* 98(2), 281-94.

Temenoff J.S., Athanasiou K.A., LeBaron R.G., and Mikos A.G. 2002. Effect of poly(ethylene glycol) molecular weight on tensile and swelling properties of oligo(poly(ethylene glycol) fumarate) hydrogels for cartilage tissue engineering. *J Biomed Mater Res.* 59(3), 429-37.

Temenoff J.S., and Mikos A.G. 2000. Review: tissue engineering for regeneration of articular cartilage. *Biomaterials.* 21(5), 431-40.

Thibault M., Poole A.R., and Buschmann M.D. 2002. Cyclic compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. *J Orthop Res.* 20(6), 1265-73.

Tognana E., Borrione A., De Luca C., and Pavesio A. 2007. Hyalograft C: hyaluronan-based scaffolds in tissue-engineered cartilage. *Cells Tissues Organs.* 186(2), 97-103.

Towbin H., Staehelin T., and Gordon J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A.* 76(9), 4350-4.

Trattnig S., Pinker K., Krestan C., Plank C., Millington S., and Marlovits S. 2006. Matrix-based autologous chondrocyte implantation for cartilage repair with HyalograftC: two-year follow-up by magnetic resonance imaging. *Eur J Radiol.* 57(1), 9-15.

Tuan R.S., Boland G., and Tuli R. 2003. Adult mesenchymal stem cells and cell-based tissue engineering. *Arthritis Res Ther.* 5(1), 32-45.

Twining S.S., Wilson P.M., and Ngamkitidechakul C. 1999. Extrahepatic synthesis of plasminogen in the human cornea is up-regulated by interleukins-1alpha and -1beta. *Biochem J.* 339 (Pt 3), 705-12.

Umeda H., Kanemaru S., Yamashita M., Kishimoto M., Tamura Y., Nakamura T., Omori K., Hirano S., and Ito J. 2007. Bone regeneration of canine skull using bone marrow-derived stromal cells and beta-tricalcium phosphate. *Laryngoscope.* 117(6), 997-1003.

Urech L., Bittermann A.G., Hubbell J.A., and Hall H. 2005. Mechanical properties, proteolytic degradability and biological modifications affect angiogenic process

extension into native and modified fibrin matrices in vitro. *Biomaterials*. 26(12), 1369-79.

Uribe J.W. 2003. The use of radiofrequency devices for chondral debridernent. *Sports Medicine and Arthroscopy Review*. 11(4), 214-221.

Uriel S., Brey E.M., and Greisler H.P. 2006. Sustained low levels of fibroblast growth factor-1 promote persistent microvascular network formation. *Am J Surg*. 192(5), 604-9.

Van Lieshout M., Peters G., Rutten M., and Baaijens F. 2006. A knitted, fibrin-covered polycaprolactone scaffold for tissue engineering of the aortic valve. *Tissue Eng*. 12(3), 481-7.

Vangsness C.T. 2005. Radiofrequency use on articular cartilage lesions. *Orthopedic Clinics of North America*. 36(4), 427-31.

Veilleux N., and Spector M. 2005. Effects of FGF-2 and IGF-1 on adult canine articular chondrocytes in type II collagen-glycosaminoglycan scaffolds in vitro. *Osteoarthritis Cartilage*. 13(4), 278-86.

Vickers S.M., Squitieri L.S., and Spector M. 2006. Effects of cross-linking type II collagen-GAG scaffolds on chondrogenesis in vitro: dynamic pore reduction promotes cartilage formation. *Tissue Eng*. 12(5), 1345-55.

Vinardell T., Thorpe S.D., Buckley C.T., and Kelly D.J. 2009. Chondrogenesis and integration of mesenchymal stem cells within an in vitro cartilage defect repair model. Ann Biomed Eng. 37(12), 2556-65.

Vinatier C., Gauthier O., Masson M., Malard O., Moreau A., Fellah B.H., Bilban M., Spaethe R., Daculsi G., and Guicheux J. 2009. Nasal chondrocytes and fibrin sealant for cartilage tissue engineering. *J Biomed Mater Res A*. 89(1), 176-85.

