

Human Recombinant Collagen Hydrogel for Control of Ventricular Remodeling and Repair after Myocardial Infarction

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Thesis submitted to the University of Ottawa in partial fulfillment of the requirements for
the Doctorate in Philosophy Cellular and Molecular Medicine

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Acknowledgements

I would sincerely like to thank all the members of the BEaTS research group at the University of Ottawa Heart Institute for their help and support during my graduate research. In particular, I would like to thank: Dr. Brian McNeill, James Podrebarac, Dr. Veronika Sedlakova, and Dr. Katsuhiko Hosoyama for their collaboration during my research studies. I would also like to thank Richard Seymour and the members of the University of Ottawa Animal Care and Veterinary Services for their help running mouse trials for this project. In addition, I would like to thank the individuals who assisted with the RNA sequencing portion of this project at Genome Quebec and the Canadian Centre for Computational Genomics. Finally, I would like to thank all those who provided me with training and advice during my research including: Suzanne Crowe, Dr. Nick Blackburn, Dr. Branka Vulesevic, Dr. Manuel Ahumada, Xiaoling Zhao, Dr. David Smyth, Dr. Gregory Cron, Dr. Christopher McTiernan, Cagla Cimenci, Dr. Marcelo Munoz, Dr. Godwin Dogbevia, and Nadya Morrow.

I would like to thank all of the members of my thesis advisory committee including: Dr. Darryl Davis, Dr. Duncan Stewart, and Dr. Wenbin Liang. I appreciate all the feedback and guidance you have provided me with throughout my graduate research.

I am deeply grateful for the guidance and support of my supervisors Dr. Erik Suuronen and Dr. Emilio Alarcon, without whom this research project would not have been possible. They have always provided me with great direction for experimental work, have been open to my research ideas, and supported my growth as a scientist. I would also like to thank Dr. Marc Ruel for answering the email of an undergraduate student interested in working in cardiovascular research, which allowed me to interview for a graduate position in the BEaTS research group.

Last but not least I would like to thank my family for their unending support over the course of the inevitable highs and lows of graduate research.

Sources of funding: This work was supported by a Collaborative Research Grant from the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council (NSERC) (CPG-158280 to Erik Suuronen, Emilio Alarcon, and Marc Ruel), a CIHR operating grant (MOP-77536 to Marc Ruel and Erik Suuronen), an NSERC Discovery Grant (2015-06235 to Emilio Alarcon) and a UOHI start-up grant (1255 to Emilio Alarcon). Sarah McLaughlin was funded by a Frederick Banting and Charles Best Canada Graduate Scholarship.

Disclosure: Emilio Alarcon, Erik Suuronen, and Marc Ruel are listed inventors on a patent application for the recombinant human collagen materials presented in this study. Patent applicant: Ottawa Heart Institute Research Corporation, 40 Ruskin Street H2406 Ottawa, Ontario K1Y 4W7, Canada. Application number: PCT/CA2018/050537. Status of the application: Published as WO/2018/201260. The specific aspect of this thesis covered in the patent application: Composition of matter for the hydrogel, a method for regenerating or repairing heart tissue, the method for preparing the composition of matter or the hydrogel, and the use of the hydrogel.

Abstract

Myocardial infarction (MI) leads to permanent loss of cardiac muscle due to the limited regenerative potential of the mammalian heart. The affected heart muscle is replaced by a fibrotic scar; however, the scar is not able to offset the increase in wall stress placed on the remaining myocardium. This distending pressure can lead to dilative remodeling of the ventricle, progressive loss of cardiac function, and heart failure. Despite current medical therapy, heart failure continues to have a high mortality rate. Therefore, there is a clinical need for treatments that can both improve cardiac function post-MI and reduce ventricular remodeling to prevent progression to heart failure.

Injectable biomaterials aim to provide a scaffold to stimulate infarct repair by mimicking the healthy cardiac extracellular matrix (ECM). The ECM plays a critical role in tissue regeneration but after a MI it is pathologically modified. Injection of biomaterials post-MI can provide a scaffold that better stimulates infarct repair. In this study, hydrogels were developed from recombinant human type I and type III collagen (rHCI and rHCIII), the two most prevalent structural proteins in the cardiac ECM. Injection of rHCI and rHCIII hydrogels in a mouse model of MI improved cardiac function and reduced infarct size 28 days post-treatment. Infarcted hearts treated with rHCI exhibited improved myocardial salvage in the region bordering the scar with improved capillary density. rHCI hydrogel was also superior to rHCIII in reducing ventricular remodeling.

The injection of rHCI hydrogel into the border zone post-MI resulted in an acute improvement of contractile function two days after treatment that was maintained long-term. At two days post-injection, rHCI treated animals had reduced apoptotic cardiomyocytes and lower levels of oxidative stress. Methylglyoxal modifies and crosslinks collagen in the ECM, leading to oxidative stress. Two days after injection, the rHCI hydrogel at the epicardial

surface was modified by methylglyoxal, while methylglyoxal-derived advanced glycation end-product levels in the underlying myocardium were lower than in control animals. It appears that rHCI hydrogel injection is soaking up free methylglyoxal from the myocardium, reducing levels of oxidative stress in cardiac muscle and improving contractility of cardiomyocytes bordering the scar. These results suggest that rHC therapy is a promising approach to improve cardiac contractility, and limit ventricular remodeling post-MI.

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Acronyms and Abbreviations

1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride	EDC
4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic Acid	HEPES
4-Hydroxynoneal	4HNE
Adenosine Triphosphate	ATP
Advanced Glycation End Products	AGEs
Aldo-Keto Reductases	AKRs
Alpha cyclodextrin	α -CD
Alpha Smooth Muscle Actin	α -SMA
Aminoactinomycin D	7-AAD
AMPA Type Subunit 1	GRIA1
Angiotensin Converting Enzyme	ACE
Angiotensin II	Ang2
Aortic Valve Closure	AVC
Apoptotic Signal Related Kinase 1	ASK1
Bone Marrow Derived Macrophages	BMDMs
Canadian Centre for Computational Genomics	C3G
Carboxymethyllysine	CML
Cardiac Output	CO
Chemokine Ligand 2	CCL2

Chemokine Ligand 22/Macrophage Derived Chemokine	CCL22
Chemokine Receptor 2	CCR2
Circulating Angiogenic Cells	CACs
Connexin 43	Cx43
Coronary Artery Bypass Grafting	CABG
Coronary Heart Disease	CHD
Damage Associated Molecular Patterns	DAMPs
Differential Scanning Calorimetry	DSC
Dulbecco's Modified Eagle Medium	DMEM
End Diastolic Volume	EDV
End Systolic Volume	ESV
Endothelial Cell Growth Basal Medium	EBM
Erythroid Differentiation Regulator 1	ERDR1
Extracellular Matrix	ECM
Fetal Bovine Serum	FBS
Fibroblast Growth Factor 2	FGF2
Foreign Body Response	FBR
Forkhead Box P3	FOXP3
Forward Scatter	FSC
Fractional Area Change	FAC

Fragments per Kilobase of Exon per Million Fragments Mapped	FPKM
Gene Ontology	GO
Generalized Linear Model	GLM
Glutathione-S-Transferase	GST
Glyceraldehyde 3-Phosphate Dehydrogenase	GAPDH
Glycosaminoglycan	GAG
Glyoxalase 1	GLO1
Good Manufacturing Practice	GMP
Green Fluorescent Protein	GSP
Guideline Directed Medical Therapy	GDMT
Hank's Balanced Salt Solution	HBSS
Heat Shock Protein 60	HSP60
Immunoglobulin Kappa Chain Variable 8-30	IGKV8-30
In situ Hybridization	ISH
Induced Pluripotent Stem Cells	iPSCs
Insulin-Like Growth Factor	IGF-1
Interferon Gamma	IFN- γ
Interleukin 1	IL1
Interleukin 6	IL6
Ischemia Reperfusion Injury	I/R

Kyoto Encyclopedia of Genes and Genomes	KEG
Left Anterior Descending Coronary Artery	LAD
Left Ventricle	LV
Left Ventricular Assist Devices	LVAD
Left Ventricular Ejection Fraction	LVEF
Likelihood Ratio Tests	LRT
Lysyl Oxidase	LOX
Matrix metalloproteinase	MMP
Methylglyoxal-Derived Hydroimidazolone 1	MG-H1
Minimum Essential Medium	MEM
Mitochondrial Aldehyde Dehydrogenase	ALDH2
Mitogen Activated Protein Kinase	MAPK
Myocardial Infarction	MI
Natural Kill Cells	NK cells
Neonatal Rat Ventricular Myocytes	NRVMs
New York Heart Association Functional Class	NYHA
N-Hydroxysuccinimide	NHS
Nicotinamide adenine dinucleotide phosphate oxidase	Nox2
Nicotinamide Adenine Dinucleotide	NAD ⁺
N-Isopropylacrylamide	NIPAAm

Nuclear Factor Kappa-Light-Chain-Enhance of Activated B Cells	NF- κ B
Optimal Cutting Temperature Compound	OCT
Paraformaldehyde	PFA
Pattern Recognition Receptors	PRRs
Percutaneous Coronary Intervention	PCI
Phosphate Buffered Saline	PBS
Platelet Endothelial Cell Adhesion Molecule 1	PECAM1
Platelet-Derived Growth Factor-BB	PDGF-BB
Polyethylene Glycol	PEG
Quantitative Polymerase Chain Reaction	qPCR
Reactive Oxygen Species	ROS
Receptor For Advanced Glycation End Products	RAGE
Recombinant Human Collagen Type I	rHCI
Recombinant Human Collagen Type III	rHCIII
Recombinant Human Collagen	rHC
Red Blood Cells	RBCs
Renin-Angiotensin-Aldosterone System	RAAS
Serpin Family A Member 1 Serine Protease Inhibitor	Serpina1e
Small Intestinal Submucosa ECM	SIS-ECM
Standard Operating Protocol	SOP

Stroke Volume	SV
Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling	TUNEL
Tissue Culture Polystyrene	TCPS
Tissue Inhibitor of Metalloproteinase	TIMP
Toll-Like Receptors	TLRs
Transforming Growth Factor Beta	TGF- β
Tumor Necrosis Factor Alpha	TNF- α
Type I Angiotensin II Receptors	AT ₁ R
Vascular Endothelial Growth Factor	VEGF
Vascular Endothelial Growth Factor	VEGF
Wheat Germ Agglutinin	WGA

Chapter 1: Introduction

1.1 Heart Failure after Myocardial Infarction

Coronary heart disease (CHD) remains one of the leading causes of death globally, accounting for 20% of the yearly deaths in Canada¹. CHD results from build up of plaque inside coronary arteries, which supply the myocardium with oxygenated blood². CHD increases the risks for the formation of a blood clot, thrombosis, within the coronary artery. Thrombosis of the coronary arteries can cause an acute myocardial infarction (MI)². An MI occurs when blood flow to the myocardium is obstructed and results in cardiomyocyte death, scar formation and loss of contractile function². The prevalence of MI in Canada is 2.0% and is increasing as the population ages, although the 30-day death rate is declining over the past decade to 12.1% of patients^{1,3,4}. The reduction in early deaths post-MI is due in part to the increase in timely revascularization strategies that restore blood flow to limit cardiac muscle loss⁴. The regenerative potential of the human heart is limited and insufficient to replace the cardiac muscle that is lost post-MI⁵. As a result, many patients with MI, 14-36% based on the clinical studies, will develop systolic heart failure that leads to worse clinical outcomes including higher levels of hospitalization and death^{6,7}. Heart failure is a condition in which the heart is no longer able to pump out enough oxygenated blood to meet the demands of the body⁸. Systolic heart failure after an MI is often characterized by declining heart function, dilated ventricle chambers, peripheral edema, and fatigue⁸. While current guideline directed medical therapy (GDMT) for heart failure can reduce the decline in cardiac function to improve survival, the improvements in outcomes are leveling off with time and the 5-year mortality rate remains high at 50%^{9,10}. Heart failure is a chronic condition which at its end-stage can only be treated with heart transplantation, and there are not enough donor hearts to meet the demand¹⁰. Heart failure is the leading cause of hospitalization in Canada, the United

States, and Europe^{11,12}. Due to the growing age of the population in Canada, the number of patients with heart failure is expected to increase over the next decade and the costs of heart failure hospitalization are expected to reach \$720 million in Canada by 2030^{1,10,13}. Therefore, there is a clinical need to develop new therapeutic options to prevent the progression to heart failure following MI.

1.2 Wound Healing after Myocardial Infarction

During an MI, loss of blood flow to an area of the myocardium for more than 15-20 min leads to irreversible loss of cardiomyocytes¹⁴. The regenerative and proliferative potential of human cardiomyocytes is not sufficient to replace lost cardiac muscle post-MI^{5,15}. Therefore, wound healing post-MI results in the formation of a fibrotic scar to prevent ventricular rupture during contraction and to stabilize cardiac function^{16,17}. The formation of a mature collagen scar takes 2-3 weeks in rodent models, 4-6 weeks in larger animal models (such as dogs, pigs, and rabbits), and 3-6 months in humans¹⁷. There are three distinct but overlapping phases of wound healing post-MI: the inflammatory phase (also called the necrotic phase), the proliferative phase, and the maturation phase¹⁸ (Figure 1.1).

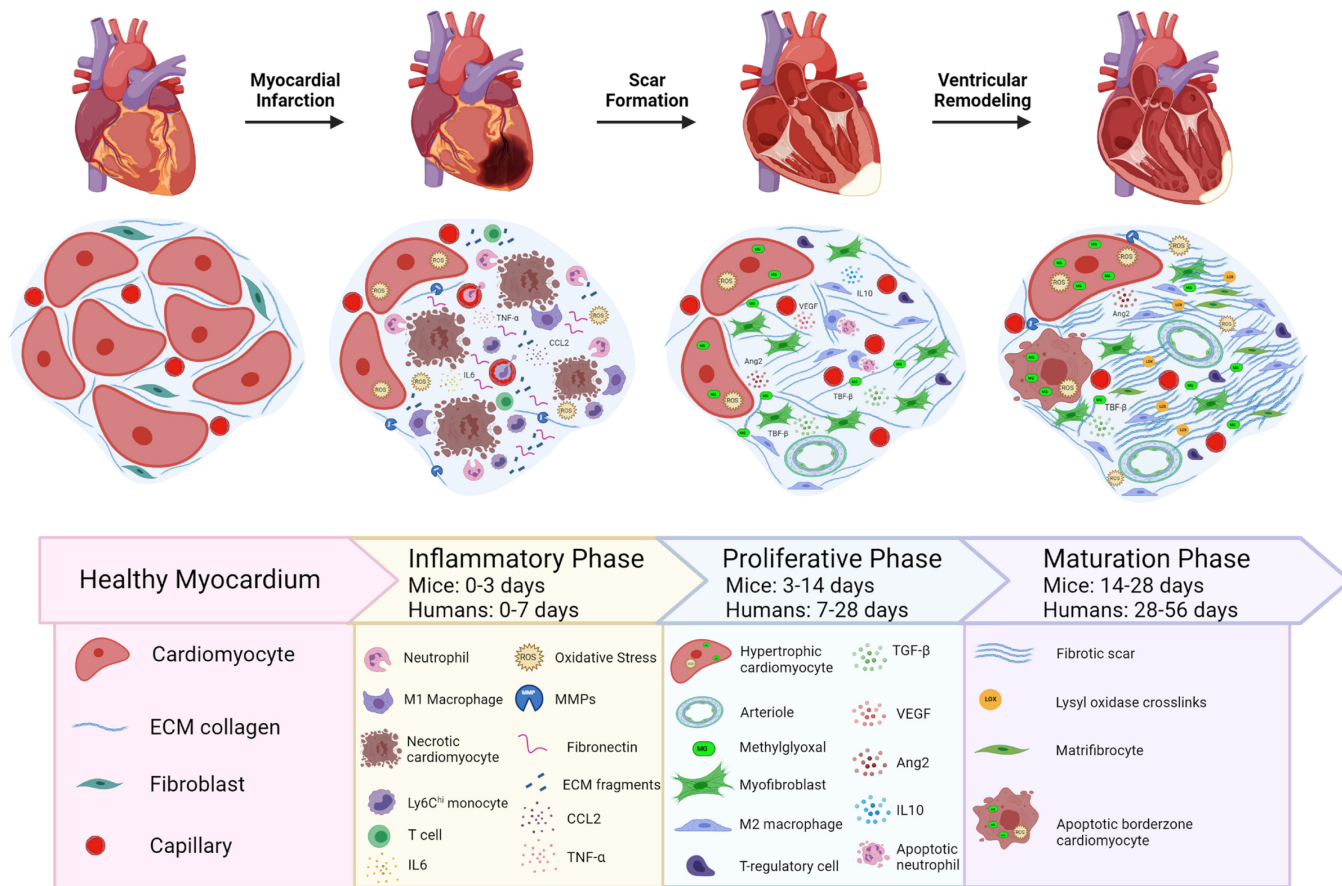


Figure 1.1. Phases of wound healing after myocardial infarction. Following the loss of blood flow during myocardial infarction there is a 3-phase wound healing process to remove necrotic cardiomyocytes and form a collagen scar. These phases occur sequentially as: inflammatory, proliferative, and maturation phases of infarct healing. Abbreviations: extracellular matrix (ECM), tumor necrosis factor α (TNF- α), interleukin 6 (IL6), transforming growth factor β (TGF- β), vascular endothelial growth factor (VEGF), angiotensin II (Ang2), interleukin 10 (IL10), and chemokine ligand 2 (CCL2). Created with BioRender.com

1.2.1 The Inflammatory Phase of Wound Healing Post-MI.

The inflammatory phase begins within the first day of an MI and ends within 3 days in mice and 7 days in humans^{18,19}. In the early stages of MI, loss of oxygenated blood to cardiac muscle leads to: loss of oxidative phosphorylation in affected cells, a decrease in adenosine triphosphate (ATP) production, and accumulation of $\text{Na}^+/\text{Ca}^{2+}$ ions in the cytosol and the mitochondria¹⁴. This creates an infarct region with high levels of necrosis and apoptosis¹⁸. The levels of neurohormonal activation and oxidative stress in the heart are immediately increased following an MI^{20,21}. Neurohormonal activation involves an increase in norepinephrine and angiotensin II levels within the heart²². This is triggered by loss of blood volume from impaired contractile function²². Angiotensin II (Ang2) in particular, leads to an increase in inflammatory cytokine and chemokine production as well as an increase in reactive oxygen species (ROS) through increased nicotinamide adenine dinucleotide phosphate oxidase 2 (Nox2) activity^{21,22}. ROS including: the superoxide radical-ion, peroxy radicals, hydrogen peroxide, and hydroxyl radicals, are also produced during ischemia from electron leakage from the mitochondrial electron transport chain (ETC)²³. These ROS species can irreversibly modify protein activity and damage nucleic acids²⁴. Elevated ROS lead to an increase in inflammation, activation of latent matrix metalloproteinases (MMPs) that degrade the extracellular matrix (ECM), cardiomyocyte hypertrophy, and cell death^{21,25,26}. During the inflammatory phase, a sterile inflammatory response is triggered by damage associated molecular patterns (DAMPs) such as necrotic cardiomyocyte debris, degraded ECM peptides, and heat shock proteins (HSP60)^{27,28}. These ligands bind to pattern recognition receptors (PRRs) on local inflammatory cells, including toll-like receptors (TLRs) and receptor of advanced glycation end products (RAGE)¹⁸. Binding of DAMPs to PPRs leads to upregulation of transcription factors mitogen activated protein kinase (MAPK)

and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)¹⁸. The result is an upregulation of cytokine and chemokine production within the infarction region and recruitment of leukocytes from the circulation²⁹. CC chemokines recruit lymphocytes, while CXC chemokines recruit neutrophils to the ischemic zone^{18,30,31}. Critical inflammatory cytokines produced in the infarct include: tumor necrosis factor alpha (TNF- α), interleukin (IL) 6, and IL-1¹⁸. The primary goal of the inflammatory phase post-MI is to clear the infarct region of necrotic cells and debris in order to facilitate scar formation. Neutrophils are the main phagocytic cell that clear dying cells and debris at this time point; they also secrete MMPs that breakdown the collagen ECM²⁹. Neutrophils invade the heart tissue through leaky vasculature within the first day of MI and peak by day 3 post-MI in mice^{32,33}. In addition, Ly6C⁺ monocytes are recruited from the circulation and splenic reservoirs through chemokine ligand 2 (CCL2) /chemokine receptor 2 (CCR2) signaling³⁴. Once in the heart, Ly6C⁺ monocytes contribute to the inflammatory and phagocytic response in part by differentiating into M1 (CCR2⁺) macrophages that secrete inflammatory mediators^{18,35}. TNF- α and vascular endothelial growth factor (VEGF) increase vascular permeability and facilitate the formation of a provisional matrix from the extravasated proteins fibrin and fibronectin¹⁴.

1.2.2 The Proliferative Phase of Wound Healing Post-MI

The proliferative phase follows the inflammatory phase post-MI and lasts up to day 14 post-MI in mice and 28 days post-MI in humans^{18,19}. The proliferative phase involves the resolution of inflammation, collagen scar formation, and angiogenesis¹⁸. Neutrophil apoptosis leads to anti-inflammatory M2 macrophage polarization, peaking at 5 days post-MI in mice, and restoration of the resident macrophage population begins^{33,35,36}. The predominant monocyte subtype also becomes the non-classical Ly6C⁻CCR2⁻ cell through

differentiation of Ly6C⁺ monocytes within the heart and recruitment from the circulation^{34,37}. The M2 macrophages and non-classical monocytes secrete anti-inflammatory/pro-resolution factors including IL10 and transforming growth factor β (TGF- β)³⁸. Finally, regulatory T-cells expressing forkhead box P3 (FOXP3) peak at 7 days post-MI and are critical for M2 macrophage differentiation and resolution of inflammation in the infarct³⁹. Angiogenesis begins 4 days after infarction and peaks within 14 days, leading to vascularization of the infarct and the restoration of nutrient flow to improve healing^{40,41}. Endothelial cells proliferate in the region of the myocardium bordering the infarct, increasing blood vessel formation and nutrient flow⁴². Resident fibroblasts and differentiated epicardial progenitor cells transform into myofibroblasts in response to TGF- β beginning at 4 days post-MI in mice⁴³⁻⁴⁵. Myofibroblasts express contractile protein alpha smooth muscle actin (α SMA) and secrete collagen⁴⁶. Collagen expression from myofibroblasts is also stimulated by Ang2 binding to type I angiotensin II receptors (AT₁R), further increasing fibrosis⁴⁰. MMP expression is reduced, and tissue inhibitor of metalloproteinase (TIMP) expression predominates in the proliferative phase transitioning from net ECM degradation to synthesis^{20,47}. The fibronectin provisional matrix is replaced by type III (expression elevated day 2 to 3 weeks) then type I collagen (expression elevated day 4 to 3 months) by myofibroblasts⁴⁷. Type I collagen provides the tensile strength required to stabilize the infarct scar¹⁹. The collagen expression plateaus at 7 days post-MI in mice and 3 weeks post-MI in humans¹⁹. Matricellular proteins, such as tenascin C, and collagen V play a role in confining fibrosis to the infarct zone and not extending into the bordering myocardium^{32,48}. During the proliferative phase, Ang2, ROS, and wall stress stimulate myocyte hypertrophy through increased synthesis of the sarcomere contractile unit within the cardiomyocytes^{22,49,50}.

1.2.3 The Maturation Phase of Wound Healing Post-MI

The final phase of wound healing post-MI, the maturation phase, lasts out to 28 days post-MI in mice and 56 days post-MI in humans^{18,19}. Collagen continues to accumulate in the infarct region and the infarct matures through crosslinking, contraction by myofibroblasts, and loss of cellular density¹⁹. Myofibroblasts within the infarct region either undergo apoptosis or differentiate into matrifibrocytes expressing tendon genes⁵¹. Collagen fibers are crosslinked to improve mechanical strength of the infarct region, including enzymatic crosslinks through lysyl oxidase (LOX) and non-enzymatic crosslinks through AGE adducts that build up on collagen due to oxidative stress^{52,53}. In the myocardium bordering the infarct and the remote myocardium, hypertrophy increases, blood vessel density declines, and there is an increase in fibrosis^{42,54}. Fibrosis is classified as either replacement or reactive fibrosis. Replacement fibrosis occurs to replace apoptotic cardiomyocytes; myocyte apoptosis occurs at consistent low rate in the remote and borderzone myocardium during the maturation phase⁵⁵. Reactive fibrosis is the accumulation of collagen fibers in the ECM without cell loss due to overactive fibrotic signaling of TGF- β and Ang2 in the myocardium⁵⁵. Formation of the collagen scar post-MI provides mechanical stabilization to the infarcted heart wall but is often insufficient to maintain contractile function post-MI¹⁹. Pathological remodeling of the myocardium begins during the wound healing process and continues post-MI, leading to progressive loss of cardiac function and heart failure⁵⁶. Therefore, improving infarct repair and wound healing post-MI is a critical therapeutic opportunity to prevent heart failure progression.

1.3 Ventricular Remodeling and Progression to Heart Failure

1.3.1 Increased Wall Stress Post-MI Stimulates Dilative Ventricular Remodeling

Dilative remodeling of the left ventricle (LV) impairs contractile function post-MI, and can lead to systolic heart failure and death⁵⁶. This ventricular remodeling is marked by: an increase in LV volume, hypertrophy of cardiomyocytes, thinning of the infarcted LV wall, fibrosis in the remote myocardium, and progressive loss of contractile function⁵⁷. The extent of dilative ventricular remodeling increases with time and is proportional to the size of the infarct region and the level of LV wall stress⁵⁸. Ventricular remodeling is exacerbated by compensatory mechanisms, such as neurohormonal activation and myocyte hypertrophy, which are activated post-MI to maintain blood volume²². The loss of cardiac muscle following MI impairs the contractile function of the LV myocardium, which is responsible for delivering oxygenated blood to meet the metabolic demands of the body. Neurohormonal signaling is activated in response to the sudden drop in cardiac function post-MI. This can be detected by a drop in blood pressure or volume, but is also activated in response to hypoxia, increased metabolic demand, and increased stress on cardiomyocytes²². The neurohormonal response post-MI can help to maintain stroke volume (SV) and cardiac output (CO), which are the volumes of blood pumped out of the heart per beat and per minute, respectively²². The two neurohormonal pathways upregulated post-MI are the sympathetic nervous system through norepinephrine/ β -adrenergic receptor signaling, and the renin-angiotensin-aldosterone (RAAS) system, that results in an increase in Ang2 signaling within the myocardium⁵⁶. β -adrenergic receptor stimulation leads to increases in heart rate and contractility, while RAAS activation leads to sodium resorption in the kidney, and vasoconstriction that elevates blood volume and the filling pressures in the LV²². In response

to elevated filling pressures and the reduction in oxygenated blood being pumped out of the heart, the LV will dilate to increase CO through the Frank Starling mechanism⁵⁹. Dilation can lead to increased contractile force as myocyte stretching allows for more optimal overlap of contractile proteins. However, this compensatory mechanism can be overwhelmed post-MI. The dilation of the LV becomes progressive and reduces contractile function due to elevation of wall stress⁵⁶. As described by the law of Laplace, wall stress in the LV is proportional to cavity radius and wall thickness. Thereby, dilation of the LV and thinning of the LV wall post-MI leads to increases in wall stress that begets more dilation and thinning of the LV myocardium⁶⁰. Dilation is measured as the volume at the end of the relaxation or filling phase of the cardiac cycle, diastole, and at the end of the contraction phase of the cardiac cycle, systole (EDV and ESV, respectively)⁶¹. Both measurements are increased through ventricular remodeling⁵⁰. SV is the difference between EDV and ESV, while CO is the produce of SV and heart rate⁶¹. Compensatory mechanisms post-MI increase LV filling and EDV leading to improved SV and CO⁶². Left ventricular ejection fraction (LVEF), which is the percentage of blood pumped out of the LV per beat, remains unchanged and is reduced in patients that develop systolic heart failure⁵⁶. EDV, LVEF and ESV are significant predictors of survival post-MI and therapies that aim to reduce ventricular remodeling improve these measurements^{63,64}.

1.3.2 Early Infarct Expansion and Late Dilative Remodeling Post-MI

Clinically, there are two main types of ventricular remodeling: early infarct expansion, which occurs within 4 days of an MI, and late dilative remodeling that can continue for months to years post-MI⁶⁵ (Figure 1.2A). Infarct expansion occurs during the inflammatory phase of wound healing, prior to the deposition of the collagen scar¹⁷. MMP activation by

leukocytes and ROS in the infarct region degrades the ECM, which is the main structural support for the LV wall⁶⁶. The distending pressure of blood on the infarcted LV wall induces myocyte slippage, infarct thinning and LV dilation⁶⁷. The thin infarct region leads to greater wall stress and increases the likelihood of infarct rupture⁶⁸. Past the acute phase of infarct expansion, late dilative remodeling occurs in more than 40% of patients¹⁷. Elevated wall stress leads to progressive LV dilation and mechanical coupling of the borderzone myocardium to the infarct region, furthering contractile dysfunction^{19,69}. The infarct borderzone refers to the region of the myocardium adjacent to the scar. The scar tissue behaves as a passive elastic material during myocardial contraction and either shows minimal displacement or paradoxical bulging at end systole¹⁹. Contractile force of the borderzone myocardium can be lost in lengthening the infarct region⁷⁰. The borderzone myocardium has depressed shortening compared to more remote regions and higher levels of wall stress^{59,69}. The extent of the contractile dysfunction extends to more remote regions over time⁷¹.

1.3.3 Cellular and Molecular Hallmarks of Ventricular Remodeling

Ventricular remodeling also refers to the cellular and molecular changes that occur in the LV post-MI (Figure 1.2B). The myocardium of failing hearts has elevated levels of cardiomyocyte hypertrophy, apoptosis, and contractile dysfunction⁴⁹. Isolated cardiomyocytes from heart failure patients show reduced contractility and poor calcium handling⁷². Myocardial contractility is determined by the association of actin and myosin filaments within the sarcomeres of cardiomyocytes⁶⁰. An increase in cytosolic calcium, released from the sarcoplasmic reticulum, can increase contractility. However, sustained calcium elevation through Ang2 or ROS stimulation can stimulate calcium insensitivity, hypertrophy, and apoptosis in cardiomyocytes⁷³. The contractility of cardiomyocytes is also

reduced in heart failure by altering the phosphorylation state and increasing glycosylation of sarcomeric proteins^{60,74,75}. Elevated wall stress in the remaining myocardium leads to greater energy demand and metabolic dysfunction⁵⁹. In heart failure, the production of ROS overwhelms cellular redox capability and antioxidant defense systems, leading to oxidative stress⁷⁶. ROS react with proteins through, for example, S-nitrosylation, causing nucleic acid damage, and lipid peroxidation⁷⁷. These ROS adducts can lead to altered protein activity, loss of mitochondrial membrane potential, modification of contractile proteins, calcium overload, and cardiomyocyte apoptosis²¹. For example, the lipid peroxidation product, 4-hydroxynoneal (4HNE), increases cardiomyocyte hypertrophy and apoptosis post-MI; and this contributes to a reduction in contractile function and increased LV dilation^{78,79}. An elevated level of cardiomyocyte apoptosis leads to progressive cardiomyocyte dropout that contributes to declining contractile function⁸⁰. These lost myocytes are replaced by reparative fibrosis. In failing hearts, cardiomyocytes also exhibit hypertrophic growth by parallel replication of contractile units⁸¹. In dilative remodeling, cardiomyocyte hypertrophy does not increase LV wall thickness to offset wall stress and reduces contractility⁴⁹. Elevated Ang2 levels contribute to cardiomyocyte hypertrophy in addition to increasing collagen production from myofibroblasts leading to fibrosis and elevating levels of oxidative stress²². In myocytes experiencing elevated mechanical stress, there is also re-activation of the fetal gene program with the consequence of reducing contractility^{82,83}. Finally, chronic inflammation is also detected in heart failure patients with elevated levels of inflammatory cytokines IL-6, TNF- α , and interferon- γ ⁸⁴. Persistent inflammation not only adds oxidative stress but can lead to persistent elevation of MMPs which can degrade the myocardial ECM and contribute to LV dilation^{18,85}.

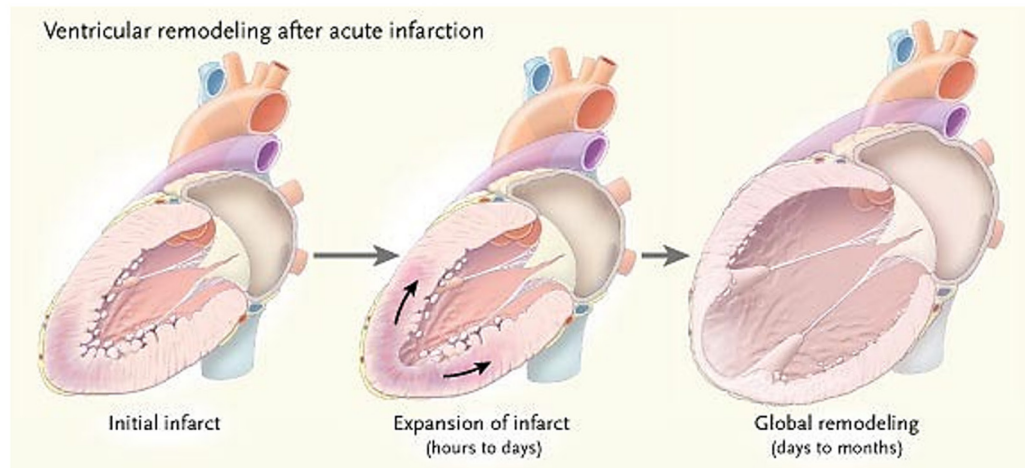
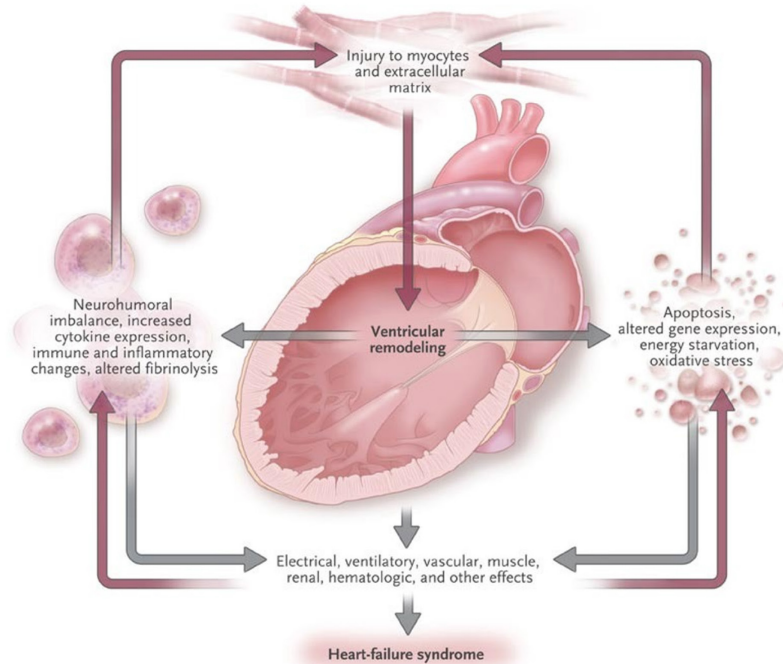
A**B**

Figure 1.2. Ventricular remodeling leads to heart failure post-myocardial infarction. A) The increase in wall stress following cardiac muscle loss from myocardial infarction leads to dilation and thinning of the left ventricle. The dilated left ventricle has reduced cardiac function and results in systolic heart failure. B) Progression to systolic heart failure has several hallmarks including: sustained neurohormonal activation, elevated inflammatory cytokine production, myocyte dropout through apoptosis, and high levels of oxidative stress. (A) Reproduced with permission from Jessup, M., and Borenza, S. (2003). Heart Failure. *N Engl J Med* 348, 2007-2018⁸⁶. Copyright Massachusetts Medical Society. (B) Reproduced with permission from McMurray, J. (2010). Systolic Heart Failure. *N Engl J Med* 362, 228-238⁸⁷. Copyright Massachusetts Medical Society.

1.3.4 Current Therapies that Improve Survival by Limiting Ventricular Remodeling Post-MI

Therapies that improve survival and reduce heart failure post-MI aim to reduce ventricular remodeling⁵⁴. The first aim is to reduce scar size through timely revascularization procedures, such as percutaneous coronary intervention (PCI)⁸⁸. Restoration of blood flow in the infarct-related coronary artery can salvage viable myocardium and leads to the greatest functional recovery if completed within ~90 minutes post-MI⁸⁹. Timely revascularization reduces scar size, limits infarct expansion, reduces LV dilation, and improves survival⁸⁹. However, PCI can also be effective to salvage hibernating myocardium in patients that are delayed more than 90 min in receiving treatment post-MI^{90,91}. One limitation of revascularization therapy is that, based on US data, only 48.9% of patients arrive at hospital within 2 hours of MI and about 23% of MIs are silent¹⁰. Silent MIs result in patients that only access medical care when experiencing symptoms of heart failure. The 30-day mortality rate is greater for patients that receive late revascularization compared to those that received PCI within 90 minutes¹⁰. In addition, revascularization results in ischemia reperfusion (I/R) injury that induces cardiomyocyte death and can account of 50% of the final scar size⁹². I/R causes the rapid restoration of oxygenated blood flow to the ischemic myocardium. This leads to myocardial salvage but also increases ROS and impairment of the microcirculation⁹².

Survival post-MI is also improved by the use of GDMT anti-remodeling drugs such as angiotensin converting enzyme (ACE) inhibitors and β -adrenergic receptor blockers⁶. Both types of drugs reduce the neurohormonal activation post-MI, lower wall stress, and limit LV dilation^{54,93}. ACE inhibitors prevent conversion of angiotensin 1 to Ang2, resulting in lower

distending pressure on the LV wall by lowering blood volume^{94,95}. Additional benefits can be attributed to the reduction in fibrotic and hypertrophic signaling of Ang2⁹³. Anti-remodeling drugs improve cardiac function (LVEF), reduce ESV/EDV, and reduce the risk of all cause mortality post-MI⁶. The increased use of early revascularization and GDMT has helped reduce the 30-day mortality rate post-MI from 20% to 12.4% over the past 20 years¹⁰. However, the progression to heart failure post-MI and 5-year mortality rate remains high¹⁰. GDMT can lead to cardiac functional recovery, ~10% improvement in LVEF, in one-third of heart failure patients^{96,97}. However, this recovery is transient with only partial normalization of gene expression for pathways involved in oxidative stress and cardiomyocyte contractility⁹⁶. This GDMT-based remission in heart failure begins to decline after 8 years and could explain some of the modest outcomes in long-term survival post-MI^{9,98,99}.

The success of current clinical therapies to improve survival post-MI indicate that myocardial salvage, reduction of LV wall stress, and limiting LV dilation can lead to improvement in cardiac function and a reduction ventricular remodeling. However, additional therapies are required to improve long-term survival, reduce progression to heart failure, and improve heart failure symptoms. Hallmarks of ventricular remodeling that have not been targeted by current therapies include: chronic inflammation, oxidative stress, and ECM remodeling¹⁴. Clinical trials for antioxidants and anti-inflammatory therapies have thus far been unsuccessful¹⁴. Anti-remodeling therapies do not aim to restore the active or passive properties of the LV that are lost post-MI, namely cardiomyocytes and the healthy ECM³². Tissue engineering strategies present a promising opportunity to replace lost myocardium or stimulate endogenous regeneration and improve infarct repair¹⁰⁰. While regeneration of lost

cardiomyocytes has been explored with stem cell therapy, another tissue engineering strategy uses biomaterials to restore the healthy ECM and reverse ECM remodeling post-MI.

1.4 Pathological Remodeling of the Extracellular Matrix after Myocardial Infarction

In addition to providing structural support for the myocardium, the cardiac ECM plays a critical role in cardiac regeneration and repair. The ECM provides topographical cues to stimulate cellular homing and organization. This was demonstrated by Ott et al. when they showed that the reseeded of decellularized healthy ECM with cardiac cells led to formation of functional cardiac constructs¹⁰¹. In response to MI, heart failure or other cardiomyopathies, the cardiac ECM undergoes pathological remodeling that impacts heart structure and function³². For example, engineered heart tissue seeded on cardiac ECM from pigs with hypertrophic cardiomyopathy have impaired contractile activity¹⁰². The cardiac ECM is also implicated in cardiac regeneration during development. The adult cardiac ECM is less permissive to cardiomyocyte proliferation by limiting cytokinesis¹⁰³. The stiffness of the cardiac ECM, measured as Young's modulus, increases during development from 12 kPa in neonatal hearts to 39 kPa for adult hearts¹⁰⁴. The increase in stiffness of the cardiac ECM parallels the loss of proliferative potential in cardiomyocytes and is inhibitory to cardiomyocyte regeneration¹⁰⁵.

1.4.1 Structure and Function of the Healthy Cardiac ECM

ECM is a viscoelastic scaffold that maintains myofiber bundle alignment during contraction, allows for optimal force development, and distributes this force evenly over the LV wall⁴⁷. There are three layers to the ECM: the endomysium that surrounds individual cardiomyocytes, the perimysium that surrounds groups of myocytes, or myofibrils, and the

epimysium that surrounds the heart¹⁰⁶. The cardiac ECM is primarily composed of structural proteins including: fibril forming collagens type I and type III as well as elastin⁶⁰. The fibril forming collagens are predominant in the ECM, which is composed of 85% type I collagen and 11% type III collagen¹⁰⁷. The ECM also contains non-structural proteins including: matricellular proteins, non-fibrillar collagens, proteases, fibronectin, growth factors, and proteoglycans⁶⁰. Glycosaminoglycans, such as chondroitin sulfate and heparin sulfate, are also present in the ECM and provide hydration and structural support to the tissue¹⁰⁸. Type I collagen is the primary structural component of the cardiac ECM and forms the majority of the ECM in the epimysium and perimysium, while type III collagen is mainly localized in the endomysium surrounding the cardiomyocytes¹⁰⁸.

Type I and type III collagen are mainly synthesized by cardiac fibroblasts. Collagen amino acid sequences are composed of repetitive sequences of glycine (GLY)-X-Y, where X is commonly proline, and Y is commonly hydroxyproline¹⁰⁹. Post-translational hydroxylation of proline is mediated by prolyl-4-hydroxylase¹¹⁰. Three alpha chains form a triple helical domain that is stabilized by interchain hydroxyproline hydrogen bonding with unfolded regions at each end of the helix¹⁰⁹. Collagen precursors for type I collagen are two $\alpha 1(I)$ and one $\alpha 2(I)$ chain, while type III collagen has three identical $\alpha 1(III)$ chains³². These procollagen monomers are secreted into the extracellular space. The unfolded ends of the triple helix, named propeptides, are removed outside the cells and trigger spontaneous fibril formation¹⁰⁹. Fibrils are supramolecular assemblies of monomers arranged in parallel but staggered by one-quarter of their length (67 nm)¹⁰⁹. Fibrils aggregate into fibers through lysyl-oxidase crosslink formation, which stabilizes and stiffens the growing fibers¹¹⁰. Type I collagen forms thick rod-like fibers, whereas type III collagen fibers are shorter and finer with greater elasticity³². Collagen fibers have a non-linear stress-strain profile. Uncoiling of

the coiled alpha helix structure requires little force but the fibers show greater resistance to deformation once straight¹¹¹. Collagen fibers uncoil during diastolic filling but resist further deformation. Collagen fibers in the perimysium run parallel to myofibrils and protect them from being overstretched beyond the limits that the sarcomere can tolerate¹⁰⁶. The elasticity of the ECM is also critical to allow for recoil during myocardial relaxation, or diastole, so as not impair ventricular filling¹⁰⁶.

Collagen interacts with cells primarily by binding to integrin heterodimers, which are cell membrane receptors¹⁰⁶. Integrins bind to specific recognition sequences, such as the GFOGER and GLOGER peptides in collagen type I fibers¹¹². Similar sequences have been found in the C-terminal region of type III collagen¹¹³. Discoidin domain receptors also can recognize collagen¹⁰⁵. Integrin receptors on cardiomyocytes and capillaries bind to collagen in the endomysium, which maintains their alignment¹⁰⁶. Myofibroblasts sense changes in wall stress or myocardial stretch through integrin receptors and opening of stretch-gated ion channels⁶⁰. Myofibroblasts synthesize new ECM in areas of increased wall stress, parallel to the direction of mechanical stretch¹¹⁴. Mechanical stress determines collagen organization and alignment during ECM remodeling⁶⁰. Collagen is also deposited by myofibroblasts in the infarct region parallel to the direction of mechanical stretch through mechanotransduction in response to wall stress⁷¹. Collagen deposition in the infarct region is necessary to prevent rupture post-MI^{16,19}. Collagen content is not always correlated with stiffness during dilative remodeling in the remote myocardium. Patients with end stage heart failure and dilated ventricles can exhibit increased levels of collagen but low levels of crosslinked collagen in the remote myocardium¹⁰⁶. This may be due in part to persistent inflammation, MMP activation, and immature reparative fibrosis in end stage failing hearts³².

1.4.2 Pathological Modification of the Cardiac ECM

ECM remodeling post-MI is marked by MMP degradation of the healthy ECM, formation of a dense, crosslinked infarct scar, and reparative fibrosis in the remote myocardium³². In dilative heart failure collagen degradation by MMPs can overwhelm collagen synthesis¹¹⁵. Macrophages and oxidative stress increased by mechanical stretch induce collagen degradation and further dilation³². Pathological ECM remodeling was characterized by Perestrelo et al. using decellularized ECM from human heart failure patients that exhibited dilative remodeling¹¹⁶. The ECM from these heart failure patients had aligned, compact, flat collagen fiber bundles with reduced organizational complexity. Pathological remodeling of the ECM disrupted cell homing, limited nutrient diffusion, and stimulated α SMA expression in cardiac fibroblasts¹¹⁶. The ECM in patients with dilative heart failure had increased collagen and proteoglycan content with a loss of perimysial-like fibers that were replaced by a compact structure with straight collagen fibers. The interconnected honeycomb structure of the healthy ECM is lost along with its organizational complexity¹¹⁶. The stiffness of the ECM is three-fold lower in the remote myocardium of dilation heart failure patients with less resistance to deformation¹¹⁶.

In addition to these pathological modifications, the cardiac ECM accumulates crosslinks through advanced glycation end products (AGEs) in response to elevated oxidative stress¹¹⁷. AGEs were first characterized in diabetic patients with diastolic dysfunction and were found to result in crosslinks that increased LV stiffness and limited LV filling^{118,119}. AGE levels also correlate with the severity of systolic heart failure post-MI^{120,121}. AGEs are formed from non-enzymatic crosslinking of glucose or a reactive dicarbonyl, such methylglyoxal, to terminal amines of lysine or arginine residues¹¹⁹. The initial product is a Schiff base which rearranges into an Amadori product then finally a stable AGE¹²². AGEs such as

carboxymethyllysine (CML) are nearly irreversible, whereas the most prominent AGE, methylglyoxal-derived hydroimidazolone 1 (MG-H1), has a shorter half-life of 2-3 weeks¹²². AGEs are cleared by proteolysis or the glyoxalase system in the presence of reduced glutathione¹²². A primary target for AGE formation is collagen, as it has a half-life 10 times longer than other myocardial proteins¹²³. AGEs stimulate inflammation, ROS production, apoptosis, and reduce cellular adhesion to the ECM¹²⁴⁻¹²⁶. AGEs can also reduce progenitor recruitment and homing for therapeutic cell delivery^{127,128}. In mice with MI, MG is upregulated within 6 hours of infarction and methylglyoxal-modified collagen remained upregulated for 28 days⁵³. MG-H1 levels were associated with reduced cardiac function and cardiomyocyte apoptosis post-MI⁵³. MG-H1 modification of myocardial contractile proteins has also been linked to reduced contractility in diabetic patients with heart failure⁷⁵. In patients with systolic heart failure, AGEs induce: ROS, cardiomyocyte death, replacement fibrosis, and NFkB pro-inflammatory pathways¹²⁹.

Current clinical therapies to reduce heart failure post-MI, namely revascularization and neurohormonal blockade, improve heart function but disrupt the collagen matrix¹⁷. The pathological modification of the ECM contributes to impaired mechanical support and infarct repair post-MI. Therefore, therapies that restore the healthy ECM post-MI could lead to improved infarct repair and limit LV dilation to prevent the progression to heart failure.

1.5 Tissue Engineering for Cardiac Repair

Tissue engineering was originally described as “an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function”¹³⁰. Tissue engineering

strategies use a combination of stem or progenitors cells, growth factors, and/or an ECM scaffold to develop tissue constructs for organ repair¹³¹. Tissue engineering strategies for cardiac repair post-MI aim to develop biomaterials that: provide mechanical support for the infarct tissue, improve infarct healing, and regenerate vasculature and cardiomyocytes¹⁰⁰. The end goal is the improvement of cardiac function and the limitation of ventricular remodeling post-MI to prevent heart failure. The ideal biomaterial therapy would be a full thickness cardiac graft which could replace the infarct scar with functional heart muscle. Considerable preclinical progress has been made in generating cardiac grafts using induced pluripotent human stem cells (iPSC) grown in bioreactors with growth factors and ECM scaffolds¹³². There are limitations to thickness imposed by limited blood supply for the graft *in vivo*, however with improved bioreactors, full thickness grafts are currently being tested in large animals of MI^{132,133}. The limitations still persist in the functional and electrical integration of the grafts within the remaining myocardium post-MI, and arrhythmias can occur¹³⁴. The risk of tumorigenicity is also present with pluripotent stem cells¹³². Despite the pre-clinical progress in developing cardiac grafts, they have yet to be translated to clinical trials.