Visna P., Pasa L., Cizmar I., Hart R., and Hoch J. 2004. Treatment of deep cartilage defects of the knee using autologous chondrograft transplantation and by abrasive techniques--a randomized controlled study. *Acta Chir Belg.* 104(6), 709-14.

Visna P., Pasa L., Hart R., Kocis J., Cizmar I., and Adler J. 2003. [Treatment of deep chondral defects of the knee using autologous chondrocytes cultured on a support--results after one year]. *Acta Chir Orthop Traumatol Cech.* 70(6), 356-62.

von Heimburg D., Zachariah S., Heschel I., Kuhling H., Schoof H., Hafemann B., and Pallua N. 2001. Human preadipocytes seeded on freeze-dried collagen scaffolds investigated in vitro and in vivo. *Biomaterials.* 22(5), 429-38.

Waas E.T., Lomme R.M., DeGroot J., Wobbes T., and Hendriks T. 2002. Tissue levels of active matrix metalloproteinase-2 and -9 in colorectal cancer. *Br J Cancer.* 86(12), 1876-83.

Wakitani S., Goto T., Young R.G., Mansour J.M., Goldberg V.M., and Caplan A.I. 1998. Repair of large full-thickness articular cartilage defects with allograft articular chondrocytes embedded in a collagen gel. *Tissue Eng.* 4(4), 429-44.

Wang P.P., and Badley E.M. 2003. Consistent low prevalence of arthritis in quebec: findings from a provincial variation study in Canada based on several canadian population health surveys. *J Rheumatol.* 30(1), 126-31.

Watanabe K., Miyazaki T., and Matsuda R. 2003. Growth factor array fabrication using a color ink jet printer. *Zoolog Sci.* 20(4), 429-34.

Wechselberger G., Russell R.C., Neumeister M.W., Schoeller T., Piza-Katzer H., and Rainer C. 2002. Successful transplantation of three tissue-engineered cell types using capsule induction technique and fibrin glue as a delivery vehicle. *Plast Reconstr Surg.* 110(1), 123-9.

Wei X., Gao J., and Messner K. 1997. Maturation-dependent repair of untreated osteochondral defects in the rabbit knee joint. *J Biomed Mater Res.* 34(1), 63-72.

Weinand C., Gupta R., Huang A.Y., Weinberg E., Madisch I., Qudsi R.A., Neville C.M., Pomerantseva I., and Vacanti J.P. 2007a. Comparison of hydrogels in the in vivo formation of tissue-engineered bone using mesenchymal stem cells and beta-tricalcium phosphate. *Tissue Eng.* 13(4), 757-65.

Weinand C., Gupta R., Weinberg E., Madisch I., Jupiter J.B., and Vacanti J.P. 2007b. Human shaped thumb bone tissue engineered by hydrogel-beta-tricalciumphosphate/poly-epsilon-caprolactone scaffolds and magnetically sorted stem cells. *Ann Plast Surg.* 59(1), 46-52; discussion 52.

Weinand C., Pomerantseva I., Neville C.M., Gupta R., Weinberg E., Madisch I., Shapiro F., Abukawa H., Troulis M.J., and Vacanti J.P. 2006. Hydrogel-beta-TCP scaffolds and stem cells for tissue engineering bone. *Bone.* 38(4), 555-63.

Weiser L., Bhargava M., Attia E., and Torzilli P.A. 1999. Effect of serum and platelet-derived growth factor on chondrocytes grown in collagen gels. *Tissue Eng.* 5(6), 533-44.

Westreich R., Kaufman M., Gannon P., and Lawson W. 2004. Validating the subcutaneous model of injectable autologous cartilage using a fibrin glue scaffold. *Laryngoscope.* 114(12), 2154-60.

Whang K., Tsai D.C., Nam E.K., Aitken M., Sprague S.M., Patel P.K., and Healy K.E. 1998. Ectopic bone formation via rhBMP-2 delivery from porous bioabsorbable polymer scaffolds. *J Biomed Mater Res.* 42(4), 491-9.

Wilkins A., Kemp K., Ginty M., Hares K., Mallam E., and Scolding N. 2009. Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro. *Stem Cell Res.* [Epub ahead of print]

Willers C., Chen J., Wood D., Xu J., and Zheng M.H. 2005. Autologous chondrocyte implantation with collagen bioscaffold for the treatment of osteochondral defects in rabbits. *Tissue Eng.* 11(7-8), 1065-76.

Willerth S.M., Arendas K.J., Gottlieb D.I., and Sakiyama-Elbert S.E. 2006. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials.* 27(36), 5990-6003.

Williams C., Johnson S.L., Robinson P.S., and Tranquillo R.T. 2006. Cell sourcing and culture conditions for fibrin-based valve constructs. *Tissue Eng.* 12(6), 1489-502.

Williams C.G., Kim T.K., Taboas A., Malik A., Manson P., and Elisseeff J. 2003. In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. *Tissue Eng.* 9(4), 679-88.

Williams G.M., Klein T.J., and Sah R.L. 2005. Cell density alters matrix accumulation in two distinct fractions and the mechanical integrity of alginate-chondrocyte constructs. *Acta Biomater.* 1(6), 625-33.

Williams L.R., Longo F.M., Powell H.C., Lundborg G., and Varon S. 1983. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. *J Comp Neurol.* 218(4), 460-70.

Williams R.J., 3rd, Ranawat A.S., Potter H.G., Carter T., and Warren R.F. 2007. Fresh stored allografts for the treatment of osteochondral defects of the knee. *J Bone Joint Surg Am.* 89(4), 718-26.

Wittkampf A.R. 1989. Fibrin glue as cement for HA-granules. *J Craniomaxillofac Surg.* 17(4), 179-81.

Woessner J.F., Jr. 1961. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys.* 93, 440-7.

Wright J.M., Crockett H.C., Slawski D.P., Madsen M.W., and Windsor R.E. 2005. High tibial osteotomy. *J Am Acad Orthop Surg.* 13(4), 279-89.

Wu M.H., Urban J.P., Cui Z.F., Cui Z., and Xu X. 2007. Effect of extracellular ph on matrix synthesis by chondrocytes in 3D agarose gel. *Biotechnol Prog.* 23(2), 430-4.

Xiang Y., Zheng Q., Jia B.B., Huang G.P., Xu Y.L., Wang J.F., and Pan Z.J. 2007. Ex vivo expansion and pluripotential differentiation of cryopreserved human bone marrow mesenchymal stem cells. *J Zhejiang Univ Sci B.* 8(2), 136-46.

Xiangdong W., Ar'Rajab A., Ahren B., Andersson R., and Bengmark S. 1991. The effect of pancreatic islets on transplanted hepatocytes in the treatment of acute liver failure in rats. *Res Exp Med (Berl).* 191(6), 429-35.

Xu J., Wang W., Ludeman M., Cheng K., Hayami T., Lotz J.C., and Kapila S. 2008. Chondrogenic differentiation of human mesenchymal stem cells in three-dimensional alginate gels. *Tissue Eng Part A.* 14(5), 667-80.

Xu T., Gregory C.A., Molnar P., Cui X., Jalota S., Bhaduri S.B., and Boland T. 2006. Viability and electrophysiology of neural cell structures generated by the inkjet printing method. *Biomaterials.* 27(19), 3580-8.

Xu T., Jin J., Gregory C., Hickman J.J., and Boland T. 2005a. Inkjet printing of viable mammalian cells. *Biomaterials.* 26(1), 93-9.

Xu X.L., Lou J., Tang T., Ng K.W., Zhang J., Yu C., and Dai K. 2005b. Evaluation of different scaffolds for BMP-2 genetic orthopedic tissue engineering. *J Biomed Mater Res B Appl Biomater.* 75(2), 289-303.