1.5.1 Stem Cell Therapy to Improve Cardiac Function After Myocardial Infarction

In clinical trials, stem cell delivery to the infarct region has been the most prevalent tissue engineering strategy. Originally, allogenic progenitor cells isolated from patient bone marrow were delivered to the heart post-MI in an attempt to promote angiogenesis and revascularize the infarct region^{135,136}. Pre-clinical studies in animal models of MI showed promising results with improvement in LVEF and reduction of scar size and ventricular volumes with stem cell injection¹³⁷. However, clinical trial results have been mixed¹³⁸. The

injection of stem cells was safe and could be completed in a minimally invasive manner using a catheter to deliver cells through the infarct related coronary artery¹³⁸. Meta analyses of clinical trials for stem cell therapy post-MI showed significant but modest increase in LVEF (~3% change from placebo) when injected less than 14 days post-MI and following up more than 3 months later¹³⁹. This resulted in significant reduction in the risk of mortality and hospitalization for heart failure¹⁴⁰. However, individual trial results are variable with 7 trials showing a 7.2% increase in LVEF at 6-month follow-up and 14 trials showing no effect of stem cell therapy on cardiac function¹⁴¹. In addition, the two most recent randomized, controlled clinical trials for stem cell therapy post-MI, BAMI and ALLSTAR, did not show a significant effect of treatment on primary outcomes, which were improved survival and infarct scar size reduction, respectively^{142,143}.

Critical limitations to bone marrow or myocardium derived progenitor cell injection post-MI are that they exhibit minimal engraftment in the heart and do not appreciably differentiate into new cardiomyocytes or vasculature¹⁴⁴. About 0.1% to 10% of cells are retained within the infarct a few weeks post-injection¹³⁸. The cells that do have more favorable engraftment, skeletal myoblasts, and human iPSC (pre-clinical models), form isolated myocyte islands in the infarct and led to an increased risk of arrhythmia in patients due to poor electrical and functional integration with the myocardium^{134,145}. Minimal cell engraftment is due to wash out from the beating heart post-injection and apoptosis of cells in the harsh microenvironment of the infarct region^{146,147}. The majority of the benefits of cell therapy are derived from a paracrine response to cell injection¹⁴⁸. The paracrine hypothesis was initially proposed when conditioned media from mesenchymal stem cells resulted in a similar improvement in cardiac function post-MI compared to cell injection¹⁴⁹. Stem cells

secrete growth factors, miRNAs, and exosomes, and interact with the immune system to: reduce inflammatory cytokine signaling, improve cardiomyocyte contractility, induce angiogenesis, promote cardiomyocyte proliferation, and limit fibrosis^{150–153}. Understanding the role of paracrine signaling in stem cell therapy mediated improvement of cardiac function has led to a trend towards utilizing acellular materials, such as: exosomes, growth factors, or biomaterials, to improve cardiac function post-MI^{100,144}.

1.5.2 The Emergence of Biomaterials for Cardiac Repair to Improve Stem Cell Retention

Biomaterials that mimic the healthy cardiac ECM, such as epicardial patches or injectable hydrogels, were originally used to improve retention of therapeutic stem cells post injection¹⁴⁷. Biomaterials are engineered materials that aim to interact with the body to restore function or promote repair of damaged tissue¹⁵⁴. Biomaterials can protect cells from the pathologically remodeled ECM environment in the infarct region. Pathologically modified ECM post-MI can reduce cell engraftment, cause anoikis (death from cell detachment), and elevate oxidative stress, inflammation, and apoptosis¹⁴⁷. Preclinical evaluation of stem or progenitor cells embedded in patches, injectable hydrogels, or cocooned in micelles showed benefits of combined therapy^{155–165}. These observed benefits were derived from either increased cell persistence in the infarct or preconditioning that yields a more potent paracrine response¹⁴⁷. Combined biomaterial and cell delivery of bone marrow cells in a collagen graft was used in the MAGNUM clinical trial¹⁶⁶. In a small sample of 20 patients, the epicardial patch was placed during coronary artery bypass grafting (CABG) surgery and patients treated with the combination of cells within a patch showed less dilation in EDV than cells alone¹⁶⁷. The improvements in cardiac function observed in

this study are difficult to interpret due to the combination of patch implantation and CABG, which itself restores cardiac function.

Intriguingly, initial studies by Christman et al. showed that a biomaterial scaffold alone, namely a fibrin matrix, could preserve cardiac function, limit LV dilation, and reduce infarct size to the same extent as skeletal myoblasts or the combined therapy^{168,169}. Since these initial studies, a number of biomaterials including cardiac patches and injectable hydrogels have successfully been applied to reduce dilation of EDV/ESV, improve cardiac function, and reduce scar size in pre-clinical models of MI^{170,171}. Successful biomaterials for infarct repair and preventing ventricular remodeling are composed of synthetic polymers, natural polymers, or a combination of the two. Synthetic polymers that limited ventricular remodeling and improved cardiac function post-MI include: degradable polyethylene glycol (PEG), α -cyclodextrin (α -CD)/MPEG-PCL-MPEG, and co-polymerizations of N-isopropylacrylamide (NIPAAm)^{172–174}. Natural polymers used for infarct repair include: collagen, alginate, platelet gel, hyaluronic acid, and decellularized ECM^{155,175–179}. Biomaterials have also been used to have localized delivery of growth factors and anti-inflammatory molecules to stimulate infarct repair such as vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), insulin-like growth factor 1 (IGF-1), IL10, and TIMP3^{180–184}. However, increasing the complexity of tissue engineered constructs can potentially limit clinical translation, especially if the construct contains bioactive components such as cells and growth factors¹⁸⁵. Biomaterials alone can provide a simple approach while still being effective in mediating infarct repair and improving cardiac function post-MI. While clinical regeneration of functional cardiac tissue may be a few decades away, there is still a possibility to stabilize cardiac function post-MI to prevent

ventricular remodeling¹⁸⁶. To this end, acellular biomaterials that mechanically stabilize the infarct region and improve infarct repair can reduce remodeling and limit progression to heart failure¹⁸⁶. This could have a significant impact on clinical outcomes post-MI even without inducing cardiomyocyte regeneration or proliferation.

1.6 Acellular Biomaterial Therapies for Cardiac Repair

1.6.1 Biomaterials for Structural Support and Infarct Repair post-MI

Biomaterial scaffolds can restore tissue mechanical properties, provide cell adhesion domains, modulate inflammation, and can release bioactive peptides upon degradation¹⁸⁷. All of these factors can stimulate endogenous wound repair from resident cells. Acellular biomaterials create a new microenvironment within the infarcted heart and can lead to salvage of reversibly damaged tissue, blood vessel regeneration and infarct repair by modulating cell behaviour¹⁸⁸. There are two main mechanisms proposed to explain the improvement of cardiac function with biomaterial injection post-MI: mechanical support through bulking the infarct wall and endogenous repair by a bioactive scaffold that restores the healthy cues of the cardiac ECM¹⁷¹ (Figure 1.3).

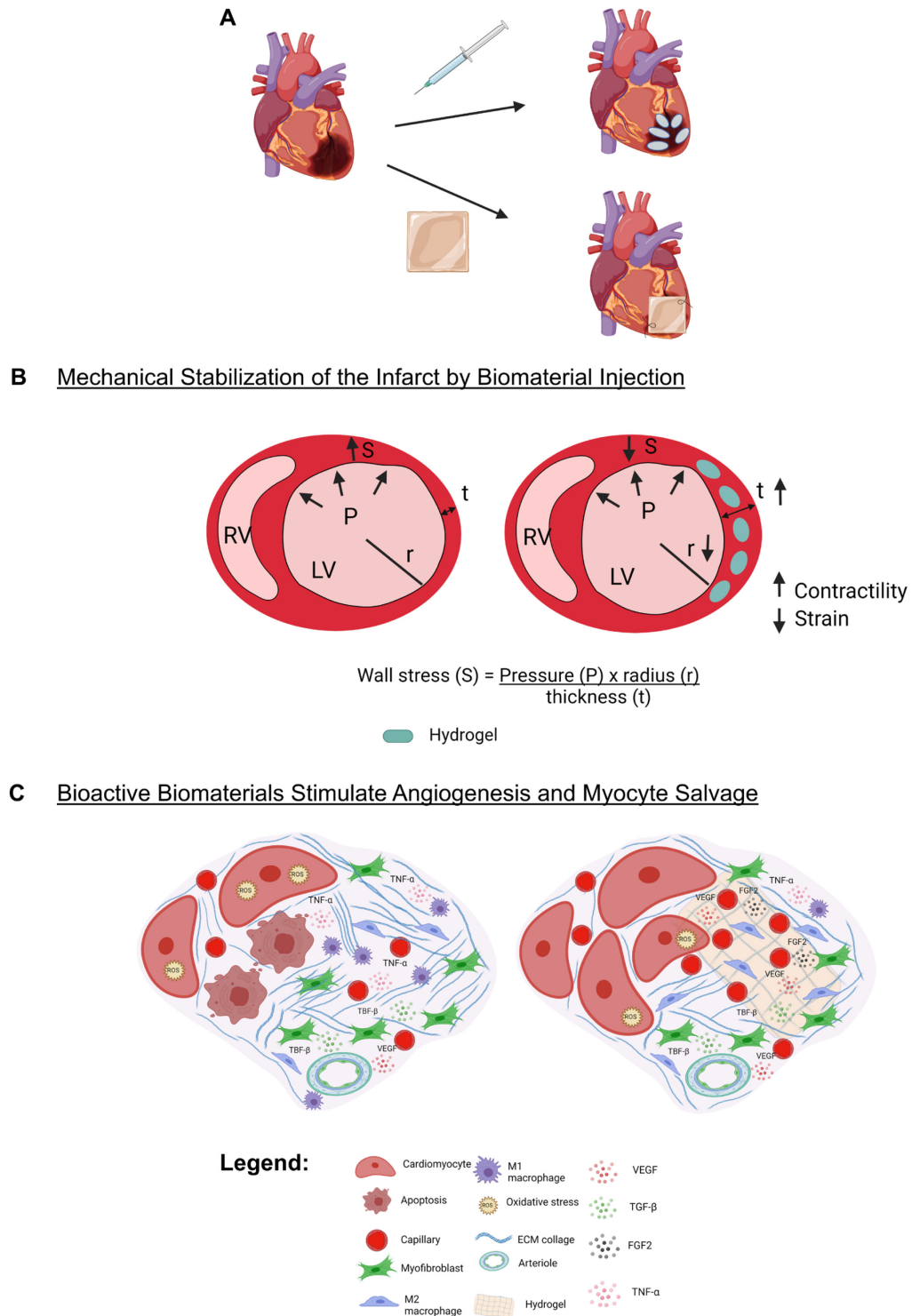


Figure 1.3. Biomaterial therapy to prevent ventricular remodeling and stimulate infarct repair after myocardial infarction. A) Biomaterials can be delivered to the borderzone and infarct regions after myocardial infarction as injectable hydrogels or as epicardial patches. B) Biomaterial delivery can thicken the infarct wall and offset wall stress in the infarct and borderzone through LaPlace's law. Borderzone strain is reduced, indicating improved

contraction of borderzone myocardium with biomaterial delivery. The mechanism of action was observed when delivering alginate hydrogel Algisyl-LVR to the infarct region. C) Bioactive biomaterials can improve infarct repair by increasing angiogenesis, reducing pro-inflammatory cytokines, reducing oxidative stress, and reducing cardiomyocyte apoptosis. This can lead to greater myocardial salvage. This mechanism of action was observed when delivering CorMatrix epicardial patch, decellularized porcine ECM and collagen-chondroitin hydrogels to the infarct region. Abbreviations: extracellular matrix (ECM), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), fibroblast growth factor 2 (FGF2), and tumor necrosis factor α (TNF- α). Created with BioRender.com

Computational modeling using a finite element model of the heart showed that an increase of 4.5% in volume of the infarct wall can lead to reduction in stress in borderzone myocardial fibers similar to that of the more remote regions¹⁸⁹. Increasing the thickness of the infarct region can offset wall stress through the relationship of LaPlace's law, thereby limiting dilation of the LV. Current clinical therapies such as drugs that block neurohormonal blockade lead to reduced wall stress in part by decreasing blood volume, which is a distending pressure on the heart⁹³. Mechanical unloading of hearts using a left ventricular assist device (LVAD) as a bridge to transplant can improve contractility of cardiomyocytes¹⁹⁰. Mechanical support by injectable biomaterials such as calcium hydroxyapatite microspheres, alginate, and hyaluronic acid hydrogels, have been studied in large animal models using magnetic resonance imaging (MRI) and finite element model analysis^{191–195}. In these models, increasing the infarct thickness through injectable materials can offset infarct and borderzone wall stress and inhibit dilation^{192,194}. In addition, injectable hydrogels, can reduce dyskinetic motion of the heart and improve borderzone strain, or contractility through infarct stabilization^{191,193}. Hydrogel injection was predicted to improve cardiac function (LVEF) by ~14%, which was confirmed through intramyocardial injection of Algisyl-LVR hydrogel in a canine model of heart failure^{176,193,196}.

The second mechanism by which injectable hydrogels can improve cardiac function post-MI is through bioactive properties of the material to stimulate infarct repair¹⁷⁰. Biomaterials composed of natural polymers such as collagen, platelet derived hydrogel, or decellularized matrices can provide sites for cell adhesion and recognition^{155,197,198}. These materials have been shown to modulate infarct repair through: inducing angiogenesis in the infarct region, reducing cardiomyocyte apoptosis, inducing pro-healing inflammatory responses (M2 or T_H2 cell polarization), and reducing fibrotic signaling from myofibroblasts¹⁹⁹. CorMatrix material, an epicardial patch made of decellularized small intestinal submucosa ECM (SIS-ECM), improves contractility and reduces dilation post-MI¹⁷⁹. The CorMatrix therapy mediates this improvement in cardiac function and ventricular remodeling through stimulation of angiogenesis and expression of angiogenic growth factors from infarct myofibroblasts (VEGF and fibroblast growth factor 2 (FGF2))²⁰⁰. A CorMatrix that was inactivated through a high degree of crosslinking did not show improvement in cardiac function even though infarct wall thickness was increased²⁰⁰. A decellularized cardiac ECM hydrogel promotes salvage of cardiomyocytes in the infarct region through an increase in cell survival pathways, reduction in apoptosis, and increase in angiogenesis^{178,198}. Therefore, the injection of biomaterials can improve cardiac function by promoting wound healing in the infarct region, reducing oxidative stress, and improving contraction in the borderzone myocardium to better stabilize cardiac function post-MI.

1.6.2 Delivery Strategies for Biomaterials

For clinical translation of biomaterials for cardiac repair, the method of delivery must be considered. Epicardial patches require opening of the chest wall through sternotomy and are applied after CABG surgery which will limit the eligible patient population^{167,201}.

Hydrogels are injectable materials that can be designed to be delivered by a catheter using similar techniques as currently used in PCI revascularization^{147,202}. Intramyocardial injection through the endocardial surface can be accomplished through a specialized catheter using NOGA mapping technology currently used for arrhythmia ablation^{178,203,204}. Epicardial delivery of injectable materials may be possible in the future through newly developed catheters that can deliver injectable materials in the pericardial space^{205,206}. Hydrogels must be designed to meet the physical (degradation and mechanical properties) and biological (cell adhesion, blood vessel regeneration, and salvage of reversibly damaged myocardium) needs of the injured tissue²⁰². Hydrogels are designed to gel *in situ* and provide a unique means for non-invasive delivery²⁰⁷. To be suitable for non-invasive delivery, a hydrogel needs to be able to be stable within the catheter, rapidly gel once exposed to the heart, and be hemocompatible as there is a possibility of leak into the circulation²⁰⁸. Several stimulus-responsive hydrogels undergo sol-to-gel transition in response to physiological stimuli including: shear stress during injection, temperature, and ion concentration²⁰⁹. Therefore, biomaterial therapies can be designed to be delivered through minimally invasive procedures post-MI, similar to the methods used for stem cell therapies.

1.6.3 Biomaterial Properties that Influence Cardiac Repair

Biomaterial properties such as degradation rate and material stiffness are important considerations as they can impact performance in pre-clinical models of MI. The effectiveness of non-degradable hydrogels made from synthetic polymers has been limited to transient prevention of LV dilation^{210,211}. Studies with non-degradable PEG hydrogels demonstrated that increases in wall thickness alone do not produce long term limitation of

ventricular remodeling^{210,211}. Biomaterials and mechanical devices made from synthetic non-degradable polymers can exhibit a foreign body response (FBR) with fibrosis capsule formation around the device²¹². The device is considered stable if the FBR resolves within a month, limiting chronic inflammation. However, as currently constructed, these materials do not stimulate wound healing²¹². Degradable synthetic polymers including PEG hydrogels with MMP degradable sequences and NIPAAm copolymers show superior performance to non-degradable PEG for improvement of cardiac function in pre-clinical models^{172,173,213,214}. The properties of synthetic polymers are easier to tune than natural materials, allowing careful design of degradation time and stiffness¹⁷⁰. Degradable synthetic polymers improve the biocompatibility compared to permanent implants but can be limited due to the toxicity of their degradation products¹⁴⁹.

Studies of degradable synthetic polymers and hyaluronic acid hydrogels have shown benefits with injection of more stable hydrogels that have a slower degradation rate (>10 weeks on average)^{170,214}. While hydrogel-mediated benefits on cardiac function did not change with degradation rate, parameters of infarct thickness and preservation of ventricular volumes were improved with more stable hydrogels. One study of natural hydrogels alginate and fibrin showed that cardiac function improved 2 days post-injection for both treatments but declined for fibrin due to degradation prior to the five-week endpoint²¹⁵. However, hydrogels designed to induce endogenous repair may not require persistence in the infarct region to improve cardiac function^{155,175}. Another property of biomaterials that can affect performance *in vivo* is material stiffness. For cardiac repair, a wide range of material stiffness from 1-100 kPa has been used¹⁷¹. For hyaluronic acid hydrogels that limit dilation post-MI, 43 kPa hydrogels better preserved infarct thickness and ventricular volumes than 8 kPa

hydrogels²¹⁶. In contrast, alginate showed improvements in cardiac function and reduced end systolic wall stress with a soft elastic modulus of $\sim 3\text{-}5\text{ kPa}$ ²¹⁷. A material that has too high of an elastic modulus can impede diastolic filling²¹⁸.

The design of a biomaterial and its intended mechanism of action, mechanical stabilization, or endogenous repair, can determine its optimal delivery timepoint post-MI²⁰². The goal is to maximize the benefits of material delivery while not interfering with the temporal phases of wound healing in the infarct region. A collagen-chondroitin hydrogel showed superior improvement in cardiac function when delivered earlier, 3 hours vs. 7 days post-MI¹⁷⁵. Three hours post-MI, the material reduced cardiomyocyte apoptosis and increased capillary density in the infarcted region. An early delivery timepoint therefore improved myocardial salvage. For synthetic hydrogels, NIPAAm and degradable PEG, delayed delivery during the proliferation phase of MI wound healing, i.e. 3 days or 1-week post-MI, improved performance compared to immediate injection^{172,213}. The immediate injection timepoint, which is during the inflammatory phase of wound healing post-MI, resulted in more rapid degradation of the material. Greater persistence of these materials was associated with greater improvement in cardiac function and limitation of ventricular remodeling.

1.6.4 Acellular Biomaterials in Clinical Trials to Improve Cardiac Function Post-MI

There have been four types of biomaterials that have progressed to clinical trials: a polyester mesh support device (Acorn CorCap), injectable alginate hydrogels (IK-5001 and Algisyl-LVR), a decellularized SIS-ECM epicardial patch (CorMatrix), and a decellularized porcine pericardial ECM hydrogel (VetriGel)¹⁷¹. The CorCap mesh was surgically implanted as a ventricular restraint device to prevent dilation in patients with dilated

cardiomyopathy. The device reduced LV dilation and heart failure symptoms at 5-year follow-up but was discontinued due to concerns of pericardial constriction and abrasions²¹⁹. Alginate hydrogels can be delivered into the infarct region and will gel there due to high levels of calcium ions²²⁰. Alginate biomaterials have low bioactivity for natural polymers but are not thrombogenic or immunogenic²²⁰. In the PRESERVATION 1 trial, the IK-5001 alginate hydrogel was delivered to patients at 2-5 days post-MI through a catheter into the infarct-related coronary artery²²¹. The material was safe, however there was no detected improvement in limiting dilative remodeling or the prevalence of heart failure at 6-month follow-up²²². Implementation of IK-5001 in porcine models showed reduction in LV volumes but no improvement in systolic function²²³. The difference in results may be attributed to the volume of material delivered or the fact that the benefit of hydrogel injection in addition to current pharmaceutical therapies to reduce remodeling are not tested in pre-clinical models¹⁷¹.

In contrast, another injectable alginate hydrogel, Algisyl-LVR, was delivered through epicardial, intramyocardial injection in patients through a left anterior limited thoracotomy²⁰¹. Patients enrolled in this study, AUGMENT-HF, had advanced heart failure²²⁴. At 12-month follow-up patients with Algisyl-LVR therapy had improved VO₂ max, 6-min walk distance, and New York Heart Association Functional Class (NYHA)²²⁵. While Algisyl-LVR improved clinical symptoms and exercise capacity, there was no change in ventricular volumes²²⁵. This clinical trial was not powered to detect changes in LV echocardiography changes. In pre-clinical studies, Algisyl-LVR injection led to improvements in cardiac function and wall stress within 3 hours that were sustained for several months in dogs with heart failure^{176,196}. The bioactivity of Algisyl-LVR is not well

characterized but alginate hydrogels do improve blood vessel density in pre-clinical models²¹⁵.

The bioactivity of decellularized ECM materials CorMatrix and VentriGel were integral to their capacity to improve cardiac function in pre-clinical models²⁰⁰. The CorMatrix ECM scaffold is currently in a Phase I clinical study to establish safety (NCT02887768). CorMatrix has previously been used patients for repair of cardiovascular tissues including main artery defects and valve repair^{226–228}. In many cases the material is well tolerated, stimulates tissue remodeling and can promote a pro-healing inflammatory response^{229,230}. However, one trial of semilunar valve repair in children showed graft rejection and inflammatory cell infiltration^{228,231}. These results were confirmed in an ovine model, with chronic inflammation and with little signs of tissue remodeling²³². Some trials showed good mechanical support but no endogenous tissue repair^{233,234}. The application of CorMatrix in a porcine model showed promising results for improved infarct repair and cardiac function post-MI^{179,200}.

The second decellularized ECM, VentriGel, is a cardiac ECM injectable hydrogel which is delivered through endocardial injection²³⁵. In a porcine model, injection of VentriGel two weeks post-MI was able to better preserve cardiac function, ventricular volumes, and reduced scar expansion compared to placebo¹⁷⁸. In a rat model, injection of VentriGel improved angiogenesis, reduced cardiomyocyte apoptosis, and reduced oxidative stress in the infarct region¹⁹⁸. The first-in-man study of VentriGel injection established the safety and feasibility of catheter-guided intramyocardial injection of the hydrogel in patients 60 days to 3 years post-MI²⁰⁴. This trial had only 15 patients and was not powered to detect changes in cardiac functional parameters. A significant improvement in 6 min walk distance

from baseline with VentriGel injection was detected. Decellularized ECM materials have the benefit of containing the diverse proteins that make up the tissue ECM¹⁸⁷. Cardiac ECM hydrogels are primarily composed of type I and type III collagens with smaller concentrations of matricellular proteins and growth factors²³⁶. The response to decellularized ECM materials *in vivo* is determined by the decellularization procedure (removal of cell debris that can act as DAMPs and trigger an inflammatory response), source tissue, and cross-linking density (Ref). One limitation of decellularized porcine ECM is the concern that xenotransplantation of materials from other species can lead to an acute or chronic inflammatory reaction and rejection of the material^{229,237,238}. Human cardiac ECM materials are limited by donor source variability and pre-existing conditions which creates variability in hydrogel formation, therefore porcine cardiac ECM is preferred²³⁹. In contrast to decellularized ECM, ECM mimicking hydrogels can be prepared from purified collagen solutions providing a bioactive scaffold for tissue repair.

1.7 Collagen Glycosaminoglycan Hydrogels for Infarct Repair Post-MI

As collagen is the major structural component of the ECM, collagen has been used extensively in tissue engineering applications¹⁰⁹. Collagen can be extracted from animal sources and contains collagen monomers with various concentrations of crosslinked collagen²⁴⁰. For cardiac repair, unmodified collagen injection into the infarct region increased scar thickness and limited ventricular dilation in a rodent model of MI²⁴¹. Collagen-glycosaminoglycan (GAG) crosslinked materials show improved mechanical properties and hydration²⁴². Collagen-GAG scaffolds or hydrogels exhibit an open porous structure with crosslinks between collagen fibers and GAGs within the pore struts²⁴³. Chondroitin sulfate is the most common GAG in the human body and is composed of D-glucuronic acid and N-

acetyl galactosamine units and is commonly used in collagen-GAG materials²⁴². Collagen-chondroitin sulfate patches were one of the first bioactive biomaterials tested in humans and are successfully used to regenerate full thickness skin wounds²⁴⁴. In approximately 1500 cases of collagen-chondroitin sulfate skin graft use there has not been an adverse response in humans²⁴³. Collagen-chondroitin sulfate scaffolds are also used clinically to stimulate peripheral nerve regeneration²⁴⁵. These scaffolds guide Schwann cell migration and axon formation between nerve stumps to stimulate peripheral nerve regeneration²⁴⁶. Collagen-chondroitin sulfate biomaterials have also been used to fabricate bioartificial corneas for *in vitro* characterization of factors that stimulate corneal nerve regeneration²⁴⁷.

Collagen-chondroitin sulfate hydrogels have also showed promising results as biomaterials for cardiac repair. Collagen hydrogels can be prepared on ice and are designed to gel at physiological temperature, allowing *in situ* hydrogel formation within the infarct region and the possibility of minimally invasive delivery²⁴⁸. In our lab collagen-chondroitin hydrogels were originally investigated as a delivery vehicle for circulating angiogenic cells (CACs), which are a highly angiogenic cell population that showed promise to revascularize the infarct region²⁴⁹. An injectable hydrogel composed of rat tail collagen type I and chondroitin sulfate (0.25% collagen and 1.1% chondroitin sulfate w/v) crosslinked with glutaraldehyde increased CAC retention and capillary formation in the hindlimb²⁴⁹. This collagen hydrogel formulation also improved the angiogenic potential of CACs when used as a culture substrate instead of fibronectin²⁵⁰. In a mouse model of MI, delivery of CACs and hydrogel 1-week post-MI led to improved cardiac function and greater myocardial perfusion compared to controls at the 28-day follow-up¹⁹⁷. The hydrogel alone maintained cardiac function at baseline levels while the phosphate buffered saline (PBS) control group

declined in function over time. There was also smaller infarct size with the hydrogel alone. However, the combination therapy was superior, and the benefits of cell encapsulation were mediated by $\alpha 2$ integrin interactions of CACs with the collagen hydrogel. The acute retention of the hydrogel was also confirmed 2 hours post-injection and was located within the infarct with minimal leakage into the lungs²⁴⁸. In a porcine model of hibernating myocardium, the combination of collagen hydrogel and CAC delivery reduced the level of myocardial hibernation and improved perfusion¹⁵⁶.

Collagen-chondroitin sulfate hydrogels have also been investigated as a standalone therapy for cardiac and striated muscle repair. The original hydrogel formation was modified from a glutaraldehyde crosslinking system to a carbodiimide crosslinking mechanism using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). The EDC/NHS crosslinking mixture results in the formation of covalent bonds between collagen fibers and between collagen and chondroitin sulfate^{251,252}. With this reaction, the stoichiometry of the crosslinker and protein minimizes potential toxicity²⁵³. The EDC/NHS crosslinked collagen-chondroitin hydrogel was able to restore perfusion, recruit bone marrow derived progenitors, and induce blood vessel formation in a hind limb ischemia model^{254,255}. The collagen-chondroitin sulfate hydrogels also induced myogenesis in response to a necrotic stimulus in a mouse model of muscular dystrophy, which improved hindlimb function²⁵⁶. In a mouse model of MI, the EDC/NHS crosslinked rat tail collagen hydrogel improved cardiac function at 4-week follow-up compared to PBS injected mice when delivered 3 hours and 7 days post-MI, but not 14 days post-MI¹⁷⁵. The improvement in cardiac function was greatest when delivered at the earlier, 3-hour timepoint. The injection of the collagen hydrogel increased infarct capillary density, reduced

cardiomyocyte apoptosis, and reduced pro-inflammatory macrophage infiltration. A modified collagen-chondroitin hydrogel with the addition of chitosan was able to improve cardiac function 4 weeks post-injection from baseline at 2 weeks post-MI²⁵⁷. This collagen-chitosan-chondroitin hydrogel reduced MMP/TIMP ratio reducing excess ECM degradation by myofibroblasts. Therefore, collagen-chondroitin sulfate hydrogels have shown promise as a therapy for cardiac repair post-MI. One drawback of the material was that it used animal-derived collagen. There remain concerns about hypersensitivity reactions to animal-derived collagens (~3% of patients), batch-to-batch variability of the collagen solutions, and the possibility of viral contamination²⁵⁸.

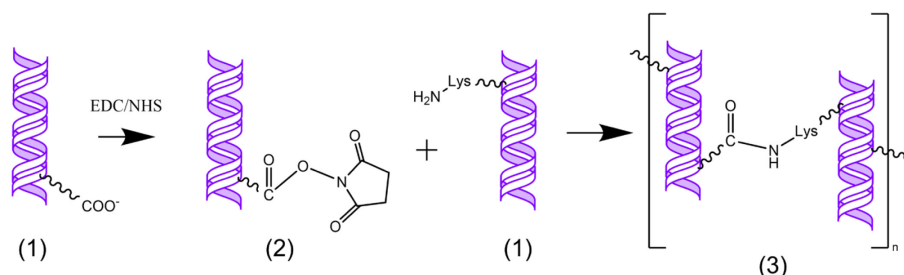
1.8 Recombinant Human Collagen Hydrogels for Cardiac Repair

Recombinant human collagens (rHC) can be produced in a number of genetically modified cells including yeast and tobacco plants²⁵⁸. The cells can be engineered to express the human collagen peptides and P4H which is necessary for triple helix formation in collagen monomers²⁵⁸. Collagen biomaterials for corneal replacement have been made with rHCI type I (rHCI) and rHC type III (rHCIII) and crosslinked with EDC/NHS²⁵⁹. In a pre-clinical model, both rHCI and rHCIII corneal implants had similar mechanical properties to a healthy cornea and were both able to regenerate the cornea through reepithelization and nerve growth²⁵⁹. This was surprising as rHCIII is only a minor component in human corneas. The rHCIII hydrogel progressed to a clinical trial due to its superior optical properties²⁵⁹. In 10 patients, rHCIII corneal implants led to regeneration of corneal tissue and improved vision at 4-year follow-up²⁶⁰. The implants did not stimulate an immune response and did not require chronic immunosuppressive therapy, unlike human donor corneas^{260,261}. The rHC hydrogels are manufactured in a closed mixing system and meet good manufacturing practice

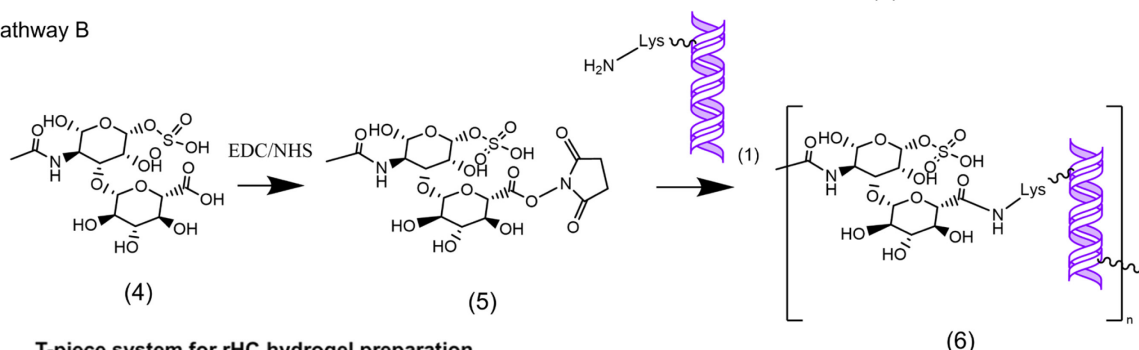
(GMP) guidelines for material preparation²⁶². To facilitate clinical translation, preparation of collagen-chondroitin hydrogels for cardiac repair will require a scalable, GMP quality standard operating protocol (SOP). Due to the success of the GMP procedure for preparation of rHC corneal substitutes in clinical studies, a similar procedure was used to develop the first rHC-chondroitin sulfate hydrogels for cardiac repair (Figure 1.4). Both rHCI and rHCIII hydrogel formulations were optimized, as these are the most prevalent proteins in the cardiac ECM¹⁰⁸.

rHC Hydrogel Preparation

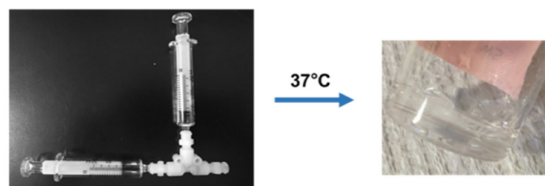
Pathway A



Pathway B



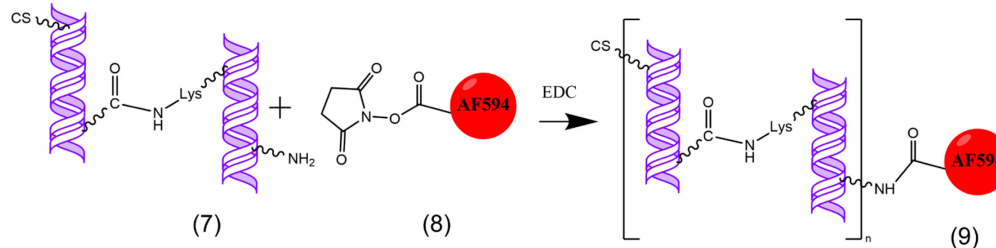
T-piece system for rHC hydrogel preparation



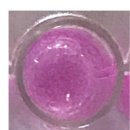
At 37°C an ECM-mimicking, porous collagen scaffold is obtained

- (1) 1% rHC collagen
 - (2) rHC collagen-NHS intermediate
 - (3) Crosslinked rHC collagen matrix
 - (4) Chondroitin sulfate
 - (5) Chondroitin sulfate-NHS intermediate
 - (6) Chondroitin sulfate modified rHC collagen matrix
 - (7) rHC collagen-chondroitin (CS) matrix
 - (8) AF594® NHS ester
 - (9) AF594® labeled rHC collagen-chondroitin matrix
- EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
NHS: N-Hydroxysuccinimide

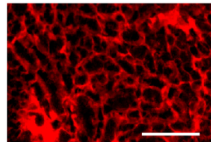
AF594 Labeled rHC Hydrogels for Tracking *In Vivo* Degradation



AF594 rHC



rHCIII



rHCI

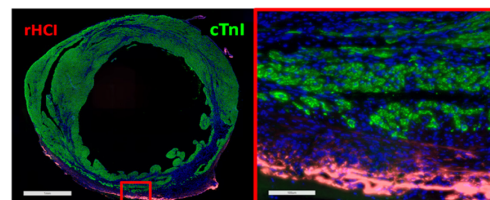
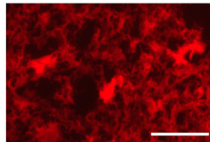


Figure 1.4. Preparation of recombinant human collagen hydrogels for infarct repair. The recombinant human collagen (rHC) hydrogels were prepared by crosslinking collagen fibrils (Pathway A) and crosslinking chondroitin sulfate to collagen fibrils (Pathway B). The crosslinking uses EDC/NHS chemistry to form an amide bond between a lysine residue in

collagen and the activated carboxy group of another collagen molecule or chondroitin sulfate. The hydrogels are prepared in a closed T-joint mixing system and gel at 37°C. Two hydrogels were prepared from rHC type I and type III (rHCI and rHCIII). The EDC/NHS chemistry was also used to label the hydrogel with AF594® fluorescent dye. An AF594®-NHS ester compound added to the hydrogel crosslinking mixture that contained EDC. The AF594® labeled hydrogels show a red fluorescence labeling the collagen fiber pores of rHCI and rHCIII. Scale bar is 50 µm. The red fluorescence can also be detected *ex vivo* in histology sections of mice injected with rHC hydrogels. Cardiac troponin is seen in green and DAPI nuclear marker in blue. The scale bar for the left histology image is 1mm and the right histology image has a scale bar of 100 µm. Chemical diagrams created using ChemDraw Professional v. 20.1.

1.9 Thesis Objective, Aims and Hypotheses

The main objective for this thesis was to characterize the effect of the first rHCI and rHCIII hydrogels on ventricular remodeling post-MI. To accomplish this objective, a mouse model of MI was used that establishes permanent occlusion of the left anterior descending coronary artery (LAD). The hydrogel was injected 7 days post-MI, during the proliferative phase of MI wound healing, which corresponds to approximately 14 days post-MI in humans. This model best represents patients that have had a large anterior-apical MI and have been delayed in getting treatment, leading to a large established scar. A 28-day follow-up was chosen for the mouse MI model because at this time-point dilative ventricular remodeling leads to a decline in cardiac function in untreated mice. The studies in this thesis were designed to address three specific aims.

Aim 1: Determine the impact of rHCI and rHCIII hydrogel injection on cardiac function and ventricular remodeling post-MI in a mouse model at the 28-day follow-up time-point. In addition, identify the differences in efficacy between rHCI and rHCIII hydrogels *in vivo* and establish the best performing treatment in this model.

Aim 2: Characterize the degradation of the rHC hydrogels *in vivo* and the immune response to hydrogel injection.

Aim 3: Based on the results of studies for aim 1 and aim 2, rHCI was the superior performing treatment in part because its injection stimulated an acute improvement in contractility within 2 days. Consequently, the final aim was to determine if rHCI injection led to acute improvement in global cardiac function within 2 days. Part of this aim was also to understand the mechanism of action behind the acute improvement of contractility with rHCI treatment. To accomplish this, an RNA sequencing (RNAseq) analysis was run on heart tissue from rHCI treated mice at 2 days post-injection and follow-up studies were done to examine the pathways of identified target genes.

Hypothesis 1: In a mouse model of MI, the injection of rHCI and rHCIII hydrogels will limit ventricular remodeling and improve cardiac function compared to PBS injection at the 28-day endpoint. The rHCI and rHCIII hydrogels will perform equally well to improve cardiac function post-MI.

Hypothesis 2: The injection of either hydrogel will not stimulate an adverse immune response *in vivo* and may increase M2 macrophage differentiation, as seen in previous *in vivo* studies of collagen hydrogel injection. Based on the *in vitro* degradation time it was anticipated that rHCI hydrogel would degrade faster than rHCIII hydrogel *in vivo*.

Hypothesis 3: The rHCI hydrogel improves cardiac function 2 days post-injection and this improvement is sustained out to the original 28-day endpoint in a mouse model of MI. The mechanism of action was anticipated to involve a reduction in stress and improved

contractility within cardiomyocytes which could lead to the increase in myocardial salvage observed 28 days after rHCI treatment.

The results for aims 1 and 2 were published as *McLaughlin and McNeill et al. Nature Communications 10 (2019) 4866*. The results of aim 3 are being drafted into a manuscript and are currently unpublished

Chapter 2: Methods

Note that common methods and methods for aims 1 and 2 (sections 2.0 and 2.1) have been published in *McLaughlin and McNeill et.al. Nature Communications 10 (2019) 4866* and are presented herein with minor modifications along with methods performed for aim 3 (section 2.2).

2.0 Methods Common to all Aims

2.0.1 Preparation of rHC Matrices

A 1% collagen solution was prepared by dissolving 0.1 g of lyophilized rHC collagen (rHCI and rHCIII, from Fibrogen) in 10 ml of ultra-pure ddH₂O. Chondroitin sulfate (CS; Wako), EDC (Sigma) and NHS (Sigma) were added to produce a final mixture with a mass ratio of 1:4:0.5:0.3 for collagen:CS:NHS:EDC. The materials were prepared on ice using an enclosed system that allows homogenous mixing without adding bubbles. After thorough mixing, the pH was adjusted to 7.4 by NaOH (1.0 N). Matrices without CS were prepared similarly but with PBS added to compensate for the volume of CS. Matrices labeled with Alexa-Fluor®594-NHS were prepared by adding 20 µL of the dye stock solution (1mg/mL in DMSO) to the gels before adding the NaOH (25 nmol of dye per hydrogel), followed by 20 mixing steps.

2.0.2 Animal Experiments

All procedures were approved by the University of Ottawa Animal Care Committee and performed according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.0.3 MI Model

MI was induced in 9-week-old female C57BL/6 mice (Charles River; number of mice/group are in the figure legends) and treatment delivery was performed using an

established protocol^{175,197}. Mice were anesthetized (2% isoflurane), intubated, and the heart was exposed via fourth intercostal thoracotomy. The LAD was then ligated just below its emergence from the left atrium. This procedure results in a large MI involving the anterolateral, posterior, and apical parts of the heart, which was confirmed at the time of surgery by myocardial blanching in the region supplied by the artery. Short acting buprenorphine was administered at least an hour prior to surgery, and long-acting buprenorphine was administered subcutaneously immediately before surgery for perioperative analgesia. At 7 days post-MI (baseline), mice were randomly assigned to receive treatment of PBS (control), rHCI or rHCIII matrices, delivered in 5 equivolumetric intramyocardial injections (10µl each site, 50µl total) through a 27G needle using an ultrasound-guided closed-chest procedure. The syringe is secured in a micromanipulator (VisualSonics), and prior to the injection procedure, both the needle and RMV scanhead probe are aligned along the heart long-axis. Via the ultrasound field-of-view, the micromanipulator is used to position the needle tip so that it is in the desired location within the myocardium for delivery of the injections (see Figure 2.1). Mice were sacrificed by terminal anesthesia at 2 days or 28 days post-treatment and hearts were collected for RNA or protein isolation, histology and/or measurements of the mechanical properties. For a schematic of endpoint sample size, see Appendix D.

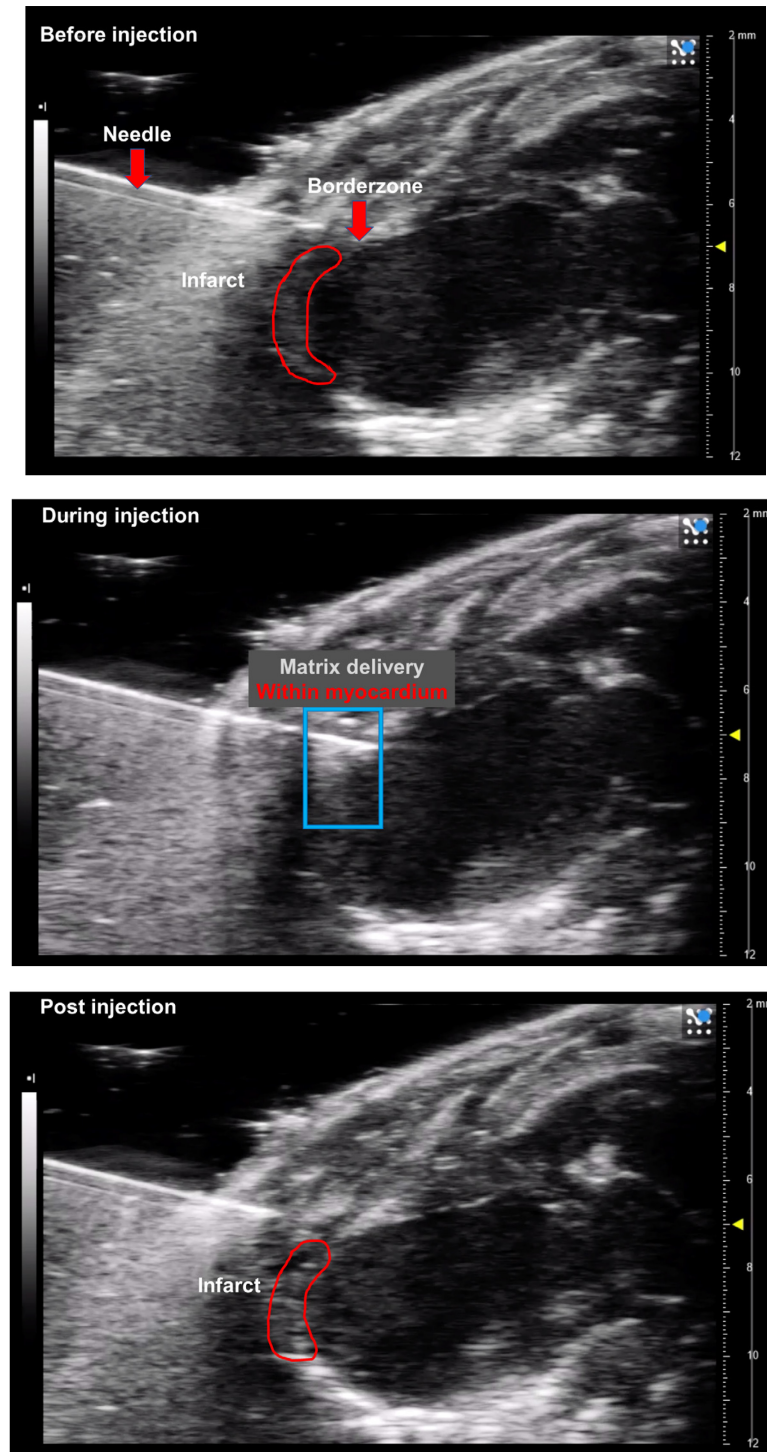


Figure 2.1. Representative images depicting the intramyocardial injection of the rHCI matrix. Images shown are at time-points prior to intramyocardial delivery (top), during the injection (middle), and post-injection (bottom). Images were captured from a video recorded using a Vevo 3100 ultrasound system operating at 30 MHz. The video is available in the video supplementary information section of this article.

2.0.4 Strain Analysis

Transthoracic echocardiography was performed on long-axis views using a Vevo3100 system in B mode with a MX400 series real-time microvisualization scan head probe (VisualSonics). Strain was analyzed using the Vevostrain application of Vevo LAB 3.1.1 software (VisualSonics)²⁶³. The imaging was performed at baseline before treatment injection (7 days post-MI), 2 days and 28 days post-injection to determine longitudinal endocardial strain at the time of aortic valve closure (representing end-systole). Endocardial strain is calculated as the percentage displacement of the endocardium at end systole (marked by the aortic valve closure) compared to its original position at end diastole. In the longitudinal direction, the myocardial segments will shorten at systole compared to diastole as the myocardium contracts. The software splits the LV myocardium into six equal segments namely the apex, mid, and base regions of the anterior and posterior LV walls. The anterior mid segment (segment 5) corresponds to the borderzone area targeted for treatment injection. Representative images for the strain measurements are included in Figure 2.2 for all the experimental groups plus a healthy animal that did not have an MI.

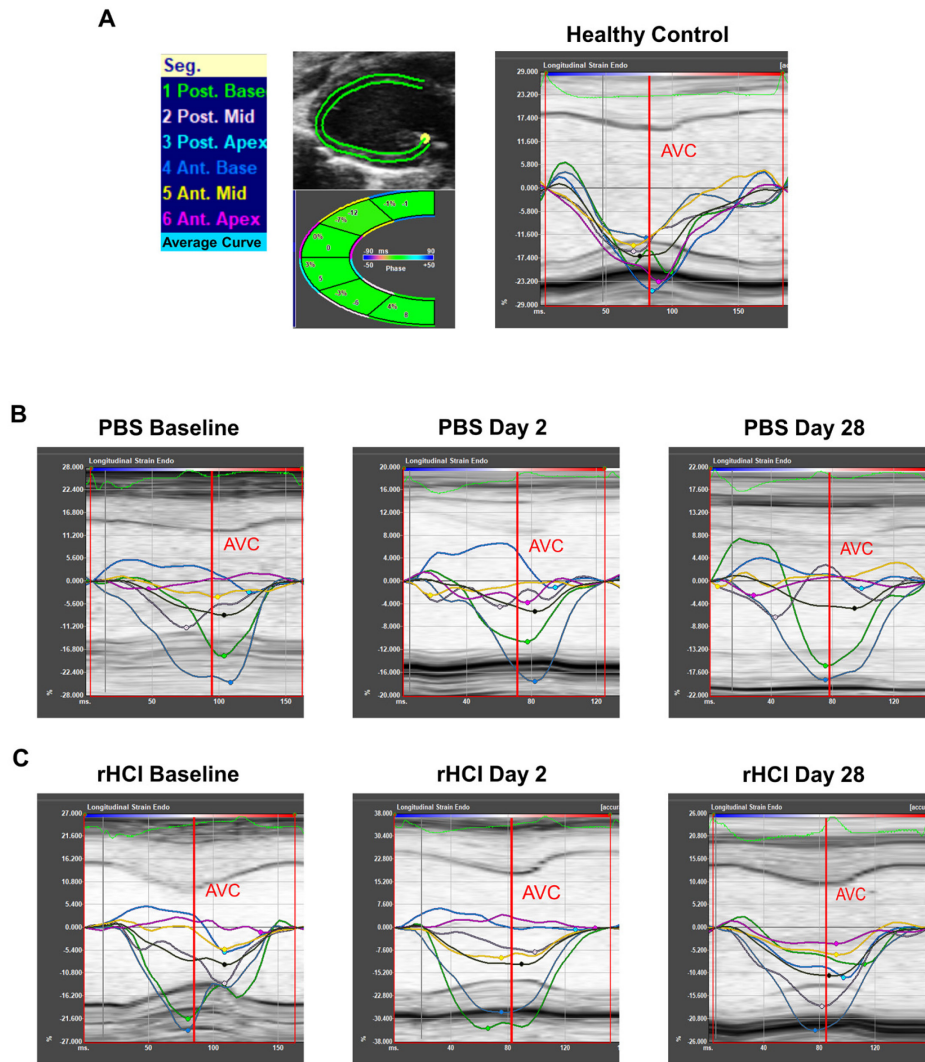


Figure 2.2. Sample images of the myocardial strain analysis using Vevostain software for day 2 and day 28 end-points. A) Long-axis left ventricular (LV) echocardiograms are divided into six segments for base, mid and apical regions of the anterior (dark blue, yellow and light pink traces) and posterior LV walls (green, white and light blue traces). Displacement of these segments is traced through the cardiac cycle and strain is recorded as the percent change from end diastole. Strain is measured using the endocardial trace in the longitudinal direction, therefore all segments in a healthy animal shorten and reach peak negative strain at end systole. For a reference point of end systole, peak strain during contraction is recorded at the time of the aortic valve closure (AVC). Example strain images from mice that received intramyocardial injection of B) PBS or C) rHCI hydrogel seven days post-myocardial infarction. Time points are baseline prior to injection as well as 2 days and 28 days post-injection.