Yakovlev S., Gorlatov S., Ingham K., and Medved L. 2003. Interaction of fibrin(ogen) with heparin: further characterization and localization of the heparin-binding site. *Biochemistry.* 42(25), 7709-16.

Yamada H., Stephens R.W., Nakagawa T., and McNicol D. 1988. Detection and partial characterization of a specific plasminogen activator inhibitor in human chondrocyte cultures. *J Biochem.* 104(6), 960-7.

Yamane S., Iwasaki N., Majima T., Funakoshi T., Masuko T., Harada K., Minami A., Monde K., and Nishimura S. 2005. Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering. *Biomaterials.* 26(6), 611-9.

Yan H., and Yu C. 2007. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. *Arthroscopy.* 23(2), 178-87.

Yan J., Qi N., and Zhang Q. 2007. Rabbit articular chondrocytes seeded on collagen-chitosan-GAG scaffold for cartilage tissue engineering in vivo. *Artif Cells Blood Substit Immobil Biotechnol.* 35(4), 333-44.

Yasuda A., Kojima K., Tinsley K.W., Yoshioka H., Mori Y., and Vacanti C.A. 2006. In vitro culture of chondrocytes in a novel thermoreversible gelation polymer scaffold containing growth factors. *Tissue Eng.* 12(5), 1237-45.

Yates K.E., Allemann F., and Glowacki J. 2005. Phenotypic analysis of bovine chondrocytes cultured in 3D collagen sponges: effect of serum substitutes. *Cell Tissue Bank.* 6(1), 45-54.

Ye Q., Zund G., Benedikt P., Jockenhoevel S., Hoerstrup S.P., Sakyama S., Hubbell J.A., and Turina M. 2000. Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 17(5), 587-91.

Yoo H.S., Lee E.A., Yoon J.J., and Park T.G. 2005a. Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. *Biomaterials.* 26(14), 1925-33.

Yoo W.J., Choi I.H., Chung C.Y., Cho T.J., Kim I.O., and Kim C.J. 2005b. Implantation of perichondrium-derived chondrocytes in physeal defects of rabbit tibiae. *Acta Orthop.* 76(5), 628-36.

Yoon D.M., and Fisher J.P. 2008. Effects of exogenous IGF-1 delivery on the early expression of IGF-1 signaling molecules by alginate embedded chondrocytes. *Tissue Eng Part A.* 14(7), 1263-73.

Young S., Wong M., Tabata Y., and Mikos A.G. 2005. Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J Control Release.* 109(1-3), 256-74.

Zehbe R., Libera J., Gross U., and Schubert H. 2005. Short-term human chondrocyte culturing on oriented collagen coated gelatine scaffolds for cartilage replacement. *Biomed Mater Eng.* 15(6), 445-54.

Zheng Z.H., Li X.Y., Ding J., Jia J.F., and Zhu P. 2008. Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis. *Rheumatology (Oxford).* 47(1), 22-30.

Zhu S.J., Choi B.H., Huh J.Y., Jung J.H., Kim B.Y., and Lee S.H. 2006a. A comparative qualitative histological analysis of tissue-engineered bone using bone marrow

mesenchymal stem cells, alveolar bone cells, and periosteal cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 101(2), 164-9.

Zhu S.J., Choi B.H., Jung J.H., Lee S.H., Huh J.Y., You T.M., Lee H.J., and Li J. 2006b. A comparative histologic analysis of tissue-engineered bone using platelet-rich plasma and platelet-enriched fibrin glue. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 102(2), 175-9.

Zuk P.A., Zhu M., Ashjian P., De Ugarte D.A., Huang J.I., Mizuno H., Alfonso Z.C., Fraser J.K., Benhaim P., and Hedrick M.H. 2002. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 13(12), 4279-95.