2.1 Methods Used for Aims 1 and 2

2.1.1 Study Design

Here, we conducted a study to (i) design clinically relevant collagen hydrogels using recombinant human collagens type I and type III; (ii) characterize and compare the physical properties of the rHCI and rHCIII materials; (iii) evaluate the therapeutic potential of the materials for treating MI in mice; and (iv) identify mechanisms underlying the observed therapeutic effects of rHC treatment. For in vivo experiments, LAD artery ligation in mice was chosen as a clinically relevant and well-established animal model of MI. For functional, histological, and molecular evaluations, the number of animals per group was minimized to three to fifteen mice, as specified in the figure legends. Mice were randomly assigned to treatment groups and all analyses were performed in a blinded fashion.

2.1.2 Material Viscosity

Viscosity measurements were carried out using a Brookfield R/S plus rheometer (Brookfield) at 37°C. A C25-2/30 conical spindle was used to compress the material (50 μ m displacement) onto a temperature-controlled pedestal. Viscosity was measured with a ramp rotational block at a speed of 1/min units pre-set at a shear rate of 5 units over a time of 30 min.

2.1.3 Degree of Crosslinking

Differential scanning calorimetry (DSC) experiments were carried out to assess the degree of crosslinking. Briefly, measurements were performed in a Q2000 differential scanning calorimeter (TA Instruments) in the range of 8 to 80°C using a scan rate of 5°C

min⁻¹. Collagen matrices (5-20 mg) were surface-dried with filter paper and hermetically sealed with an aluminum lid (Tzero; TA Instruments) in an aluminum sample pan (Tzero; TA Instruments). The denaturation temperature (T_d) was measured at the onset of the endothermic peak.

2.1.4 Water Content

Water content of the materials was measured by weighing the wet weight (W) of the sample, equilibrated in PBS for 96 h at 4°C. The material was then vacuum-dried at room temperature for 96 h to obtain the dry mass (W_0). The total water content of the hydrogels (W_t) was then calculated according to the equation:

$$(1) W_t = \frac{(W - W_0)}{W} \times 100$$

2.1.5 Degradation

Enzymatic degradation was measured using 50-100 mg of hydrogel placed in 5 ml of type I collagenase (10 U/ml in PBS) at 37°C. The remaining solid mass was measured over a period of up to 24h. The degradation rate is calculated from the initial slope of the plots of remaining mass vs. time and reported as mg/min.

2.1.6 Material Porosity

Low-temperature scanning electron microscopy (Cryo-SEM) measurements were performed at -50°C using a Tescan (Model: Vega II – XMU) equipped with a cold stage sample holder, a backscatter electron detector (BSE) and a secondary electron detector (SE).

Pore sizes were measured from at least 250 individuals from 4-6 random areas of the sample using ImageJ® software. Diameter for pores were quantified using the longitudinal axis of the pore using the straight-line tool. Area of the image was adjusted against the size scale bar obtained from the Tescan Vega II system²⁶⁴.

2.1.7 Echocardiography

Transthoracic echocardiography was performed on long-axis views using a Vevo770 system in B mode with a 707B series real-time microvisualization scanhead probe (VisualSonics). The imaging was performed at baseline before treatment injection (7 days post-MI) and at 28 days post-injection to determine LVEF, fractional area change (FAC), ESV, EDV, SV and CO. Note that LVEF, ESV, and EDV are used as clinical predictors of HF and survival after MI²⁶⁵.

2.1.8 Ex Vivo Imaging of Alexa-Fluor®594 Labeled Matrix

The chemically tagged rHC matrices (25 nmol of dye per hydrogel) were injected to the infarcted mouse heart, as described above. Animals were sacrificed at 2 h, 2 days and 7 days after treatment, and hearts were harvested and imaged ex vivo by IVIS® Spectrum (PerkinElmer) to visualize rHCI and rHCIII distribution within the hearts ($\lambda_{\text{excitation}}$: 570 nm; $\lambda_{\text{emission}}$: 640 nm). For histology, tissue sections were prepared in acetone and then stained with DAPI followed by imaging using a Leica Aperio Versa slide scanner with a final magnification of 20× and a Z-stack with 8 steps at 1.5 μm .

2.1.9 Electrocardiography

Electrocardiograms were obtained simultaneously with echocardiography using a Vevo3100 system (VisualSonics) at baseline before treatment injection (7 days post-MI)

and at 2 days post-injection. Electrocardiograms were exported from Vevo LAB 3.1.1., software (VisualSonics). The length of the PR, QT and QRS intervals was determined using ImageJ software (Table 2.1).

2.1.10 Mechanical Properties of the Myocardium

Mice were euthanized by terminal anesthesia at 2- or 28-days post-treatment and the hearts were harvested. Rectangular pieces (2.5 x 5mm) of the left ventricle comprising the scar and border zone were excised. To determine the tensile elasticity of the tissue (Young's modulus), the samples were subjected to mechanical testing in an Instron mechanical universal tester (Model 3342, Instron) equipped with Series IX/S software, using a crosshead speed of 10 mm min⁻¹.

2.1.11 Histology/Immunohistochemistry

Slides of myocardial tissue sections were prepared from a subset of hearts that were not used for mechanical properties measurements. At 28 days post-injection, hearts were harvested, perfused with PBS, embedded in optimal cutting temperature compound (OCT), and snap frozen in liquid nitrogen. Sections of 10 µm were cut using a cryostat. To assess scar size, tissue sections were fixed in 4% paraformaldehyde (PFA) for 1h and stained with Masson's trichrome procedure (Sigma). Images taken with an Olympus BX50 microscope using a 2× objective and eight sections per mouse were used to measure remote wall thickness and to determine scar size using the mid-line arc method using MIQuant software²⁶⁶. For immunohistochemistry, tissue sections were fixed in acetone for 20 min, permeabilized with 0.1% Triton for 10 min and then blocked in 10% serum for 1h at room temperature. Primary

antibodies were applied overnight at 4°C in 10% serum and then slides were rinsed and treated with secondary antibodies for 1h at room temperature, before being mounted with fluorescent mounting medium (Dako). For the detection of blood vessels and myofibroblasts, platelet endothelial cell adhesion molecule (PECAM-1) (also known as CD31; Santa Cruz 101454, 1:50) and α -SMA (Abcam 5694, 1:200) antibodies were used and detected with AF594 anti-rat (Life Technologies A11008, 1:500) and AF488 anti-rabbit (Life Technologies A11007, 1:500), respectively. M2 macrophages were detected by AF488 conjugated anti-CD206 antibody (Biolegend 141710, 1:50). For cardiac troponin I staining, sections were incubated with AF488-labeled wheat germ agglutinin (WGA) (ThermoFisher, W11261) and goat cardiac troponin I primary antibody (Abcam ab56357, 1:200) and detected by donkey anti-goat AF594 secondary antibody (Life Technologies A-11058, 1:500). Finally, for connexin 43 staining, sections were incubated with connexin 43/GJA1 (Cx43) (Abcam ab11370, 1:400) and cardiac troponin I (Abcam ab188877, 1:200) primary antibodies followed by detection with AF488 anti-rabbit (Life Technologies A11007, 1:500) and donkey anti-goat AF594 secondary antibody (Life Technologies A-11058, 1:500), respectively. Fluorescent images were obtained using a Zeiss Axio Observer microscope with a 20 $\times$ objective and for the connexin 43 stained sections a Leica Aperio Versa slide scanner with a 20 $\times$ objective was used with a Z-stack of 8 $\times$ 1.5 μ m steps. For all IHC stains, 4 sections per mouse were analyzed and for each section 2-4 images within the border zone, infarct and remote regions were used for analysis. For hematoxylin-eosin (H&E) staining, tissue sections were fixed in 10% formalin. Sections were then stained first in hematoxylin Gill's solution No. 2 for 7 min, followed by acid alcohol differentiation, and then 0.5% eosin for 7 min, followed by dehydration (all solutions from Sigma). Images were taken with an Olympus BX50 microscope. The border zone was designated as the FOV directly adjacent to either

side of the infarct region that begins when 50% of the LV is fibrotic scar tissue (see Figure 2.3 for a schematic depiction).

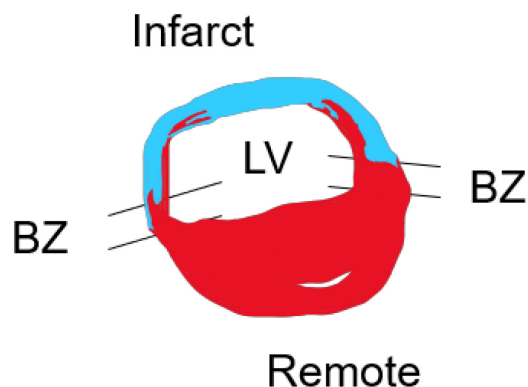


Figure 2.3. Schematic depiction for the definition of the different histological areas within the infarcted myocardium. BZ: Border zone, LV: Left ventricle lumen.

2.1.12 Cx3cr1-EGFP Mouse Experiments

To evaluate the recruitment of circulating mononuclear cells to the myocardium following rHC treatment, B6.129P-Cx3cr1^{tm1Litt/J} mice (Cx3cr1-EGFP) were purchased from the Jackson Laboratory. These mice express enhanced green fluorescent protein in monocytes, dendritic cells, natural killer (NK) cells, and brain microglia, and are a model reported for the study of mononuclear cell recruitment to the heart³⁵. At 7 days post-MI, mice received treatment of 50µl of phosphate buffered saline (PBS), rHCI or rHCIII, as described above. Animals were sacrificed 2 days after treatment and blood was collected into EDTA tubes. Also, hearts were perfused with PBS and the right ventricle, and the apical region of the left ventricle were collected. The tissues were rinsed with hank's balanced salt solution (HBSS) and digested in 2.4U/ml dispase I (Roche) and 1mg/ml Collagenase B (Roche) for 40 min at 37°C. Samples were washed 3 times with PBS, centrifuged for 5 min at 400g, and

the isolated cells were prepared for flow cytometry (FACS Aria III; Becton Dickinson). Cells were labeled with APC anti-mouse/human CD11b (Biolegend 101211), PE anti-mouse Ly-6G/6C (Biolegend 108407), PE/Cy5 anti-mouse F4/80 (Biolegend 123111), PE/Cy7 anti-mouse CD38 (Biolegend 102717) and Alexa Fluor® 700 anti-mouse CD206 (Biolegend 141733), following the manufacturer-recommended dilutions (0.25µg/L per 1×10^6 cells for CD11b, Ly-6G/6C, CD38, and CD206, and 1µg/L per 1×10^6 cells for F4/80). See Figure 2.4 for sorting/gating strategy.

2.1.13 Cell Isolation and Flow Cytometry for Monocyte Subsets

Two days post-treatment injection mice were sacrificed by CO₂ inhalation followed by cervical dislocation. Blood was collected from mice through cardiac puncture in a 50 mM EDTA solution and red blood cells (RBCs) were lysed with red blood cell (RBC) lysis buffer according to the manufacturer's protocol (Biolegend 420301). Cells were isolated from harvested mouse hearts using a digestion buffer containing: DNase I (50 U/µL; Sigma D5025), collagenase type II (400 U/mL; ThermoFisher 17101015), collagenase D (0.15 U/mL; Sigma 11088866001) and hyaluronidase (10 U/mL; Sigma H3506). Following an hour digestion at 37°C, isolated heart cells were passed through a 70 µm filter. Finally, harvested mouse spleens were mashed and triturated through a 70 µm filter followed by incubation with red blood cell (RBC) lysis buffer. Cell pellets were collected by centrifugation at 400xg for 5 min at 4°C. Isolated cells from all tissues were incubated with Zombie Aqua fixable viability dye (1:500, Biolegend 423101) for 20 min at room temperature. Next, Fc receptors were blocked with TruStain X reagent (1:100, Biolegend 103319) for 10 min at room temperature. The cells were then incubated with an antibody cocktail for 45 min at room temperature containing CD45-APC/Fire 750 (1:160, Biolegend

30-F11), CD11b-PE/Cy7 (1:80, Biolegend M1/70), Ly6G-PerCP/Cy5.5 (1:160, Biolegend 1A8), CD3-PE (1:160, Biolegend 17A2), B220-AF488 (1:50, Biolegend RA3-6B2), F480-AF647 (1:100, Biolegend BM8) and Ly6C-BV421 (1:80, BD Biosciences AL-21). Flow cytometry was performed using a BD FACS Aria III and data was analyzed with FlowJo V10.5.2 software (see Fig. 3. S12 for sorting/gating strategy). Total viable cell number for each tissue post-isolation was determined by trypan blue exclusion using a hemocytometer. Total number of cells within each leukocyte population was determined using the percentage of live cells for a given cell population and the total number of live cells counted post-isolation which was then normalized to the mg of tissue or mL of blood collected. Gating strategy: Single cells were gated based on forward scatter (FSC) gates, FSC-A and FSC-H. Live single cells were gated on low staining for Zombie Aqua live/dead dye. From this live single cell population leukocytes were CD45⁺. Leukocyte subsets were characterized as follows: neutrophils CD45⁺CD11b⁺Ly6G⁺, Ly6C low monocytes CD45⁺CD11b⁺Ly6G⁻F480⁻Ly6C⁻, Ly6C high monocytes CD45⁺CD11b⁺Ly6G⁻F480⁻Ly6C⁺, macrophages CD45⁺CD11b⁺Ly6G⁻F480⁺, T cells CD45⁺CD11b⁻Ly6G⁻CD3⁺B220⁻ and B cells CD45⁺CD11b⁻Ly6G⁻CD3⁻B220⁺. Note for blood F480 expression was not used in the gating strategy. Gates were set relative to isotype controls.

2.1.14 Neonatal Cardiomyocyte Studies

Neonatal rat ventricular myocytes (NRVMs) were freshly isolated using an established protocol²⁶⁷. Left ventricles collected from 2-day old Sprague–Dawley rats (Harlan) were digested by trypsin (Amersham Biosciences) and collagenase type II (Worthington Biochemical). Isolated cells were re-suspended in M-199 medium (Life Technologies) supplemented with 10% fetal bovine serum (FBS), 19.4 mM glucose, 2 mM l-glutamine, 2 unit/mL penicillin, 0.8 µg/mL vitamin B12, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 1× Minimum essential medium (MEM) non-essential amino acids (Sigma-Aldrich). Two rounds of 60 min pre-plating were performed, during which cardiac fibroblasts attach to the dish bottom, thus enriching the non-adherent population for NRVMs. The non-adherent cells, NRVMs, were then seeded onto the different collagen matrices in 24-well plates (40,000 cells/cm²). For the survival assay, 3-day cultured NRVMs were subjected to M-199 media containing 50µM hydrogen peroxide for 3h, after which a terminal deoxynucleotidyl transferase dUTP nick-end Labeling (TUNEL) assay was performed, and dead cells were counted in 3 random fields-of-view.

2.1.15 Cell Cultures

Using an established protocol, bone marrow-derived macrophages were isolated from 8-12 week old C57BL/6J mice²⁶⁸. Mice were euthanized by CO₂ inhalation and cervical dislocation, and tibia bones were collected and flushed with media to isolate the bone marrow. The bone marrow isolate was pipetted repeatedly to obtain a single cell suspension, which was then passed through a cell strainer. Freshly isolated cells were cultured for 1 week in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 20% L929 conditioned media and penicillin-streptomycin, and then re-plated on the rHC hydrogels for

3 days. Cells were collected from the rHC hydrogels using 3mM CaCl₂ HBSS containing 250 units of collagenase I (Gibco). Macrophage polarization was assessed by flow cytometry (FACS Aria III; Becton Dickinson) using CD86 and CD206 (Biolegend) to identify M1 and M2 macrophages, respectively. For mononuclear cell isolation, bone marrow from tibia bones was collected as described above. Mononuclear cells were purified by density gradient centrifugation using Histopaque® (Sigma) according to the manufacturer's instructions. Cells were labeled with 0.5µg/ml DAPI (Sigma) for 30 min at 37°C.

2.1.16 Macrophage Adhesion Assay

Mononuclear cells were isolated by flushing the tibias and femurs of 6- to 12-week-old mice. Cells were purified by density gradient centrifugation using Histopaque® (Sigma) according to the manufacturers' instructions. Mononuclear cells were counted and labeled with DAPI. Cells were plated on the different biomaterials at a concentration of 50,000 cells/cm² for 24h. Cells were fixed with 4% PFA for 5 min and cells were counted. Data was expressed relative to the control (non-coated wells, i.e., tissue culture polystyrene (TCPS)) to minimize the effect of inter-donor cell variability.

2.1.17 Macrophage Migration Assay

Bone marrow mononuclear cells were cultured in DMEM supplemented with 10% FBS, 20% L929-conditioned medium and Pen/Strep for 7 days to generate bone marrow-derived macrophages (BMDMs) and labeled with DAPI, as described above. BMDMs (2×10^5) were then re-suspended in endothelial cell growth basal medium (EBM) lacking growth factors and serum and loaded into the upper chamber of a Transwell plate (Life Technologies) coated with 100 µL of rHCI or rHCIII. The bottom chamber contained full

macrophage media as described above. After 24h, the inserts were removed and the number of BMDMs that migrated through the biomaterial was quantified in a blinded fashion using a Zeiss Z1 fluorescence microscope. Data was expressed relative to the control (non-coated wells, i.e., TCPS) to minimize the effect of inter-donor cell variability.

2.1.18 Macrophage Polarization Assay

BMDMs were generated in DMEM supplemented with 10% FBS, 20% L929-conditioned medium and Pen/Strep for 7 days, as described above. For macrophage activation experiments, cells were stimulated for 3 days with lipopolysaccharide (1 μ g/ml; Sigma) plus interferon gamma (IFN- γ) (50ng/ml; Sigma) for M1 activation, or with IL-4 (20ng/ml; R&D systems) for M2 activation. Total RNA was extracted using Tri Reagent (Zymo Research), according to manufacturer's protocol. First-strand cDNA was synthesized with Smartscribe Reverse Transcriptase (Takara Bio USA) and random hexamer primers (Fisher Scientific). Target gene mRNA levels were assessed by quantitative real time polymerase chain reaction (qPCR) with LightCycler 480 SYBR Green I Mastermix (Roche) and a LightCycler 480 Real-Time PCR System (Roche). Sequences for primer pairs are listed in Table 2.1. Relative changes in mRNA expression were determined by the $\Delta\Delta C_t$ method, expressed as levels relative to 18S. Data was expressed relative to the control (non-coated wells, i.e., TCPS) to minimize the effect of inter-donor cell variability.

2.1.19 Survival After H₂O₂ Exposure

Cells were cultured for 7 days in DMEM supplemented with 10% FBS, 20% L929-conditioned medium and Pen/Strep. On day 7, the media was changed to DMEM containing 0.5mM hydrogen peroxide and cells were cultured for 3h before being stained with 7

aminoactinomycin D (7-AAD) for analysis by flow cytometry. Data was expressed relative to the control (non-coated wells, i.e., TCPS) to minimize the effect of inter-donor cell variability.

2.1.20 Statistical analysis

Statistical analysis was performed using Kaleida Graph 4.5®. All data are presented as the mean $\pm$ SEM. For the comparison of *in vivo* data between treatments, an ANOVA was used followed by Holm's correction for multiple comparisons. Scar size was analyzed using a multiple regression including treatment group (rHCI, rHCIII and PBS) and baseline LVEF. rHCI and rHCIII as compared to PBS were significant predictors of infarct size, in addition to baseline LVEF. The correlation coefficient for the model is 0.6 using STATA multiple linear regression. *In vitro* NRVM, macrophage and cardiac fibroblast data was analyzed either by a Student's t-test or by a one-way ANOVA with a Holm's correction for multiple comparisons, as specified in the figure legends.

2.2 Methods for Aim 3

2.2.1 Study Design

This study was designed to test the MI heart's acute functional response to intramyocardial injection of rHCI hydrogel. A mouse model of MI was used that induces permanent occlusion of the LAD artery and treatments (rHCI hydrogel or PBS) were injected 7 days after MI into the anterior borderzone myocardium. This model was used previously for the first *in vivo* trials of rHC hydrogels to improve cardiac function post-MI²⁶⁹. The primary follow-up time-point in these trials was 28 days after treatment and rHCI improved cardiac function at that time-point. Intriguingly, there was evidence to suggest that cardiac function may have improved as early as two days post-injection. In this study, two sets of

functional studies were done. The first had an acute follow-up at 2 days after treatment injection (9 days post-MI). The second had the original follow-up time-point 28 days after injection, with echocardiography completed at both 2 and 28 days after injection. This first study was used to determine if cardiac function (LVEF) improved in rHCI-treated mice compared to PBS control group 2 days after treatment. The hearts at this follow-up were harvested to study gene expression changes between treatment groups by RNA-seq and to run follow-up histology on pathways downstream of the detected target genes. The 28-day trial was used to determine if the improvement in cardiac function over baseline for rHCI treatment group was equivalent at 2 days and 28 days post-treatment. Hearts harvested at the 28-day follow-up were used in histology experiments to test if changes in the myocardium found at 2 days are sustained out 28 days post-treatment in rHCI mice. Mice were randomly assigned to treatment groups 7 days post-MI. Intramyocardial injection and follow-up analyses were performed by an operator blinded to treatment group.

2.2.2 Echocardiography

Echocardiography was performed using a similar procedure to that described in section 2.1.7. In brief, cardiac function was analyzed by echocardiography at baseline prior to treatment (7 days post-MI) as well as 2 days and 28 days post-treatment injection. Using the Vevo3100 system with a MX400 series real-time microvisualization scan head probe (VisualSonics), transthoracic echocardiography was performed in B mode to capture the long axis view of the LV. Vevo LAB 3.1.1 software (VisualSonics) was used to calculate measures of cardiac function from long axis echocardiograms including: LVEF, SV, ESV, EDV, and CO.

2.2.3 RNA Sequencing and Differential Expression Analysis

Two days after treatment injection, hearts were harvested, perfused with PBS, and the LV tissue was snap frozen with liquid nitrogen. Frozen LV tissue was ground into a fine powder in liquid nitrogen using a mortar and pestle. From 30 mg of ground LV tissue, RNA was isolated using the RNeasy® mini kit (Qiagen 74004) according to the manufacturer's instructions. On-column DNase digestion was also completed using an RNase-Free DNase set (Qiagen 79254) following the manufacturer's protocol. RNA quality was determined using the RNA 6000 Nano kit (Agilent 5067-1511) and 2100 Bioanalyzer (Agilent). RN values for all samples were in the 8.2-8.6 range and were automatically calculated by the 2100 Expert software (Agilent). Next generation RNA sequencing was completed by Genome Quebec. An mRNAseq stranded library was prepared with NEBNext dual adaptor sequences (New England Biolabs) from 500 ng of sample RNA. Next generation RNA sequencing was performed on mRNA libraries using the NovaSeq 6000 S2 PE100 system (Illumina) with a sequencing depth of 50 million reads per sample. Differential expression analysis was completed by the Canadian Centre for Computational Genomics (C3G). The MUGIC pipeline from their Genpipes platform was used²⁷⁰. In summary, RNAseq reads were trimmed and adaptors were removed using Trimmomatic²⁷¹, trimmed reads were aligned to the reference genome (*Mus musculus* assembly GRCm38/mm10) using STAR²⁷², and aligned reads were mapped to transcripts with their abundance measured by fragments per kilobase of exon per million fragments mapped (FPKM) using the Cufflinks program²⁷³. Differential expression analysis was completed using edgeR^{274,275}. Briefly, normalization and dispersion factors were calculated, then a generalized linear model (GLM) was fitted for all treatment groups and comparisons were made between PBS vs. rHCI, rHCI vs. sham and

PBS vs. sham using the GLM likelihood ratio tests (LRT) function. A Benjamini Hochberg false discovery rate was used to determine significance of differentially expressed genes per treatment group. Using the lists of differentially expressed genes, enriched gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms were analyzed using R software for PBS vs. sham and rHCI vs. sham comparisons. Finally, Enrichr²⁷⁶⁻²⁷⁸ analysis was used to determine significantly enriched mammalian phenotypes (from the mouse genome informatics, MGI, database) and GO molecular pathways from PBS vs. sham and rHCI vs. sham differentially expressed gene lists. For PBS vs. rHCI differentially expressed genes, a heatmap was created using heatmap.2 and hierarchical clustering functions in R.

2.2.4 Quantitative Polymerase Chain Reaction to Validate Gene Candidates.

QPCR primer sequences (see Table 2.2) were designed to detect mouse mRNA transcripts of: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (NM_001289726.1), chemokine ligand 22 (CCL22) (NM_009137.2), and erythroid differentiation regulator 1 (ERDR1) (NM_13362.2) using Primer Blast (NCBI). GAPDH¹²⁷ and ERDR1^{279,280} primers have been used previously. SMARTScirbe® reverse transcriptase kit (Clontech 639538) along with random hexamer primers (ThermoFisher SO142) and a 10 mM deoxynucleotide triphosphate mix (ThermoFisher 10297018) was used to produce cDNA from 1 µg of sample RNA, according to the manufacturer's protocol. Primer sequences were validated using endpoint PCR (Figure 2.5). Endpoint PCR was run with GoTaq® Flexi DNA Polymerase kit (Promega M8291), using 0.2 µM of each forward and reverse primer, and 3 mM MgCl₂, following the manufacturer's protocol. Amplicons were run on 2% agarose gels with RedSafe™ Nucleic Acid Staining Solution (iNtRON 21141) at 120V for 30 min. Gels were imaged using a ChemiDoc Gel Imaging system (Bio-Rad). QPCR was run for each target

gene with LightCycler® 480 SYBR Green I Master reagent (Roche 04707516001). The final reaction mixture contained: 1X SYBR Green I Master Mix, 0.2 μ M each of forward and reverse primers, and 1:5 dilution of cDNA (ERDR1 assay) or 1:2 dilution of cDNA (CCL22 assay). Three replicates were run per sample. The reactions were run using a LightCycler® 480 thermocycler (Roche) with an amplification program of: initial denaturation at 95°C for 5 min and 45 cycles of 95 °C for 15 sec, 58°C for 20 sec and 72°C for 20 sec. Threshold cycle values (Ct) were calculated using the LightCycler® 480 software version 1.2.9.11 (Roche). The $\Delta\Delta$ Ct value for target gene expression in each sample was calculated by first finding the difference in Ct between the target gene and the house keeping gene in the sample (Δ Ct). Then the difference between the sample Δ Ct and the average sham sample Δ Ct was used to calculate $\Delta\Delta$ Ct for each sample. The fold change in target gene expression from sham mice for each sample can then be calculated as $2^{-\Delta\Delta\text{Ct}}$. Values were Log transformed prior to statistical analysis.

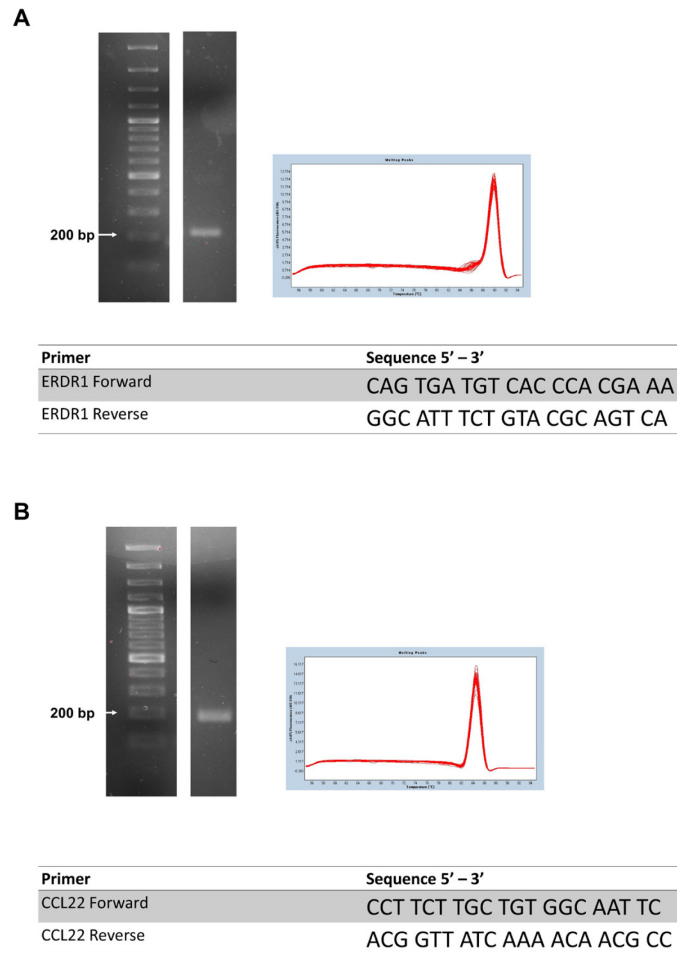


Figure 2.5. QPCR primer validation for RNA sequencing target genes that are differentially expressed between PBS and rHCl-treated mice. QPCR gene expression analysis was completed for A) erythroid differentiation regular 1 (ERDR1) and B) c-c motif chemokine ligand 2 / macrophage derived chemokine (CCL22). Gel images show the end point PCR product for each primer pair run on 2% agarose gels (expected product size of 200 bp). The melt curve analysis from the qPCR runs shows a single PCR product. Primer sequences are indicated in the lower tables.

2.2.5 In Situ Hybridization to Detect ERDR1 Within Cardiomyocytes

In situ hybridization (ISH) to detect *ERDR1* expression in cardiomyocytes of MI mouse heart tissue was performed using RNAscope® technology²⁸¹. Hearts were harvested

at the day 2 endpoint and perfused with PBS to remove blood. Hearts were incubated in OCT for 2 min, then ventricles were filled with OCT. Finally, the hearts were embedded in OCT in a cryomold and snap frozen in liquid nitrogen. Cryosectioning of the hearts was completed at -15°C starting 250 µm from the apex of the heart. Section thickness was 10 µm. One section per mouse, 2170 µm from the apex, was prepared for the RNAscope assay by following the fresh frozen section pre-treatment protocol and reagents (ACDbio 322381). Detection of *ERDR1* transcripts was completed by using the Multiplex Fluorescent v2 assay kit (ACDbio 323100) and Mus Musculus-Erdr1 probe (ACDbio 465101, in HRP-C1), following the manufacturer's instructions. Opal 620 dye (Akoyabio FP1495001KT, 1:750) was used to detect the *ERDR1* signal. Control probes used to test RNA integrity and background signal were: 3-plex Positive Control Probe Mus Musculus (ACDbio PN 320881) and 3-plex Negative Control Probe (ACDbio PN 320871). The negative control probe detects the DapB bacterial gene. The positive control probe detects DNA-directed RNA polymerase II subunit RPB1 (Polr2A) transcripts, a gene that is expressed at low levels in all cell types at 3-10 copies per cell, which is used to confirm RNA quality. Following *ERDR1* ISH, immunohistochemistry (IHC) to detect cardiomyocytes was performed. Sections were rinsed in Tris buffered saline (TBS) buffer with 0.05% Tween 20 (TBST) then blocked in 10% FBS for 1 hour at room temperature. Sections were incubated with the primary antibody for cardiac troponin I (Abcam ab188877, 1:200) and AF488-labeled WGA (ThermoFisher W11261, 1:50) overnight at 4°C. The sections were then washed in TBST, incubated with anti-goat AF647 (Life Technologies A21447, 1:250) and DAPI (10 µg/mL) and slides were mounted using ProLong® Gold antifade mountant (ThermoFisher). Slides were imaged using a Leica Aperio Versa slide scanner at 20X magnification. Image analysis was performed in QuPath software (see Figure 2.6). Briefly, a pixel classifier was trained on

sample regions from images within the data set to detect cardiac troponin⁺, WGA⁻ regions that contain cardiomyocyte nuclei and WGA⁺, cardiac troponin⁻ areas that contain all other nuclei. The cell detection algorithm was run to detect DAPI⁺ nuclei, then the subcellular detection algorithm was used to detect puncta of *ERDR1* signal. The pixel classifier was then used to classify the cells as cardiomyocytes or ‘other’. A script was then run to classify three levels of *ERDR1* expression as bins of transcripts per cell: 1-7, 8-15 and >15, in addition to negative cells. An H score was calculated as: 1x(percentage of cells 1-7 transcripts per cell) + 2x(percentage of cells 8-15 transcripts per cell) + 3x(percentage of cells >15 transcripts per cell). H Scores can range from 0-300 with 300 being 100% of cells have greater than 15 *ERDR1* transcripts.

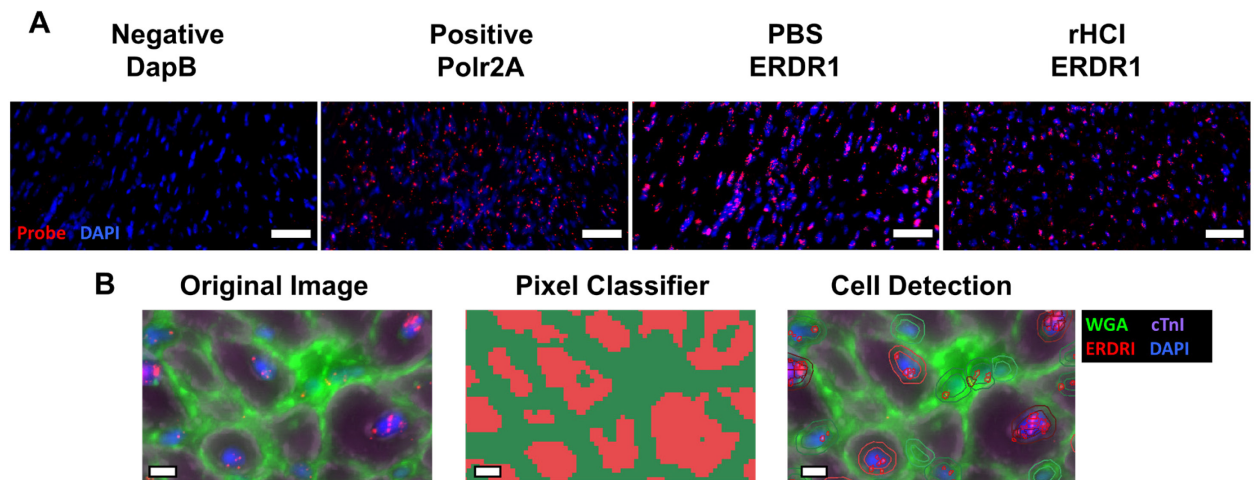


Figure 2.6. In situ hybridization detection of erythroid differentiation regulator 1 (ERDR1) in heart tissue after myocardial infarction. A) RNAscope multiplex fluorescent detection kit was used to detect ERDR1 mRNA transcripts in mouse heart tissue. Hearts were harvested two days post-treatment and fresh frozen tissue sections were prepared for in situ hybridization. The negative control probe detects the DapB bacterial gene. The positive control probe detects DNA-directed RNA polymerase II subunit RPB1 (Polr2A) transcripts, a gene that is expressed at low levels in all cell types at 3-10 copies per cell. Scale bars are 50 μ m. B) ERDR1 ISH was combined with IHC for cardiac troponin I (cTnI, purple), wheat germ agglutinin (WGA, green) and DAPI (blue). A pixel classifier was trained from multiple sample regions of images in the data set using QuPath software. The pixel classifier distinguished areas with cardiomyocytes (red) from non-cardiomyocyte areas (green). After

cell detection for DAPI positive nuclei and subcellular detection of ERDR1 signal, cells were classified as cardiomyocytes (red cell outlines) or non-cardiomyocytes (green cell outlines) using the pixel classifier areas. The level of ERDR1 expression in cardiomyocytes and non-cardiomyocytes was determined. Scale bar represents 10 μ m.

2.2.6 Histology and Immunohistochemistry

Heart tissue sections were prepared as described previously for the ISH assay. Briefly, at both the 2- and 28-days post-injection endpoints, hearts were harvested, perfused with PBS, embedded in OCT, and snap frozen in liquid nitrogen. Sections of 10 μ m were cut using a cryostat beginning 250 μ m from the apex. To examine myocardial salvage and fibrosis, tissue sections were fixed in 10% formalin for 20 min and stained with Masson's trichrome procedure (Sigma, HT15-1KT). For immunohistochemistry, tissue sections were fixed in acetone for 20 min at 4°C, permeabilized with 0.1% Triton for 10 min and then blocked in 10% fetal bovine serum for 1h at room temperature. Primary antibodies were applied overnight at 4°C in 10% serum. Slides were then washed in PBS with 0.05% Tween 20 (PBST), incubated with secondary antibodies and DAPI (10 μ g/mL) for 1h at room temperature. Finally, slides were mounted with ProLong® Gold antifade mountant (ThermoFisher). For the detection of blood vessels and myofibroblasts, PECAM-1 (also known as CD31; Santa Cruz 101454, 1:50) and α -SMA (Abcam ab5694, 1:200) antibodies were used and detected with AF594 anti-rat (Life Technologies A11007, 1:500) and AF488 anti-rabbit (Life Technologies A11008, 1:500), respectively. M2 macrophages were detected by AF488 conjugated anti-CD206 antibody (Biolegend 141710, 1:50). As a marker of oxidative stress, 4HNE antibody (Abcam ab46545, 1:100) was used and detected with AF488 anti-rabbit (Life Technologies A11008, 1:500). For T-regulatory cell IHC, primary antibodies used were anti-CD3 (Abcam ab16669, 1:400) and anti-FOXP3 (Thermofisher 14-

5773-82, 1:100) and were detected with AF488 anti-rabbit (Life Technologies A11008, 1:600) and AF594 anti-rat (Life Technologies A11007, 1:600). For methylglyoxal-derived hydroimidazolone-1 (MG-H1) detection, the IHC protocol was modified in the following ways: triton permeabilization was not used, PBST was buffer was replaced by TBS buffer with 0.05% Tween 20 (TBST) and blocking was performed for 2 hours at room temperature using anti-mouse FAB fragment antibody (Jackson ImmunoResearch AB_2307338, 1:25) in 10% FBS. Antibodies for MG-H1 (Cell Biolabs STA-011, 1:80), collagen type I (Abcam ab34710, 1:100) and cardiac troponin I (Abcam ab188877, 1:200) were used and detected by AF488 anti-mouse (Life Technologies A21202, 1:500), AF594 anti-rabbit (Life Technologies A21207, 1:500), and AF647 anti-goat (Life Technologies A21447, 1:250). For cardiomyocyte staining of day 28 tissue, sections were fixed in 4% PFA at room temperature rather than acetone. The same general immunohistochemistry procedure as described above was used. Sections were incubated with AF488-labeled WGA (ThermoFisher W11261, 1:50) and cardiac troponin I primary antibody (Abcam ab188877, 1:200) and detected by donkey anti-goat AF568 secondary antibody (Life Technologies A-11057, 1:250). TUNEL staining for apoptotic cardiomyocytes was performed using the Click-iT™ Plus TUNEL Assay with AF488 detection according to the manufacturer's protocol (ThermoFisher C10617). Following the TUNEL kit procedure, immunohistochemistry was then completed using the cardiomyocyte staining protocol described above with the following modifications: AF555 WGA (ThermoFisher W32464, 1:25) was used, slides were washed with 3% bovine serum albumin (BSA) in PBS, and the cardiac troponin antibody was detected by AF647 anti-goat secondary antibody (Life Technologies A21447, 1:250). AF594 rHCI hydrogel sections were stained with MG-H1 and collagen 1 or DAPI only using the immunohistochemistry protocols described above. Full scans of 2-4 sections per mouse were taken with the 20X objective of

a Leica Aperio Versa slide scanner with an 8x1.5 μm step Z-stack. Sections for one mouse were each separated by 480 μm . For the AF594 rHCI hydrogel sections counterstained with DAPI, 5 sections separated by 480 μm each were imaged.

2.2.7 Image Analysis for Histology Experiments

Image analysis for TUNEL, CD206, and T regulatory lymphocyte IHC experiments was done manually using Aperio Imagescope version 12.4.0.5043. For 4HNE (Figure 2.7), CD31 (Figure 2.8), MG-H1 (Figure 2.9 and 2.10), Masson trichrome (Figure 2.11A-B), and cardiomyocyte (Figure 2.11C-E), IHC image analysis was completed in QuPath software²⁸². Pixel classifiers were trained to detect positive staining areas. Training was performed on sample regions from images within a data set. To train a classifier, positive staining areas from one or multiple fluorophores were marked along with negative background staining. Either a random trees or artificial neural network classifier was used for all fluorescent signals of interest. Gaussian and Laplacian of gaussian features were included for all. For detecting cardiomyocytes from non-myocytes in the analysis of IHC staining, local mean and variance subtraction was used in the classifier. For WGA and cardiomyocyte IHC at day 28, manual correction of detected cardiomyocytes was necessary to accurately analyze the cell area. For CD31 staining the cell detection and classification algorithms in QuPath were also used to classify DAPI positive nuclei based on the CD31 pixel classifier. For all IHC stains, cell density or positive staining area was expressed relative to the infarct, borderzone, or remote tissue area. TUNEL positive cardiomyocyte density was expressed relative to the total cardiac troponin positive area in each region. The borderzone was defined as the region that began immediately adjacent to the infarct tissue, where 50% of the LV thickness is fibrosis,

and extended no further than 3 fields of view at 20X (250 μm x 250 μm) away from the infarct.

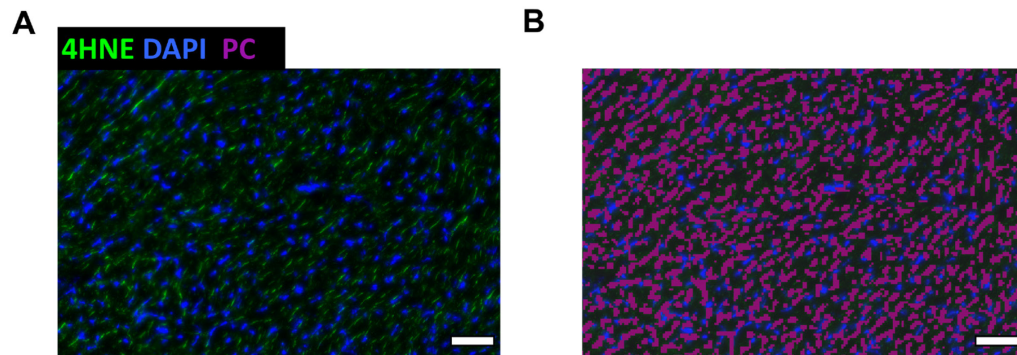


Figure 2.7. Pixel classifier used for image analysis to detect the level of 4HNE adducts in heart tissue 2 and 28 days after treatment injection in mouse MI model. A) An sample image of 4-hydroxynoneal (4HNE) staining (green) and (B) the trained pixel classifier (purple) used for automated detection of 4HNE positive area. The pixel classifier was trained and used for image analysis in QuPath software. The pixel classifier was trained by marking 4HNE positive and negative areas in sample regions from images within the dataset. Scale bar is 50 μm .

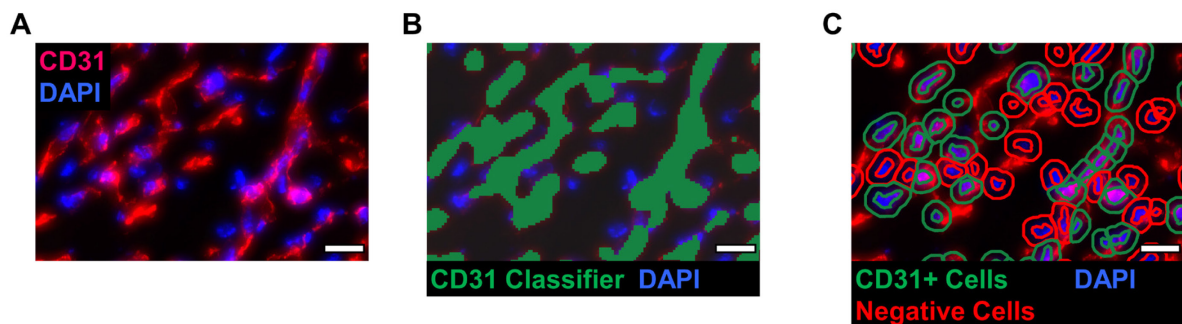


Figure 2.8. Pixel classifier used to detect CD31 positive endothelial cells in heart tissue 2 days after treatment injection in a mouse MI model. A) IHC staining was done on heart sections to detect CD31⁺ (red) capillaries with DAPI (blue) being used as a marker for nuclei. B) A pixel classifier was trained on sample regions to detect CD31⁺ areas (green). C) DAPI⁺ nuclei in those regions were classified as CD31⁺ cells (green outlines) and all other nuclei were classified as negative (red outlines). Scale bars are 20 μm . The pixel classifier training, cell detection, and object classification algorithms were used in QuPath image analysis.

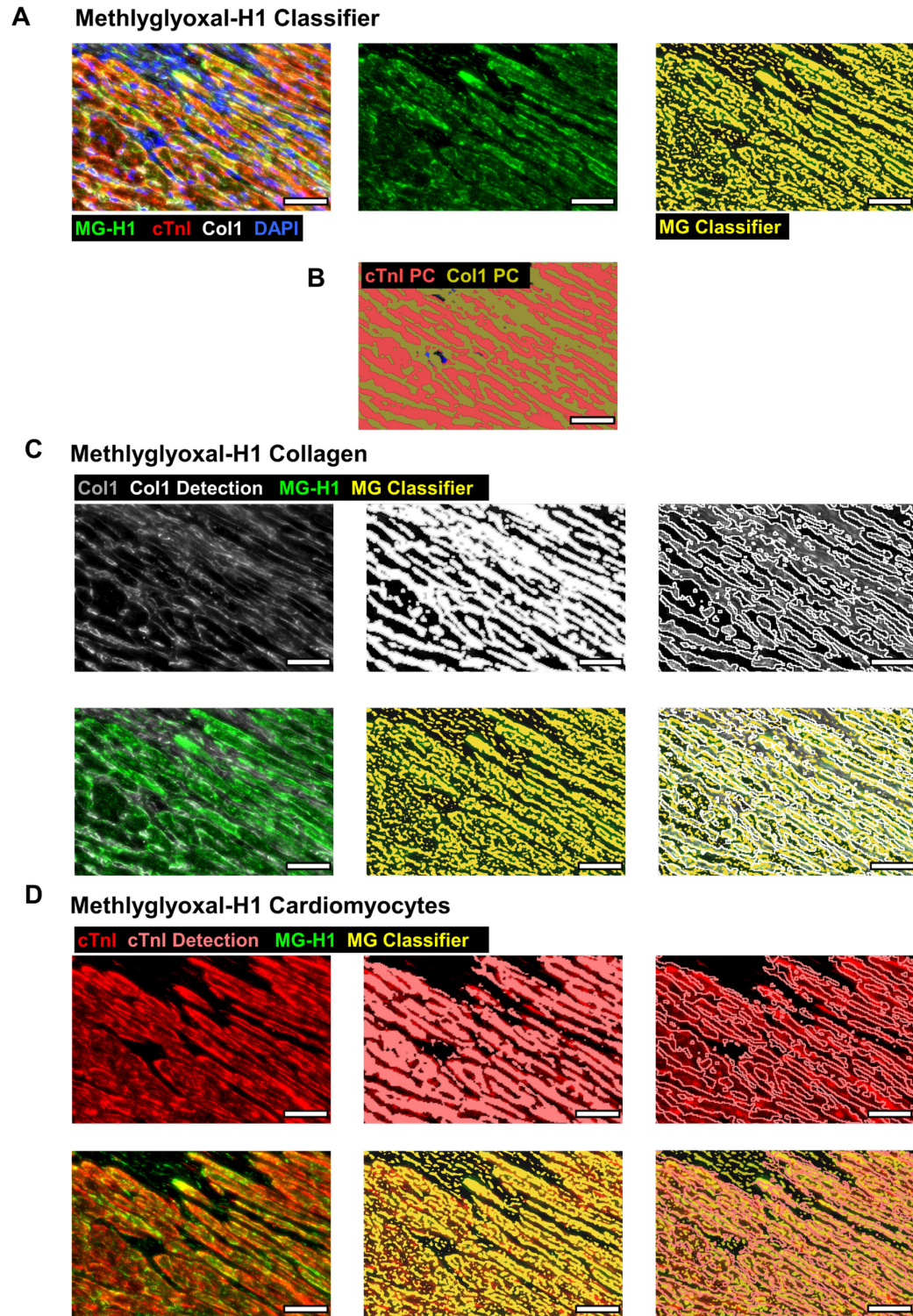


Figure 2.9. Development of a methylglyoxal adduct classifier within QuPath software. Heart tissue sections were stained with antibodies for methylglyoxal adducts (MG-H1, green), collagen type I (Col1, white), cardiac troponin (cTnI, red) and DAPI (blue). Pixel classifiers in QuPath were trained by marking positive and negative areas on sample regions from images in the dataset. A) An example of MG-H1⁺ area detection using a MG-H1 classifier

(yellow). B) Another classifier was trained to detect cardiomyocytes (light red) and collagen (olive). By combining the pixel classifiers, MG-H1 adducts on collagen (C) and within cardiomyocytes (D) were measured. Scale bars are 50 μ m.

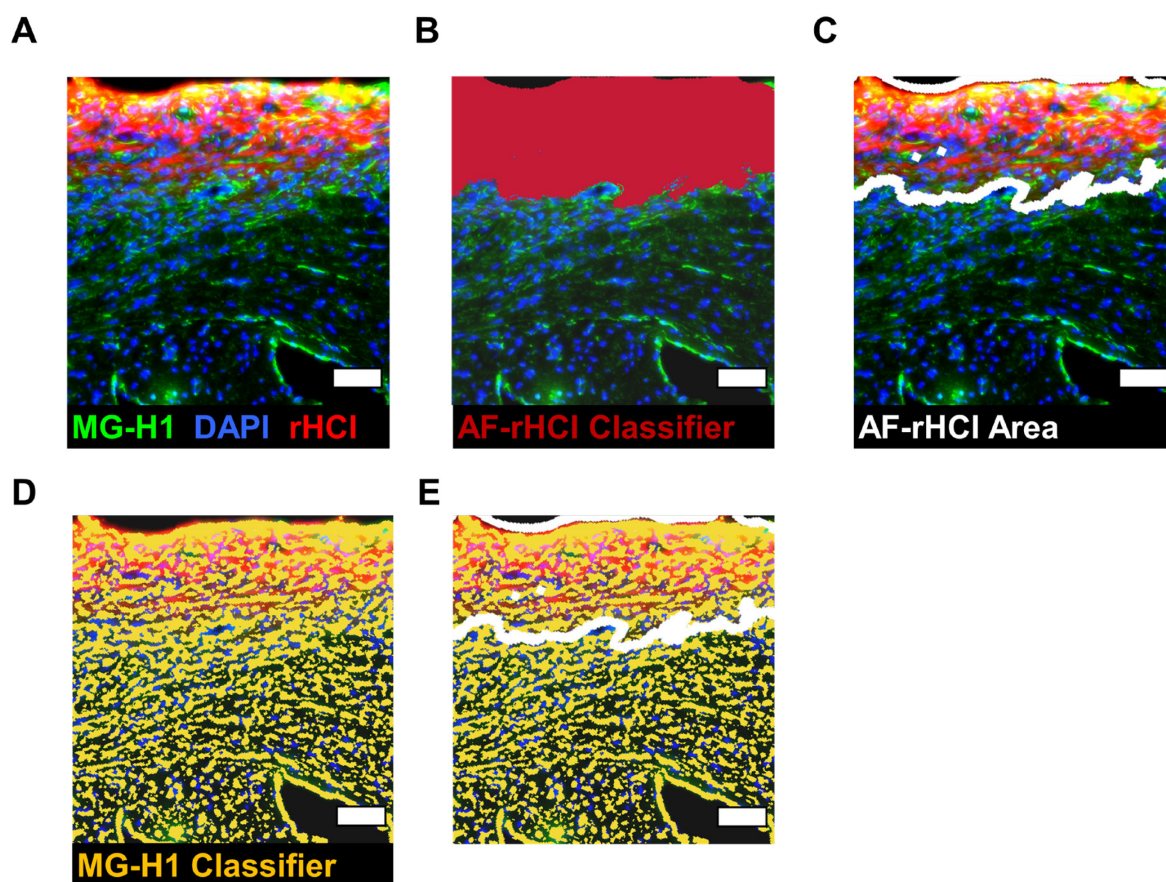


Figure 2.10. A pixel classifier was used to detect methylglyoxal modifications in rHCI hydrogel two days post-injection. Seven days after MI, mice received intramyocardial injection of rHCI hydrogel tagged with AlexaFluor 594 (AF). Hearts were harvested 2 days after treatment injection for histology. Heart sections were stained with anti-MG-H1 antibody to detect methylglyoxal adducts (green) and DAPI counterstain (blue). The rHCI hydrogel tagged with AF594 appears in red. QuPath software was used to train pixel classifiers to detect (B) AF594-rHCI (dark red). C) The area of rHCI is outlined in white. D) A pixel classifier was also trained to detect MG-H1 (yellow). E) The percentage of the rHCI hydrogel (white outline) that is positive for MG-H1 (yellow, pixel classifier trace) is then determined. Scale bars are 50 μ m.

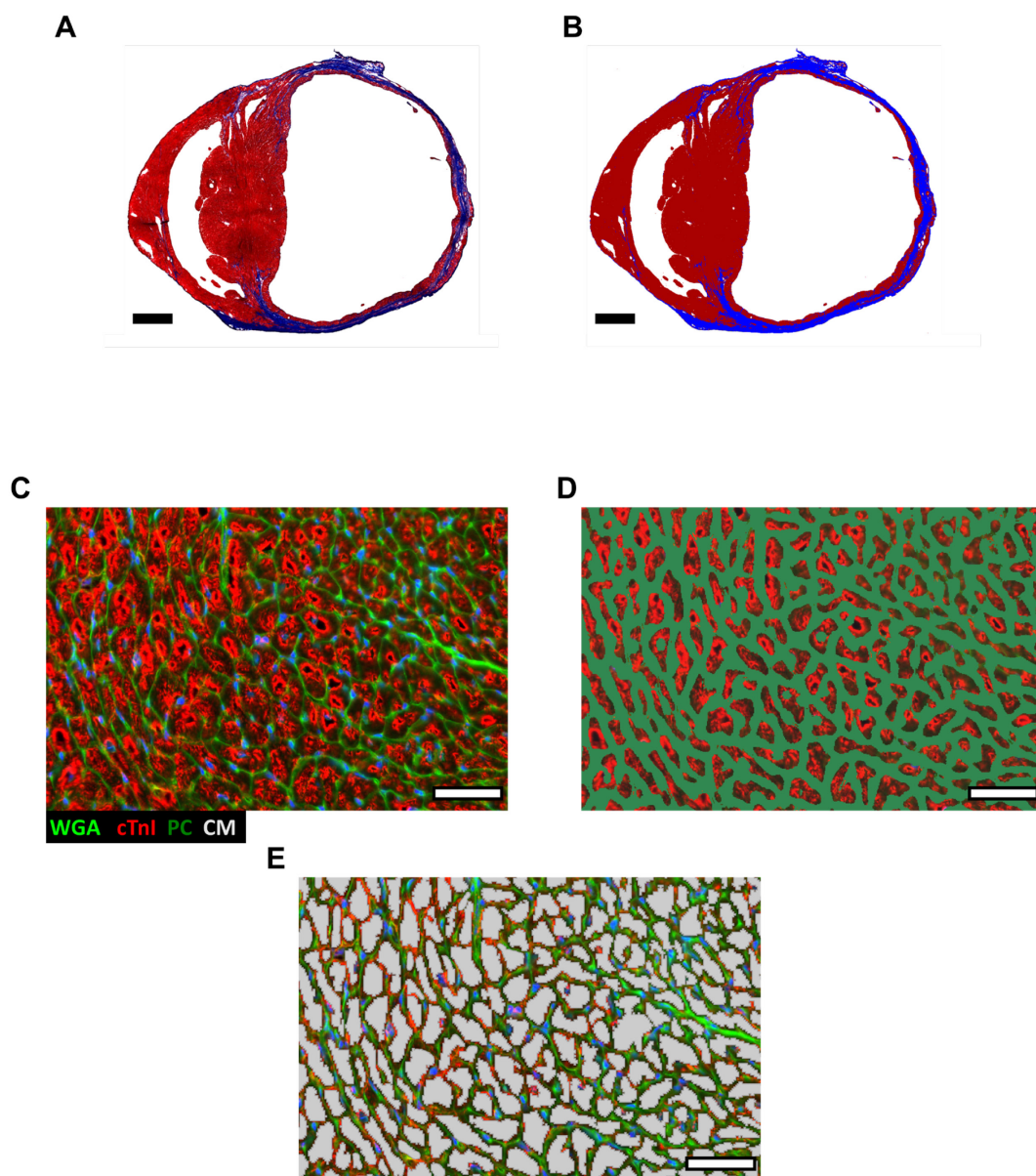


Figure 2.11. Pixel classifiers used to detect muscle and fibrosis in Masson's trichrome staining and cardiomyocytes from IHC stained heart sections. Seven days after MI, mice received intramyocardial injection of PBS or rHCI hydrogel. Twenty-eight days after treatment injection, hearts were harvested for histology. A) Heart sections were stained with Masson's trichrome procedure to detect fibrosis (blue) and muscle (red). B) A pixel classifier was trained in QuPath software to detect fibrosis (blue) and muscle (red). Scale bars are 500 μm. C) IHC staining for cardiomyocytes using cardiac troponin I (cTnI, red) and wheat germ agglutinin (WGA, green) to detect cell borders. D) A pixel classifier (PC) was used to detect WGA⁺ cardiomyocyte cell borders. E) Cardiac troponin⁺ cardiomyocytes (grey) were identified from the inverse of the PC area and manual correction. Scale bars are 500 μm. QuPath software was used for image analysis.

2.2.8 Mitochondrial Aldehyde Dehydrogenase 2 Activity Assay

Hearts were harvested two days after treatment injection and perfused with PBS. The remote myocardium was dissected from the infarct/borderzone and snap frozen in liquid nitrogen. Remote LV tissues were thawed in homogenization buffer on ice. The homogenization buffer contained cOmplete® ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor cocktail (Roche 4693132001) and PhosSTOP® phosphatase inhibitor cocktail (Roche 4906837001) dissolved in PBS. Thawed, remote LV tissue and 2 volumes of homogenization buffer were then added to Green Eppendorf Lysis kit tubes for the bullet blender homogenizer (Next Advance GREENE5). Tubes were homogenized in a blue BBX24B bullet blender homogenizer (Next Advance) at 4°C, on speed 8 for 4 min. The homogenates were then processed using the mitochondrial aldehyde dehydrogenase (ALDH2) activity assay kit from Abcam (ab115348) following the manufacturer's instructions. Briefly, protein was extracted from the homogenates by incubation with 4 volumes of kit extraction buffer on ice for 20 min. Protein extracts were isolated by centrifugation at 16000 x g for 20 min at 4°C. Protein concentration for each extract was then determined using the Pierce® BCA protein assay kit (ThermoFisher 22352). Extracts were stored at -80°C overnight. Extracts were diluted to 2000 µg/mL in incubation buffer and added to the ELISA plate for ALDH2 capture. Two replicates per sample were run. Following a 3-hour incubation at room temperature, the samples were washed and then nicotinamide adenine dinucleotide (NAD⁺), coupler and reagent dye mixture was added to each sample well. The plate was then analyzed by a kinetic reading of absorbance at 450 nm. As NAD⁺ is reduced by ALDH2, the increasing acidity of the solution leads to greater 450 nm absorbance from the reagent dye. The reaction was run for 120 min and the absorbance

at 450 nm was measured every minute. The rate of change in optical density overtime was calculated for each sample replicate. A blank sample was run that contained only incubation buffer and no protein extract to measure for background 450 nm absorbance over time.

2.2.9 Cell Isolation and Flow Cytometry for Monocyte Subsets

The cell isolation and flow cytometry procedure described previously in section 2.1.13 was used with the following specified modifications. In brief, two days post-treatment injection, cells were isolated from harvested mouse hearts. The cells were then incubated with an antibody cocktail containing CD45-APC/Fire 750 (1:160, Biolegend 30-F11), CD11b-PE/Cy7 (1:80, Biolegend M1/70), Ly6G-PerCP/Cy5.5 (1:160, Biolegend 1A8), F480-AF647 (1:100, Biolegend BM8), Ly6C-BV421 (1:80, BD Biosciences AL-21), CD86-PE (Biolegend 105008, 1:160) and anti-CD206 antibody (Biolegend 141710, 1:80). Flow cytometry was performed using a BD FACS Aria III and data was analyzed with FlowJo V10.5.2 software. M2 macrophages were detected using the gating strategy employed previously (see Figure 2.4) with minor modifications. Macrophages were detected as single live cells that were CD45⁺CD11b⁺Ly6G⁻F480⁺. The percentage of CD206⁺ macrophages was then determined using a threshold set from a CD206AF488 fluorescence minus one control sample. This percentage was then converted to M2 macrophage density per mg of heart tissue by using the known live cell density of the cell suspensions (counted by trypan blue exclusion in a hemocytometer) and the mass of tissue used to make the cell suspension.

2.2.10 Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0.0 software. The two exceptions were: the previously described differential gene expression analysis that was

performed in R and the slope for the ALDH2 activity assay was calculated in Excel. All data are presented as the mean $\pm$ SEM. For the echocardiography and strain data, a mixed-effects two-way ANOVA was used. This was followed by Holm's correction for multiple comparisons. The comparisons made were between treatment groups at a given time point and within each treatment group over time. For the qPCR and flow cytometry assays with three treatment groups, a one-way ANOVA was run with Tukey-corrected multiple comparisons between treatment groups. For the histology and ALDH2 activity data an unpaired t-test was used to compare two treatment groups at a given timepoint or region in the heart. Significance was determined by $FDR < 0.05$.

Gene	Forward	Reverse
<i>α-SMA</i>	GCCCAGCTTCGTCGTATTCC	ACGGCCGCCTCCTCTTCCTC
<i>MMP-1</i>	GTG CTC TCC TTCCACAGAGG	GGTCCACGTCTCATCAAGGT
<i>MMP-2</i>	AAGGATGGACTCCTGGCACATGCCTTT	ACCTGTGGGCTTGTCACGTGGTGT
<i>MMP-9</i>	AAGGACGGCCTTCTGGCACACGCCTTT	GTGGTATAGTGGGACACATAGTGG
<i>TIMP2</i>	CGTTTTGCAATGCAGACGTA	GAATCCTCTTGATGGGGTTG
<i>TIMP1</i>	GTAAGGCCTGTAGCTGTGCC	AGGTGGTCTCGTTGATTCGT
<i>Arg1</i>	AAAGCTGGTCTGCTGGAAAA	ACAGACCGTGGGTTCTTCAC
<i>18S</i>	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA

Table 2.1. List of qPCR primers for aims 1 and 2.

Primer	Sequence 5' – 3'
<i>ERDR1</i> Forward	CAG TGA TGT CAC CCA CGA AA
<i>ERDR1</i> Reverse	GGC ATT TCT GTA CGC AGT CA
<i>CCL22</i> Forward	CCT TCT TGC TGT GGC AAT TC
<i>CCL22</i> Reverse	ACG GTT ATC AAA ACA ACG CC
<i>GAPDH</i> Forward	TGA AGG GGT CGT TGA TGG
<i>GAPDH</i> Reverse	AAA ATG GTG AAG GTC GGT GT

Table 2.2. Primer sequences used in aim 3 for qPCR validation of genes of interest that are differentially expressed between PBS- and rHCI-treated mice. Primers were developed for qPCR detection of erythroid differentiation regulator 1 (ERDR1), c-c motif chemokine ligand 2 (CCL22; also known as macrophage derived chemokine), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All primers were designed based on the mm10 mouse genome.

Chapter 3: Results

3.0 Disclosure Statement

E.I.A., E.J.S., and M.R. are listed inventors in a patent application for the rHC materials presented in this study.

3.1 Injectable Human Recombinant Collagen Matrices Limit Adverse Remodeling and Improve Cardiac Function after Myocardial Infarction.

Results text and figures for aims 1 and 2 were published in *McLaughlin and McNeill et.al. Nature Communications 10 (2019) 4866*. The content is presented here with minor modifications.

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3.1.1 Authors Contributions

The echocardiography analysis of rHC hydrogel impact on cardiac function was done by me, with an initial trial being done by Brian McNeill. I was responsible for the Vevostrain analysis. I completed all follow-up histology analyses at day 28 post-rHC injection and for the short-term follow-up of AF594-tagged hydrogels. I designed and completed the flow

cytometry study of mouse leukocyte subsets in response to rHC injection. I also optimized and prepared all AF594-tagged hydrogels and completed the intramyocardial injection for these gels. I completed the IVIS analysis of rHC degradation *in vivo*, with help running the IVIS from Gregory Cron. David Smyth provided technical help and expertise running the flow cytometer. For the MI mouse model, MI surgeries were done by Richard Seymour and intramyocardial injection of untagged rHC hydrogels was done by Brian McNeill. The Cx3Cr1-GFP flow cytometry study as well as the BMDM cell culture analyses were completed by Brian McNeill. The NRVM studies were completed by Katsuhiko Hosoyama. The development and preparation of rHCI and rHCIII hydrogels was done by initially by James Podrebarac and then later by me. Physical characterization of the rHC hydrogels was completed by James Podrebarac. Veronika Sedlakova assisted with some of the AF594 animal studies and completed the electrocardiogram data analysis. Keshav Goel assisted in writing SOPs for the rHC hydrogels. I completed most of the statistical analysis for this study. Study design and trainee supervision was done by Wenbin Liang, Katey Rayner., Marc Ruel, Emilio Alarcon, and Erik Suuronen. Manuscript preparation was done by me, Brian McNeill, Emilio Alarcon, and Erik Suuronen, with input from all authors.

3.1.2 Brief Introduction to the Results for Aims 1 and 2

Herein, we report the first injectable biomaterials made from recombinant human collagens type I and type III in order to produce a clinically translatable material for cardiac therapy. Notably, these proteins have been previously used for the fabrication of corneal implants, which were demonstrated to be safe when transplanted in humans²⁶¹. We investigated the pre-clinical performance of our injectable rHCI and rHCIII materials in a well-established mouse model of MI²⁸³. Intramyocardial injections of rHCI or rHCIII are

administered to mouse hearts at 1-week post-MI, which is a model for patients who have a delay in getting medical attention (e.g. remote location, or ignore early signs after heart attack) or who do not adequately respond to conventional treatment, and are at greater risk of ECM remodeling and developing heart failure^{284,285}. This time-point constitutes the proliferative phase of infarction during which scar formation is initiated (mice: 2-7d; humans: 4-14d) and is a prime opportunity to intervene to limit pathological ECM remodeling and promote infarct repair^{18,286-288}. The primary outcome is cardiac function determined by echocardiography 28 days after treatment, and we assessed cardiac morphology, ventricular remodeling, vascularization, and inflammation in the MI heart in response to rHC treatment. In addition, we used *in vitro* studies to elucidate how macrophages interact with the rHC materials. Our results highlight the potential for a clinically relevant biomaterial based on human recombinant collagen to be used as therapy for the amelioration of cardiac function post-MI through the promotion of a wound healing environment, cardiomyocyte survival, and reduced pathological remodeling.

3.1.3 Synthesis and Characterization of rHC Matrices

The collagen formulations used in this work were developed as thermo-responsive matrices to ensure their retention within the myocardium upon intramyocardial injection. In designing the materials, the goal was to have a gelation time of less than 10 min to allow sufficient time for injection in liquid form with minimal time required for subsequent gelation. The same total concentration of collagens (rHCI and rHCIII) and the crosslinker agents EDC and NHS was used to maintain consistency in the chemical composition between the rHCI and rHCIII matrices. Our rHC matrices are also glycosylated, containing the glycosaminoglycan chondroitin sulfate C (Figure 3.1A). The hydrogels are then cross-linked

in situ and assembled into a 3D matrix to provide a biomimetic niche for promoting endogenous repair within the infarcted myocardium.

Once cross-linked, the resulting rHCI and rHCIII matrices had equivalent denaturation temperatures of $>45^{\circ}\text{C}$ (Figure 3.1B). Similarly, water content of both rHC hydrogels was determined to be $\approx 94\%$ ($p=0.47$). The rHCI matrices were degraded ≈ 4 times more quickly than the rHCIII matrices upon exposure to collagenase (Figure 3.1C) and also had 2.9-fold lower viscosity (Figure 3.1D). Porosity is critical for cell infiltration and engraftment, and pore sizes of 9.3 ± 0.3 and 29 ± 1.0 μm were measured for the rHCI and rHCIII matrices, respectively (Figure 3.1E). The presence of chondroitin sulfate did not affect either the denaturation temperature or the porosity of the material (Supplementary Figure S1A & B). To characterize the injection and retention properties of the collagen matrices, they were labeled with Alexa-Fluor®594-NHS dye prior to injection to the MI mouse heart. Ex vivo fluorescence imaging revealed that the injected rHCI and rHCIII matrices were localized and retained within the injection site for at least up to 7 and 2 days, respectively. This was also confirmed by histological analysis of the infarct region using the labelled matrix fluorescence emission (Figure 3.1F). Based on these labeling techniques, the injected rHC matrices appear to localize primarily within the epicardial tissue spreading across the infarct and border zone areas. This was also observed in fluorescent imaging and H&E staining of tissue sections at an earlier 2h post-delivery time-point (Supplementary Figure S2A & B). Sections at different depths from the apex to the base of the rHC-treated hearts further confirm the presence of the rHC material within the epicardium and its spread over the infarct and border zone areas at 2h post-injection (Supplementary Figure S2C & D).

In summary, the characterization of the rHC materials indicate that they possess physical properties (denaturation at higher than body temperature, degradability by collagenase and porous structure), and *in vivo* properties (they remain in the infarct area for at least 2 days post-injection) that make them good candidates as injectable materials for treating the infarcted myocardium.

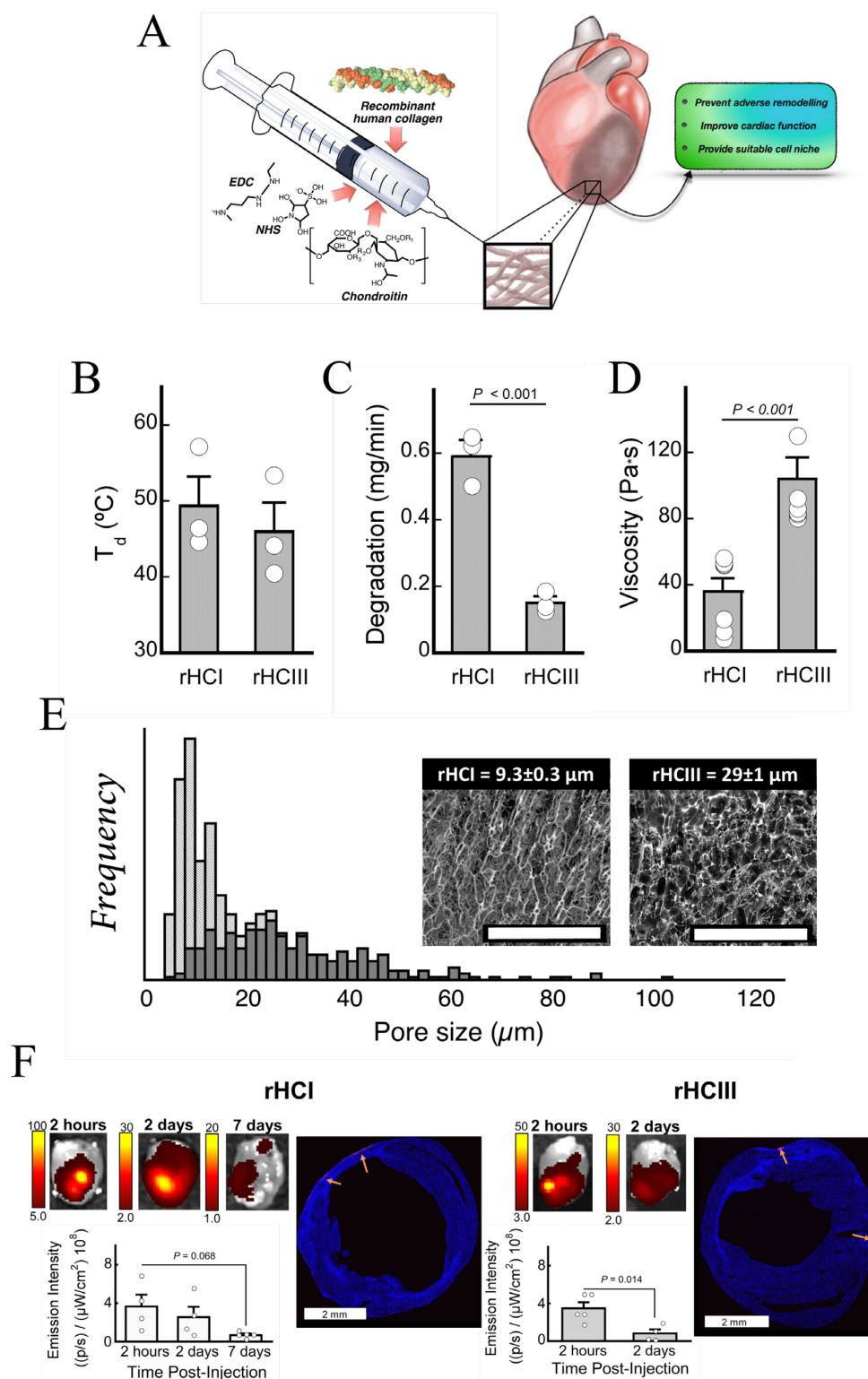


Figure 3.1. Preparation and physical characterization of injectable rHC matrices. (A) Schematic depicting the components, delivery and reparative properties of the injectable material presented in this study. (B) Denaturation temperatures (T_d) (°C; $n=3$), (C) in vitro

degradation in collagenase (mg/min; n=3), and (D) viscosity measurements (Pa·s; n=7) for rHCI and rHCIII matrices. (E) Pore size distribution for the rHCI (gray bars) and rHCIII (black bars) matrices calculated from 250 individual pores per sample. Inset: Representative Cryo-SEM images of the rHC matrices. Scale bar = 200μm. (F) Representative images of ex vivo imaging of MI mouse hearts at 2h and 2 days after treatment with rHCI and rHCIII matrices, labeled using Alexa-Fluor®594-NHS dye. Bottom histograms display the average total fluorescence emission for the different time-points (n=4). Also shown are representative histological sections of the myocardium at 2 days post-injection. Arrows indicate representative regions containing the collagen matrices labelled with the fluorescent dye. P values were determined by a 2-tailed t-test for rHCIII and ANOVA with Dunnett's correction for rHCI. For B-D $\pm$ data is presented as the mean $\pm$ SD and in F $\pm$ corresponds to SEM. Source data are provided as a Source Data file. For B-E, n indicates number of hydrogel batches. For F, n is the number of mice per group.

3.1.4 rHC Matrix Improves Post-MI Cardiac Function and Morphology

The therapeutic potential of the rHC matrices was tested using a mouse model of MI with the primary endpoint being LVEF 28 days after treatment. One week after MI, mice were randomized to receive intramyocardial injection of rHCI matrix, rHCIII matrix or PBS (control). Treatment with rHCI resulted in an improvement in LVEF from $34.6 \pm 1.6\%$ at baseline to $44.2 \pm 1.9\%$ at 28 days post-treatment; whereas LVEF between baseline and follow-up was unchanged for rHCIII-treated hearts and it deteriorated in hearts that received PBS (Figure 3.2A). At 28 days, LVEF was greater in hearts treated with rHCI or rHCIII matrix compared to PBS-treated hearts (Figure 3.2A). Similar to PBS treatment, the injection of pure non-cross-linked collagens (rHCI or rHCIII) resulted in a reduction in LVEF (Supplementary Figure S3), which indicates that rHCI and rHCIII require assembly into a crosslinked 3D matrix to confer their positive effects on post-MI cardiac function.

For other parameters of cardiac function, the FAC at 28 days relative to baseline was superior in rHCI-treated hearts compared to PBS and rHCIII treatment (Figure 3.2B). The

change in ESV was reduced in rHCI-treated hearts compared to the other 2 groups (Figure 3.2C), whereas no difference was observed for EDV (Figure 3.2D). ESV at 28 days was increased in the PBS group compared to rHCI matrix-treated mice (Supplementary Figure S4A), indicating worse remodeling and a worsening of cardiac function in the PBS-treated mice. For EDV at 28 days, no difference was observed between groups, but it was significantly increased for rHCIII-treated hearts at 28 days compared to its baseline (Supplementary Figure S4B). Also, both the rHCI and rHCIII treatments improved the fold change in SV and CO from baseline to follow-up vs. PBS-treated hearts (Figure 3.2E & F). Notably, the tensile elasticity of the infarcted myocardium was restored by rHCI and rHCIII treatment at 2 days post-injection to levels comparable to that of the healthy myocardium, and this was maintained up to 28 days for rHCI (Figure 3.2G). In contrast, elasticity of the PBS-treated infarcted myocardium was severely compromised after 2 days and was too weak to undergo testing at 28 days due to the extreme thinning and frailty of the ventricular wall. In vivo, analysis of longitudinal endocardial strain through speckle tracking echocardiography²⁶³ demonstrated a significant improvement in the strain reached by the mid anterior LV wall at end systole, which is marked by the aortic valve closure (AVC), two days after injection of rHCI (Figure 3.2H). The mid anterior LV wall is the segment of the myocardium targeted for hydrogel injection as it contains the accessible infarct borderzone. The longitudinal endocardial strain becomes more negative during systole as the heart shortens in this direction due to the stress placed on the myocardium during contraction. In healthy animals, strain peaks at the AVC, which is an indicator of end systole and strain at this point is a measurement of myocardial contractility (Supplementary Figure S5). There is no difference in contractility between treatment groups in the infarct or remote myocardium 2 days post-injection (Supplementary Figure S5). Therefore, the strain analysis provides

evidence that rHCI injection, but not rHCIII, improves contractility in the borderzone area of the LV wall where it was injected as compared to PBS treated animals. Neither rHC matrix treatment affected the heart rate or any of the electrocardiographic parameters at 2 days post-injection (Supplementary Table S1), with the exception of the PR interval for the rHCI matrix group. This indicates that the rHC matrices do not negatively interfere with electrical conductivity in the myocardium.

Cardiac remodeling can lead to enlargement of the heart in advanced stages post-MI. While mice that received PBS treatment had enlarged hearts, this was prevented in mice whose hearts were treated with rHCI matrix (Figure 3.3A & B). Coinciding with this reduced remodeling, rHCI-treated hearts had greater remote ventricular wall thickness compared to the wall thinning observed with PBS treatment (Figure 3.3C & D). Masson's trichrome staining at 28 days showed that scar size was reduced by 37% and 31% for the rHCI and rHCIII treatment groups, respectively, compared to PBS-treated hearts (Figure 3.3C & E).

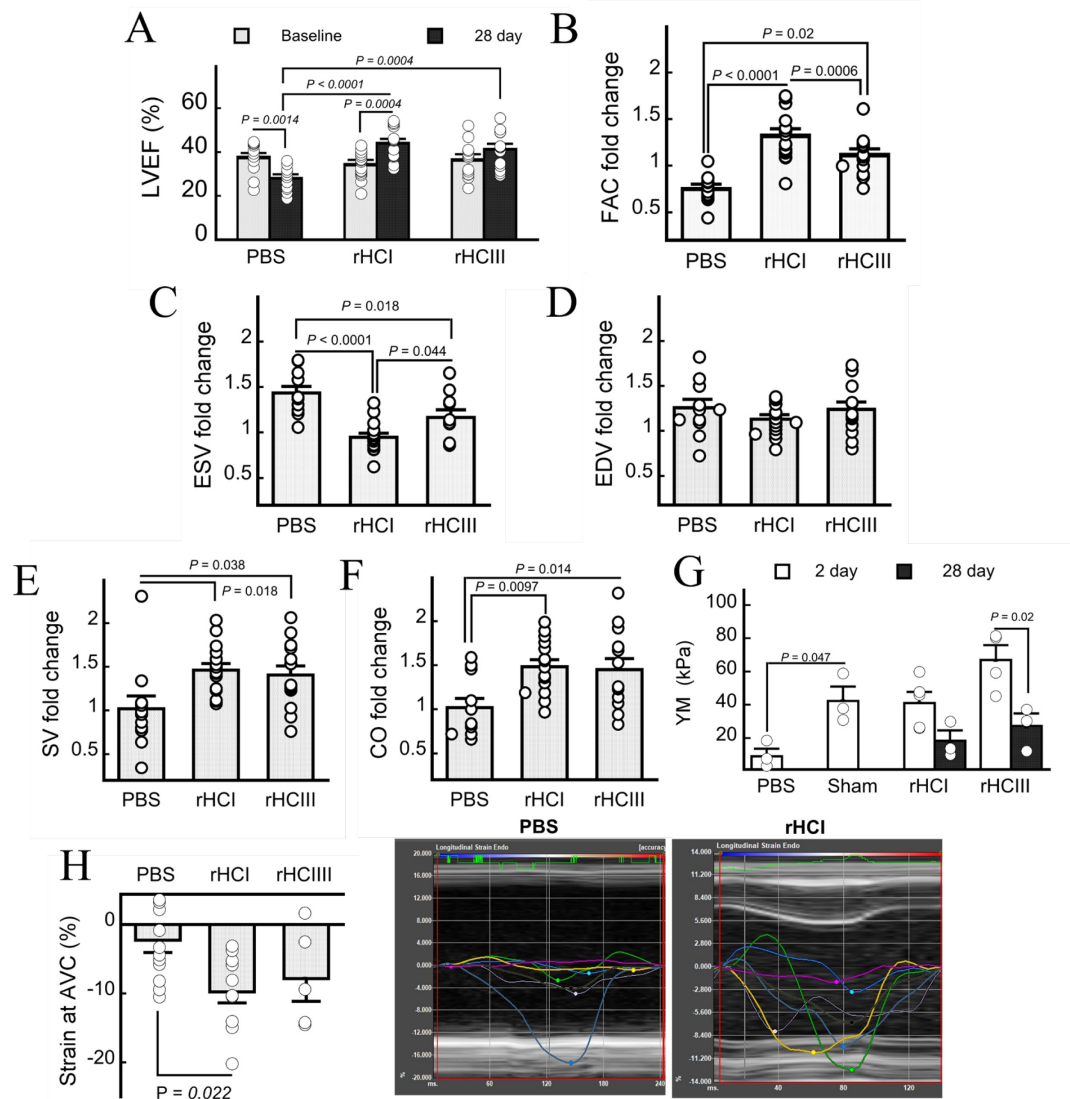


Figure 3.2. rHC matrices improve cardiac function post-MI and restore mechanical properties. (A) Left ventricular ejection fraction (LVEF) at baseline (time of treatment delivery) and at 28 days post-treatment (n=11 for PBS, n=15 for rHCI, and n=13 for rHCIII). Significance determined by a two-way ANOVA followed by Holm's correction for multiple comparisons. (B-F) The change in (B) fractional area change (FAC), (C) end-systolic volume (ESV), (D) end-diastolic volume (EDV), (E) stroke volume (SV), and (F) cardiac output (CO) between baseline and 28 days post-treatment (n=11 for PBS, n=15 for rHCI, and n=13 for rHCIII). P values were determined by an ANOVA followed by Holm corrected multiple comparisons. (G) Young's modulus (YM) for scar/border zone tissue measured at 2 and 28 days (n=3-4). (H) Left: Strain force reached at the aortic valve closure within the mid anterior LV wall at 2 days post-injection for the different experimental groups (n=5-12). Right: Representative longitudinal strain curves for the PBS and rHCI groups. The yellow traces correspond to the anterior mid region. Significance determined by a one-way ANOVA

followed by Dunnett's comparison as well as a two-tailed student's t-test within a treatment group over time. Data is presented as the mean $\pm$ SEM. Source data are provided as a Source Data file. For A-H, n indicates the number of mice per group.

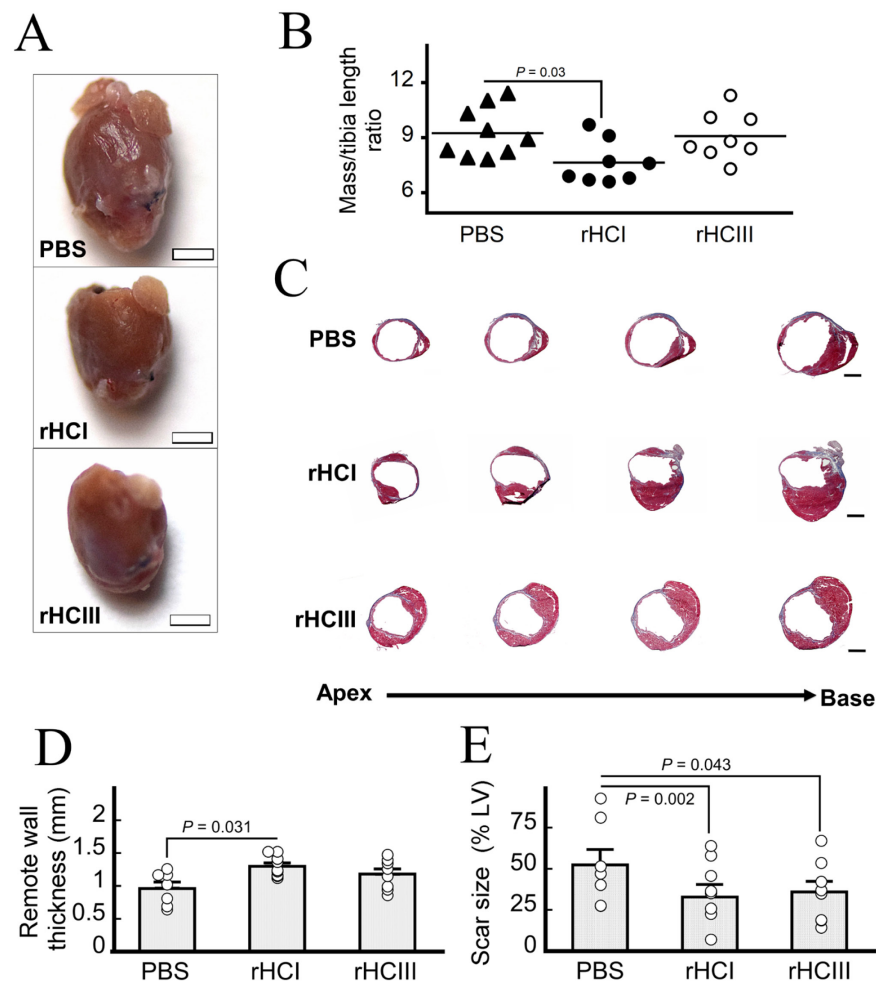


Figure 3.3. Morphology of MI hearts is improved by rHCI treatment. (A) Representative images of hearts harvested 28 days after treatment (bar = 2mm). (B) Scatter plot for the heart mass/tibia length ratio measured 28 days post-injection (n=8-9). P values were determined by a one-way ANOVA followed by Holm's multiple comparisons to PBS control. (C) Representative Masson's trichrome stained sections of hearts harvested at 28 days (bar = 1 mm). (D) Wall thickness of the remote myocardium measured in Masson's trichrome stained tissue sections at 28 days post-treatment (n=7-8). P values were determined by one-way ANOVA using Holm's multiple comparison. (E) Scar size (% of LV) measured at 28 days post-treatment in Masson's trichrome stained tissue sections (n=7-8). P values were determined by multiple regression analysis including treatment group (rHCI, rHCIII and

PBS) and baseline LVEF. rHCI and rHCIII as compared to PBS were significant predictors of infarct size, in addition to baseline LVEF. The correlation coefficient for the model is 0.6. Data is presented as the mean $\pm$ SEM. Source data are provided as a Source Data file. For A-E, n indicates the number of mice per group.

3.1.5 Benefits of rHCI Matrix on Vascularity and Cardiomyocytes

To provide insight into the mechanisms underlying the functional benefit of rHC matrix therapy, immunohistochemistry was performed to assess vascularity and cardiomyocyte preservation in the scar and border zone at 28 days post-treatment. Although no difference in vascular density (arterioles or capillaries) was observed in the scar area between groups, the number of capillaries was increased in the border zone of the hearts treated with rHCI or rHCIII matrix compared to PBS (Figure 3.4A). The area of cardiac troponin I expression (a cardiomyocyte marker) was also shown to be greater in the border zone of hearts treated with rHCI matrix compared to PBS treatment (Figure 3.4B), suggesting greater preservation of cardiomyocytes. Notably, the level of connexin 43 expression in the remote zone was greater for the rHCI matrix group compared to PBS (Figure 3.4C).

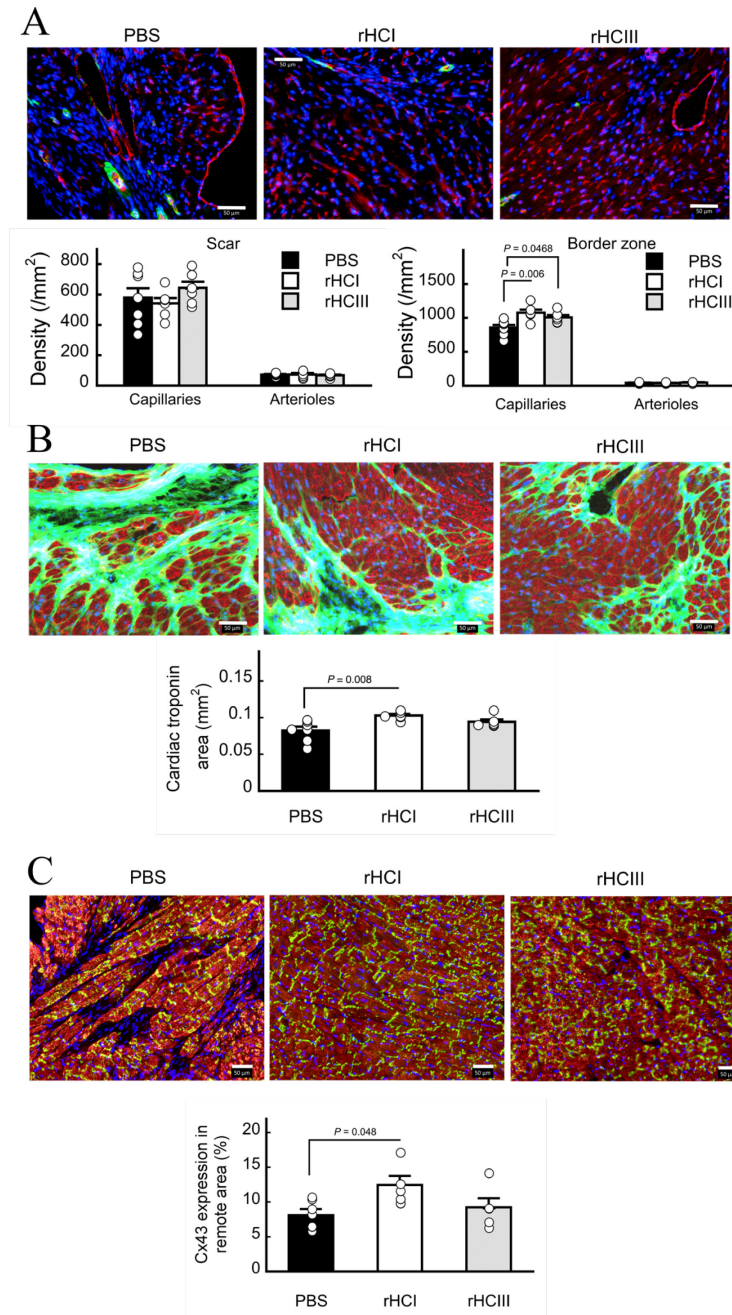


Figure 3.4. rHC matrix increases vascularity and preserves cardiomyocytes post-MI. (A) Immunohistochemistry analysis and quantification (number/ mm^2) of capillary and arteriole number in the myocardial scar and border zone after treatment with PBS, rHCI or rHCIII. CD31, red; α -SMA, green; DAPI, blue ($n=6-7$). (B) Immunohistochemistry analysis and quantification of total area (in mm^2 positive for cardiac troponin I (cTnI) staining in the border zone after treatment with PBS, rHCI or rHCIII. cTnI, red; wheat germ agglutinin, green; DAPI, blue. (C) Immunohistochemistry analysis and quantification of connexin 43 (Cx43) expression (percent area) in the remote area after treatment with PBS, rHCI or rHCIII.

Cx43, green; cTnI, red; DAPI, blue (n=6-7). P values were determined by one-way ANOVA using Holm's multiple comparison. Data is presented as the mean $\pm$ SEM. Source data are provided as a Source Data file. For A-E, n indicates the number of mice per group.

3.1.6 rHC Matrix Treatment Alters Mononuclear Cell Recruitment.

Cell death post-MI stimulates an inflammatory response in the myocardium. Promoting the polarization of pro-wound healing M2 macrophages over the pro-inflammatory M1 macrophage phenotype has been shown to attenuate chronic inflammation and improve cardiac repair post-MI¹⁰. In our study, 28 days after rHCI matrix treatment there was a $\approx$ 1.5-fold increase in the number of CD206⁺ M2 macrophages in the scar region compared to the PBS group (Figure 3.5A). In response to death signals post-MI, inflammatory cells home to the infarcted myocardium from the circulation after their mobilization from the bone marrow. Therefore, we assessed the effect of rHC treatment on the homing of bone marrow cells to the myocardium using Cx3cr1-EGFP mice, whose bone marrow cells express green fluorescent protein (GFP). Our results showed a reduced total number of recruited GFP⁺ cells in the left ventricle of hearts 2 days after treatment with rHCI matrix vs. those treated with rHCIII or PBS (Figure 3.5B). By co-staining with inflammatory cell markers, we observed that the number of recruited GFP⁺CD38⁺ and GFP⁺CD11b⁺ leukocytes was reduced in rHCI-treated hearts (Figure 3.5B).

For further characterization of mobilized monocytes, we evaluated peripheral blood mononuclear cells in wild-type mice 2 days after treatment. There was an increase in the overall number of circulating monocytes and a trend for increased numbers of Ly6C^{hi} monocytes in the rHCIII-treated mice compared to rHCI (Figure 3.6A). No change in the other types of blood monocytes was seen between groups (Supplementary Figure S6). In the

myocardium, the rHCIII matrix increased the number of Ly6C^{hi} monocytes 2 days after treatment, compared to PBS and rHCI (Figure 3.6B), whereas there was no overall change in the numbers of leukocytes, macrophages, Ly6C^{lo} monocytes, neutrophils, T-cells, or B-cells (Supplementary Figure S7). Finally, there was a higher retention of Ly6C^{lo} monocytes in the spleen for the rHCIII group compared to PBS (Supplementary Figure S8).

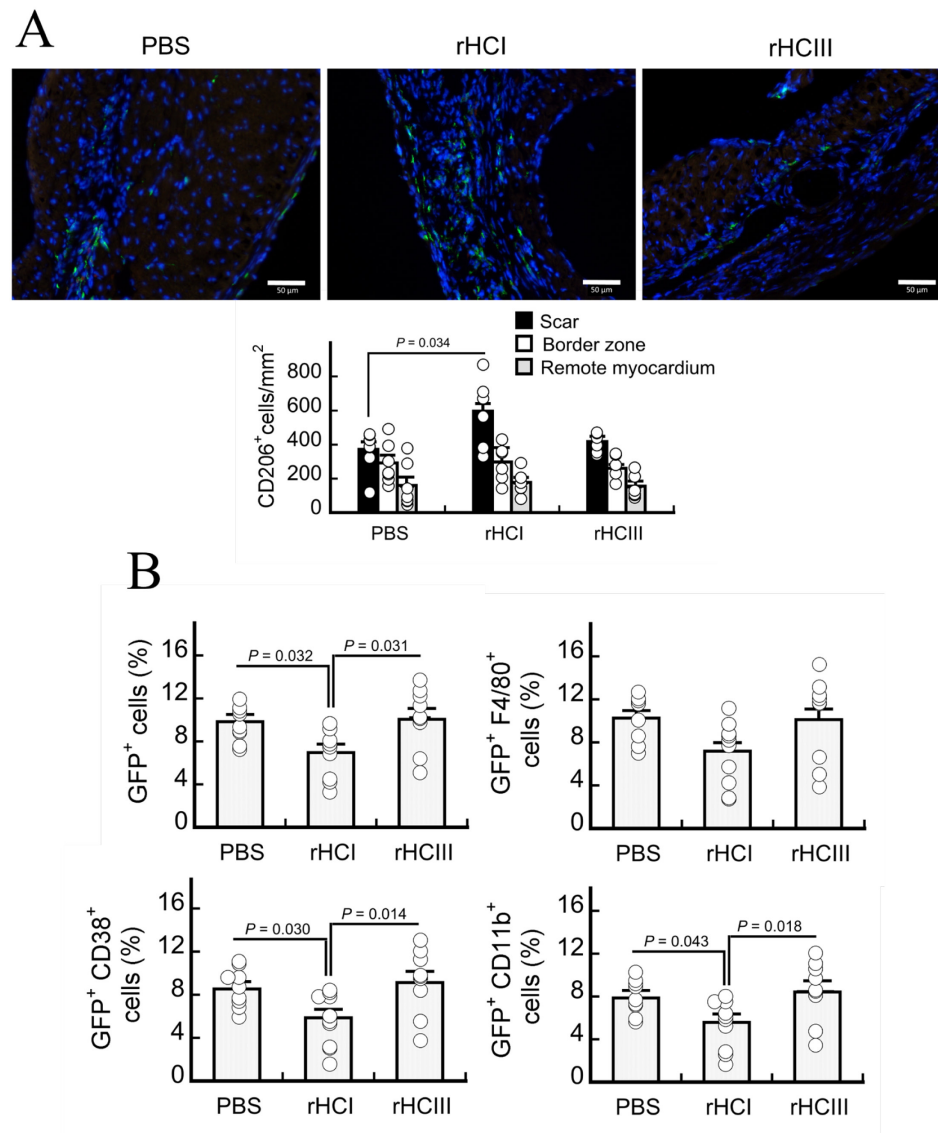


Figure 3.5. rHC matrix treatment alters bone marrow cell and macrophage response. (A) Immunohistochemistry analysis and quantification of the number of CD206⁺ macrophages (/mm²) in the scar, border zone (BZ) and remote myocardium (RM) of MI hearts treated with

PBS, rHCl or rHCIII. CD206, green; DAPI, blue (n=6-7). (B) Flow cytometry analysis for the number of GFP⁺, GFP⁺F4/80⁺, GFP⁺CD38⁺ and GFP⁺CD11b⁺ cells recruited from the bone marrow to the left ventricle of Cxcr3-EGFP MI mice 2 days after PBS, rHCl or rHCIII treatment (n=9-10). P values were determined by one-way ANOVA using Holm's multiple comparison. Data is presented as the mean $\pm$ SEM. Source data are provided as a Source Data file. For A-B, n indicates the number of mice per group.