Zwingmann J., Mehlhorn A.T., Sudkamp N., Stark B., Dauner M., and Schmal H. 2007. Chondrogenic differentiation of human articular chondrocytes differs in biodegradable PGA/PLA scaffolds. *Tissue Eng.* 13(9), 2335-43.

XI. APPENDIX I: *Supplementary Data*

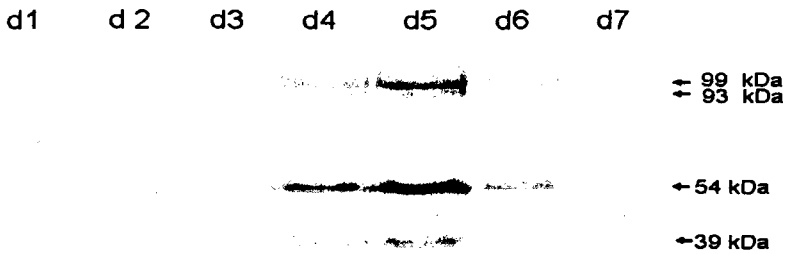

FIG.1. SDS-PAGE of the conditioned media from day 1 to day 7 (d1-d7) shows the dramatic increase of FDP at day 4, 5.

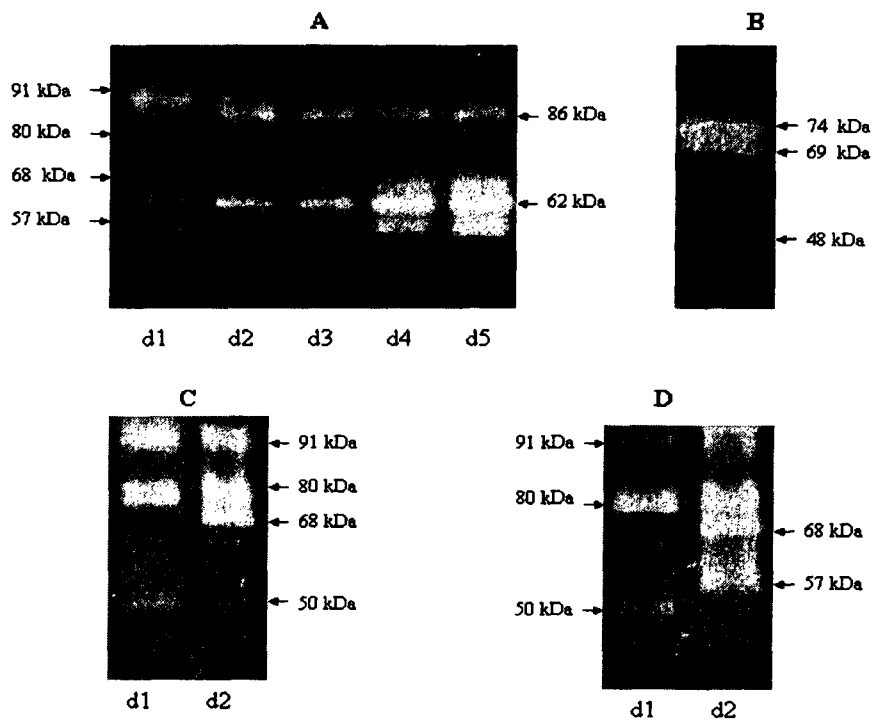

FIG.2. **A**: Gelatin zymogram shows the proteolytic bands of gelatinases MMP-2 (57,62 and 68 kDa) and MMP-9 (80, 86 and 91 kDa) in conditioned media day 1-5 (d1 to d5); **B**: Gelatin zymogram revealed purified plasmin at 74, 69 and 48 kDa; **C**: casein zymogram shows the proteolytic bands of MMP-3 (50 kDa), latent form of MMP-2 (68 kDa) in addition to active and latent forms of MMP-9 (80, 91 kDa respectively); **D**: fibrin zymogram shows the proteolytic bands of MMP-3 (50 kDa), active and latent form of MMP-2 (57, 68 kDa respectively) in addition to active and latent forms of MMP-9 (80, 91 kDa respectively),