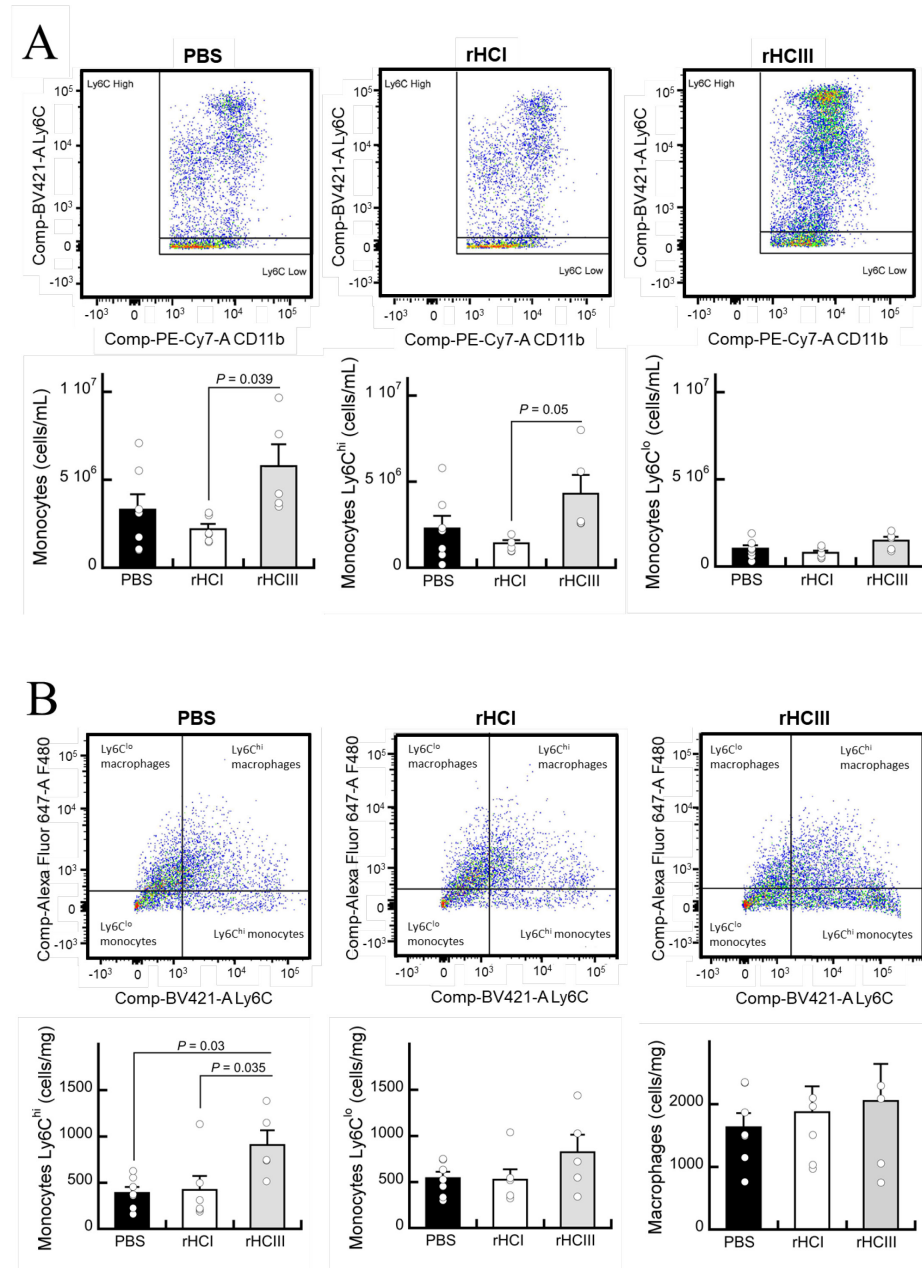


Figure 3.6. rHCIII matrix increases Ly6C^{hi} monocyte mobilization and accumulation in the heart. Flow cytometry plots show F480 and Ly6C expression of mononuclear cells (CD45⁺CD11b⁺Ly6G⁻) in the blood (A) and heart (B) of mice at 2 days post-treatment.

Macrophages were considered F480⁺ while monocytes were F480⁻ and classified based on the expression of the Ly6C marker. Data is presented as the mean $\pm$ SEM (n=5-7). P values were determined by a one-way ANOVA for effect of treatment followed by Holm's correction for multiple comparisons. Source data are provided as a Source Data file. For A-B, n indicates the number of mice per group.

3.1.7 In Vitro Assessment of rHC Matrices

We next explored the interaction of the rHC matrices with macrophages in vitro to gain insight into how the materials may be regulating inflammatory cell function and conferring therapeutic benefit in vivo.

Bone marrow-derived macrophages from mice were used to evaluate the effect of the rHC matrices on macrophage function. The adhesion of unstimulated M0 macrophages to the rHCI and rHCIII matrices was equivalent with (Figure 3.7A) or without (Supplementary Figure S9) the inclusion of chondroitin sulfate; however, the number of M0 macrophages to migrate through the rHCIII matrix was 2-fold greater than for the rHCI matrix (Figure 3.7B). The effect of the rHC matrices on macrophage polarization was also assessed. The results indicate that both matrices promote the polarization of the M2 wound healing phenotype (Figure 3.7C) compared to TCPS, with rHCIII promoting greater M2 macrophage differentiation than rHCI. Gene expression analysis for several ECM remodeling proteins including MMP1, MMP2, MMP9, TIMP1 and TIMP2, as well as the M2 phenotype marker Arg1 was performed on macrophages cultured on TCPS, rHCI and rHCIII. The expression of MMP1 was increased in macrophages cultured on the rHCIII matrices vs. TCPS, while Arg1 expression was increased in macrophages cultured on rHCI compared to TCPS (Figure 3.7D). To mimic the presence of oxidative stress post-MI, cultured macrophages were exposed to hydrogen peroxide (H₂O₂); and the results indicate that cells cultured on the rHCI and rHCIII matrices were protected from H₂O₂-induced death (Figure 3.7E). The same H₂O₂

exposure experiments were performed with NRVMs; no protective effect from the collagen matrices was observed (Figure 3.7F).

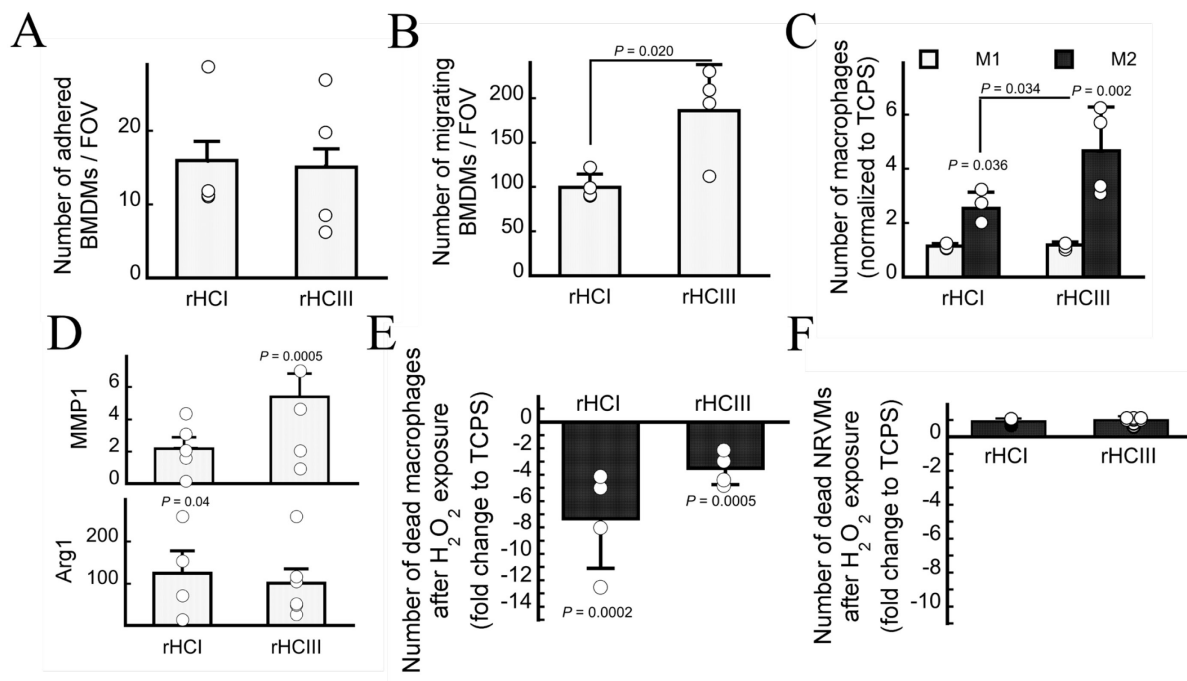


Figure 3.7. rHC matrices support wound healing macrophages. (A) Number of adherent macrophages after culture on rHC matrices (n=4). (B) Number of macrophages that migrated through 300 μ m thickness rHC matrices (n=4). (C) M1 and M2 macrophage polarization determined by CD86 and CD206 expression, respectively, after culture on rHC matrices (n=4). (D) MMP1 and Arg1 mRNA expression in macrophages cultured on rHC matrices for 72h relative to TCPS (n=4). (E) Percentage of dead macrophages (7-AAD⁺ cells) after 3h incubation with H₂O₂ (n=4). (F) Percentage of dead neonatal rat ventricular myocytes (NRVMs) after 3h incubation with H₂O₂ (n=4). P values were determined by a one-way ANOVA using Holm's multiple comparison (C, D, E, and F), or by a 2-tailed t-test (A and B). All data is presented as the mean $\pm$ SD. Source data are provided as a Source Data file. For A-E, n indicates the number of mice (mouse bone marrow) per group. For F, n indicates the number of hydrogel batches.

3.2 Recombinant human collagen type 1 hydrogel rapidly reduces methylglyoxal levels in cardiomyocytes and increases contractility of the border zone myocardium in a mouse model of myocardial infarction.

This study contains unpublished data for the results of Aim 3, for which a manuscript is currently in preparation.

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3.2.1 Authors Contributions

Preparation of rHCI hydrogel, with and without AF594 fluorescent dye, and rHCIII hydrogel was done by me. Surgery to induce myocardial infarction in mice was performed by Richard Seymour. The intramyocardial injections of all hydrogel or PBS control treatments were done by me, except for three mice that were done by Brian McNeill (he also completed the baseline echocardiography for these animals). Baseline echocardiography was completed by me and Veronika Sedlakova. Follow-up echocardiography at day 2 and day 28 was completed by me. Most of the echocardiography and Vevostrain analysis was completed by me with assistance on one trial from Veronika Sedlakova. Harvesting and sectioning of harvested hearts for histology was completed by me and Veronika Sedlakova. All histology and immunohistochemistry experiments were completed by me, except the immunohistochemistry for regulatory T-cells which was completed by Veronika Sedlakova. Isolation of RNA for sequencing was completed by me. Next generation sequencing was

completed by Genome Quebec. Differential expression analysis was completed by the Canadian Centre for Computational Genomics (C3G). Enrichr analysis of differentially expressed genes was run by me. Output figures for RNA seq were completed by C3G except for: Enrichr output in Figure 2 and the heatmap and graphs in Figure 3 which were completed by me. QPCR primer design and qPCR experiments were run by me. In situ hybridization for erythroid differentiation regulator 1 was completed by me. Protein isolation and mitochondrial aldehyde dehydrogenase activity assay were run by me. Flow cytometry assay design, cell isolation/staining and data analysis were done by me. David Smyth helped with running the flow cytometer. Image analysis and pixel classifier analysis in QuPath, statistical analysis and figure design were completed by me. Study design was done by me, Erik Suuronen and Emilio Alarcon. The written portion of this unpublished section was completed by me with revisions by Erik Suuronen and Emilio Alarcon. Supervision for this work was provided by Erik Suuronen, Emilio Alarcon, and Marc Ruel.

3.2.2 Brief Introduction to the Results of Aim 3

Remodeling of the cardiac ECM leads to progressive ventricular dilation and heart failure after MI¹¹⁰. Injectable hydrogels are promising therapeutics that can stabilize the infarct region and improve wound healing post-MI, limiting progression to heart failure¹⁰⁰. An rHCI hydrogel was previously shown to improve cardiac function 28 days post-injection in a mouse model of MI²⁶⁹. This functional improvement was maintained well after there was evidence that the majority of the hydrogel had degraded, which occurred within 7 days post-injection. There was also evidence that contractility of the infarct borderzone improved by 2 days after rHCI injection, suggesting that rHCI injection may lead to acute functional improvement. In this study, rHCI hydrogel injection post-MI was found to improve cardiac

function within 2 days and the improvements in cardiac function and contractility were sustained out to the original 28-day endpoint. The rHCI hydrogel reduced oxidative stress and cardiomyocyte apoptosis 2 days after injection in MI mice. The hydrogel soaked up a reactive dicarbonyl compound, methylglyoxal (MG), and reduced MG adducts (MG-H1) within cardiomyocytes and on ECM collagen within the myocardium. MG has been shown to be a key contributor to oxidative stress, pathological ECM remodeling, reduced vascularization, and impaired cardiac function post-MI. The reduction in the levels of MG-H1 in rHCI-treated hearts was maintained to 28 days post-injection and there was also less hypertrophy and myocyte loss in rHCI-treated mice.

3.2.3 rHCI Hydrogel Improves Cardiac Function Two Days After Injection in a Mouse Model of MI

Treatments of rHCI hydrogel and PBS were injected into the border zone myocardium at 7 days post-MI (baseline). Two studies were run to analyze both the acute changes in cardiac function with treatment, 2 days after injection, and to test if the acute changes in cardiac function are sustained out to the 28-day post-injection endpoint (Figure 3.8A). LVEF was improved 2 days after rHCI hydrogel injection ($40 \pm 2\%$) compared to baseline ($29 \pm 2\%$; $p < 0.0001$) (Figure 3.8B). Two days after treatment injection, the LVEF of rHCI hydrogel treated mice was also greater than that of PBS treated mice ($30 \pm 2\%$; $p = 0.013$). Injection of rHCI hydrogel results in a 13% reduction in ESV at 2 days post-injection compared to baseline ($p = 0.03$) (Supplementary Figure S10A). There was no change in EDV between treatment groups or within a treatment group from baseline to follow-up (Supplementary Figure S10B). SV ($p = 0.008$) and CO ($p = 0.001$) are both improved with injection of rHCI hydrogel compared to PBS two days after treatment (Supplementary Figure

S10C-D). SV ($p = 0.0001$) and CO ($p < 0.001$) also increased from baseline to follow-up, 2 days after rHCI hydrogel injection. To determine if these improvements in cardiac function are sustained out to the 28-day endpoint, an additional MI mouse trial was run to compare the fold change from baseline in functional parameters at day 2 and at day 28. The fold change in LVEF from baseline is greater in rHCI-treated mice compared to PBS-treated mice at both the day 2 (1.37 ± 0.07 vs. 0.93 ± 0.03 , $p < 0.0001$) and day 28 endpoint (1.39 ± 0.07 vs. 0.91 ± 0.03 , $p < 0.0001$) (Figure 3.8C). There is no difference in the fold change of LVEF from baseline for rHCI treated mice at day 2 compared to day 28 ($p = 0.83$). The fold change in ESV from baseline is reduced in rHCI treated mice compared to PBS treated mice at both the day 2 (0.96 ± 0.06 vs. 1.10 ± 0.03 , $p < 0.043$) and day 28 endpoint (1.15 ± 0.04 vs. 1.36 ± 0.05 , $p = 0.007$) (Figure 3.8D). Both rHCI ($p = 0.026$) and PBS ($p = 0.002$) treated mice show an increase in the fold change of ESV from baseline at day 28 compared to day 2. There is no difference in EDV fold change from baseline between either treatment group at day 2 or day 28. The fold change in EDV increases in both rHCI (1.14 ± 0.04 vs. 1.32 ± 0.03 , $p = 0.005$) and PBS (1.06 ± 0.02 vs. 1.31 ± 0.04 , $p < 0.0001$) treatment groups from day 2 to day 28 (Figure 3.8E). The fold change in both SV and CO is greater in rHCI treated mice compared to PBS treated mice by at both day 2 and day 28 endpoints (Supplementary Figure S11A-B). The fold change from baseline in both SV and CO increased from day 2 to day 28 for rHCI and PBS treatment groups.

Contractility of the myocardium was measured from echocardiograms by calculating the strain in the base, mid and apex regions of the anterior and posterior LV walls. Strain was measured in the longitudinal direction at end systole, marked by the aortic valve closure. In the healthy heart, LV segments shorten from end diastole to end systole due to contraction

of the myocardium and the average strain was -17% in healthy mice. Strain was measured in the six myocardial regions at baseline and 2 days after rHCI or PBS treatment injection (Supplementary Figure S12). As observed in previous studies, strain in the anterior mid LV wall is increased in rHCI treated mice ($-7\pm 1\%$) compared to PBS treated mice ($1\pm 3\%$) two days after treatment injection ($p = 0.028$) (Supplementary Figure S12E). In addition, strain increased in this region for the rHCI treatment group from baseline ($1\pm 2\%$) to 2 days post-treatment ($p = 0.001$). The mid anterior LV region contains the anterior infarct borderzone, which is the region targeted for treatment injection. Strain was unchanged in the posterior base, mid, and apex regions as well as the anterior base and apex regions between treatment groups or within a group over time (Supplementary Figure S12A-D,F). The average strain (Supplementary Figure S12G) and the maximum opposing wall delay (Supplementary Figure S12H) to reach peak strain were unchanged with treatment group or with time. The change in strain from baseline was also measured at day 2 and day 28 post-injection for PBS and rHCI hydrogel treated mice (Supplementary Figure S13). The change in anterior mid LV wall strain from baseline was greater in rHCI treated mice as compared to PBS treated mice at both day 2 ($-7\pm 1\%$ vs. $1\pm 1\%$, $p = 0.002$) and day 28 ($-5\pm 1\%$ vs. $2\pm 1\%$, $p = 0.002$) post-treatment injection (Figure 3.8F). There is no difference in mid anterior wall contractility for rHCI treated mice at day 2 compared to day 28 post-hydrogel injection ($p = 0.73$). The change in strain from baseline in all other LV segments, average strain, and standard deviation of strain reached by each segment are not different between treatment groups or within a treatment group over time (Supplementary Figure S13A-D, F-H).

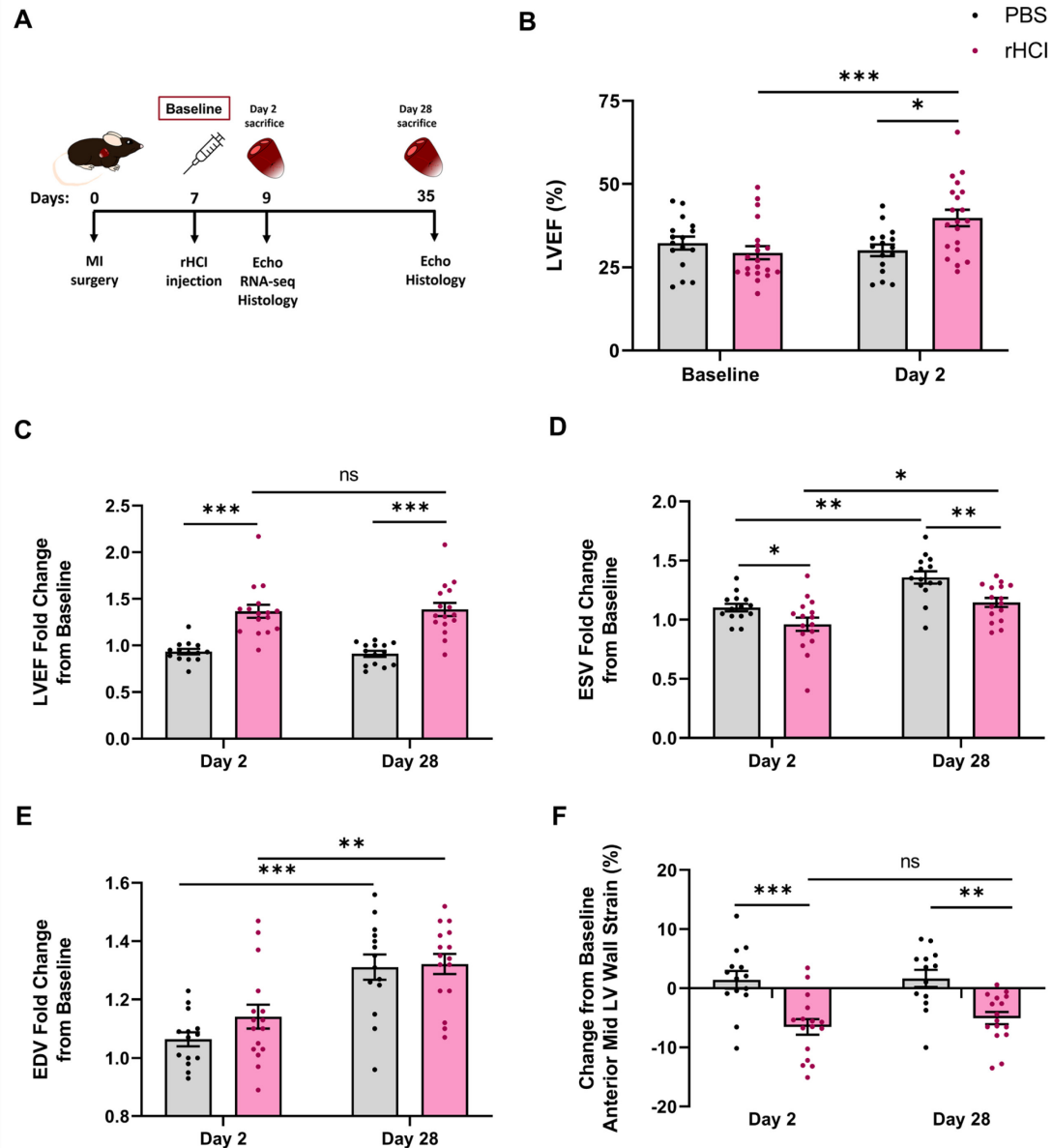


Figure 3.8. rHCI hydrogel improves cardiac function two days post-injection in a mouse model of myocardial infarction and this improvement is sustained for 28 days post-injection. A) Schematic of experimental design. Mice were injected with rHCI or PBS seven days after a myocardial infarction (MI) was induced. Echocardiography was done at baseline prior to injection and at both day 2 and day 28 post-treatment follow-ups. Mice were sacrificed either at the day 2 or the day 28 endpoint. B) Left ventricular ejection fraction (LVEF) measured at baseline prior to injection of PBS (n = 16) or rHCI (n = 20) and two days post-treatment. These mice were sacrificed at day 2 post-injection. In a separate trial, mice were sacrificed at day 28 post-injection of PBS (n = 14) or rHCI (n = 16). The fold change in echocardiography measurements of (C) LVEF, (D) end systolic volume (ESV), and (E) end diastolic volume (EDV) for day 2 and day 28 post treatment injection relative to baseline are presented. F) Change from baseline in the percent strain reached by the mid anterior LV wall

at the aortic valve closure (AVC) for both day 2 and day 28 timepoints, PBS (n = 14) or rHCI (n = 16). This is a measurement of contractility in the anterior border zone myocardium. Data is presented as mean $\pm$ SEM. Statistical significance was determined by a two-way ANOVA followed by Holm's multiple comparison corrections and was noted as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. NS indicates not significant, $p > 0.05$ for rHCI between day 2 and day 28. Statistical analysis was run in Prism software.

3.2.4 RNA Sequencing Identifies Seven Genes That Are Differentially Expressed In Hearts Treated With rHCI Hydrogel Two Days After Treatment Injection

To determine the mechanism behind the acute improvement in cardiac function post-injection of rHCI hydrogel in MI mice, RNA sequencing was done to detect changes in gene expression two days after rHCI treatment. LV tissue from MI mice injected with PBS or rHCI 2 days prior and from age-matched sham mice was processed and sent for next generation RNA sequencing. The differential expression of protein coding transcripts was compared between these three groups. The sham animals clustered away from both MI treatment groups, while there was only modest separation between PBS- and rHCI-treated MI mice (Figure 3.9A-C). There were 12,593 genes that were differentially expressed between PBS and sham LV tissue (Figure 3.9D). The top differentially expressed genes between PBS and sham groups included upregulation of collagens and inflammatory mediators (Figure 3.9E and Supplementary Table S2). The changes in gene expression in PBS compared to sham were enriched for pathways involved in increased heart weight, decreased cardiomyocyte contractility, decreased NADH dehydrogenase activity and increased collagen binding (Figure 3.9F-G). Comparing rHCI hydrogel treated LV tissue and sham LVs, there were 11,427 differentially expressed genes (Figure 3.9H). Most of these genes are the same as those differentially expressed between PBS and sham groups (Figure 3.9C). The top differentially expressed genes were also collagens and inflammatory mediators (Figure 3.9I

and Supplementary Table S3) and they were enriched for involvement in cardiac fibrosis, increased heart weight, decreased NADH oxidase activity, and increased collagen binding (Figure 3.9J-K). The GO term that was most enriched in the differentially expressed gene signature of both PBS vs. sham and rHCI vs. sham comparisons was the mitochondrion part (Supplementary Tables S4-S5). The KEGG pathway most downregulated in the PBS vs. sham and rHCI vs. sham comparisons was oxidative phosphorylation (Supplementary Figure S14 and Tables S6-7). The KEGG pathways most unregulated in the PBS vs. sham and rHCI vs. sham comparisons were cytokine receptor interactions and cell adhesion molecule interactions (Supplementary Figures S15-16 and Supplementary Tables S6-7).

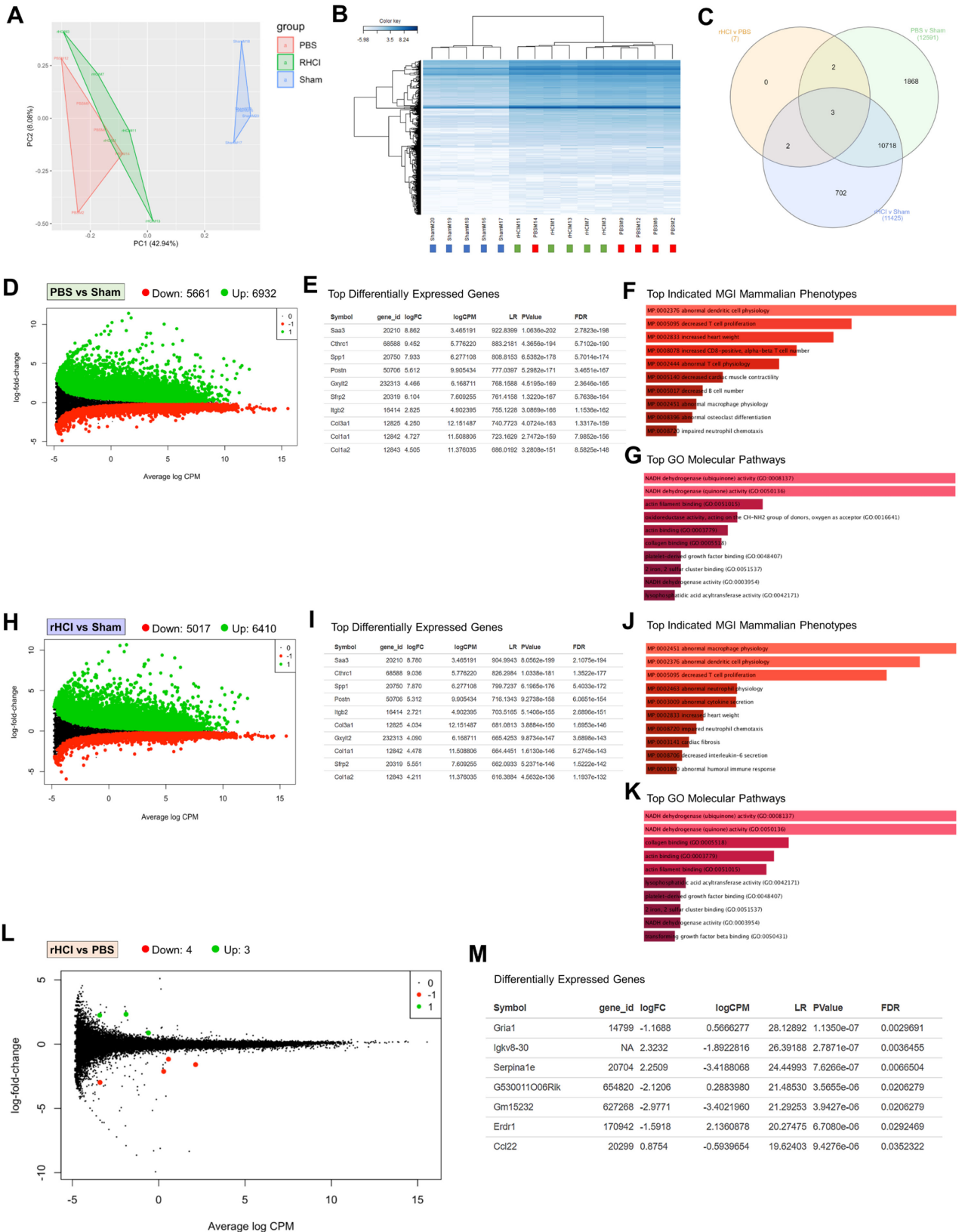


Figure 3.9. Summary of RNA sequencing results for rHCI hydrogel, PBS and sham mouse left ventricle tissue at day 2 post-treatment injection. Mice received treatment injection of rHCI (n = 5) or PBS (n = 5) seven days post-myocardial infarction. RNA was isolated from the left ventricles (LV) of mice two days post-treatment and sent for RNA sequencing of protein coding genes, which was performed by Genome Quebec. An age-matched, sham operated control group was also included (n = 5). RNAseq data analysis output was generated by C3G bioinformatics group. A) Principal component analysis and B) heatmap clustering analysis of the raw read count matrix from the edgeR gLM function. C) Differential expression analysis with a Benjamini-Hochberg false discover rate (FDR) was used to identify significant gene expression changes between the three treatment groups. (D-G) Differential expression analysis output for PBS vs. sham including: scatter plot showing significantly altered genes, top differentially expressed gene information table as well as gene ontology (GO) analysis of significantly associated phenotypes and molecular pathways. Similar outputs are shown for the rHCI vs. sham and rHCI vs. PBS comparisons (H-K and L-M). No significant GO pathways were altered between PBS and rHCI LV tissue. CPM indicates gene read counts per million normalized to the total number of reads in a sample.

Seven genes were differentially expressed in the LV tissue of PBS- and rHCI-treated mice 2 days after treatment (Figure 3.9L-M and Table S8). RNAseq was performed on whole LV tissue and sequences from >20,000 genes were detected. Three genes were upregulated, and 4 gene were downregulated in rHCI hydrogel treated mice compared to PBS treated mice (Figure 3.10A). The genes downregulated with rHCI injection were: erythroid differentiation regulator 1 (*ERDRI*), glutamate ionotropic receptor AMPA type subunit 1 (*GRI1I*), predicted gene Gm15232 (*GMI5232*), and RIKEN cDNA G530011O06 gene (*G530011O06Rik*) (Figure 3.10B-E). The genes upregulated with rHCI injection were: immunoglobulin kappa chain variable 8-30 (*IGKV8-30*), serpin family A member 1 serine protease inhibitor (*SERPINA1E*), and C-C motif chemokine ligand 22/ macrophage-derived chemokine (*CCL22*) (Figure 3.10F-H). *ERDRI* and *CCL22* were the target genes chosen for follow-up analysis as they were the most highly expressed downregulated and upregulated genes, respectively that had known protein products. *ERDRI* downregulation with rHCI hydrogel injection was validated by quantitative polymerase chain reaction (qPCR) (Figure

3.11A). *ERDR1* expression was 3.5-fold lower in rHCI treated LV tissue compared to PBS treated LV tissue 2 days post-treatment injection ($p = 0.0016$). *ERDR1* is also downregulated 2.6-fold in rHCI treated LVs compared to sham LVs ($p = 0.016$). The 1.9-fold upregulation of *CCL22* with rHCI injection detected by RNA-seq was not confirmed by qPCR (Figure 3.11B). Macrophage expression of *CCL22* has been shown to recruit FOXP3 positive T-regulatory cells²⁸⁹. At day 2 post-treatment there is no difference in T-regulatory cell density between PBS- and rHCI-treated MI mice (Supplementary Figure S17A). However, there was a 2.8-fold increase in T-regulatory cell density in the borderzone of rHCI treated hearts 28 days after treatment injection ($p = 0.011$) (Supplementary Figure S17B).

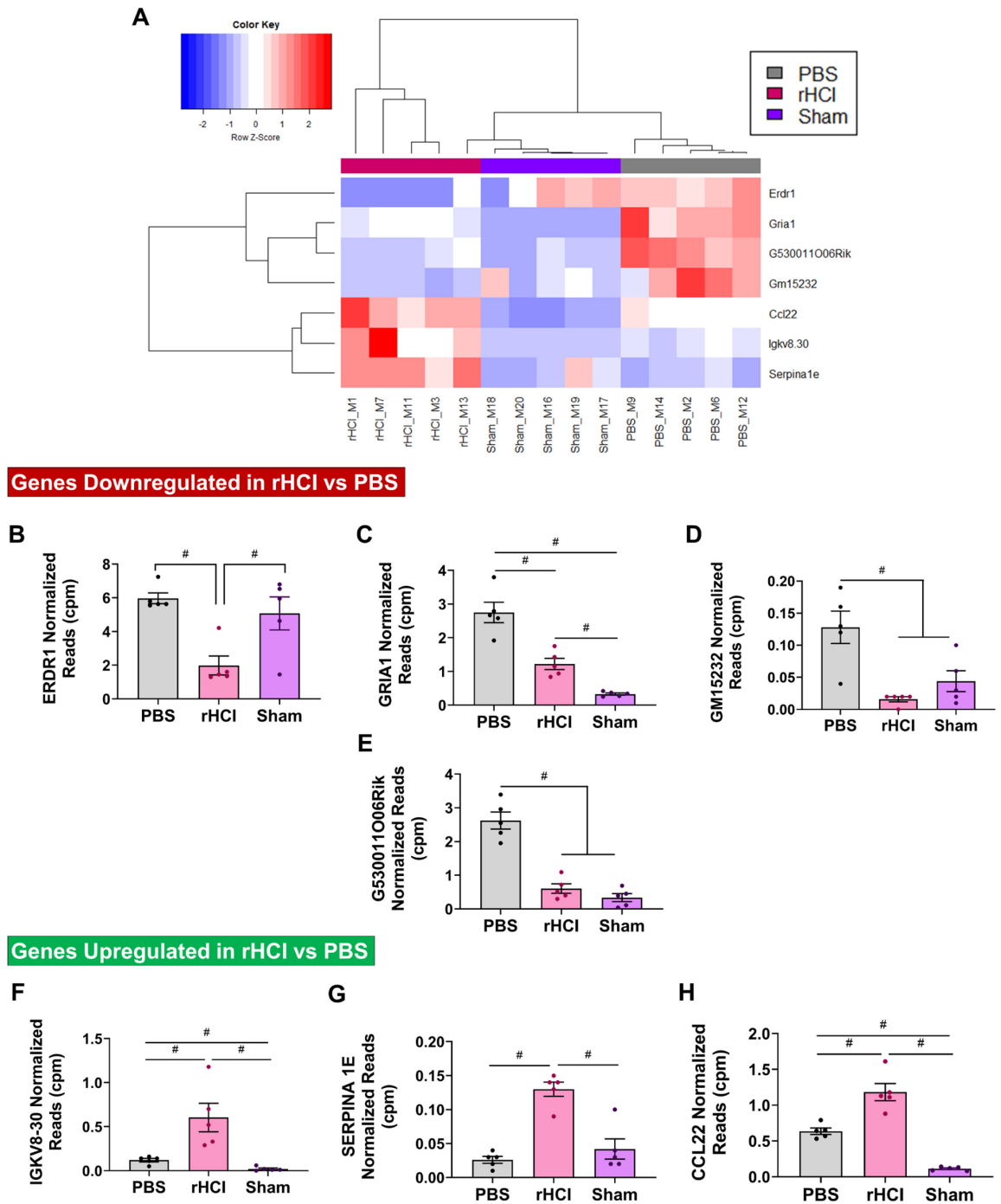


Figure 3.10. RNA sequencing of PBS- and rHCl-treated MI hearts detected 7 genes that are differentially expressed at day 2 post-treatment injection. Mice were injected with rHCl hydrogel (n = 5) or PBS (n = 5) at 7 days post-myocardial infarction. RNA was isolated from left ventricle tissue at 2 days post-injection, the time-point when a significant improvement in cardiac function is observed with rHCl injection. An age-matched sham group was also

included in the analysis (n = 5). Differentially expressed genes were determined using a gLM function in edgeR with a Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons. A) Clustering heatmap showing the expression level in all samples of the 7 genes that are differentially expressed between PBS and rHCI treated MI hearts. The colour scale indicates expression level from low to high as blue to red. (B-E) Genes significantly downregulated in rHCI hydrogel-treated hearts vs. PBS hearts were: erythroid differentiation regulator 1 (ERDR1), glutamate ionotropic receptor 1 (GRIA1), GM1532 and G530011O06Rik. (F-H) Genes significantly upregulated in rHCI treated hearts vs PBS were: immunoglobulin kappa chain variable 8-30 (IGKV8-30), serpin family A member 1e (SERPINA 1E) and macrophage derived chemokine (CCL22). Data is presented as mean $\pm$ SEM in read counts per million of the gene normalized to the total number of reads in the sample. # indicates significant difference in gene expression between treatment groups determined by FDR < 0.05.

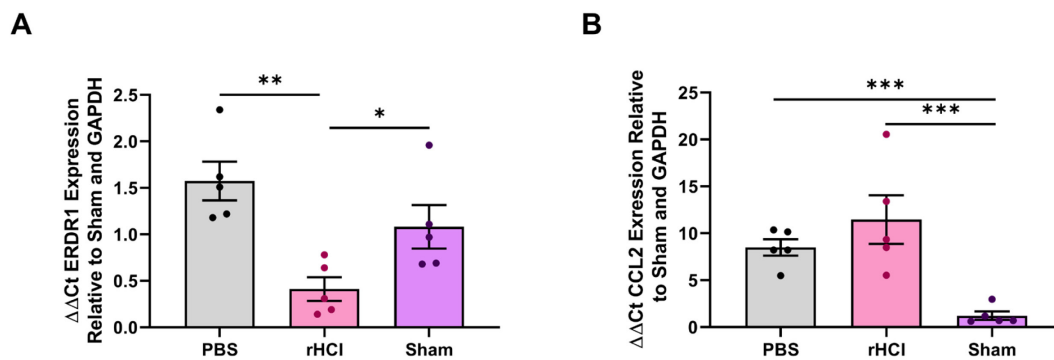


Figure 3.11. ERDR1 expression is significantly reduced in the hearts of rHCI-treated mice as compared to PBS at 2 days post-treatment injection. QPCR was used to validate the top differentially expressed genes between PBS and rHCI treated hearts from RNAseq analysis. QPCR gene expression analysis was completed for A) erythroid differentiation regular 1 (ERDR1) and B) macrophage derived chemokine (CCL22). Change in cycle threshold (Ct) for each gene from GAPDH house keeping gene control is presented for PBS and rHCI groups relative to sham. RNA was isolated from the left ventricles of hearts injected two days prior with rHCI (n = 5) or PBS (n = 5). Mice were injected 7 days post-myocardial infarction and an age matched sham control group was also included (n = 5). Data are presented as mean $\pm$ SEM. Significance was determined by a one-way ANOVA followed by Tukey multiple comparison corrections and noted as * p < 0.05, ** p < 0.01 or *** p < 0.001.

3.2.5 *ERDR1* Expression Is Downregulated In Cardiomyocytes Two Days After rHCI Injection in MI Mice

A combined ISH and IHC assay was used to measure *ERDR1* target gene expression in cardiomyocytes of rHCI- and PBS-treated hearts 2 days after treatment injection in MI mice (Figure 3.12A). The expression of *ERDR1* was studied in cardiomyocytes due to the acute improvement in myocardial contractility with rHCI injection in MI mice. Two days after treatment injection, MI mice injected with rHCI hydrogel had a lower percentage of *ERDR1* positive cardiomyocytes in the remote ($69\pm 5\%$) and borderzone ($71\pm 5\%$) myocardium compared to PBS-injected MI mice ($83\pm 2\%$ ($p = 0.016$) and $83\pm 2\%$ ($p = 0.045$), respectively) (Figure 3.12B-C). There was no difference in *ERDR1* expression in the infarct region cardiomyocytes between rHCI and PBS treatment groups ($p = 0.54$) (Figure 3.12D). The expression level of *ERDR1* within cardiomyocytes, expressed as an H-score (with a range of 0-300), is lower in the remote myocardium of rHCI-treated MI mice (105 ± 13) compared to PBS-treated MI mice (148 ± 7 ; $p = 0.0099$) (Figure 3.12E). There was a trend towards lower expression of *ERDR1* in borderzone cardiomyocytes of rHCI compared to PBS treatment group ($p = 0.069$) (Figure 5F). There was no difference in *ERDR1* expression level in the infarct region of rHCI- and PBS-treated MI mice (Figure 3.12G). In all other cells, referred to as non-cardiomyocytes, there was a reduction in the percentage of cells that express *ERDR1* ($p = 0.037$) and a trend for reduced *ERDR1* H-score ($p = 0.10$) in the remote myocardium of rHCI compared to PBS-treated MI mice (Supplementary Figure S18A and D). There was no difference between treatment groups in *ERDR1* expression by non-cardiomyocytes of the borderzone and infarct region (Supplementary Figure S18B-C and E-F).

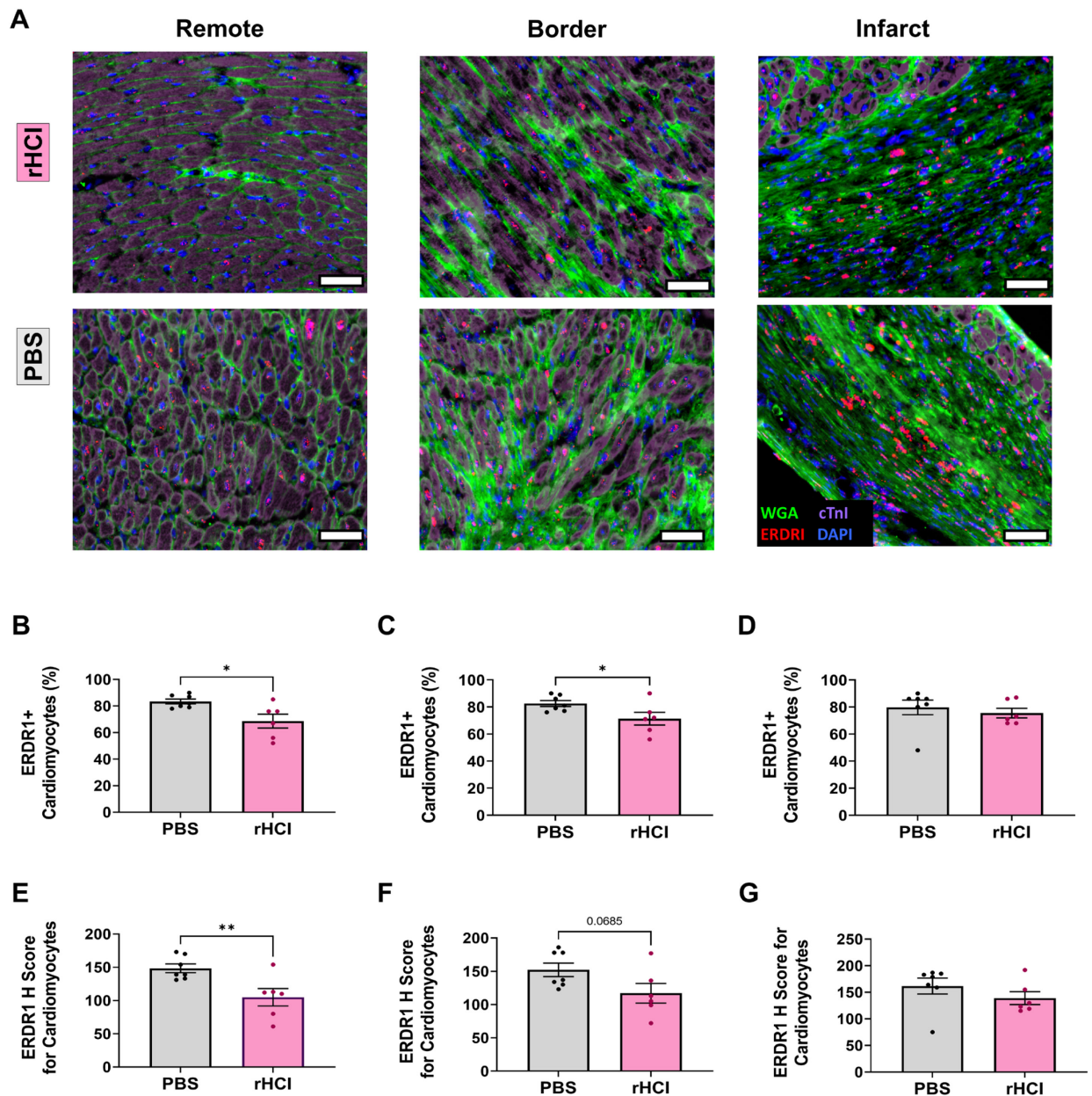


Figure 3.12. Erythroid differentiation factor 1 expression (ERDRI) is downregulated in border and remote cardiomyocytes of rHCI-treated mice two days post-treatment. Mice were injected with rHCI (n = 6) or PBS (n = 7) hydrogel seven days post-myocardial infarction. Hearts were harvested two days post-treatment and fresh frozen tissue sections were prepared for in situ hybridization. A) Representative RNAscope images of combined in situ hybridization for ERDRI (red) and IHC for cardiac troponin I (cTnI, purple), wheat germ agglutinin (WGA, green) and DAPI (blue). Scale bars are 50 μ m. B-D) Percentage of cardiomyocytes that express ERDRI for remote, borderzone and infarct regions. E-G) H

score of ERDR1 expression levels in remote, borderzone (border) and infarct cardiomyocytes. Three levels of expression are defined as bins of transcripts per cell: 1-7, 8-15 and >15, in addition to negative cells. H Scores can range from 0-300 with 300 being 100% of cells have greater than 15 ERDR1 transcripts. Image analysis was performed in Qupath software. Data are presented as mean $\pm$ SEM. Significance was determined by an unpaired t-test noted as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. Statistics were run in prism software.

3.2.6 Cardiomyocyte Apoptosis Is Reduced 2 Days After rHCI Injection In MI Mice

ERDR1 expression has not been extensively studied post-MI, however, in models of T-cell immunity and skin cancer, *ERDR1* upregulation has been associated with apoptosis^{279,280,290,291}. In addition, *ERDR1* is upregulated in several models of oxidative stress and with NADPH oxidase upregulation^{292,293}. Therefore, IHC staining was performed to detect markers of myocardial oxidative stress and cardiomyocyte apoptosis in MI mouse heart tissue two days after injection of PBS or rHCI hydrogel. Immunohistochemistry for the 4HNE adduct (Figure 3.13A) was used to detect the level of oxidative stress in the borderzone, remote, and total LV myocardium (Figure 3.13B). MI mice treated with rHCI hydrogel had lower levels of 4HNE staining in the remote myocardium ($16 \pm 1\%$) and within the total LV ($13 \pm 2\%$) compared to PBS-treated MI mice ($23 \pm 1\%$ ($p = 0.0031$) and $19 \pm 1\%$ ($p = 0.034$), respectively). There was no difference in 4HNE levels between treatment groups in the borderzone myocardium ($p = 0.11$). The same 4HNE staining was run on heart tissue sections from PBS- and rHCI-treated MI mice 28 days post-treatment (Supplementary Figure S19A). There was no difference in 4HNE staining levels between treatment groups in borderzone ($p = 0.61$), remote ($p = 0.73$) or total LV myocardium ($p = 0.64$) (Supplementary Figure S19B). The level of ALDH2 activity, a key enzyme involved in 4HNE adduct removal^{178,294}, was measured in the remote myocardium two days after treatment injection.

There was no difference in ALDH2 activity in the remote myocardium between PBS- and rHCI-treated MI mice (Supplementary Figure S20). Cardiomyocyte apoptosis was detected by TUNEL staining (Figure 3.13C). MI mice treated with rHCI had lower apoptotic cell density in the borderzone (41 ± 8 cells/mm²), and remote myocardium (13 ± 3 cells/mm²) two days post-treatment compared to MI mice injected with PBS (75 ± 9 cells/mm² ($p = 0.009$) and 26 ± 5 cells/mm² ($p = 0.023$), respectively) (Figure 3.13D). Apoptotic cardiomyocyte density was lower in the borderzone (8 ± 1 cells/mm² vs. 18 ± 3 cells/mm², $p = 0.012$) and remote myocardium (3 ± 1 cells/mm² vs. 7 ± 2 cells/mm², $p = 0.033$) of rHCI- compared to PBS-treated mice (Figure 3.13E). The level of apoptotic non-cardiomyocytes was also lower in the borderzone ($p = 0.024$) and remote ($p = 0.002$) regions of rHCI-treated mice (Supplementary Figure S21). *ERDR1* has also been linked to macrophage migration post-MI. However, there was no difference in macrophage density or CD206 positive density in rHCI- compared to PBS-treated MI mouse hearts two days post-treatment (Supplementary Figure S22). This contrasted with an rHC type III (rHCIII) hydrogel that reduced the percentage of CD206 positive macrophages in the heart two days post-injection compared to rHCI ($p = 0.005$) or PBS ($p = 0.02$) treated hearts (Figure S22).