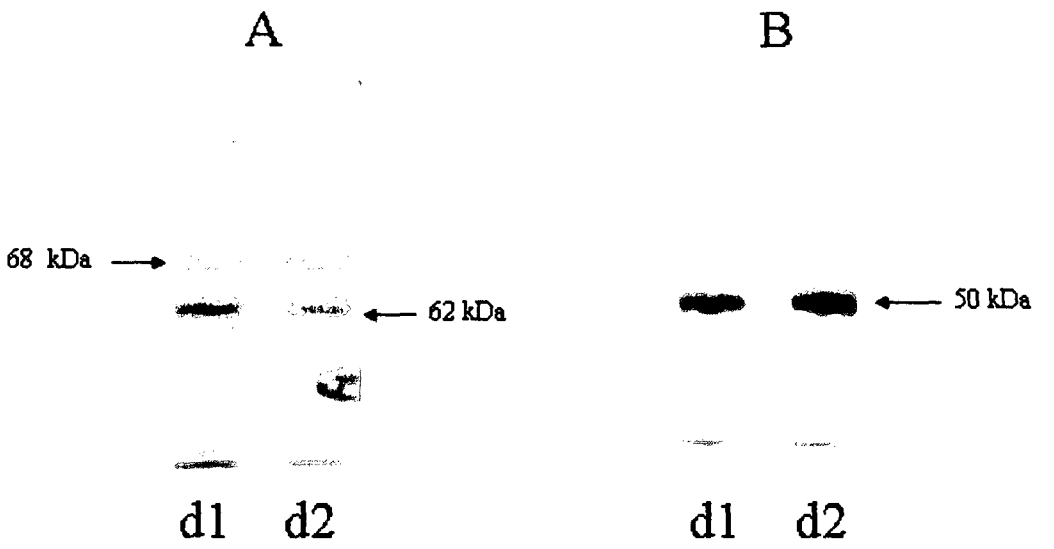

FIG.3. Western blot for MMP-2 **(A)** and MMP-3 **(B)** in conditioned media day 1, 2 (d1 and d2).

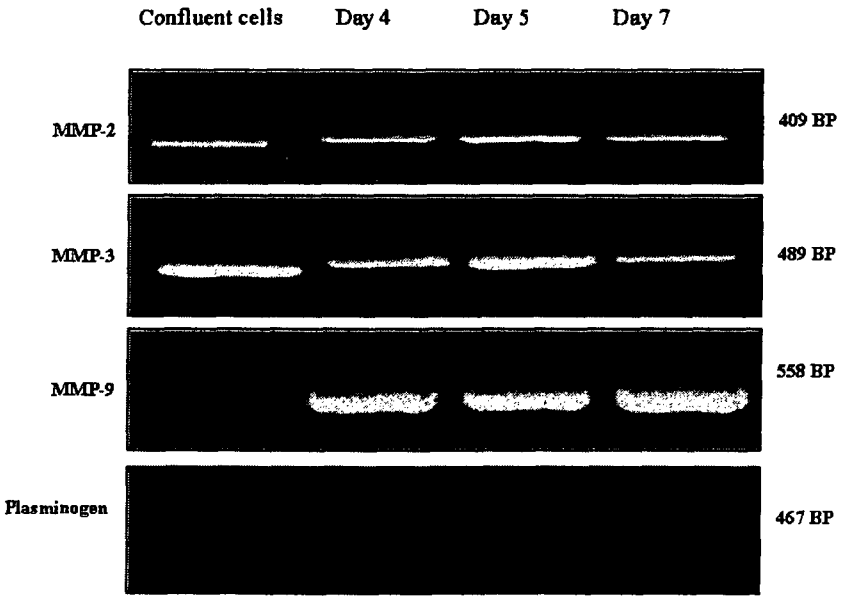

FIG.4. RT-PCR results for MMP-2, MMP-3, and MMP-9 as well as plasminogen expression for C5.18 cells before and after encapsulation in fibrin hydrogels. Gels are mainly broken down by day 5.

XII. APPENDIX II: *Curriculum Vitae*

Tamer Anwar Esmail Ahmed, M.Sc.

Current address (Work)

(Home)

Born 1975, Alexandria, Egypt.

Citizenship Egyptian, permanent resident of Canada

Awards

1- Egyptian government, Mission department Ph.D. Scholarship (Feb 2005 – Feb 2009)
2- Ontario Graduate Scholarship (OGS, May 2009-December 2009)

Education

2005-present Ph.D. Candidate, Faculty of Medicine University of Ottawa, Ottawa, Ontario, Canada (2005-present).

1- CMM 5304 (Introduction to Developmental Biology): Grade **(A)**

2- CMM 8325S (Seminar II): Grade **(A-)**

3- CMM 5001 (Pathological Basis of Disease): Grade **(A+)**

4- CMM 9998 (Comprehensive Exam) entitled "A Tissue Engineering Approach for the Correction of Diabetes - Associated Hyperglycemia: Encapsulation of

Mesenchymal Stem Cells in Collagen-Polyethylene Glycol": **Pass**

2001 M.Sc. Applied Medical Chemistry (Excellent), Medical Research Institute, Alexandria, Egypt. "Paraoxonase Enzyme activity and its relation to lipid profile in uremic patients undergoing haemodialysis"

1996 B.Sc. Special Biochemistry (Very good), Faculty of Science, Alexandria University, Alexandria, Egypt.

General Experience

- Tissue engineering of articular cartilage and bone using mesenchymal stem cells.
- Enzyme kinetics of arylhydrolase.
- Culturing of anchrogenous and unanchrogenous cells (mammalian and insect cells) and these include Chinese Hamster Ovary (CHO K1) cells, Human Embryonic Kidney (HEK-293) cells, SF-9, and High five.
- Adaptation of cell lines to serum-free media.

Positions

2001-2005 Assistant Researcher, Medical Biotechnology Dept. Genetic Engineering and Biotechnology Research Inst. Mubarak City for Scientific Research, New burg Al-Arab, Alexandria, Egypt.

1999-2001 Researcher Assistant, Medical Biotechnology Dept.

Genetic Engineering and Biotechnology Research Inst. Mubarak City for Scientific Research, New burg Al-Arab, Alexandria, Egypt (1999- 2001).

Peer-reviewed publications

1. Ahmed TA, Giulivi A, Griffith M, and Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. Tissue Engineering Part A (In revision).
2. Ahmed TA and Hincke M. Strategies for articular cartilage lesion repair and functional restoration. Tissue Eng. Part B. 2010 (Epub ahead of print)
3. Ahmed TA, Dare EV, and Hincke M. Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng. Part B (Reviews) 2008; 14(2): 199-215.
4. Ahmed TA, Griffith M, and Hincke M. Characterization and inhibition of fibrin hydrogel-degrading enzymes during development of tissue engineering scaffolds. Tissue Eng. 2007; 13(7): 1469-77.
5. El-Demellawy M, Esmail TA (Ahmed TA), and El-Enshasy HA. Kinetics of Cell Growth and Metabolic Activity of Bone Marrow Derived Stem Cells under Different Osmotic Stresses. Egypt J Biomed. Sci. 2005; 17: 330-340.