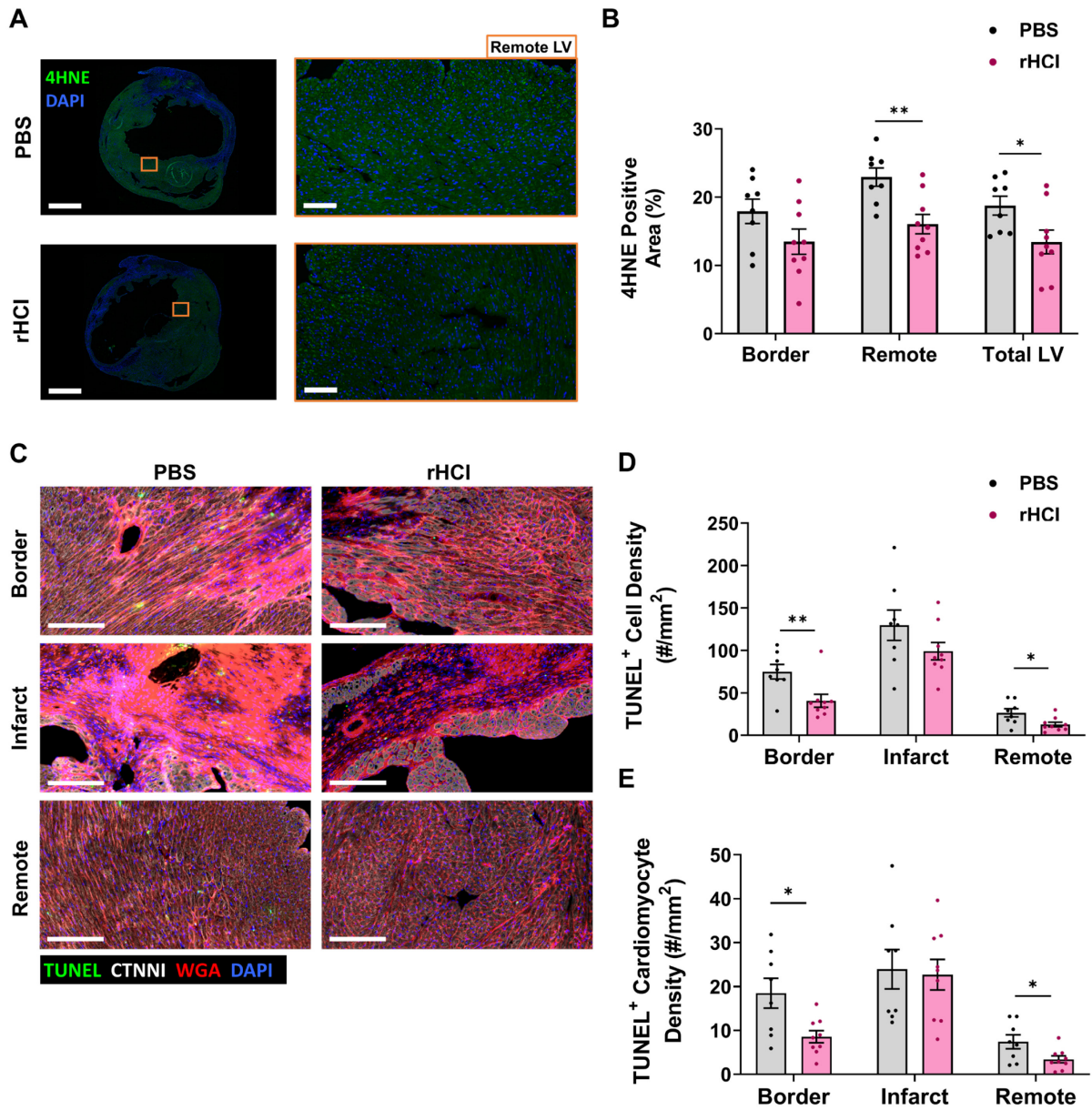


Figure 3.13. rHCI hydrogel injection reduces markers of oxidative stress and cardiomyocyte apoptosis at 2 days post-treatment. Histological analysis of possible downstream pathways related to ERDR1, including oxidative stress and DNA damage. Seven days after myocardial infarction, mice were injected with rHCI (n = 9) or PBS (n = 8). Hearts were harvested for histology two days after treatment. A) Representative images for 4-hydroxynoneal (4HNE) IHC staining (green), scale bars indicate 1mm for full slide scans and 100 μ m for remote left ventricle (LV) 20X magnification. B) 4HNE positive area as a percentage of the borderzone (border), remote and total LV area in PBS and rHCI treated hearts. C) Example images from TUNEL (green) IHC scans, scale bars indicate 200 μ m. Cardiomyocytes were defined as cardiac troponin I (CTNNI)⁺ (white) and cell borders were identified with wheat germ agglutinin (WGA) stain (red). D) TUNEL⁺ positive cell density and (E) TUNEL⁺

cardiomyocyte in the border, infarct, and remote regions of PBS and rHCI treated hearts. Data are presented as mean $\pm$ SEM. Significance between treatments was determined for each region by an unpaired t test and noted as * $p < 0.05$ or ** $p < 0.01$. Statistics were run in prism software.

3.2.7 rHCI Hydrogel Soaks Up Methylglyoxal At The Epicardial Surface And Reduces Methylglyoxal Adducts Within The Myocardium Two Days Post Injection In MI Mice

Two days after intramyocardial injection of rHCI hydrogel into the borderzone, it spreads over the epicardial surface of the infarct and borderzone myocardium (Supplementary Figure S23). Most of the rHCI hydrogel does not interact with cardiomyocytes in the borderzone (Supplementary Figure S24A-B). Cells infiltrate the gel one day post-injection including Wilm's tumor protein 1 (WT1) epicardial cells, endothelial cells, and myofibroblasts (Supplementary Figure S24C-E). There is minimal infiltration of M2 macrophages (Supplementary Figure S24F). Given that rHCI hydrogel injection lowers oxidative stress and apoptosis in cardiomyocytes, a mechanism is required by which the hydrogel at the epicardial surface could lower oxidative stress levels in the myocardium below without direct contact with cardiomyocytes. Methylglyoxal is a reactive dicarbonyl that is produced in conditions of oxidative stress including post-MI^{53,295}. MG-H1 adducts build up on extracellular proteins, primarily collagens, and intracellular proteins, including sarcomeric proteins within cardiomyocytes where it limits contractility^{75,122}. Levels of MG-H1 adducts within cardiomyocytes and on collagen type 1 were measured by IHC in MI mouse hearts two days post-treatment injection (Figure 3.14A). There was a significant reduction of MG-H1 adducts within cardiomyocytes of rHCI-treated mice in the infarct ($47\pm4\%$ vs. $68\pm4\%$, $p = 0.0033$), borderzone ($37\pm4\%$ vs. $57\pm4\%$, $p = 0.0037$), and remote myocardium ($36\pm4\%$ vs. $51\pm3\%$, $p = 0.0095$) compared to PBS-treated mice (Figure 3.14B-

D). A similar reduction in MG-H1 modified collagen type 1 was observed in the infarct ($58\pm3\%$ vs. $67\pm2\%$, $p = 0.015$), borderzone ($45\pm4\%$ vs. $58\pm2\%$, $p = 0.0060$), and remote ($38\pm2\%$ vs. $50\pm3\%$, $p = 0.0031$) regions of rHCI-treated mice compared to PBS-treated mice (Figure 3.14E-G). The rHCI hydrogel is modified by methylglyoxal two days post-injection (Supplementary Figure S25). On average, $52\pm2\%$ of the rHCI hydrogel stains positive for MG-H1 (Figure 3.15A-B). MG-H1 modified collagen type 1 can cause endothelial cell dysfunction and apoptosis in models of diabetic cardiomyopathy¹²⁷. There is no effect of rHCI injection on capillary density in the heart two days post-treatment (Supplementary Figure S26).

The level of MG-H1 modification in cardiomyocytes and on collagen type 1 was also measured in hearts 28 days after rHCI or PBS injection in MI mice (Figure 3.16A). There was still a reduction of MG-H1⁺ area within cardiomyocytes of the infarct ($40\pm2\%$ vs. $51\pm4\%$, $p = 0.033$) and borderzone ($32\pm1\%$ vs. $38\pm3\%$, $p = 0.037$) regions of rHCI-treated mice compared to PBS-treated mice (Figure 3.16B-C). There was no difference in MG-H1 levels within remote cardiomyocytes between treatment groups ($p = 0.29$) (Figure 3.16C). There was also a trend towards a reduction of MG-H1 modified collagen in the infarct region of rHCI-treated mice compared to PBS ($53\pm2\%$ vs. $60\pm2\%$, $p = 0.055$) (Figure 3.16E). No difference in MG-H1 modified collagen was detected in the borderzone ($p = 0.14$) or remote ($p = 0.58$) myocardium between treatment groups at 28 days post-treatment.

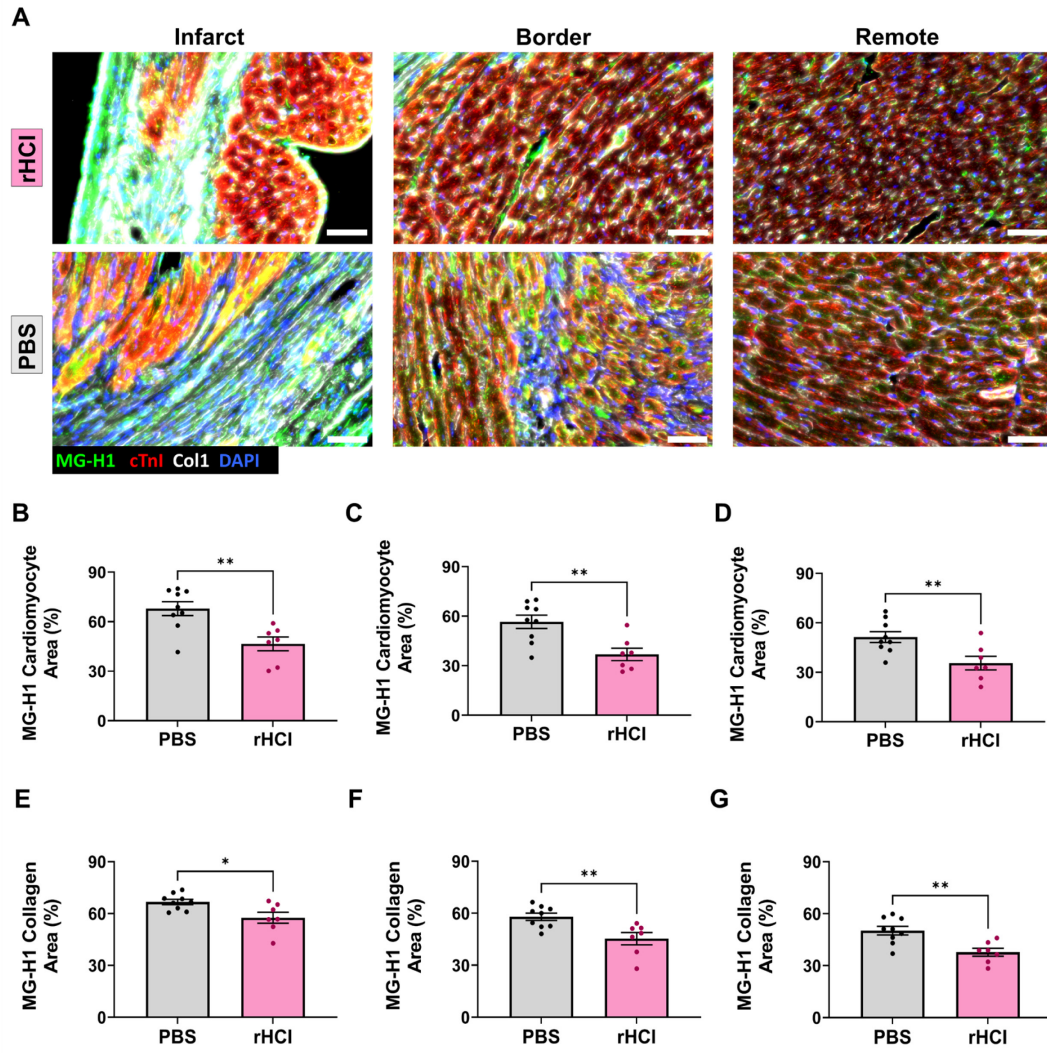


Figure 3.14. rHCI hydrogel reduces methylglyoxal adducts on collagen and within cardiomyocytes two days after treatment in mice with myocardial infarction. Treatments of PBS (n = 9) and rHCI (n = 7) were injected 7 days after myocardial infarction in mice. Hearts were harvested 2 days after treatment for histology. A) Representative images of IHC staining for methylglyoxal adducts (MG-H1, green), collagen type I (Col1, white), cardiac troponin 1 (cTnI, red) and DAPI (blue). Scale bar is 50 μ m. B-D) Area of collagen that was modified by MG-H1 in the infarct, borderzone (border) and remote myocardium of PBS- and rHCI-treated mice. E-G) Area of cTnI⁺ cardiomyocytes that is modified by MG-H1 in the infarct, borderzone (border) and remote myocardium of PBS- and rHCI-treated mice. Image analysis was performed in Qupath software. Data are presented as mean $\pm$ SEM. Significance between treatments was determined for each region by an unpaired t test and noted as * $p < 0.05$ or ** $p < 0.01$. Statistics were run in prism software.

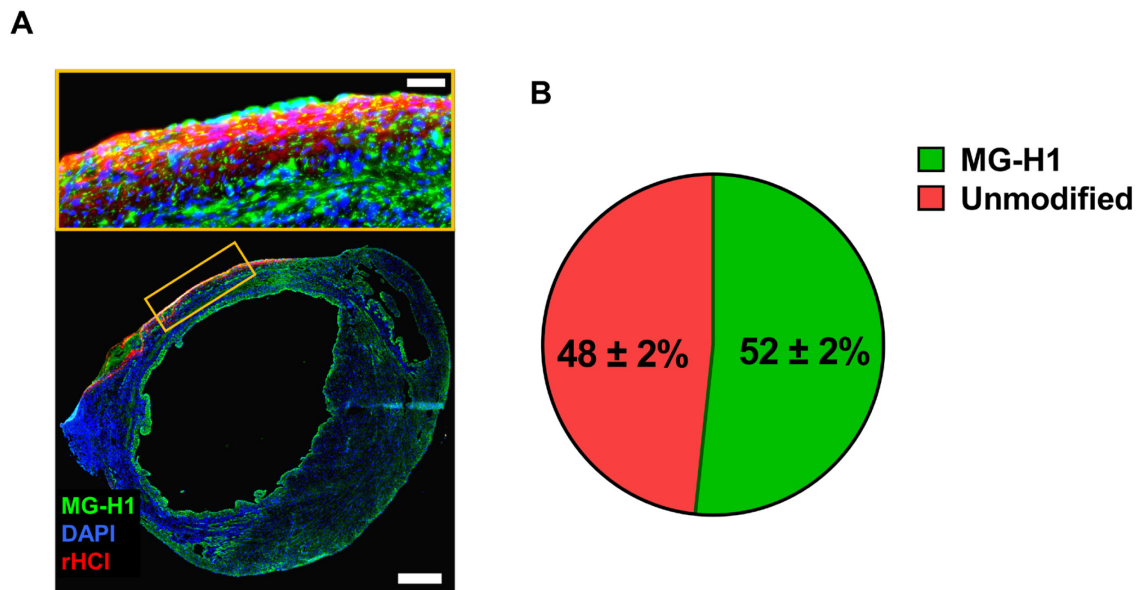


Figure 3.15. rHCI hydrogel is modified by methylglyoxal two days after treatment injection into the border region of the infarcted myocardium. Seven days after myocardial infarction, mice were treated with intramyocardial injection of rHCI hydrogel ($n = 5$). The rHCI hydrogel was tagged with AlexaFluor® 594 (AF594, red) fluorescent dye prior to injection. A) Hearts were harvested two days post-injection for IHC detection of methylglyoxal adducts (MG-H1, green). DAPI counterstain is in blue. B) MG-H1 modified area in the rHCI hydrogels was determined using QuPath software. Data are presented as mean $\pm$ SEM and was analyzed using Prism software.

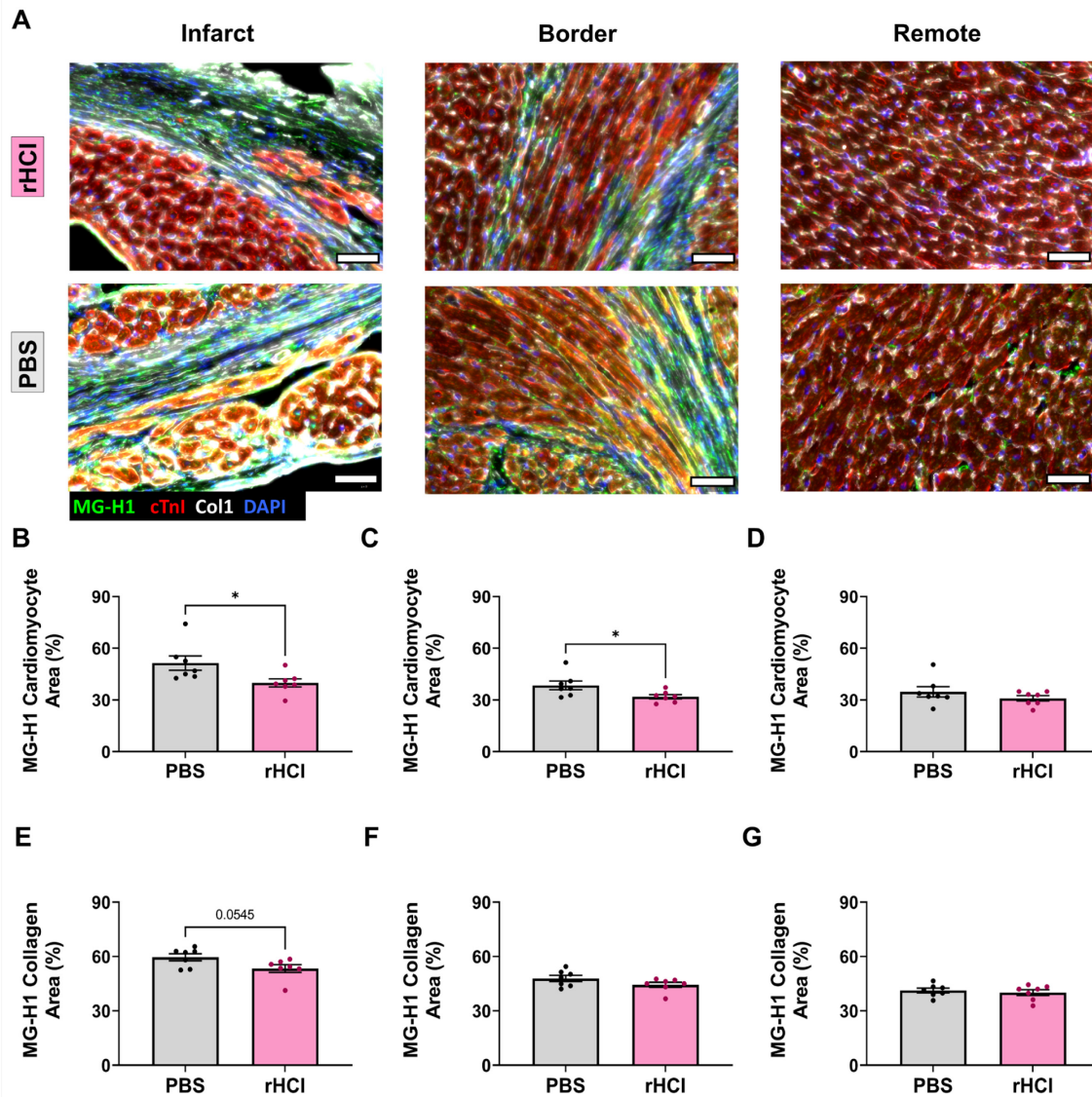


Figure 3.16. Methylglyoxal adducts within cardiomyocytes remain reduced in the infarct and borderzone of rHCI treated hearts 28 days after treatment injection. Treatments of PBS (n = 7) and rHCI (n = 7) were injected seven days after myocardial infarction in mice. Hearts were harvested 28 days after treatment for histology. A) Representative images of IHC staining for methylglyoxal adducts (MG-H1, green), collagen type I (Col1, white), cardiac troponin 1 (cTnI, red) and DAPI (blue). Scale bar is 50 μ m. B-D) Area of collagen that was modified by MG-H1 in the infarct, borderzone (border) and remote myocardium of PBS- and rHCI-treated mice. E-G) Area within cTnI⁺ cardiomyocytes that is modified by MG-H1 in the infarct, borderzone (border) and remote myocardium of PBS and rHCI treated mice. Image analysis was performed in Qupath software. Data are presented as mean $\pm$ SEM. Significance between treatments was determined for each region by an unpaired t test and noted as * $p < 0.05$. Statistics were run in prism software.

3.2.8 rHCI Hydrogel Injection Leads To Myocardial Salvage And Reduced Cardiomyocyte Hypertrophy 28 Days Post-Treatment In MI Mice

Masson's trichrome staining was used to detect fibrosis and cardiac muscle in heart sections of MI mice 28 days after injection of rHCI hydrogel or PBS (Figure 3.17A, C). The level of myocardial salvage in the infarct region increased with rHCI treatment compared to PBS ($60\pm3\%$ vs. $50\pm3\%$, $p = 0.049$) (Figure 3.17B). There was also a decrease in fibrosis within the borderzone myocardium of rHCI-treated mice as compared to PBS-treated mice ($13\pm1\%$ vs. $17\pm2\%$, $p = 0.049$) (Figure 3.17D). The density of cardiomyocytes and the cardiomyocyte cell area were measured in the borderzone and remote myocardium (Figure 3.17E). In the borderzone region of rHCI-treated mice there was an increase in cardiomyocyte density (1427 ± 93 cells/mm² vs. 1119 ± 95 cells/mm², $p = 0.040$) and a reduction in cardiomyocyte cell size (308 ± 26 μm^2 vs. 400 ± 33 μm^2 , $p = 0.049$) compared to PBS-treated mice (Figure 3.17F-G). There was also an increase in cardiomyocyte density (1984 ± 145 cells/mm² vs. 1583 ± 81 cells/mm², $p = 0.032$) and reduction in cardiomyocyte cell area (229 ± 12 μm^2 vs. 293 ± 15 μm^2 , $p = 0.0061$) in the remote myocardium of rHCI-treated mice compared to PBS-treated mice (Figure 3.17H-I).

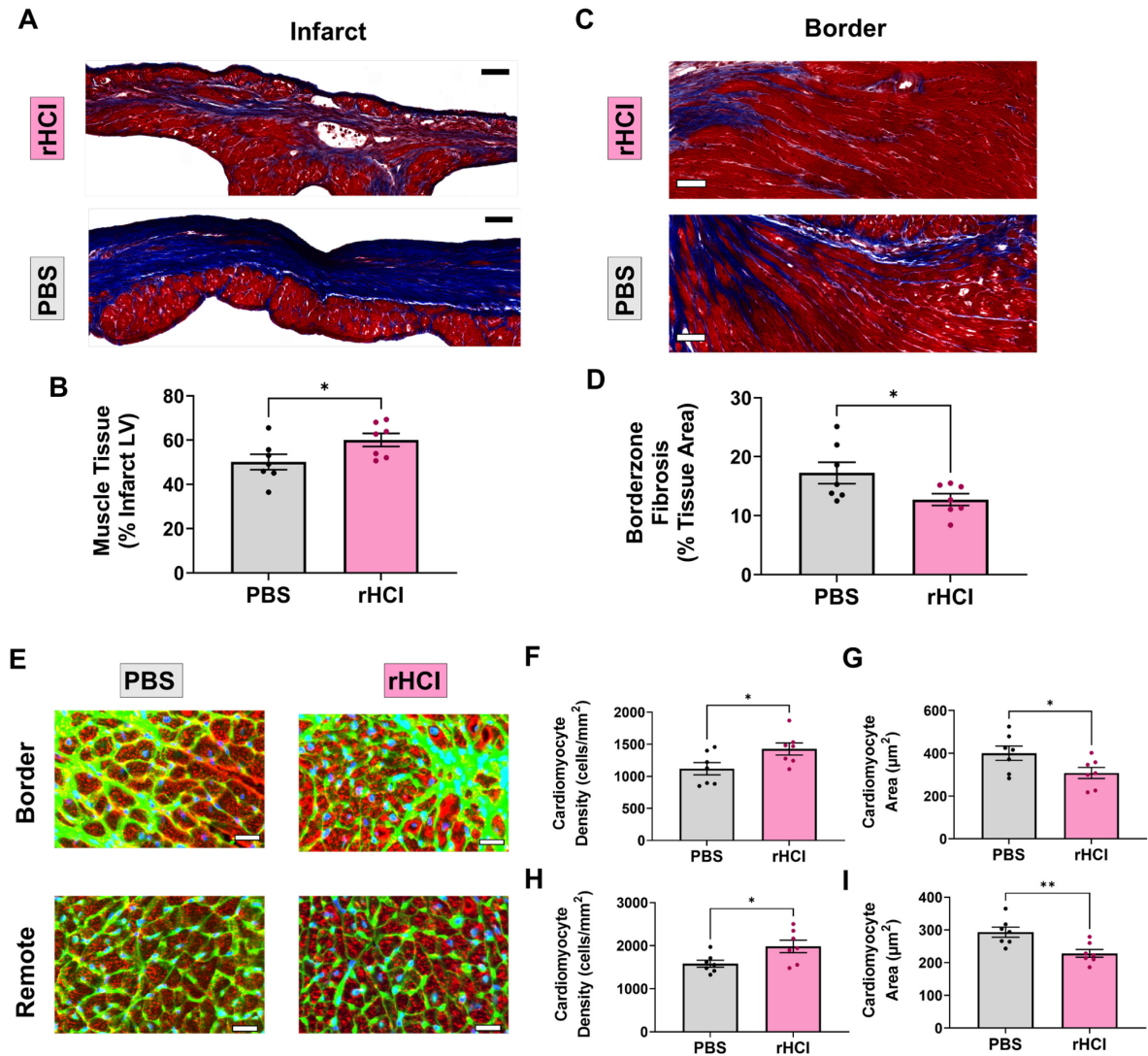


Figure 3.17. Mice treated with rHCI hydrogel after myocardial infarction have improved myocardial salvage twenty-eight days after treatment injection. Seven days after a myocardial infarction was induced in mice, rHCI hydrogel ($n = 7$) or PBS ($n = 7$) was injected into the borderzone myocardium (border). Twenty-eight days after treatment injection, mouse hearts were harvested for histology. A) Masson's trichrome stained images of the infarct region in rHCI- and PBS-treated hearts. B) Percentage of myocardial salvage in the infarct region. C) Masson's trichrome stained borderzone myocardium; and D) percentage of fibrosis in the borderzone. Scale bars are 50 μm . E) Images of IHC to detect cardiomyocytes expressing cardiac troponin I (cTnI, red) and cell borders with wheat germ agglutinin (WGA, green). Scale bars are 25 μm . Cardiomyocyte density (F-G) and cardiomyocyte cell area (H-I) were quantified in the infarct and borderzone myocardium of PBS- and rHCI-treated mice. Image analysis was performed in Qupath software. Data are presented as mean $\pm$ SEM. Significance between treatments was determined for each region by an unpaired t test and noted as * $p < 0.05$ or ** $p < 0.01$. Statistics were run in prism software.

Chapter 4: Discussion

4.1 Summary

In this thesis the first rHC hydrogels developed for infarct repair post-MI were tested in a clinically relevant mouse model of MI. The goal was to determine their therapeutic efficacy and understand their mechanism of action in order to facilitate clinical translation. The first aim of this research was to determine if the rHC hydrogels improved cardiac function and limited ventricular remodeling at the day 28 follow-up timepoint. Both rHCI and rHCIII hydrogels had greater cardiac function, smaller ESV, and smaller infarct size compared to the PBS control group. The rHCI hydrogel showed the additional benefits of: improved cardiac function at day 28 as compared to baseline prior to injection, better preserved myocardial wall thickness, and borderzone myocardial salvage. The second aim was to evaluate the *in vivo* degradation and inflammatory response for both hydrogels. In comparison to rHCIII, rHCI hydrogels persisted longer (7 days vs. 2 days) *in vivo* and did not stimulate an inflammatory response in the heart. When injected 7 days post-MI, in the proliferative phase of MI wound healing, rHCI hydrogel exhibited more benefits of treatment without the risk of an inflammatory monocytic response. At this delivery timepoint the rHCI hydrogel was the superior performing treatment.

The third aim of this research was to understand the mechanism of action for rHCI hydrogel injection post-MI. To accomplish this aim, the acute functional response to rHCI hydrogel was studied 2 days post-injection. Cardiac function improved in rHCI-treated MI mice 2 days post-injection and was the result of increased contractility in the anterior borderzone myocardium that was sustained out to the 28-day endpoint. The rHCI hydrogel reduces the level of adducts generated by oxidative stress in the myocardium 2 days post-

injection, namely 4HNE and MG-H1. The level of methylglyoxal modifications within cardiomyocytes is reduced by rHCI hydrogel, perhaps in part because the hydrogel soaks up free methylglyoxal from the tissue. The rate of cardiomyocyte apoptosis is also reduced at day 2 post-rHCI injection and leads to greater cardiomyocyte density with less hypertrophic cardiomyocytes at day 28. Methylglyoxal has been implicated in decreasing cardiomyocyte contractility and stimulating cardiomyocyte death and hypertrophy. Therefore, the proposed mechanism of action for rHCI injection post-MI is that rHCI hydrogel stimulates improved contractility within 2 days by reducing the level of methylglyoxal in the myocardium leading to sustained improvement of cardiac function and myocardial salvage.

4.2 Discussion of Aims 1 and 2

Note that the discussion for aims 1 and 2 has been published in: *McLaughlin and McNeill et.al. Nature Communications 10 (2019) 4866*. It is presented here with minor modifications.

4.2.1 rHC Hydrogels Were Designed as Injectable Therapeutics for Infarct Repair Post-MI

Injectable hydrogels are attractive for cardiac applications due to their ease of use and the possibility of minimally invasive delivery. Therapeutically, they can provide physical stability to the infarcted myocardium, and can act as a biomimetic matrix for supporting cell function²⁹⁶. Several biomaterials composed of natural ECM proteins have been tested in animal models of MI and heart failure. Many have been shown to reduce adverse remodeling and limit the loss of function post-MI; but more materials are needed that can increase cardiac function when used as a stand-alone therapy for treating myocardial scar at the late proliferative stage^{186,199,297}. Furthermore, clinical translation of the materials tested so far can be limited and more challenging due to their animal origin^{229,298}.

We have assessed the performance of our therapy at the end of the proliferative phase post-infarction (i.e., 7 days in mice, which is the equivalent of 14 days post-MI in humans)^{18,265,286–288}. This time-point represents a clinical opportunity to intervene to limit pathological ECM remodeling and promote infarct repair in patients who have not responded to surgical interventions and other therapeutics. Using the two most abundant types of collagen found in the healthy heart, we developed injectable rHCI and rHCIII matrices. The rHCI and rHCIII matrices exhibited differences in their biophysical properties (viscosity, porosity, and enzymatic degradation), but both demonstrated good injectability and gelation at 37°C, making them suitable for delivery to the heart that has sustained an MI. Although injecting collagen post-MI may seem counterintuitive given the presence of the collagenous scar, the composition and mechanical properties of the scar are vastly different from the normal myocardium^{299,300}. Supporting their use as a therapy post-MI, (animal-derived) collagen materials have been shown to improve angiogenesis and tissue integration, reduce inflammation and apoptosis, and limit negative remodeling and the loss of cardiac function^{100,175,197,210,241,250,254,301–304}. Furthermore, the safety and efficacy of a porcine myocardial ECM hydrogel, composed primarily of collagens, was demonstrated in a pre-clinical pig MI model¹⁷⁸.

4.2.2 rHC Hydrogels Improve Cardiac Function and Limit Ventricular Remodeling Post-MI

In the present study, improved cardiac function between baseline (i.e., time of treatment delivery) and 28 days post-treatment was observed only in mice treated with the rHCI matrix; whereas cardiac function was preserved with rHCIII treatment, and worsened in the control group. The improvement in LVEF for the rHCI group was similar to that

observed in MI mice that received rat-tail collagen matrix treatment delivered acutely at 3h post-MI¹⁷⁵. The recovery of LVEF function was accompanied by reduced ESV, as well as improved fractional area change, stroke volume, and cardiac output, which are all indicators of overall improvement in cardiac function. In contrast, EDV was not improved in hearts that received rHC treatment. However, this prognostic indicator is typically used in assessing adverse remodeling months after MI has occurred (4-6 months in humans; equivalent to ~2-3 months in mice), and our furthest time-point evaluated is at 5 weeks post-MI (28 days post-treatment). Furthermore, while the use of EDV alone as a clinical endpoint has been questioned, ESV has been shown to be an early predictor for recovery of the MI heart^{50,67,305-307}.

The mechanical properties of the myocardium, measured *ex vivo* (tensile strength), were comparably restored within days of receiving rHCI or rHCIII treatment, but only rHCI treatment improved the longitudinal endocardial strain in the borderzone measured *in vivo* (strain at the aortic valve closure), and both rHC treatments were accompanied by a decrease in the scar size. Notably, rHCI-treated hearts were smaller, and the thickness of the remote myocardium was greater in the rHCI group. This suggests that rHCI treatment can reduce dilation and adverse ventricular remodeling, which has been linked to superior clinical outcomes³⁰⁸. Thus, the capacity of our materials to improve cardiac function post-MI after the scar has formed (late proliferative phase), particularly the rHCI formulation, presents a desirable and clinically translatable platform for treating MI patients beyond the initial inflammatory phase¹⁹⁹.

Vascularization of the infarct and peri-infarct regions after MI is critical to limiting necrosis and preserving cardiac function^{309,310}. Although no differences in vascular density

(arterioles or capillaries) were observed in the scar region between treatment groups, the capillary density was greater in the border zone for both rHCI and rHCIII groups. Increasing the microvasculature in the peripheral area of the infarct can lead to salvage of surrounding myocardium, limit expansion of the infarct and prevent the progression to heart failure³¹¹. This may be an explanation for the increased cardiac troponin I positive area in the borderzone of rHCI treated mice, indicating greater myocardial salvage in the peri-infarct area. There is also greater Cx43 staining in the remote region of rHCI-treated hearts compared to either PBS or rHCIII.

4.2.3. rHC Hydrogels Elicit Different Responses from the Immune System Post-Injection

Modulating inflammation and macrophage phenotype has also been shown to limit adverse remodeling in the MI heart^{312,313}, and is a possible mechanism that may be linked to the observed benefits of rHCI matrix treatment. At 28 days post-MI, the number of M2 macrophages in the scar region was ≈ 1.5 fold greater for rHCI vs. PBS treatment. Interestingly, experiments using Cx3cr1-EGFP mice showed that the rHCI matrix reduced the total number of GFP⁺ monocytes that were recruited from the marrow to the infarct site, 2 days after treatment delivery (9 days post-MI). Therefore, it appears that the rHCI matrix can reduce inflammatory cell recruitment early on and promote wound healing in the long-term. The analysis of monocyte populations 2 days post-treatment in wild-type mice showed an increase in the mobilization of monocytes into the blood and greater accumulation of Ly6C^{hi} cells in the myocardium for the rHCIII group. Also, an increased number of Ly6C^{lo} cells was seen in the spleen for the rHCIII group, suggesting some degree of inflammatory response to the rHCIII matrix, but not the rHCI matrix. The increase in Ly6C^{hi} monocytes

with rHCIII injection may in part explain the shorter degradation rate of rHCIII *in vivo*, due to increased MMP levels produced from these inflammatory monocytes³¹⁴. A further study of rHCIII hydrogel demonstrated that greater functional improvement was seen when injected 3 hours post-MI, compared to 7 days post-MI³¹⁵. Due to the higher level of inflammation at this timepoint the monocytic recruitment by rHCIII may be less impactful. Altogether, the effects of the rHCI matrix on vascularity, anti-inflammatory/pro-wound healing, and cardiomyocyte viability may play a role in its ability to improve cardiac function in the MI mouse heart. To gain further insights on the biological activity of the matrices, we performed *in vitro* experiments.

Upon injection, monocytes/macrophages will be recruited to the rHC matrices, and cell adhesion will dictate the ensuing initial response. *In vitro* experiments showed no difference in the adhesion of murine BMDMs between the two rHC materials; however, BMDM invasion into the rHCIII matrix was 2-fold greater compared to the rHCI matrix. Since the pore size for rHCIII was considerably larger than that of rHCI, greater cell permeation for rHCIII is not surprising. Both rHC matrices promoted greater M2 polarization and expression of M2 markers (MMP1 and Arg1) than TCPS, but more so with the rHCIII matrix than the rHCI matrix. This is in contrast to our *in vivo* observations, in which only the rHCI matrix, but not rHCIII, led to greater numbers of M2 macrophages in the infarcted heart long-term compared to PBS control treatment. This may be related to differences between the *in vitro* and *in vivo* conditions. For example, it has been shown that M2 polarization is stimulated to a greater extent in 3D vs. 2D environments³¹⁶. Thus, it is plausible that, although cells are seeded on the surface of the materials *in vitro* (*i.e.*, in 2D), the larger pore size of the rHCIII material may result in more cells being exposed to a 3D-like environment leading

to greater M2 macrophage polarization. In the in vivo setting, the injected material is subject to enzymatic degradation, which was shown to be greater for the rHCIII material; thus, we speculate that the slower clearance of rHCI leads to an increased number of M2 macrophages over time in the rHCI treatment group.

Oxidative stress post-MI is considered to be a major factor in cardiac remodeling²⁶. We therefore sought to examine the protective effect of the matrix against oxidative stress-induced death of macrophages (BMDMs), which were increased in number within the myocardium of rHCI-treated hearts. Both rHCI and rHCIII matrices were able to reduce the number of dead BMDMs in the presence of hydrogen peroxide. However, the NRVMs remain susceptible to H₂O₂-induced death when cultured on the rHC matrices. This suggests that our rHC materials may act as a protective niche limiting the loss of wound healing macrophages in conditions of oxidative stress.

4.2.4. Conclusions from Aims 1 and 2

In summary, we have demonstrated that our rHCI matrix made from clinical grade human collagen is able to prevent adverse cardiac remodeling, and to improve cardiac function in the MI heart when applied during the late proliferative phase. Our results are the first step in the development of a stand-alone collagen biomaterial therapy that is clinically translatable. In terms of the potential cost savings from our new therapy, it should be considered that in Canada alone there are 600,000 patients living with advanced heart failure, whose healthcare costs amounts to more than \$2.8B every year³¹⁷. These patients require long and frequent hospital stays. Thus, a therapy that is able to reduce the progression to clinical heart failure would greatly improve both quality and quantity of life for many MI patients and potentially save millions in healthcare spending. We expect these findings will

pave the way for providing a biomaterial therapy suitable for combination with cell therapies or surgical procedures aimed at restoring cardiac function in MI patients.

4.3 Discussion of Aim 3

4.3.1 rHCI hydrogel Improves Cardiac Function by Increasing Borderzone Contractility Two Days Post-Injection.

The previous study determined that injection of rHCI hydrogel 7 days post-MI leads to acute improvement of contractility of the borderzone myocardium compared to PBS-treated mice. The next objectives were to determine if the increase in borderzone contractility was accompanied by an improvement in cardiac function 2 days post-rHCI injection and to uncover the mechanism underlying this acute therapeutic effect. Within 2 days of treatment, mice treated with rHCI hydrogel show increased LVEF, SV and CO compared to baseline and mice receiving PBS injection. A decrease in ESV underlies this improvement in global cardiac function with rHCI injection. The reduction in ESV is likely a result of increased shortening, measured by longitudinal strain, in the mid anterior LV wall. The accessible borderzone myocardium, which is targeted for injection, is located in the mid anterior LV wall. Therefore, at 2 days post-rHCI injection the increase in borderzone myocardium contractility improves global cardiac function.

The borderzone myocardium can become functionally coupled to the infarct region¹⁹. The infarct region is marked by scar tissue that does not contract or can bulge during contraction (dyskinesis)⁵⁹. The myocardium bordering the scar experiences elevated wall stress, greater stretching, and can become functionally impaired overtime^{69,71,318}. Strain imaging can be used clinically to determine the extent of contractile impairment in

myocardial segments⁶⁰. Normal myocardial strain in the longitudinal direction ranges from -17% to -28.9% in humans⁶⁰. This is similar to the average strain measured in healthy mouse hearts in this study, which is -17%. Strain values closer to zero indicate contractile dysfunction and values greater than zero indicate dyskinetic segments⁶⁰. In the mid anterior borderzone myocardium, baseline measurements for both groups and PBS treated animals at day 2 exhibit functional impairment, with an average strain of - 2.5 to +1.3%. The injection of rHCI hydrogel increases the shortening of these segments to closer to normal levels of contraction at -6.9%. This improvement in contractility in the anterior borderzone myocardium with treatment is not accompanied by an increase in lengthening of the apical infarct region or desynchrony of contraction. An increase in borderzone contraction can be expended in lengthening the infarct region, thereby not resulting in forward propulsion of blood⁷⁰. However, with rHCI hydrogel injection this is not the case and the increase in borderzone contractility leads to greater volume of blood pumped out of the heart, and greater global cardiac function post-MI.

4.3.2 The Improvement in Cardiac Function Two Days After rHCI injection is Equivalent to the Improvement Originally Observed at the Day 28 Endpoint

The acute improvement in cardiac function is maintained out to the 28-day post-injection endpoint. Over time, both PBS and rHCI treated mice have increases in EDV and ESV, with rHCI treated mice exhibiting less dilation in ESV at both day 2 and day 28 timepoints. There is also an increase in SV and CO from day 2 to day 28 in both treatment groups, and again rHCI treated mice have higher SV and CO compared to PBS at each timepoint. The increase in SV and CO in the PBS treatment group results from increased filling at end diastole. This allows the volume of blood pumped out per beat (SV) to increase

through the Frank Starling mechanism to meet the demands of the body post-MI^{56,59}. However, this compensation mechanism will not maintain cardiac function or prevent heart failure long-term as dilation begets more dilation through increased wall stress and myocardial thinning^{47,56}. There is no improvement in LVEF in the PBS group with time. LVEF is predictor of survival outcomes in patients with heart failure and post-MI^{64,98,319}. LVEF is improved in rHCI treated animals compared to PBS at 2 days and this improvement is maintained out to day 28. The improvement in borderzone contractility is also maintained in rHCI treated animals as compared to PBS at the day 2 to day 28 timepoint.

4.3.3 Mechanisms of Acute Functional Improvement Following Hydrogel Injection Post-MI

Two possible ways that rHCI hydrogel injection could result in an acute improvement in borderzone contractility are mechanical stabilization of the infarct region or improving the contraction of borderzone cardiomyocytes. Mechanical stabilization of the infarct region with a hydrogel or patch can reduce systolic bulging of the infarct region and limit the amount of borderzone contractile force that is expended in infarct stretching^{193,217}. Stabilization of the infarct region can reduce the wall stress in the borderzone myocardium^{320,321}. There is a precedent for acute improvement of cardiac function with alginate hydrogel injection (Algisyl-LVR), measured at 3 hours post-injection in a pre-clinical dog model of heart failure (LVEF <30%) and this improvement was sustained for 17 weeks^{176,196}. The hydrogel increased wall thickness and modeling suggested that it reduced dyskinesis in the infarct both of which had an impact in reducing wall stress in the borderzone. Using a finite element model it was proposed that the reduction in wall stress led to an acute improvement in contractility within the borderzone¹⁹³. In support of the infarct stabilization theory, the results

of aim 1 showed that the stiffness of the infarct region increased with rHCI hydrogel injection to levels more similar to healthy myocardium 2 days post-injection²⁶⁹. However, the stiffness increase in the infarct region with rHCI hydrogel injection does not change the strain in the infarct region compared to PBS control. Therefore, the hydrogel does not reduce dyskinesia in the infarct region. In addition, while the hydrogel comprised 8% of the infarct thickness in treated animals, this did not lead to an increase in infarct wall thickness at day 2 or day 28 compared to the control group (data not shown). Therefore, the rHCI hydrogel does not offset wall stress by increasing infarct thickness in accordance with Laplace's law¹⁸⁹.

From these observations, it seems unlikely that mechanical stabilization of the infarct region is the primary mechanism behind the acute functional improvement with rHCI injection. Contractility of cardiomyocytes is influenced by factors other than wall stress, including modification of contractile proteins by oxidative stress. These factors could be affected by the bioactivity of the rHCI hydrogel. While the acute effects of bioactive hydrogels on cardiac function post-MI have not been well studied, Mewhort et al. demonstrated that the bioactivity of their submucosal ECM epicardial patch (CorMatrix-ECM) was necessary for the improvement of LVEF from baseline to 3 weeks post-treatment in a rat I/R model²⁰⁰. Inactivated patches exhibited no improvement on cardiac function. Another factor to consider is that the improvement in cardiac function is maintained after the majority of the rHCI hydrogel has been degraded 7 days post-injection²⁶⁹. In addition to the trend in reduced AF594-rHCI signal intensity at 7 days post-injection (80% reduction compared to 2 hours), there is no histological evidence of rHCI in the heart at the 28-day endpoint. The alginate hydrogel mentioned previously did not degrade during the follow-up period and also has minimal bioactivity compared to collagen. In a study comparing alginate

to a bioactive fibrin hydrogel, both improved LVEF by day 2 post-injection, but the improvement in the fibrin group was lost 5 weeks post-injection due to the degradation of the gel²¹⁵. Therefore, acute molecular or cellular changes within the myocardium must occur in response to rHCI injection to retain the improvement in contractility when the hydrogel is gone. RNA sequencing of LV tissue two-day post-treatment injection was run to detect any changes in gene expression that may be associated with the acute functional improvement with rHCI injection.

4.3.4 RNA Sequencing Analysis of LV Tissue Two Days post-rHCI Injection

The RNAseq analysis in this study was able to detect well known pathways that are differentially regulated post-MI compared to healthy hearts. These include: a downregulation in oxidative phosphorylation, increase in cytokine production, and an increase in collagen production with a concomitant increase in collagen binding proteins^{322–324}. These changes are expected in the proliferative phase of scar formation at 7 days post-MI, when collagen deposition and ECM remodeling of the maturing scar occur²⁸⁷. There were only 7 genes (out of > 20,000 detected) that were differentially expressed between rHCI and PBS treated LVs 2-day post-injection in MI mice. This was unexpected given the magnitude of the functional improvement. However, given the short time period from treatment injection to follow-up it is likely that the majority of changes occur at the post-transcriptional level, including protein activity changes or reduction of ROS and ROS-derived adduct protein modification. In addition, bulk RNAseq was performed on LV tissue, as the exact cell type or region where the hydrogel exerts its potential bioactive effects was not known. It is possible that cardiomyocyte specific RNAseq or separation of infarct, borderzone, and remote myocardium may improve the detection of gene expression changes at the short-term time

point. More specific tissue region or cell specific RNAseq limits the variability in the cell sources of transcripts and can improve sensitivity to resolve specific differential gene expression in a target region or cell type of interest³²⁵.

In comparison to the modest changes in gene expression seen with rHCI injection, Wassenaar et al. showed that in the infarct region of mice treated with a cardiac ECM hydrogel there were 129 known genes with differential expression 3 days after injection, even though the samples did not cluster separately¹⁹⁸. Functional data for the 3-day timepoint was not provided. This difference in impact on gene expression may result from the localization of the hydrogels within the heart. The cardiac ECM hydrogel is located within the infarct region post-injection and there are multiple cell types that invade the hydrogel post-injection^{198,326}. The hydrogel leads to changes in expression of a number of genes involved in angiogenesis and the inflammatory response. This could be due, in part, due to the cellular invasion and remodeling of the hydrogel within the infarct region. The rHCI hydrogel in this study is located on the epicardium of the infarct and borderzone. There are cells within the hydrogel, but these are mainly epicardial cells, which represent a small percentage of the overall cell population in the heart.

4.3.5 Follow-up Studies on CCL-22 Target Gene

The two target genes that were chosen for follow-up analysis were *ERDR1* and *CCL22*. The expression of *Ccl22* by macrophages can lead to the recruitment of regulatory T-cells, and FOXP3⁺ T-regulatory cells improve the resolution of inflammation post-MI^{39,289,327}. The 1.9-fold upregulation of *CCL22* expression detected by sequencing could not be detected by qPCR. These results were confirmed by IHC detection of T-regulatory cells, in which there was no difference in cell density at 2 days post-injection. However, there was

an increase in FOXP3⁺ T-regulatory cell density 28 days post-injection in the borderzone myocardium of rHCI treated MI mice. This observation highlights that some initial changes in gene expression that are modest at the day 2 timepoint could become greater and have a functional consequence by day 28. Future studies could perform RNAseq at the day 28 timepoint. However, this would not simply detect gene expression changes underlying the initial functional improvement with rHCI hydrogel but would also detect changes in gene expression that result from the maintained functional improvement and the associated responses that are activated after the hydrogel has degraded.

4.3.6 Erythroid Differentiation Regulator 1 is Downregulated in Cardiomyocytes post-rHCI Injection

The second target gene from RNA-seq, *ERDR1*, was confirmed by qPCR to be downregulated 3-fold in LVs injected with rHCI compared to PBS. Two days after rHCI injection, there is decreased expression of *ERDR1* by remote and borderzone cardiomyocytes as well as non-cardiomyocytes within the remote region. Erdr1 protein has not been widely investigated and there is currently no commercially available antibody. It is expressed on the X and Y chromosomes and in knockout models is embryonic lethal²⁹⁰. Erdr1 was initially discovered as a soluble factor that stimulated hemoglobin synthesis in human erythroleukemia cells in culture³²⁸. In further investigations, it was found to be a cytokine that is released under stress conditions in the culture of Burkitt's lymphoma cell line (BL-70)³²⁹. At high cell density and high Erdr1 concentrations, Erdr1 promoted BL-70 cell apoptosis. However, at low cell density and low Erdr1 concentrations, Erdr1 promoted cell survival. Upregulation of *ERDR1* has promoted apoptosis in a mouse model of melanoma²⁸⁰, mediates UVB-induced apoptosis of keratinocytes²⁷⁹, and leads to Fas-mediated cell death in

T-cells in an autocrine manner^{290,330}. *ERDR1* expression has pleiotropic effects in other *in vivo* models including: suppressing inflammation in mouse models of rheumatoid arthritis and psoriasis^{331–333}, increasing survival of chronic lymphocytic leukemia cells in culture³³⁴, and increasing the regeneration of intestinal epithelial cells³³⁵.

In the heart, *ERDR1* knockdown rescues macrophage migration post-MI in prostaglandin E₂ knock-out mice³³⁶. *ERDR1* expression is downregulated in NADPH oxidase 2 knock-out mice²⁹². NADPH oxidase increases ROS levels post-MI and increases dilative remodelling³³⁷. In *mdx* mice, which developed severe dilated cardiomyopathy, *ERDR1* expression is upregulated in addition to NADPH oxidase 4 and Lysyl oxidase, two major sources of oxidative stress²⁹³. Finally, an RNAseq study by Duijvenboden et.al. investigated the differences between borderzone and remote myocardium and identified *ERDR1* as a biomarker for borderzone cardiomyocytes³³⁸. *ERDR1* was upregulated 15-fold in borderzone compared to remote cardiomyocytes. Borderzone cardiomyocytes were shown to switch from a cardiomyocyte lineage gene program to a stress response program.

In aim 3 of this thesis, follow-up analyses were performed to detect the impact of rHCI hydrogel on oxidative stress 2 days post-injection. This decision was based on the known role of *ERDR1* in apoptosis in other systems, its upregulation in response to oxidative stress in models of cardiovascular disease, and the finding that *ERDR1* is downregulated when rHCI hydrogel improves cardiac function post-MI. However, it is interesting that *ERDR1* expression is downregulated in rHCI treated MI mice compared to both PBS treated MI mice and sham animals, with no difference between PBS and sham. Similar results were found in the Duijvenboden et al. study, where there was no difference in *ERDR1* expression in bulk-sequencing between healthy or borderzone myocardium 7 days post-MI³³⁸. The

difference between borderzone and remote *ERDR1* expression was only seen in cardiomyocyte specific RNAseq. The *ERDR1* expression levels specifically in healthy cardiomyocytes were not investigated in this study, so it is unknown if *ERDR1* expression is elevated in cardiomyocytes post-MI compared to healthy mice. The receptor(s) for Erdr1 have also not been established. Given the pleiotropic roles of *ERDR1* in multiple systems it is possible that post-MI a different receptor profile on Erdr1 responsive cells or interaction with other pathways upregulated under oxidative stress leads to different responses to Erdr1 post-MI compared to healthy conditions.