Conferences

1. Ahmed TA*, Halpenny M, Giulivi A, Dervin G, Griffith M, Hincke M, and Atkins H. Incorporation of heparin-binding delivery system into fibrin Glue for tissue engineering of articular cartilage. Stem Cell Network annual general meeting. Vancouver, British Columbia, Canada. Nov 5-7, 2008. (Poster)

2. Ahmed TA*, Halpenny M, Atkins H, Giulivi A, Dervin G, Griffith M, and Hincke M. Stabilization of fibrin-mesenchymal stem cells (MSCs) constructs under hypoxic conditions during tissue engineering of articular cartilage. Second combined meeting of the American Orthopaedic Association (AOA) and the Canadian Orthopaedic Association (COA). Quebec city, Quebec, Canada. June 4-7, 2008. (Invited podium presentation)

3. Ahmed TA*, Halpenny M, Atkins H, Giulivi A, Griffith M, and Hincke M. Effect of hypoxia on the stability of fibrin hydrogel-mesenchymal stem Cell (MSCs) constructs during articular cartilage tissue engineering. Ottawa hospital research day: Regenerative medicine - developing the therapies for tomorrow. Ottawa, Ontario. June 2, 2008. (Poster)

4. Ahmed TA*, Halpenny M, Atkins H, Giulivi A, Dervin G, Griffith M, and Hincke M. Stabilization of fibrin-mesenchymal stem cells (MSCs) constructs under hypoxic conditions during tissue engineering of articular cartilage.HK Uhthoff Research day. Ottawa, Ontario. April 17, 2008. (Invited podium presentation)

5. Ahmed TA*, Halpenny M, Atkins H, Giulivi A, Griffith M, and Hincke M. Effect of hypoxia on the stability of fibrin hydrogel-mesenchymal stem Cell (MSCs) constructs during articular cartilage tissue Engineering. Stem Cell Network annual general meeting. Toronto, Ontario, Canada. Nov 7-9, 2007. (poster)

6. Ahmed TA*, Halpenny M, Griffith M, and Hincke M. Stabilization of Fibrin Hydrogel for Tissue Engineering of Articular Cartilage. 13th Annual Canadian Connective

Tissue Conference (CCTC 2007). Toronto, Ontario, Canada. May 24-26, 2007. (poster)

7. Esmail TA (Ahmed TA), Dare E, Griffith M, and Hincke M*. Characterization and Inhibition of Fibrin Hydrogel-Degrading Enzymes during Tissue Engineering for Articular Cartilage. Canadian Federation of Biological Societies Annual Meeting, Saskatoon, Saskatchewan, Canada. June 13-18, 2006. (Poster)

8. Esmail TA (Ahmed TA)*, Griffith M, and Hincke M. Stabilization of fibrin hydrogels using protease inhibitors that are specific for MMP's and plasmin. 12th Annual Canadian Connective Tissue Conference (CCTC 2006). Ottawa, Ontario, Canada. May 25-27, 2006. (Invited podium presentation)

9. El-Enshasy HA*, Esmail TA (Ahmed TA), and El-Demellawy MA. Kinetic of growth of bone marrow derived stem cells under different osmotic stress. 5th Annual Meeting of the Tissue Engineering Society International. Kobe & Japan. December 8-10, 2002. (poster)

*: the presenter of the poster and the presentation.

Projects

2005-present

Tissue engineering for articular cartilage. Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

2002-2004

Tissue Engineering in dentistry project entitled "regeneration of alveolar bone by tissue engineered implants" The project is established and funded under the international agreement between the government of USA

and government of the Arab Republic of Egypt on science and technology cooperation, Research team.

2001-2005

Mubarak city for scientific research and technology applications Internal project entitled "Isolation and characterization of bone marrow derived mesenchymal stem cells. Potential medical applications" Research Team (2001-2005).

Training courses and attended conferences

- 11th Annual Canadian Connective Tissue Conference (CCTC 2005), Montreal, Quebec, Canada. May 26-28, 2005.
- Flow cytometry: Applications in medicine and biology. BD workshop. March 18-20, Molecular Immunology Unit (MIU), Al-Azhar university, Cairo, Egypt.
- Tissue Engineering: Dental application and future promises. 13th International Alexandria Dental Congress, October 29- November 1, 2002. Alexandria, Egypt.

Volunteering activities

- Reviewer of manuscripts submitted to the journal Tissue Engineering (2007, 2008); 3 manuscripts.
- Safety representative for Dr. Griffith Lab (2008).
- Let's talk science organization, 2008.