4.3.7 rHCI Hydrogel Injection Reduces Oxidative Stress and Cardiomyocyte Apoptosis Two Days Post-Injection

The injection of rHCI hydrogel post-MI results in lower levels of 4HNE adducts in the remote myocardium and lower levels of apoptotic cardiomyocytes in the borderzone and remote myocardium 2 days post-treatment. 4HNE is a reactive aldehyde formed by ROS-mediated lipid peroxidation^{339,340}. Mitochondrial dysfunction is a major source of ROS-derived 4HNE formation in the failing heart, such as increased hydrogen peroxide production from mitochondrial monoamine oxidase post-MI^{79,341}. 4HNE can form adducts on a variety of proteins including: mitochondrial proteins, sarcomeric proteins, and enzymes responsible for 4HNE detoxification, such as ALDH2, thus modifying their function^{342–344}. In addition, elevated levels of 4HNE post-MI lead to calcium influx into the mitochondria, membrane depolarization and cardiomyocyte death⁷⁹. The level of 4HNE is negatively correlated with cardiac function and it increases overtime in heart failure patients³⁴⁵. The reduction in 4HNE levels by rHCI hydrogel injection suggests that this treatment acutely reduces oxidative stress levels in the remote myocardium. Injection of rHCI did not increase ALDH2 activity in the

remote myocardium, therefore it is not involved in removing the 4HNE adducts with rHCI treatment. 4HNE has a short half-life under physiological conditions, around 2 min, therefore limiting 4HNE accumulation over 2 days may be sufficient to produce a significant decrease in 4HNE from PBS-treated mice without an increase in clearance³³⁹. There are also other enzymes that can detoxify 4HNE including glutathione-*S*-transferase (GST) and aldo-keto reductases (AKRs)³⁴⁰. Therefore, rHCI hydrogel could both reduce 4HNE accumulation and increase 4HNE clearance by an ALDH2 independent mechanism.

The reduction in cardiomyocyte apoptosis occurs concurrently in the regions which have reduced *ERDR1* expression post-rHCI injection, namely borderzone and remote myocardium. This implicates *ERDR1* as a factor upregulated in cardiomyocytes experiencing higher levels of oxidative stress, and in regions of the myocardium with higher apoptotic rate. However, a knockdown experiment will be required in future studies to determine if *ERDR1* downregulation can lead to a reduction of oxidative stress and apoptosis post-MI. As previous research in the gut has shown that *ERDR1* knockout is embryonic lethal, and conditional knockdown is difficult to achieve based on the flanking sequences on the chromosome, a short hairpin RNA (shRNA) knockdown could be used in future studies to understand the role of *ERDR1* post-MI.^{290,330}

An increase in cardiomyocyte apoptotic rate is observed in heart failure patients and is correlated with LV function in experimental MI models^{80,346,347}. Persistent, elevated oxidative stress and mitochondrial dysfunction post-MI is also involved in cardiomyocyte hypertrophy and eventually apoptosis downstream of apoptotic signal related kinase 1 (ASK1)^{49,73,76}. At the 28-day endpoint rHCI treated MI mice have less hypertrophic cardiomyocytes and greater cardiomyocyte density within the borderzone and remote

myocardium. This may be the result of lower levels of apoptosis and oxidative stress adducts within the myocardium starting at the 2-day timepoint post-treatment.

The reduction in oxidative stress, detected by 4HNE adducts, could underlie the reduction in apoptotic cardiomyocyte rate in the remote myocardium. However, the lower rate of apoptosis in the borderzone myocardium, the key area of contractile improvement, is independent of 4HNE levels. Furthermore, 4HNE levels appear to increase in both groups over time and are not different between treatment groups at day 28, while the improvement in cardiac function is maintained at this timepoint in rHCI treated animals. Therefore, the acute reduction in 4HNE with rHCI hydrogel injection most likely does not directly mediate its immediate impact on contractility and cardiac function post-MI.

4.3.8 rHCI Hydrogel Soaks Up Free Methylglyoxal and Reduces MG-H1 Adducts Within Cardiomyocytes

The data indicates that the improvement in cardiac function 2 days after rHCI injection is associated with *ERDRI* downregulation, reduction in oxidative stress, and reduction in apoptosis of cardiomyocytes. However, the question remained how the hydrogel, located at the epicardial surface, could reduce the level of oxidative stress in the myocardium below. In addition, the link between acute reduction in oxidative stress and increase in borderzone contractility with rHCI injection had not been determined.

Methylglyoxal adducts were studied due to their ability to modify collagens, modify contractile proteins and stimulate ROS production^{122,125,348}. Methylglyoxal is a reactive dicarbonyl that is primarily generated as an endogenous by-product of glycolysis^{119,122}. Methylglyoxal modifies arginine and lysine residues on proteins. The main MG-AGE formed

is the MG-H1 adduct, which can compromise protein function¹²². Methylglyoxal was initially studied in complications associated with diabetes. For example, in patients with diabetic cardiomyopathy. High glucose levels led to impaired diastolic filling due to MG-H1 crosslinks in ECM collagen that increased chamber stiffness^{124,127,349}. MG-H1 levels increase as early as 6h post-MI and have been shown to contribute to a decline in cardiac function, impaired angiogenesis, and cardiomyocyte apoptosis⁵³. MG-H1 levels are increased by high levels of oxidative stress, such as ischemia/reperfusion injury, and in turn can increase ROS through RAGE signaling in a positive feedback loop^{350,351}. Two days after injection of rHCI hydrogel there was a reduction in MG-H1 levels within cardiomyocytes in the infarct, borderzone, and remote myocardium. The MG-H1 levels in cardiomyocytes showed a characteristic sarcomere banding pattern. Previous studies have shown that MG-H1 modifies contractile proteins within cardiomyocytes leading to impaired calcium handling, less actin-myosin interactions, and lower contractile force^{75,352}. Therefore, the reduction in MG-H1 within cardiomyocytes, may underlie the improvement in contractility and reduction in cardiomyocyte apoptosis observed two days post-rHCI injection. There was also a reduction in MG-H1 modified collagen of the infarct, borderzone, and remote myocardium of rHCI treated mice. MG-H1 can modify RGD integrin binding sites on collagen, leading to cell anoikis, and can upregulate ROS production in cells through RAGE^{125,353}. This may explain the reduction in apoptosis in both cardiomyocyte and non-cardiomyocytes within the borderzone and remote myocardium with rHCI treatment. The levels of MG-H1 on collagen and within cardiomyocytes remain reduced within the infarct and borderzone myocardium at day 28 post-rHCI injection. The level of myocardial salvage is higher in both infarct and borderzone myocardium at day 28 endpoint in the rHCI treatment group as well. The reduction in borderzone MG-H1 levels within borderzone cardiomyocytes is concurrent with

and may contribute to the improved contractility in the borderzone myocardium at both day 2 and day 28 post-MI.

The reduction in MG-H1 levels in the heart tissue of rHCI treated mice may be in part due to the uptake of free methylglyoxal into the hydrogel at the epicardial surface. MG-H1 adducts are estimated to have a half life of 2-3 weeks under physiological conditions¹²². Therefore, the reduction in MG-H1 levels on native collagen and within cardiomyocytes, is most likely due to reduced production of new MG-H1 adducts or the enzymatic removal of MG-H1 adducts through the glyoxalase system. The high level of MG-H1 modification of the rHCI hydrogel, ~50% of its total area, suggests that the level of free methylglyoxal within the myocardium is high. The hydrogel prevents this free methylglyoxal from modifying proteins within the myocardium and suggests that limiting the build up of further MG-H1 modifications is a major component of the difference in MG-H1 levels between PBS and rHCI injection 2 days post-treatment. It was observed that the MG-H1 levels in the control group appear to be lower at day 28 compared to day 2. Therefore, MG-H1 adducts may be cleared overtime as the inflammation and oxidative stress from the initial MI injury is resolved. The acute reduction of MG-H1 with rHCI treatment persists compared to PBS in the infarct and borderzone. This suggests the uptake of methylglyoxal by rHCI hydrogel may speed up the resolution of MG-H1 stress in these areas. Further studies will need to establish whether the uptake of MG-H1 by rHCI hydrogel is sufficient to reduce MG-H1 levels in the myocardium. Overexpression of glyoxalase-1 (GLO1), the rate limiting enzyme involved in MG-H1 removal, has previously been shown to reduce MG-H1 build up and improve cardiac function within a week post-MI⁵³. There is no upregulation of *GLO1* detected by RNA-seq,

but further studies will need to establish whether or not increased GLO1 activity plays a role in MG-H1 reduction with rHCI hydrogel injection.

4.3.9 Proposed Mechanism for the Improvement in Cardiac Function with rHCI Hydrogel Injection Post-MI

The injection of rHCI hydrogel leads to an acute improvement in borderzone contractility and cardiac function within 2 days of injection that is maintained out to 28 days. Due to the limited increase in infarct thickness and the lack of improvement in contractility of the scar, rHCI injection likely does not achieve greater cardiac function through infarct stabilization. At the day 2 timepoint there is a concurrent reduction in ROS intermediates, 4HNE and MG-H1, within the myocardium and a lower cardiomyocyte apoptosis rate indicating a lower level of oxidative stress with rHCI treatment. In particular, the uptake of methylglyoxal into rHCI hydrogel at the epicardial surface may lower the level of MG-H1 adducts within cardiomyocytes. MG-H1 adducts within cardiomyocytes bind to sarcomeric proteins and have been previously shown to impair contractility⁷⁵. The proposed mechanism of action is that methylglyoxal uptake into the epicardial rHCI layer reduces the MG-H1 build up in the borderzone cardiomyocytes, leading to improved cardiomyocyte contractility and an acute improvement in cardiac function. The reduction in MG-H1 in borderzone cardiomyocytes is sustained after the hydrogel is degraded and allows for maintained improvement in cardiac function out to the 28-day endpoint. The lower levels of MG-H1 found in all regions at day 2, both on collagen and within cardiomyocytes, result in a reduction in oxidative stress, leading to lower rates of cardiomyocyte apoptosis and greater myocardial salvage at the 28-day endpoint. The level of MG-H1 within borderzone and

infarct cardiomyocytes remains reduced out to day 28 and there is improved cardiomyocyte salvage in rHCI-treated mice.

4.4 General Discussion

4.4.1 Comparison to Other Hydrogel Therapies

There are several key differences between the *in vivo* performance of rHCI hydrogel and that of previously studied type I animal collagen gels studied in our lab. The two previous studies used 0.375% rat tail type I collagen, one with glutaraldehyde crosslinker and the other with EDC/NHS crosslinking system^{175,197}. Both hydrogels were injected 7 days post-MI in the mouse LAD ligation model. The glutaraldehyde-crosslinked rat tail collagen hydrogel preserved LVEF from 1 week to day 28 post-MI but showed no improvement over baseline. The EDC-crosslinked rat tail collagen had more modest improvement in LVEF compared to PBS at 4-week follow-up (~7%) compared to rHCI hydrogel (~20%). Comparisons to baseline function 1-week post-MI were not presented. The improvement in LVEF over PBS was similar to rHCI hydrogel when the EDC crosslinked rat tail gel was injected 3 hours post-MI. Therefore, rHCI hydrogel provides a greater functional benefit in an established scar. The functional benefit observed with rHCI hydrogel injection is equivalent to or greater than that seen in small animal studies of hydrogels and patches currently being tested in clinical trials for to treat heart failure post-MI^{176,235,354}. The materials currently in clinical trials include alginate and decellularized porcine ECM^{171,204,225}. Some of the benefits of rHCI hydrogel is that it provides greater bioactivity than alginate without the concerns of immunogenicity of decellularized animal ECM materials currently being test in the clinic (see SWOT analysis Figure 4.1). While rHCI hydrogels do not have the native tissue

complexity that decellularized ECM materials possess, the simple composition of rHCI hydrogels limits batch to batch variability.

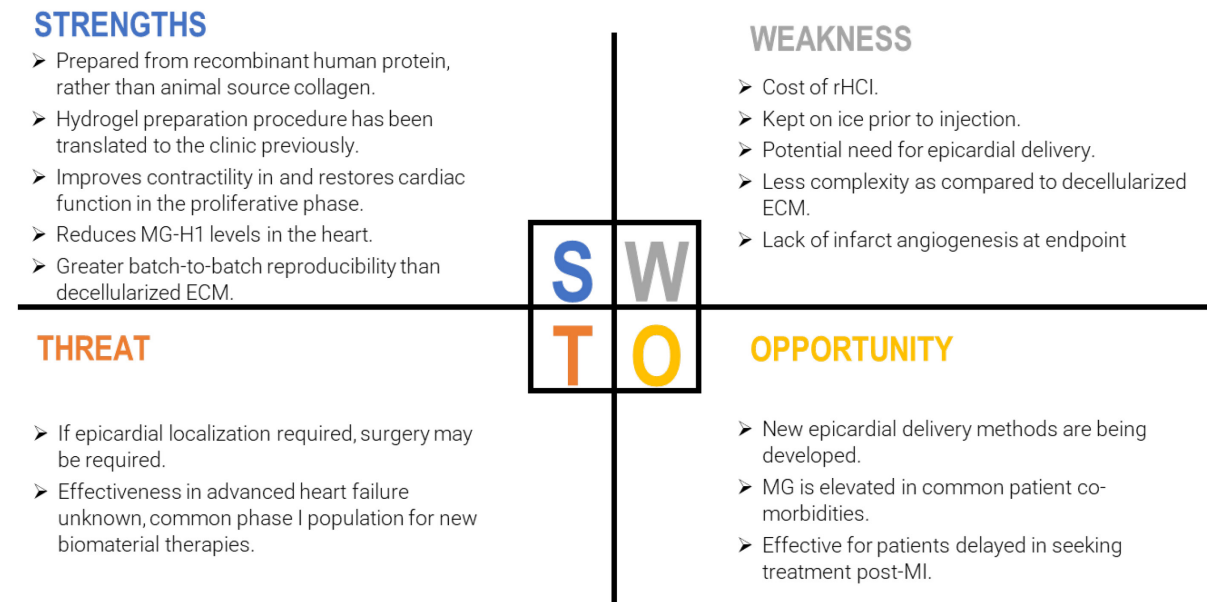


Figure 4.1. SWOT analysis of rHCI hydrogel as compared to current acellular biomaterial options in clinical trials.

One of the key differences between rHC and animal-derived collagen gels previously developed in our lab is that rHCI is located on the epicardial surface of the infarct and borderzone post-injection. The animal-derived collagen hydrogels were detected within the infarcted myocardium²⁴⁸. The anterior borderzone myocardium was targeted for injection for both hydrogel trials and the same mouse model of MI was used. The difference in hydrogel localization may result from increased viscosity of the rHCI injectate compared to the animal derived collagens. The increased viscosity is due to a 3-fold greater concentration of collagen compared to both rat tail collagen hydrogels and a 6-fold greater concentration of EDC crosslinker in solution, when compared to the EDC-crosslinked rat tail collagen gel. The viscosity of the injectate may lead to it being pushed onto the epicardial surface post-

injection. In contrast to the animal derived collagens, the rHCI hydrogel does not improve vascular density within the infarcted myocardium. The collagen hydrogels that are located within the infarct region will be better able to stimulate angiogenesis compared to rHCI at the epicardial surface. However, the epicardial localization of rHCI may be critical to soak methylglyoxal radicals out of the myocardium below, leading to improved myocardial contractility. The importance of epicardial localization will need to be determined in order to optimize hydrogel delivery in larger animal models. An rHCI hydrogel can be designed with viscosity similar to the previous animal-derived collagen hydrogels and *ex vivo* fluorescent imaging of tagged hydrogels can determine if this leads to intramyocardial localization of the hydrogel. The functional impact of rHCI hydrogels on the epicardial surface and within the infarct region can be compared to determine if epicardial localization is required for functional improvement.

If epicardial localization is required to maximize the improvement in cardiac function with treatment, the rHCI hydrogel will need to be delivered by epicardial intramyocardial injection rather than catheter-based endomyocardial injection in the clinic. Clinical trials have been performed with Algisyl-LVR injection through ministernotomy in patients with advanced chronic heart failure^{224,225}. The procedure was safe; however, it will limit the patient population that is eligible for hydrogel injection due to the invasiveness of the procedure. In addition, it cannot be completed at the 14-day post-MI timepoint unless injection is done alongside CABG²⁰¹. Future pre-clinical studies of rHCI hydrogel can be designed to test if rHCI injection can improve cardiac function in more advanced heart failure models, such as 5 weeks post-MI in rodents. There have been developments in catheter technology that allow minimally invasive delivery of hydrogels through the pericardial space

onto the epicardium post-MI^{205,206}. However, they have only been successfully used to delivery biomaterials in large animal models in current publications and clinical data is not yet available. Finally, several synthetic polymer hydrogels have been designed to scavenge ROS and reduce oxidative damage post-MI³⁵⁵. To our knowledge, this is the first description of radical scavenging by a natural hydrogel.

4.4.2 Study Limitations and Future Directions

A limitation of the animal model used in this study is that the mice do not have common comorbidities seen in patients with MI in clinic such as older age and diabetes¹⁰. These comorbidities can reduce the capacity of bioactive biomaterials to stimulate endogenous repair due to reduced regenerative capacity within the tissue and defective progenitor cell populations^{356,357}. In addition, permanent ligation models do not replicate the increase in ROS stimulated by reperfusion injury. Reperfusion is performed in the majority of patients post-MI^{10,11}. However, the effect of rHCI hydrogel therapy may not be limited by these patient characteristics, as elevated MG-H1 modifications are associated with the damage caused by both comorbidities and reperfusion injury^{350,352,358}. An additional limitation to the mouse model of MI is that the mice are not given current guideline directed medical therapy, such as β -blockers and ACE inhibitors¹⁰. These drugs are not administered in most pre-clinical models of MI as the goal is to determine the effect of a novel therapy on cardiac function. However, the interaction and/or possible overlap in benefits with current therapy and rHCI hydrogel will need to be tested in large animal model of MI. Given that β -blockers and ACE inhibitors reduce distending pressures on the heart and reduce neurohormonal stimulation to limit dilation post-MI, the overlap with rHCI hydrogel injection is likely to be small⁴⁷. There is some data to suggest that β -blocker therapy does not

reduce ROS adducts in congenital heart disease³⁴³, indicating rHCI hydrogel-mediated reduction of MG-H1 may exert additional benefits to the current guideline-directed medical therapy post-MI. The final limitation in the mouse model was the impact of rHCI hydrogel on cardiac function was only monitored for 4 weeks. At this timepoint deterioration in function from pathological remodeling can be seen in control animals. However, further studies will need to establish sustained improvement out to 3-4 months post-injection at which point a survival benefit can be determined.

The studies in this thesis establish several key features of rHCI hydrogel injection post-MI that can inform potential translation to large animals and clinical trials. These discoveries include: the epicardial localization of the hydrogel, the minimal inflammatory response to rHCI injection, and the ability of rHCI to reduce MG-H1 modification levels in the myocardium. However, the proposed mechanism of action for rHCI hydrogel injection raises further research questions. First, is methylglyoxal uptake into the rHCI hydrogel required for cardiac functional improvement post-MI? To answer this, the rHCI hydrogel solution could be pre-modified by methylglyoxal prior to injection, such that no methylglyoxal uptake is possible. These MG-H1 modified hydrogels would be expected to have a reduced impact on cardiac function. In addition, the dose-response relationship needs investigated for rHCI hydrogels and could be done by injecting lower volumes of material into the heart post-MI and measuring cardiac function in response to dose delivered. Also, the effect of rHCI hydrogel on GLO1 activity and MG-H1 clearance was not addressed in this thesis work. Another question raised is whether MG-H1 reduction from 7 to 9 days post-MI in mice is sufficient to improve cardiac function? To test this hypothesis an inducible

over-expression model of GLO1 in heart tissue could be created to reduce methylglyoxal levels from day 7 to day 9 post-MI while cardiac function is measured by echocardiography.

The role of *ERDR1* downregulation in the reduction of MG-H1 and cardiomyocyte apoptosis has also not been established in this work. Little is known about the signaling pathways activated by *Erdr1* post-MI. Future research studies can establish the change in cardiomyocyte expression of *ERDR1* in response to MG-H1 and whether *Erdr1* can stimulate cardiomyocyte ROS production, hypertrophy, and oxidative stress. One final limitation to be considered for these studies is that rHC hydrogel degradation was measured *ex vivo* by a fluorescent reporter. *In vivo* imaging would provide a more accurate degradation rate for the hydrogels. In particular monitoring hydrogel degradation overtime by MRI would be beneficial as one can simultaneously measure cardiac function, myocardial wall stress and strain¹⁷⁷. Differential gene expression post-rHCI degradation could also be informative to understand the remodeling response of the heart to sustain functional improvement once the rHCI hydrogel is no longer present.

4.4.3 Conclusion

The results of this research have established rHC hydrogels as effective treatments to limit ventricular remodeling post-MI. The rHCI hydrogel is the superior performing treatment when injected during the proliferative phase of healing post-MI. Injection of rHCI stimulates an increase in borderzone contractility that improves cardiac function. Further studies determined that rHCI hydrogel scavenges free methylglyoxal to reduce MG-H1 modification of collagen and cardiomyocyte contractile proteins in the myocardium. The role of elevated MG-H1 in exacerbating ischemia-reperfusion injury and heart failure highlights the potential for rHCI hydrogel injection post-MI to address an unmet clinical need.

Appendices

Appendix A: Supplementary Data

Supplementary Data for Aims 1 and 2.

Supplemental figures and tables for aims 1 and 2 were published in *McLaughlin and McNeill et.al. Nature Communications 10 (2019) 4866*. They are presented here with minor modifications.

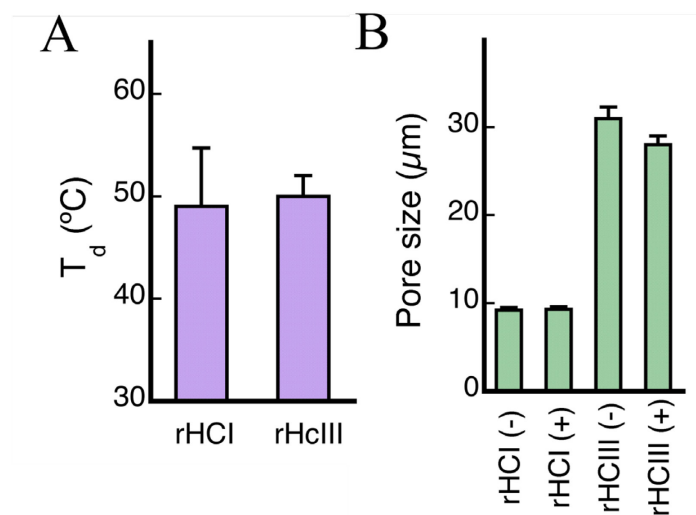


Figure S1. Effect of chondroitin sulfate on the collagen matrices' physical properties. (A) Denaturation temperatures for collagen matrices prepared without chondroitin sulfate (T_d) (°C; $n=3$). Bars represent mean $\pm$ SEM. (B) Mean pore size measured for collagen matrices with (+) or without (-) chondroitin sulfate. Bars represent mean $\pm$ SD. For A-B, n indicates the number of hydrogel batches.

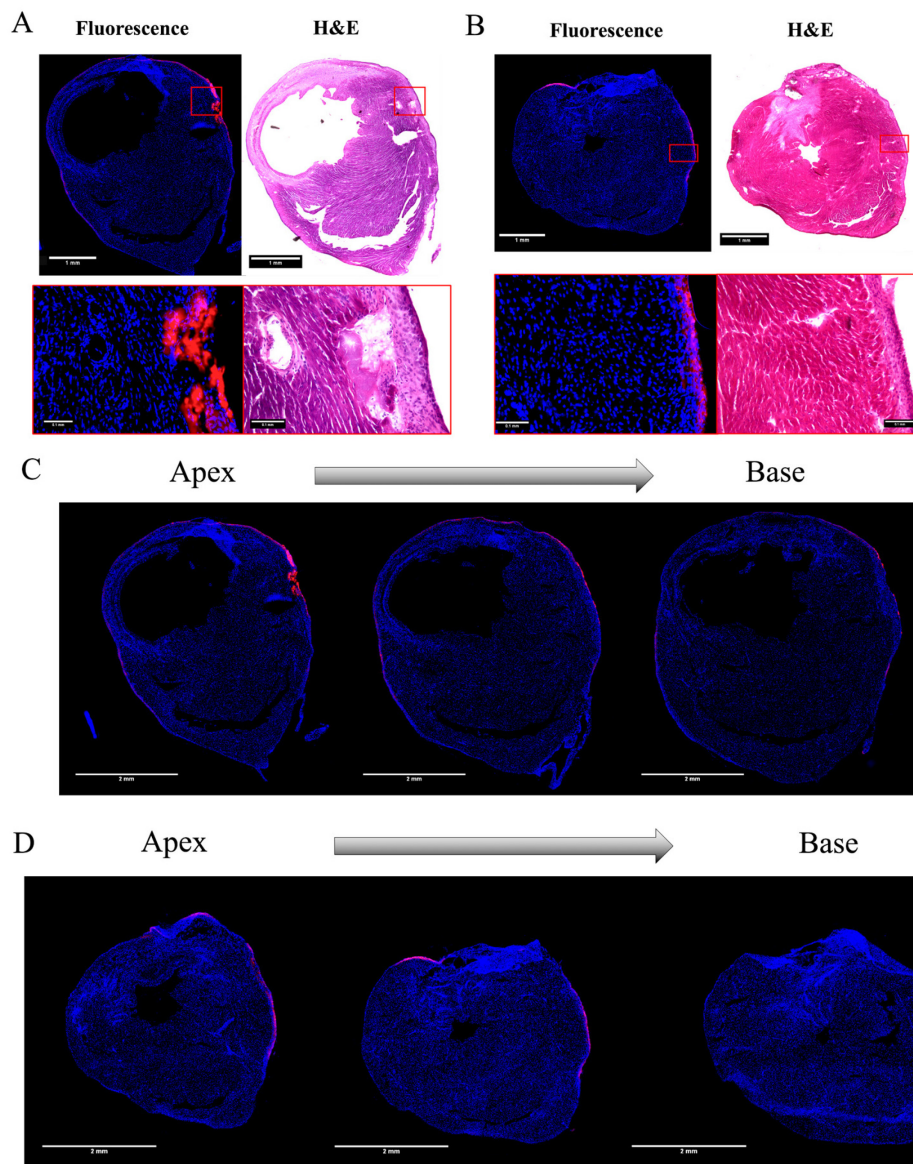


Figure S2. Histological examination of rHC hydrogel-treated MI mouse hearts at 2 hours post-injection. The sections show fluorescently labeled (A) rHCI hydrogel and (B) rHCIII hydrogel. Fluorescence images show AF594 labeled rHCI/III hydrogels (red) and DAPI-stained cell nuclei (blue). Corresponding serial sections were stained with H&E. Scale bars for top images = 1 mm. Red boxes in the top images identify the area that is magnified below (scale bars = 0.1 mm). (C) and (D) depict fluorescence images of sections at different depths for the hearts that received the AF594 labeled (C) rHCI or (D) rHCIII matrices. The sections start at around 1.44 mm from the Apex (denoted as Apex) and increase in 0.48 mm increments towards the base of the heart (aorta/valve). Scale bars = 2 mm.

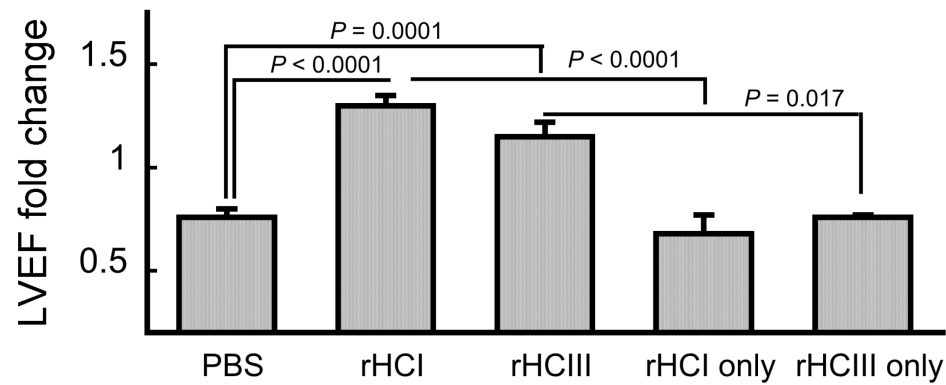


Figure S3. Changes in left ventricle ejection fraction for collagen matrices and non-crosslinked collagen solutions. Change in left ventricular ejection fraction (LVEF) from baseline to day 28 follow-up after injection of collagen matrices (rHCI and rHCIII) and non-crosslinked human recombinant collagen solutions (rHCI only and rHCIII only) in mice with myocardial infarction. Samples sizes for PBS, rHCI and rHCIII were $n = 11-15$. Sample sizes for rHCI only and rHCIII only were $n=3-5$. P values were calculated from a one-way ANOVA for effect of treatment followed by Holm's corrected multiple comparisons. The n values indicate the number of mice per group.

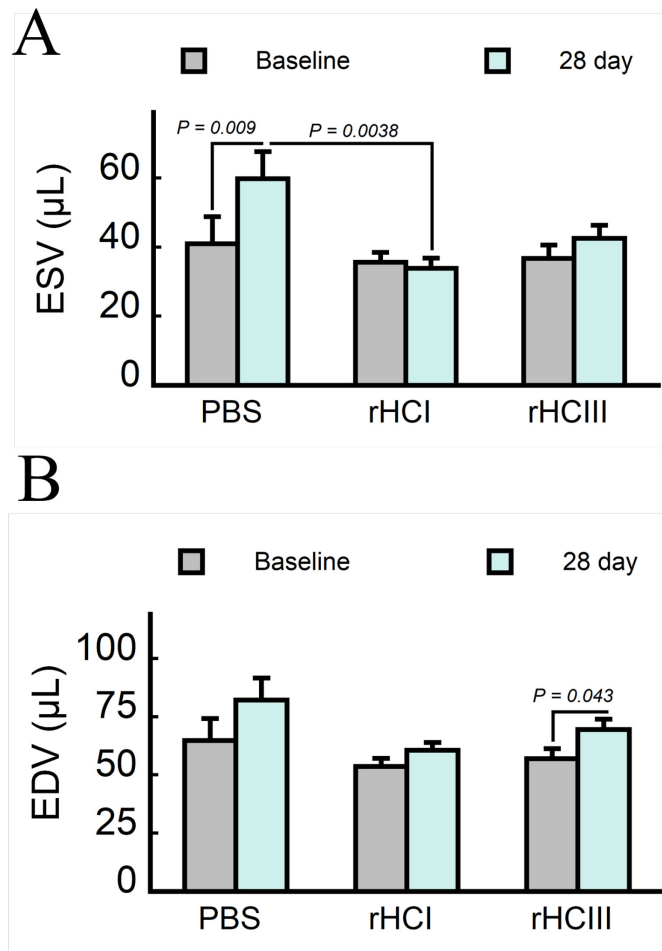


Figure S4. The change in end systolic volume and end diastolic volume from baseline to day 28 follow-up post-rHC injection in mice with myocardial infarction. Change in (A) end-systolic volume (ESV) and (B) end-diastolic volume (EDV) for the different treatment groups. Bars represent mean $\pm$ SEM (n=11 for PBS, n=15 for rHCl, and n=13 for rHCIII). P values were calculated from a two-way ANOVA followed by Holm's corrected multiple comparisons. For A-B, n indicates the number of mice per group.

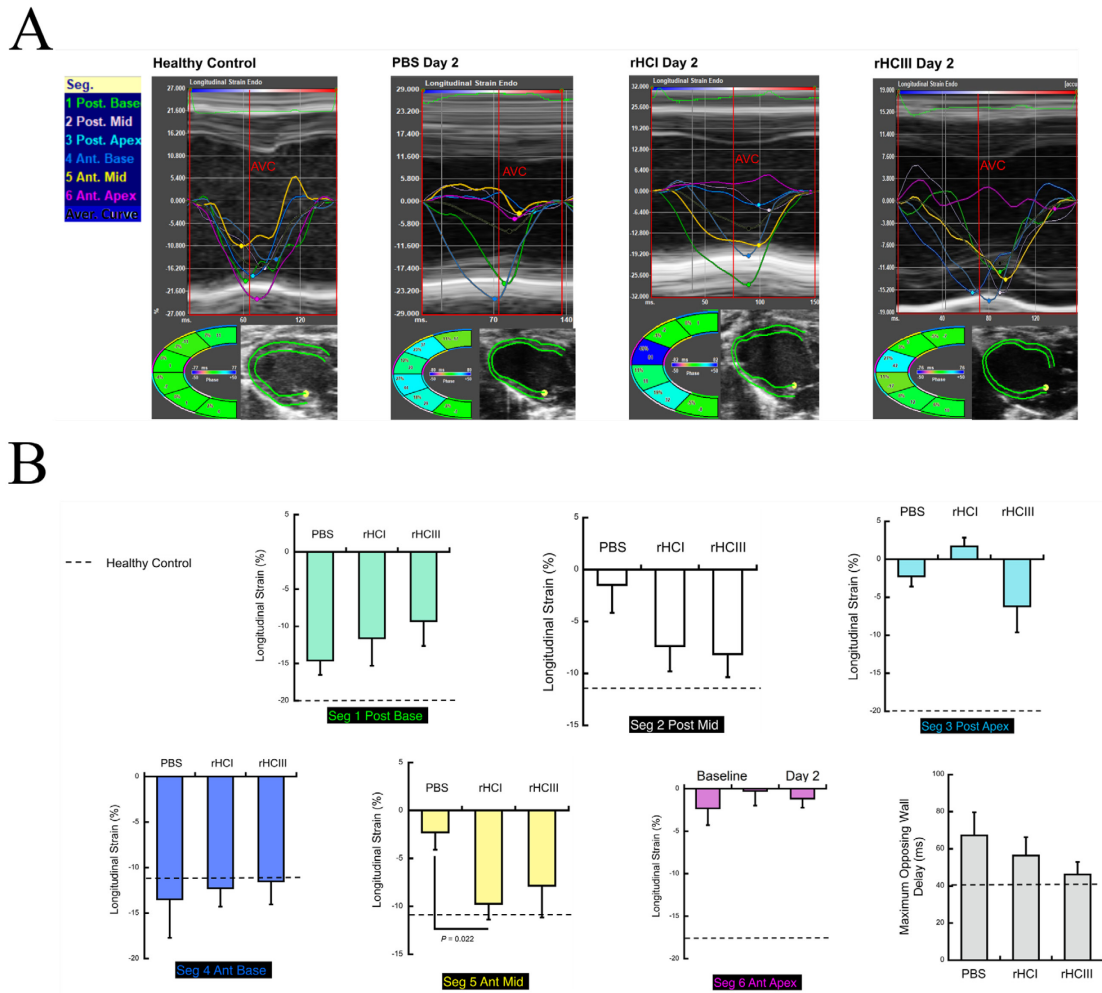


Figure S5. Myocardial strain is improved in rHCI mice two days post-injection within the mid anterior LV wall which is the targeted injection region. (A) Example images from Vevostrain analysis of long axis B mode echocardiograms taken at two days post-injection in mice which were treated seven days post-MI. A healthy age-matched control in addition to PBS and rHCI treated mice are shown. For the strain analysis, the LV is split into six segments indicated in the legend. The region where the treatments are injected is highlighted (seg 5 anterior mid). The strain curves in each segment indicate the displacement of the endocardium over time through the cardiac contractile cycle. Strain is reported as a percentage indicating the change in position of the endocardium relative to its initial position at end diastole. The heart shortens in the longitudinal direction as it contracts, therefore the strain will become more negative during systole and in a healthy animal should peak at the aortic valve closure (AVC). To determine the impact of rHCI injection on cardiac contractility the strain reached at the time of the AVC (red line) was used for analysis. Below the strain curves is an example of the segmentation beside the echocardiogram of the heart. The values within the segmentation diagram correspond to the peak percent strain achieved in that region along with the phase of the cardiac cycle when that strain was reached. (B) Data are presented as mean $\pm$ SEM for longitudinal strain at the time of aortic valve closure

within each segment of the LV. The final graph indicates the maximum opposing wall delay which is the difference in the time to reach peak strain between anterior and posterior regions of the LV myocardium. PBS (n=12), rHCI (n=11), rHCIII (n=5). Significance was determined with a one-way ANOVA for effect of treatment followed up with Dunnett-corrected post-hoc tests between treatment groups and PBS control. The dashed line indicates the average value of aged matched healthy control mice. The n value indicates the number of mice per group.

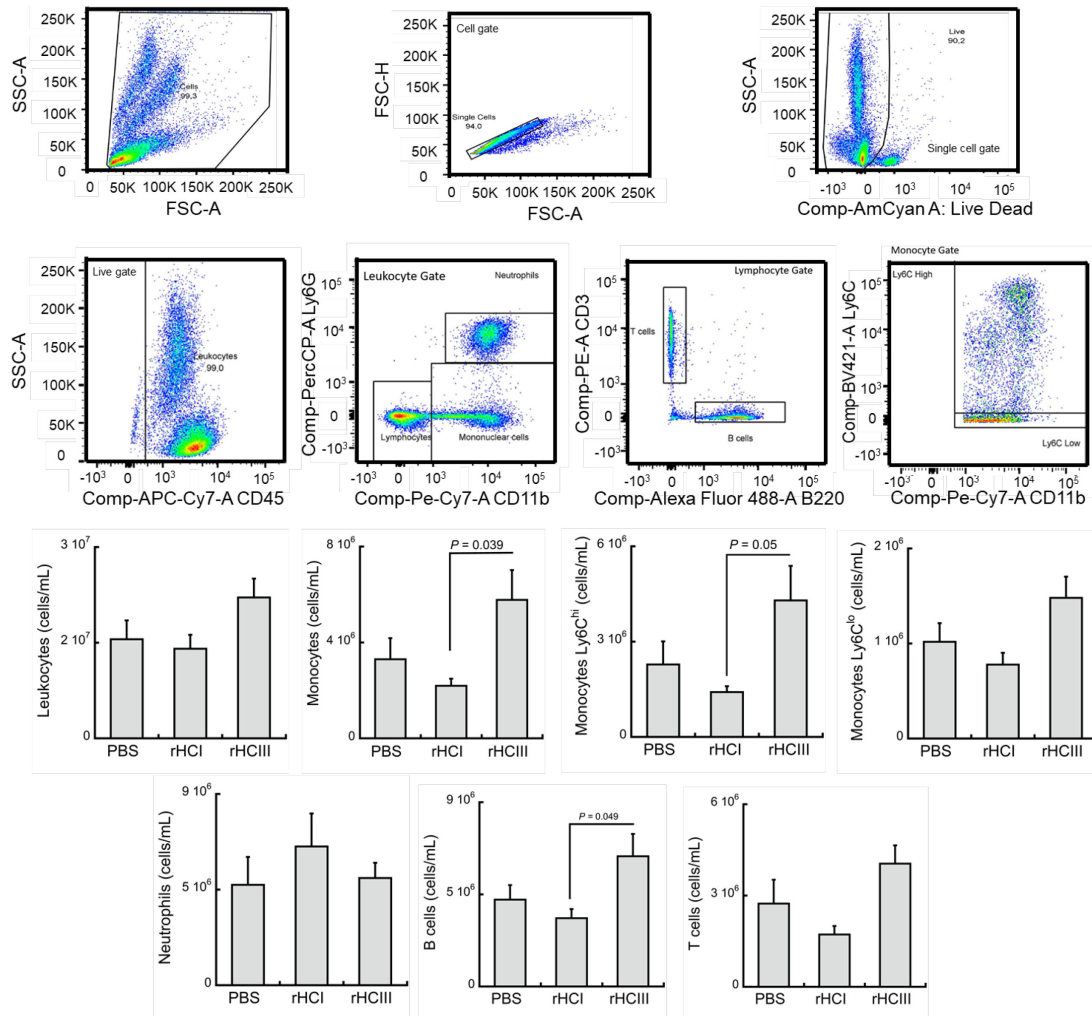


Figure S6. Flow cytometry analysis of mouse blood at 2 days post-treatment. Live single cells were sorted into leukocyte subsets based on the expression of CD45, CD11b, Ly6G, CD3, B220, and Ly6C. P values were determined by a one-way ANOVA for effect of treatment followed by Holm's correction for multiple comparisons. Data is represented as the mean $\pm$ SEM (n=5-7). The n values indicate the number of mice per group.

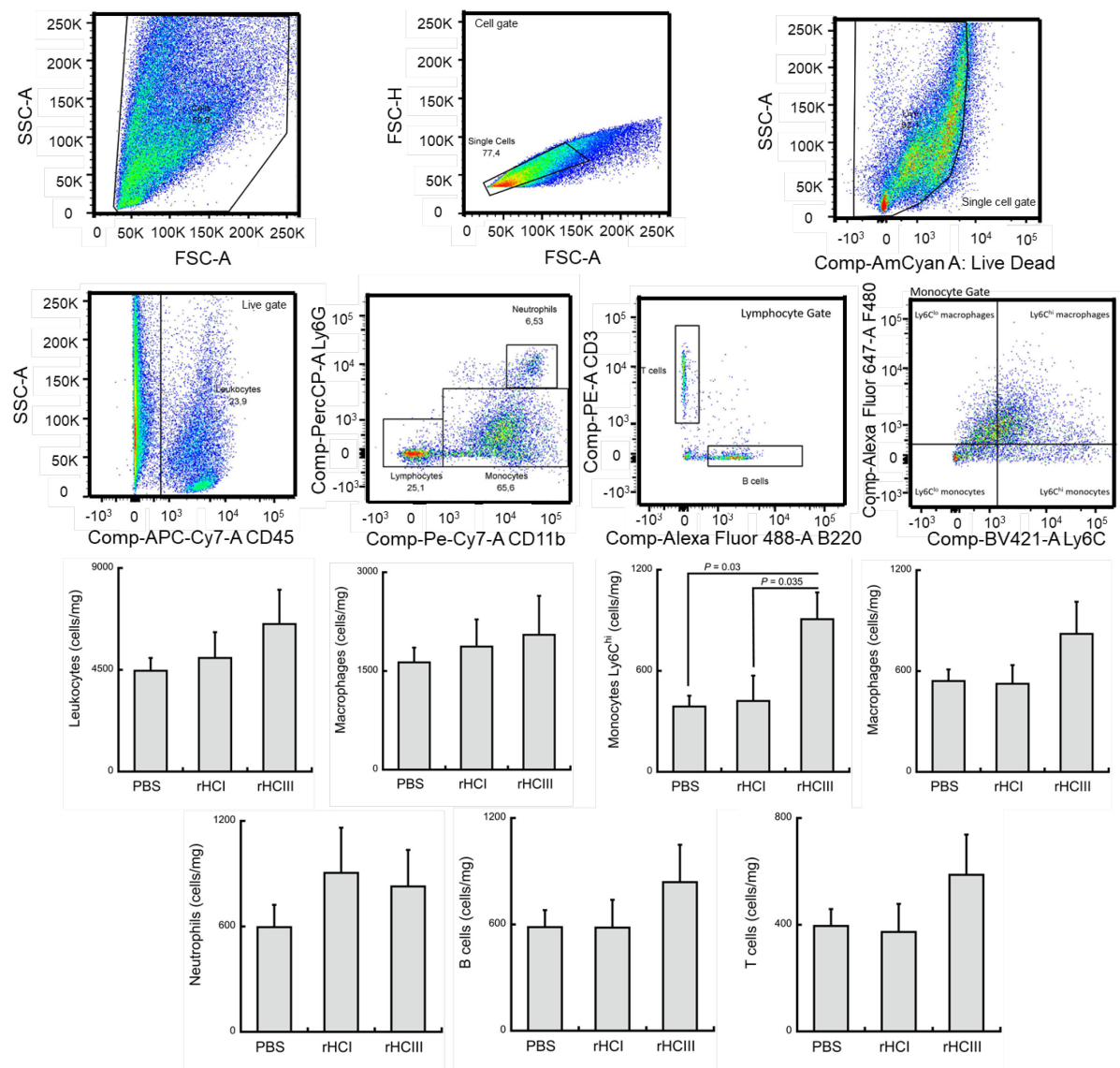


Figure S7. Flow cytometry analysis of mouse heart cells at 2 days post-treatment. Live single cells were sorted into leukocyte subsets based on the expression of CD45, CD11b, Ly6G, CD3, B220, F480 and Ly6C. P values were determined by a one-way ANOVA for effect of treatment followed by Holm's correction for multiple comparisons. Data is represented as the mean $\pm$ SEM (n=5-7). The n values indicate the number of mice per group.

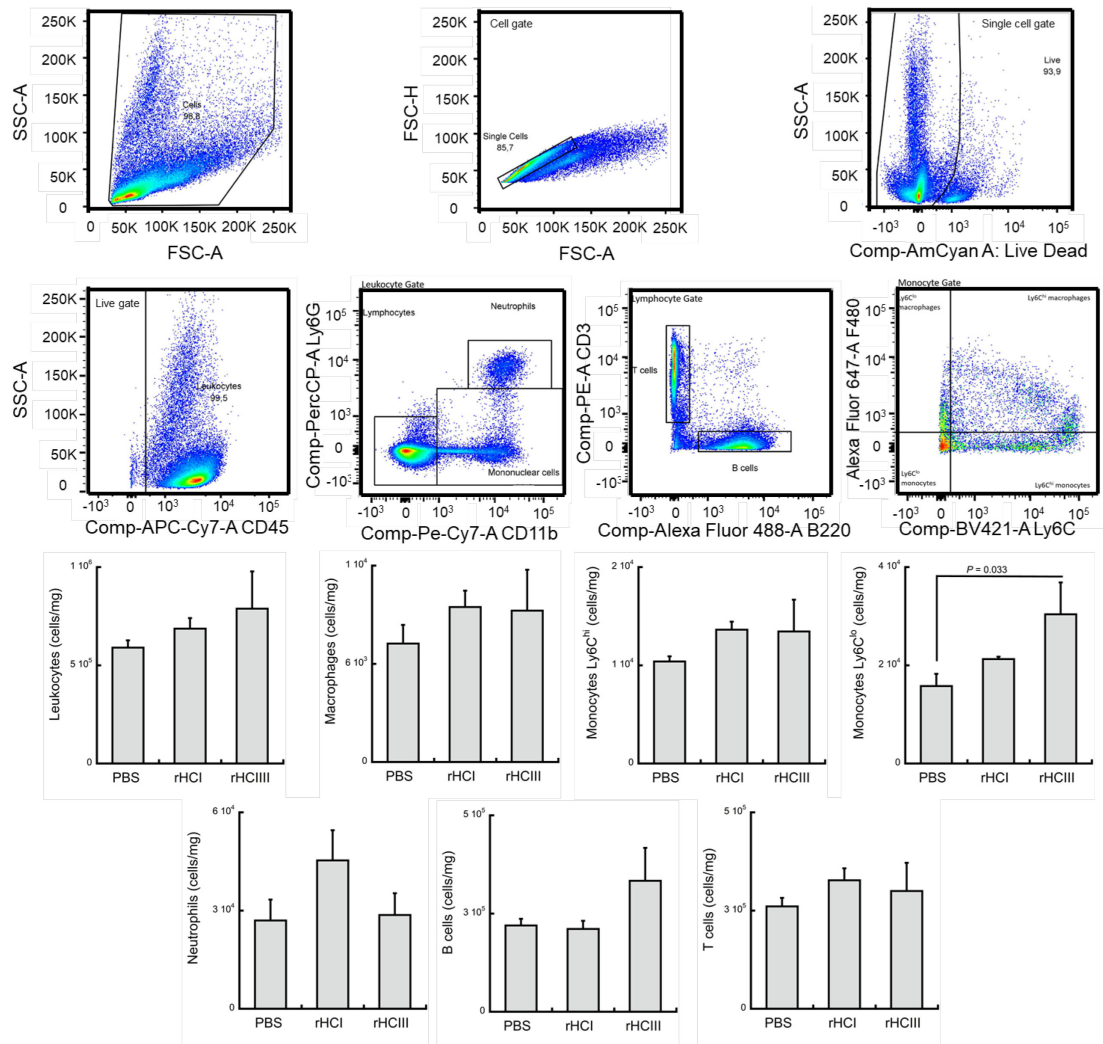


Figure S8. Flow cytometry analysis of mouse spleen cells at 2 days post-treatment. Live single cells were sorted into leukocyte subsets based on the expression of CD45, CD11b, Ly6G, CD3, B220, F480 and Ly6C. P values were determined by a one-way ANOVA for effect of treatment followed by Holm's correction for multiple comparisons. Data is represented as the mean $\pm$ SEM (n=5-7). The n values indicate the number of mice per group.

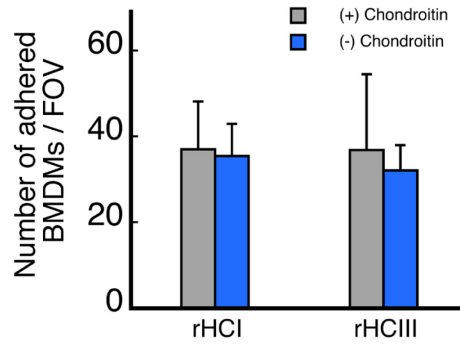


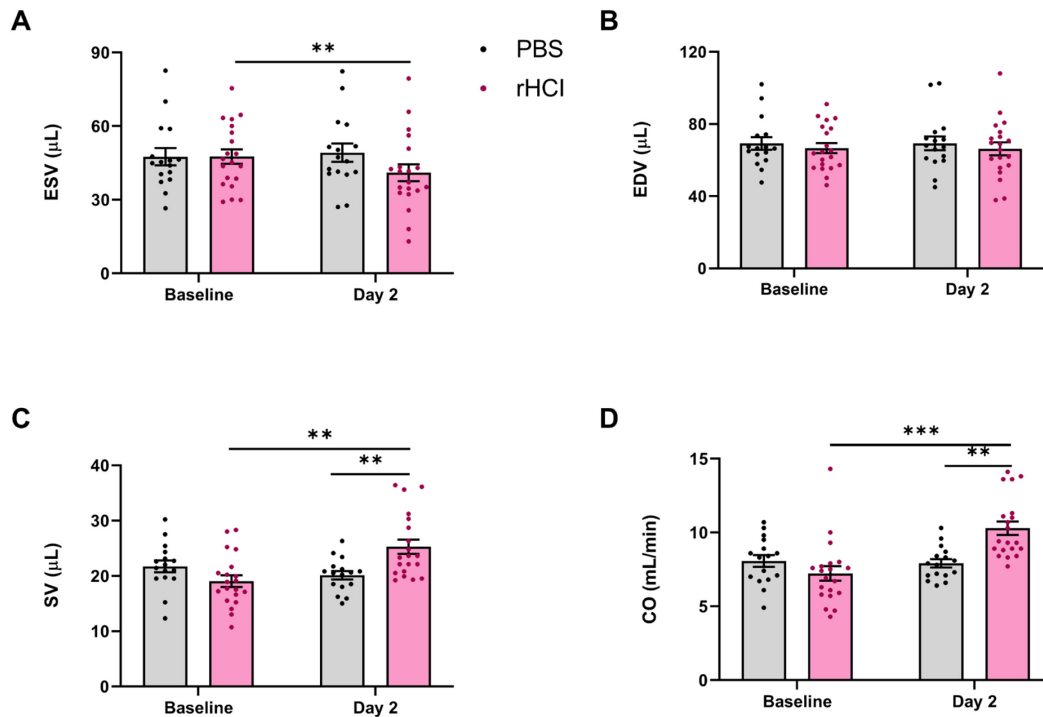
Figure S9. Number of adherent macrophages after culture on rHC matrices. rHCI and rHCIII coated wells were seeded with bone marrow derived macrophages (BMDMs) (n=4). Bars represent mean $\pm$ SD. n indicates the number of mice (mouse bone marrow) per group.

<i>Group</i>	<i>n</i>	<i>Heart rate</i>	<i>PR interval</i>	<i>QRS interval</i>	<i>QT interval</i>	<i>QT (%)</i>
<i>PBS</i>	11	13.6 $\pm$ 20.2	-0.4 $\pm$ 28.1	2.3 $\pm$ 27.9	-7.0 $\pm$ 24.3	5.5 $\pm$ 32.9
<i>rHCI</i>	12	22.0 $\pm$ 10.6	-36.7 $\pm$ 31.4 *	-12.3 $\pm$ 22.6	-14.6 $\pm$ 28.5	4.3 $\pm$ 37.1
<i>rHCIII</i>	5	16.6 $\pm$ 12.0	16.7 $\pm$ 18.1	9.8 $\pm$ 21.7	9.4 $\pm$ 15.0	27.7 $\pm$ 22.1

* $p < 0.05$, vs PBS and rHCIII, calculated from Tukey's HSD test

Table S1. ECG-derived parameters measured at 2 days post-treatment. Values represented as % change to baseline.

Supplementary Data for Aim 3



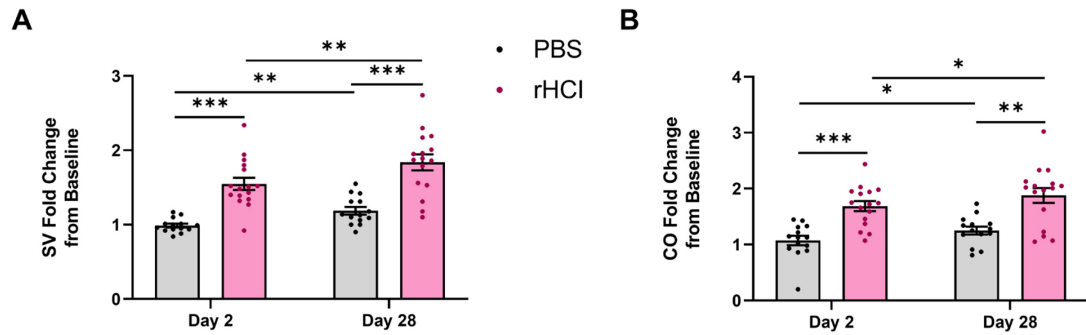


Figure S11. The improvement in stroke volume and cardiac output observed 2 days post-rHCI injection is maintained at the 28-day endpoint. Fold change in echocardiography measurements of (A) stroke volume (SV) and (B) cardiac output (CO) for day 2 and day 28 post-treatment injection relative to baseline prior to injection. Mice received intramyocardial injection of rHCI (n = 16) or PBS (n = 14) seven days post-myocardial infarction. Data presented as mean $\pm$ SEM. Statistical significance was determined by a two-way ANOVA followed by Holm's multiple comparison corrections and was noted as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$. No difference, between rHCI at day 2 and day 28 is highlighted by ns ($p \geq 0.05$).

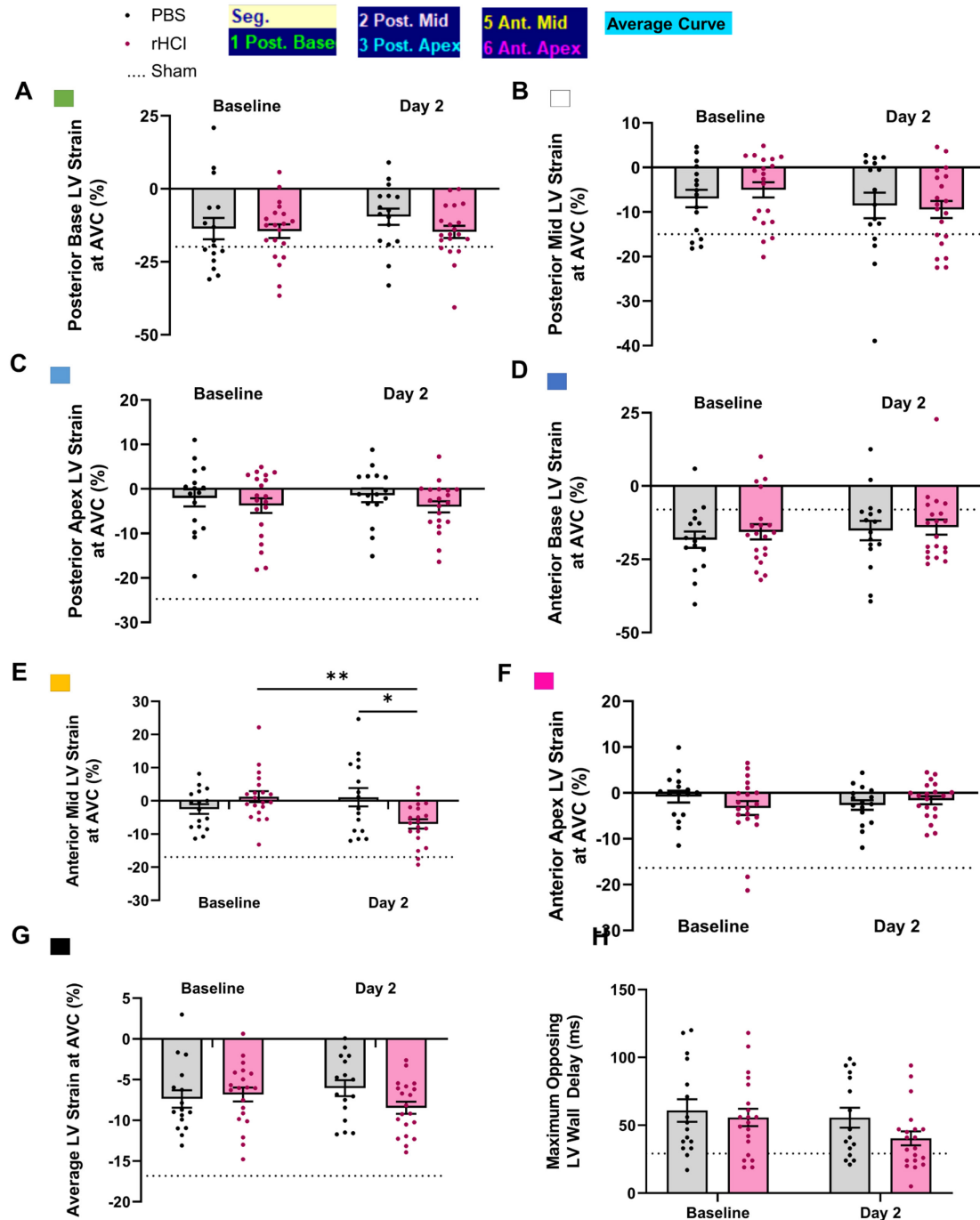


Figure S12. Longitudinal endocardial strain at baseline and day 2 post-treatment in a mouse model of myocardial infarction. As a measure of contractility, peak myocardial strain was calculated from long axis B mode echocardiograms of the left ventricle (LV) using Vevostrain software. At seven days post-myocardial infarction, mice were injected with rHCl hydrogel (n = 20) or PBS (n = 16) in the anterior border zone myocardium. Echocardiography was performed at baseline prior to injection and at day 2 post-injection. Longitudinal endocardial percent strain at the aortic valve closure (AVC) was measured in base, mid and

apical regions of the posterior and anterior LV wall (A-F). The average percent strain of the LV (G) and maximum delay in peak strain reached by opposing LV segments (H) were also recorded. At day 2 post-injection, mice treated with rHCI hydrogel had improved strain in the mid anterior LV wall segment. This segment includes the anterior border zone region where the treatments are injected at baseline. The average strain values for each region in sham control mice are noted for reference by a dashed line. Colours of the corresponding segment strain curve from the software output graphs (Figure S3) are noted above the plots. Data presented as mean $\pm$ SEM. Statistical significance was determined by a two-way ANOVA followed by Holm's multiple comparison correction and was noted as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

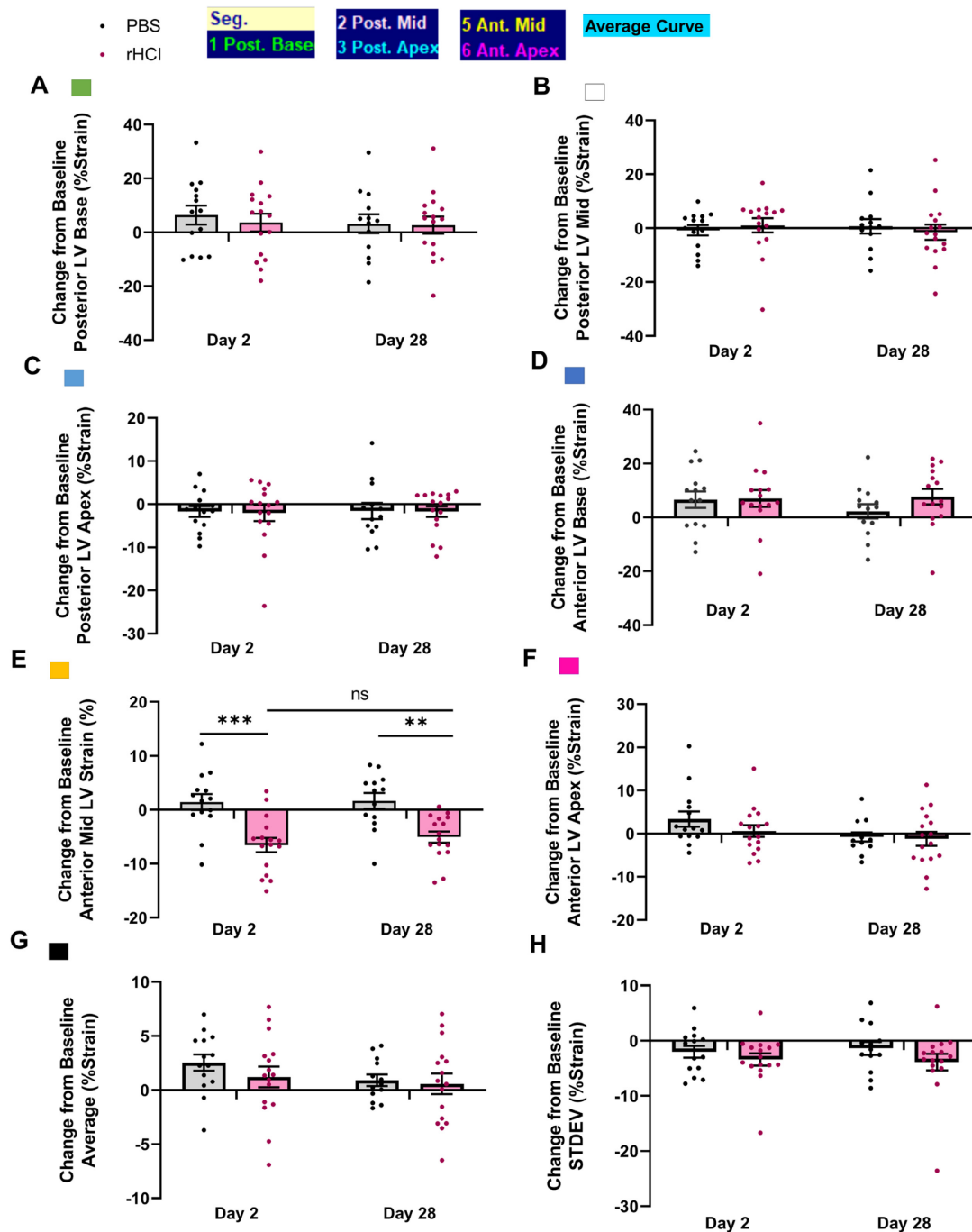
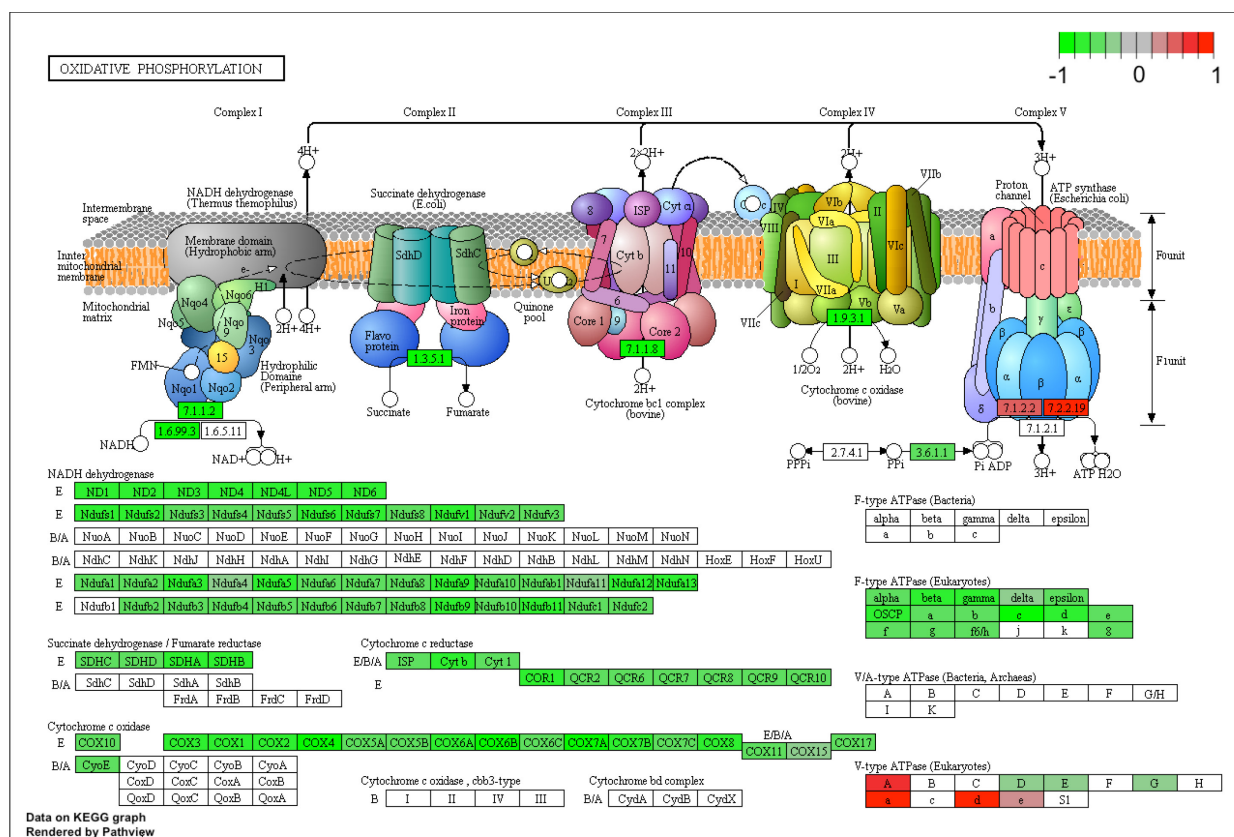


Figure S13. Fold change in longitudinal endocardial strain from baseline to day 2 and day 28 post-treatment endpoints in a mouse model of myocardial infarction. As a measure of contractility, peak myocardial strain was calculated from long axis B mode echocardiograms of the left ventricle (LV) using Vevostain software. At seven days post-myocardial infarction, mice were injected with rHCI hydrogel (n = 16) or PBS (n = 14) in the anterior border zone myocardium. Echocardiography was performed at baseline prior to injection as well as day 2 and day 28 post-injection. Change from baseline in longitudinal endocardial percent strain at the aortic valve closure (AVC) was measured in base, mid and apical regions

of the posterior and anterior LV wall (A-F) at day 2 and day 28 post-treatment. The change from baseline in average percent strain of the LV (G) and standard deviation of strain values in all regions (H) were also recorded. The mid anterior wall segment includes the anterior border zone region where the treatments are injected at baseline. Colours of the corresponding segment strain curve from the software output graphs (Figure S3) are noted above the plots. Data presented as mean $\pm$ SEM. Statistical significance was determined by a two-way ANOVA followed by Holm's multiple comparison correction and was noted as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

rHCl v. Sham



PBS v. Sham

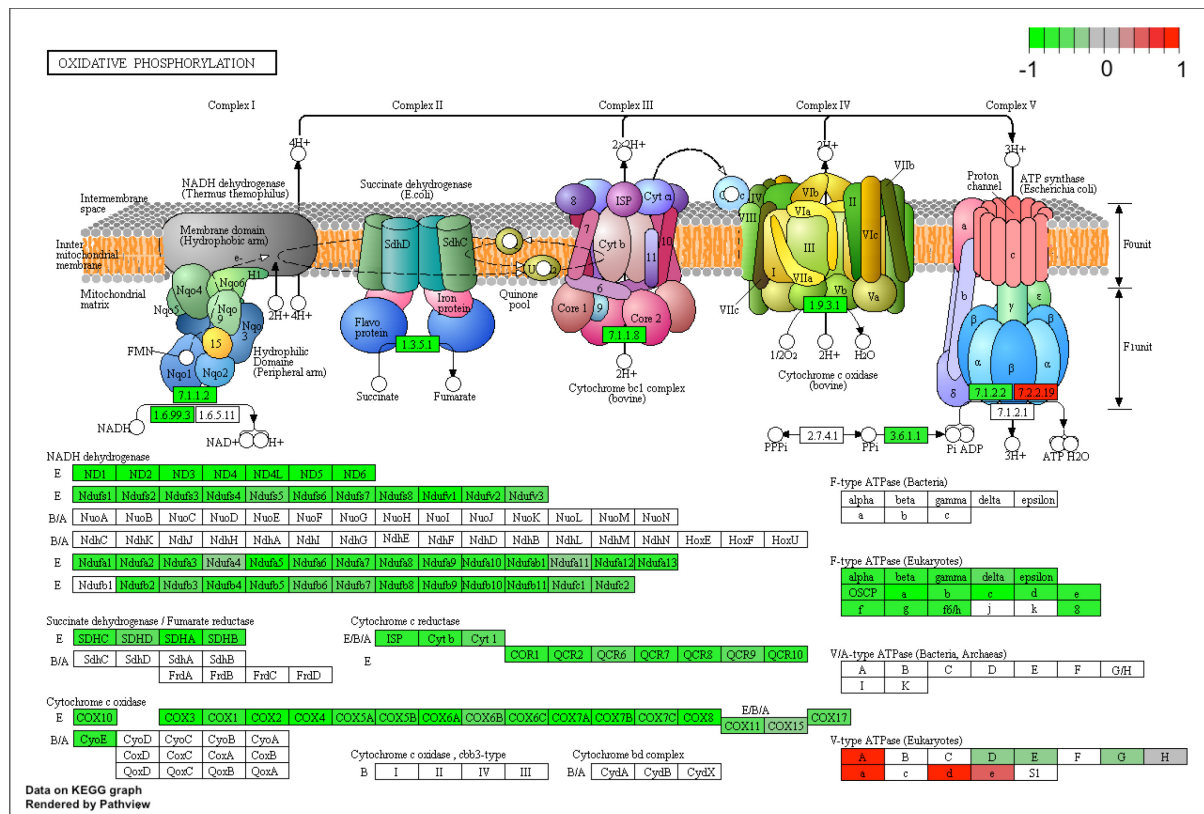
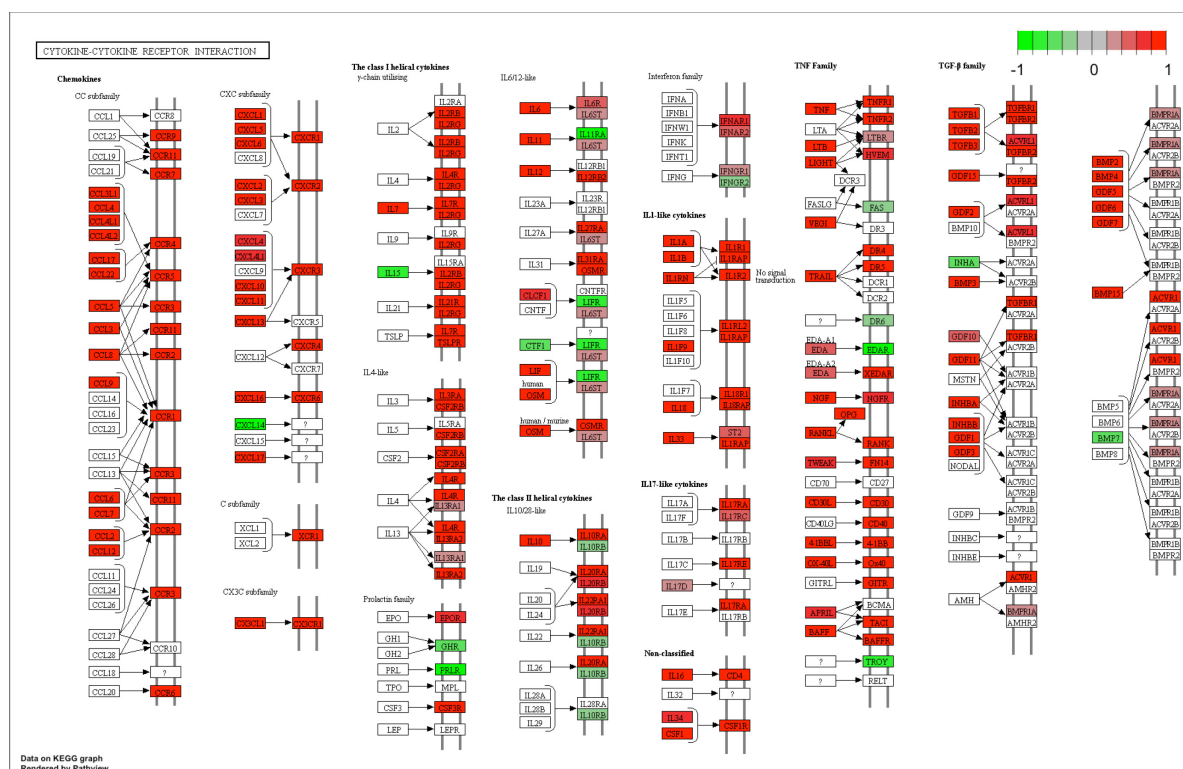


Figure S14. KEGG pathway analysis schematic for differentially expressed genes involved in the oxidative phosphorylation pathways between PBS and sham mice as well as rHCI and sham mice. Mice received intramyocardial injection of PBS (n = 5) or rHCI (n = 5) seven days after myocardial infarction. A myocardial infarction was not induced in sham animals. Two days after treatment injection, RNA was isolated from left ventricle tissue of PBS, rHCI and sham (n = 5) mice. Next generation sequencing was followed by differential expression analysis and KEGG enrichment analysis. Downregulated genes in comparison to sham are indicated in green. Upregulated genes in PBS relative to sham are in green and downregulated genes are in red. This analysis was completed by C3G bioinformatics group.

rHCl v Sham



CYTOKINE-CYTOKINE RECEPTOR INTERACTION

The diagram illustrates the interactions between various cytokines and their receptors, categorized into several families. The color scale at the top right indicates the strength of the interaction, ranging from -1 (green) to 1 (red).

Chemokines

- CC subfamily**: CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9, CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CCL29, CCL30, CCL31, CCL32, CCL33, CCL34, CCL35, CCL36, CCL37, CCL38, CCL39, CCL40, CCL41, CCL42, CCL43, CCL44, CCL45, CCL46, CCL47, CCL48, CCL49, CCL50, CCL51, CCL52, CCL53, CCL54, CCL55, CCL56, CCL57, CCL58, CCL59, CCL60, CCL61, CCL62, CCL63, CCL64, CCL65, CCL66, CCL67, CCL68, CCL69, CCL70, CCL71, CCL72, CCL73, CCL74, CCL75, CCL76, CCL77, CCL78, CCL79, CCL80, CCL81, CCL82, CCL83, CCL84, CCL85, CCL86, CCL87, CCL88, CCL89, CCL90, CCL91, CCL92, CCL93, CCL94, CCL95, CCL96, CCL97, CCL98, CCL99, CCL100, CCL101, CCL102, CCL103, CCL104, CCL105, CCL106, CCL107, CCL108, CCL109, CCL110, CCL111, CCL112, CCL113, CCL114, CCL115, CCL116, CCL117, CCL118, CCL119, CCL120, CCL121, CCL122, CCL123, CCL124, CCL125, CCL126, CCL127, CCL128, CCL129, CCL130, CCL131, CCL132, CCL133, CCL134, CCL135, CCL136, 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[illegible]

rHCI v sham

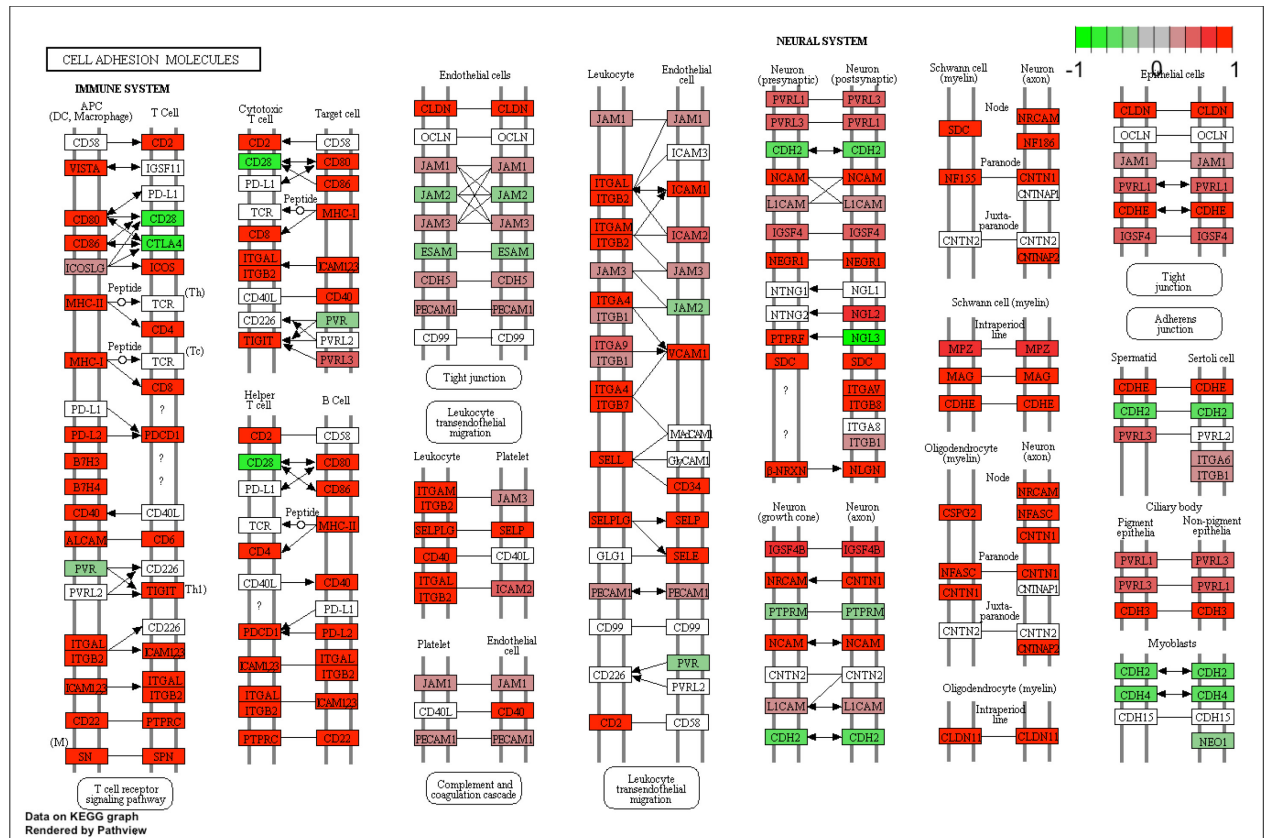


Figure S16. KEGG pathway analysis schematic for differentially expressed genes involved in cell adhesion molecule interactions a between PBS and sham mice as well as rHCI and sham mice. Mice received intramyocardial injection of PBS (n = 5) or rHCI (n = 5) seven days after myocardial infarction. A myocardial infarction was not induced in sham animals. Two days after treatment injection, RNA was isolated from left ventricle tissue of PBS, rHCI and sham (n = 5) mice. Next generation sequencing was followed by differential expression analysis and KEGG enrichment analysis. Downregulated genes in compared to sham are indicated in green. Upregulated genes in PBS relative to sham are in green and downregulated genes are in red. Analysis was completed by C3G bioinformatics group.

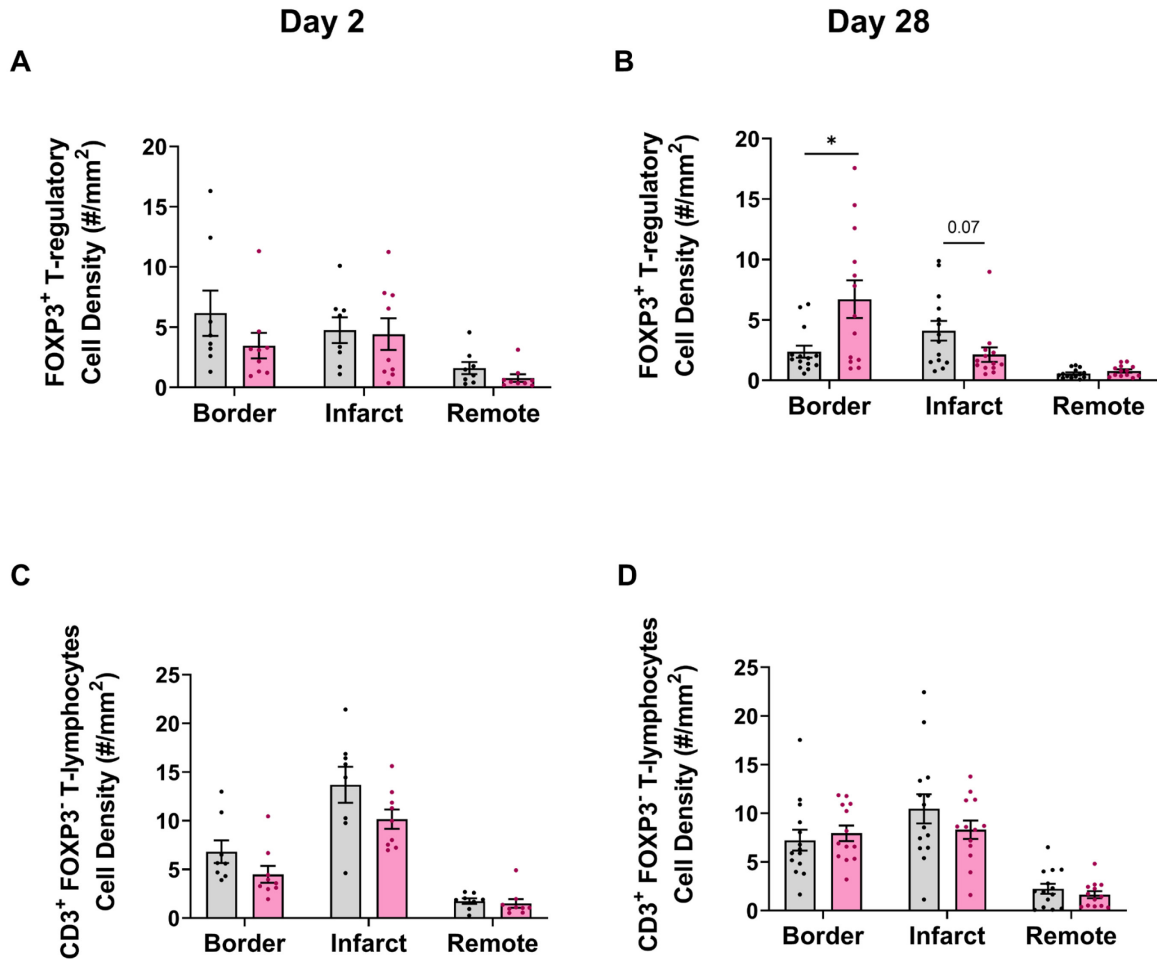


Figure S17. Intramyocardial injection of rHCI hydrogel into the infarct borderzone does not change regulatory T cell density 2 days after treatment, however there is increased T regulatory cell density in the borderzone at 28 days post-injection. Seven days after myocardial infarction was induced, mice received intramyocardial injection of PBS or rHCI hydrogel. Hearts were harvested at 2 days (PBS n = 8 and rHCI n = 9) or 28 days (PBS n = 14 and rHCI n = 13) after treatment. IHC staining for CD3 and FOXP3 was used to detect T regulatory cells (CD3⁺FOXP3⁺) and all other T cell populations (CD3⁺FOXP3⁻) in the infarct, borderzone (border) and remote myocardium 2 days (A, C) and 28 days (B, D) after treatment. Data are presented $\pm$ SEM. A significant difference between treatments for a given region was determined by an unpaired t test and denoted as * $p < 0.05$. Statistical analysis was done in Prism software.

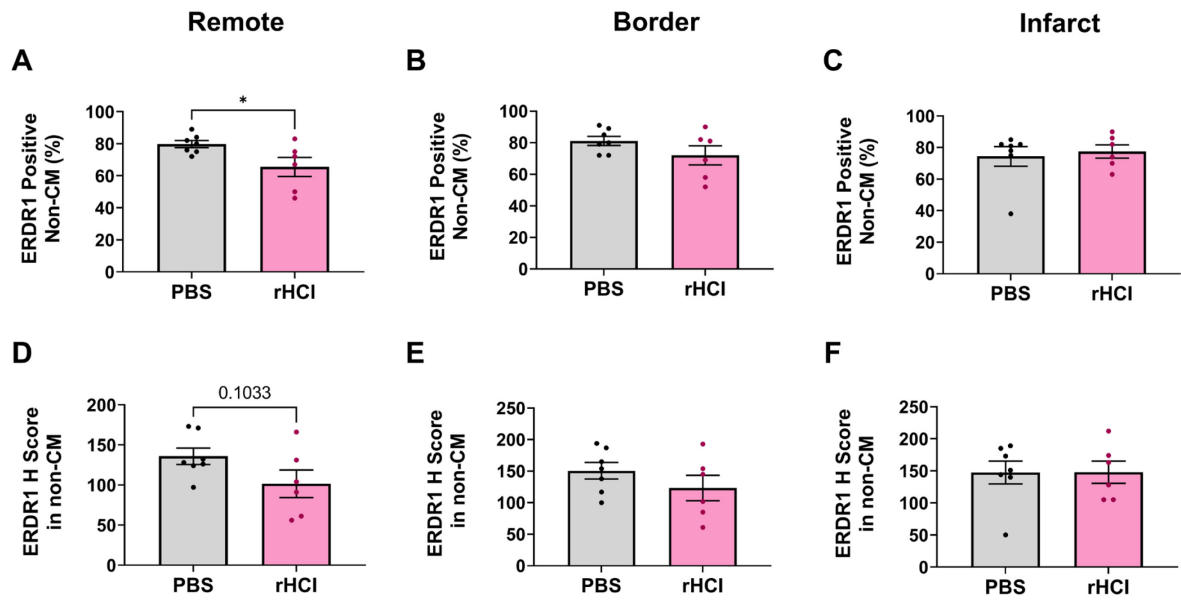


Figure S18 Erythroid differentiation regulator 1 (*ERDR1*) expression is lower in non-cardiomyocytes 2 days after rHCl treatment injection in a mouse model of myocardial infarction. RNAscope multiplex fluorescent detection kit was used to detect *ERDR1* mRNA transcripts in mouse heart tissue. Mice received intramyocardial injection of rHCl (n = 6) or PBS (n = 7) at seven days post-myocardial infarction. Hearts were harvested two days post-treatment and fresh frozen tissue sections were prepared for in situ hybridization. A-C) Percentage of non-cardiomyocytes that express *ERDR1* for remote, borderzone and infarct regions. D-F) H score of *ERDR1* expression levels in remote, borderzone and infarct cardiomyocytes. Three levels of expression are defined as bins of transcripts per cell: 1-7, 8-15 and >15, in addition to negative cells. H Scores can range from 0-300 with 300 being 100% of cells have greater than 15 *ERDR1* transcripts. Data are presented as mean $\pm$ SEM. Significance was determined by an unpaired t-test noted as * $p < 0.05$. Statistics were run in prism software. Image analysis was run in QuPath software.

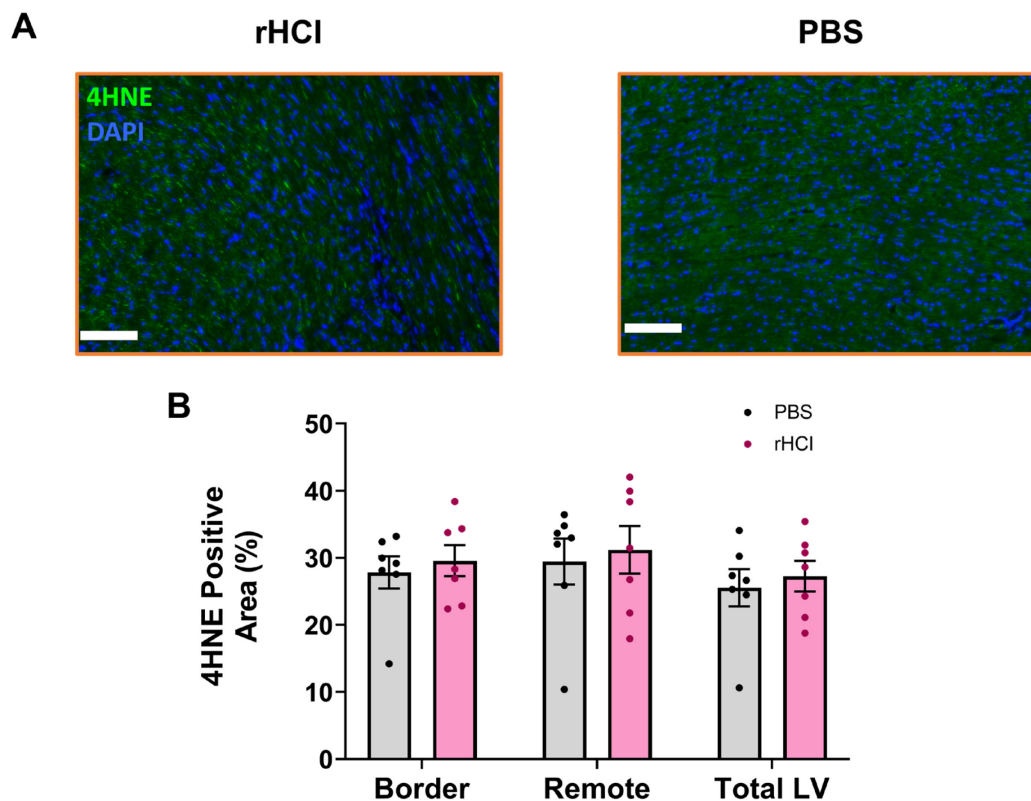


Figure S19. The level of 4HNE adducts is not different between rHCI and PBS treatment groups 28 days post-injection in a mouse model of myocardial infarction. Seven days after myocardial infarction, mice received intramyocardial injection of PBS (n = 7) or rHCI hydrogel (n = 7). Hearts were harvested 28 days after treatment. A) Example images for 4-hydroxynoneal (4HNE) IHC staining (green) in the remote myocardium, scale bars indicate 100 μ m. B) 4HNE⁺ area in the borderzone (border), remote myocardium and total left ventricle (LV) of PBS and rHCI treated mice. Image analysis was run in QuPath software. Data are presented as mean $\pm$ SEM. Significance between treatments in a region was determined by an unpaired t-test. Significance threshold was $p < 0.05$. Statistics were run in Prism software.

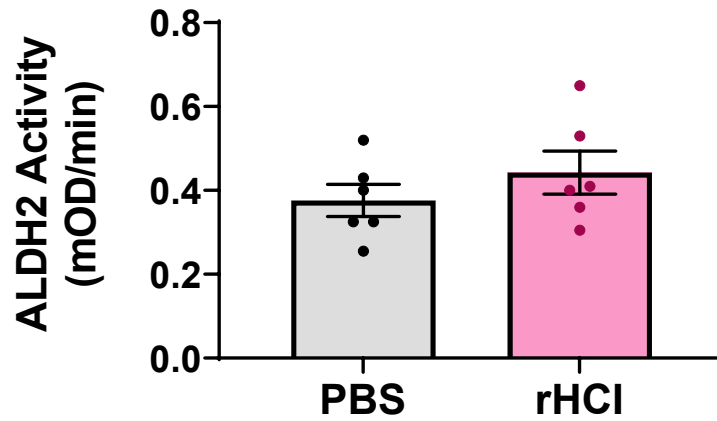


Figure S20. Mitochondrial aldehyde dehydrogenase activity in the remote region was not affected by rHCl hydrogel treatment 2 days after treatment in mice with myocardial infarction. Seven days after myocardial infarction, mice received intramyocardial injection of PBS (n = 6) or rHCl (n = 6). Left ventricle tissue was harvested 2 days after treatment injection, and the remote myocardium was separated from infarct and borderzone. Mitochondrial aldehyde dehydrogenase (ALDH2) was immunocaptured from remote tissue homogenates using an ALDH2 activity assay kit. ALDH2 activity was measured by reagent dye absorption (450 nm) due the increased acidity of solution with nicotinamide adenine dinucleotide reduction. Activity is measured as the rate of change in optical density (mOD) at 450 nm per minute. The blank sample was 0.17 mOD/min. Data are presented as mean $\pm$ SEM. P value determined by an unpaired t test. Statistical analysis run in excel and Prism software.

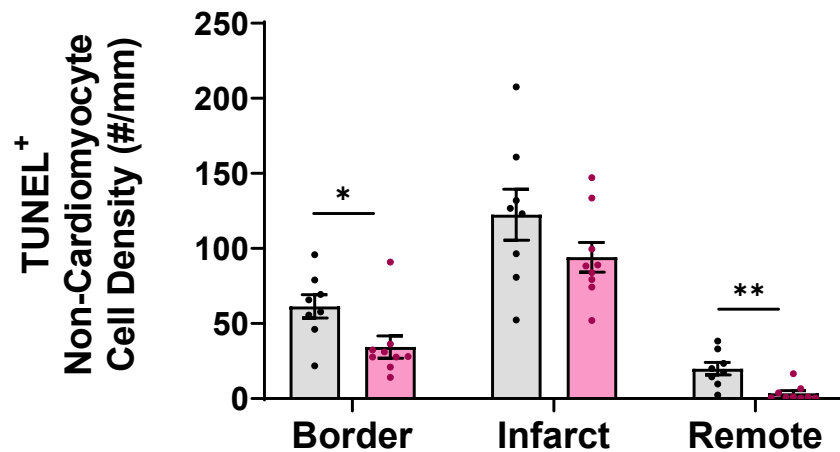


Figure S21. Apoptosis is reduced in non-cardiomyocyte cells two days after injection of rHCI hydrogel in a mouse model of myocardial infarction. Mice received intramyocardial injection of PBS (n = 8) or rHCI (n = 9) 7 days after myocardial infarction. Hearts were harvested for histology 2 days post-treatment injection. IHC staining was completed for detecting TUNEL⁺ apoptotic cells and cardiac troponin⁺ cardiomyocytes. Cardiomyocyte cell borders were identified with wheat germ agglutinin, and all nuclei not contained within the borders are classified as non-cardiomyocytes. Data are presented as mean $\pm$ SEM. Significance was determined between treatments by an unpaired t test within infarct, borderzone (border) and remote myocardium. Significance is denoted by * $p < 0.05$ or ** $p < 0.01$. Image analysis was completed in QuPath. Statistical analysis was done in Prism.

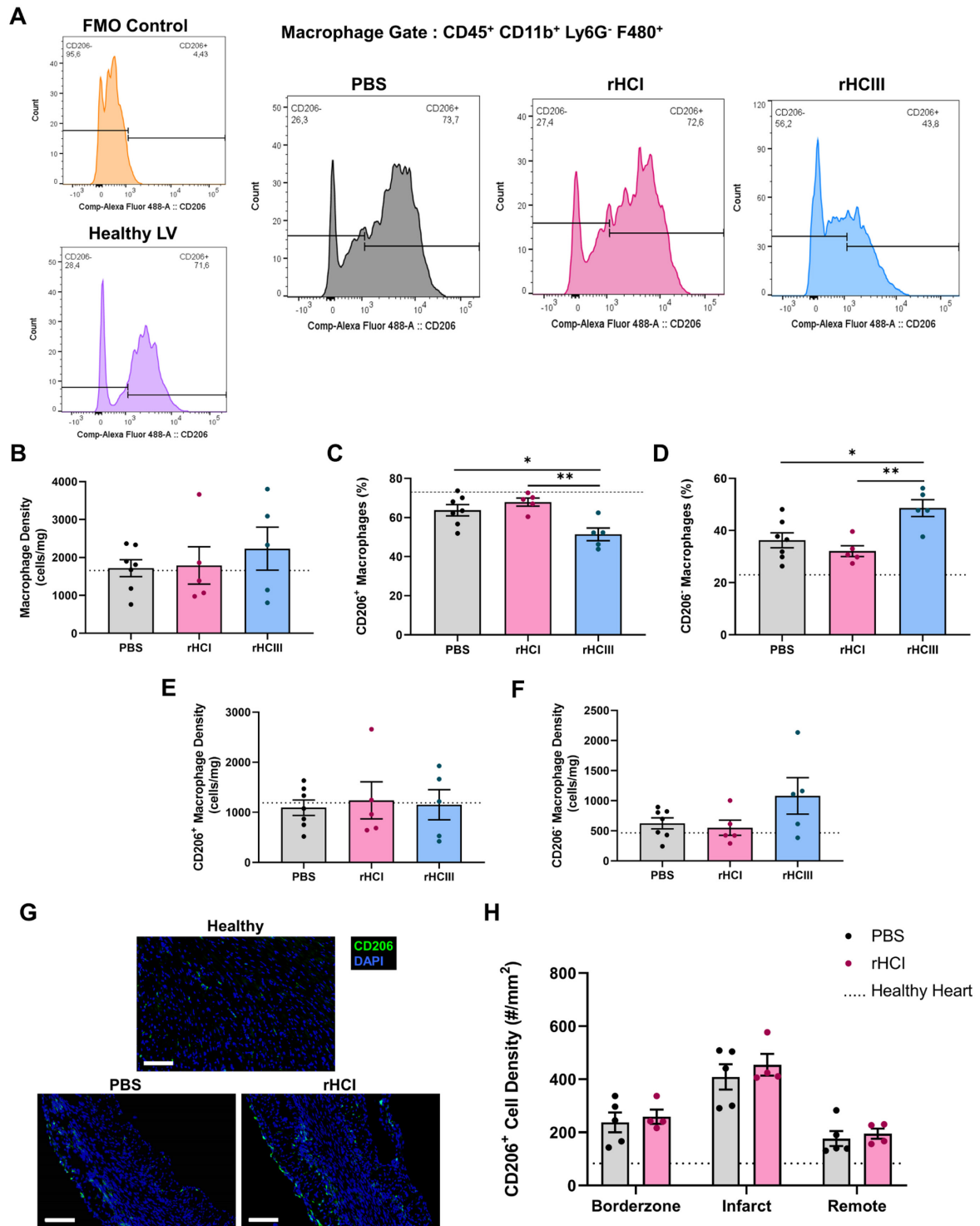


Figure S22. There is no change in M2 macrophage density or localization at day 2 post-rHCI injection, while rHCIII hydrogel injection reduces the percentage of M2 macrophages in the heart. A) Flow cytometry was used to detect CD206⁺ M2 macrophages in the heart two days after intramyocardial injection of rHCI (n = 5), rHCIII (n = 5) or PBS (n = 7). Treatments were injected at 7 days post-myocardial infarction in mice. The histograms display the

percentage of the macrophage population that is CD206⁺. The macrophage population was previously gated as CD45⁺CD11b⁺Ly6G⁺F480⁺. The CD206⁻ gate was set using a CD206 fluorescence minus one (FMO) control. B) Total macrophage density as well as E) CD206⁺ and F) CD206⁻ macrophage density was unchanged between treatment groups. C) rHClIII treated animals showed a significant reduction in the percentage of CD206⁺ macrophages and D) an increase in the percentage of CD206⁻ macrophages as compared to the other treatment groups. Significance was determined by a one-way ANOVA followed by Tukey's multiple comparisons between treatment groups. G) Heart sections of mice sacrificed at 2 days post-injection and stained for CD206⁺ positive cells. Scale bar indicates 100 μ m. H) In each region of the heart there is no difference in macrophage density between rHCl (n = 4) and PBS (n = 5) treated mice day 2 post-injection. Significant differences between treatment groups were determined by unpaired t tests for each region. Data are presented as mean $\pm$ SEM. Significance is noted as * p < 0.05, ** p < 0.01 or *** p < 0.001. Data analysis was done in FlowJo and Prism.

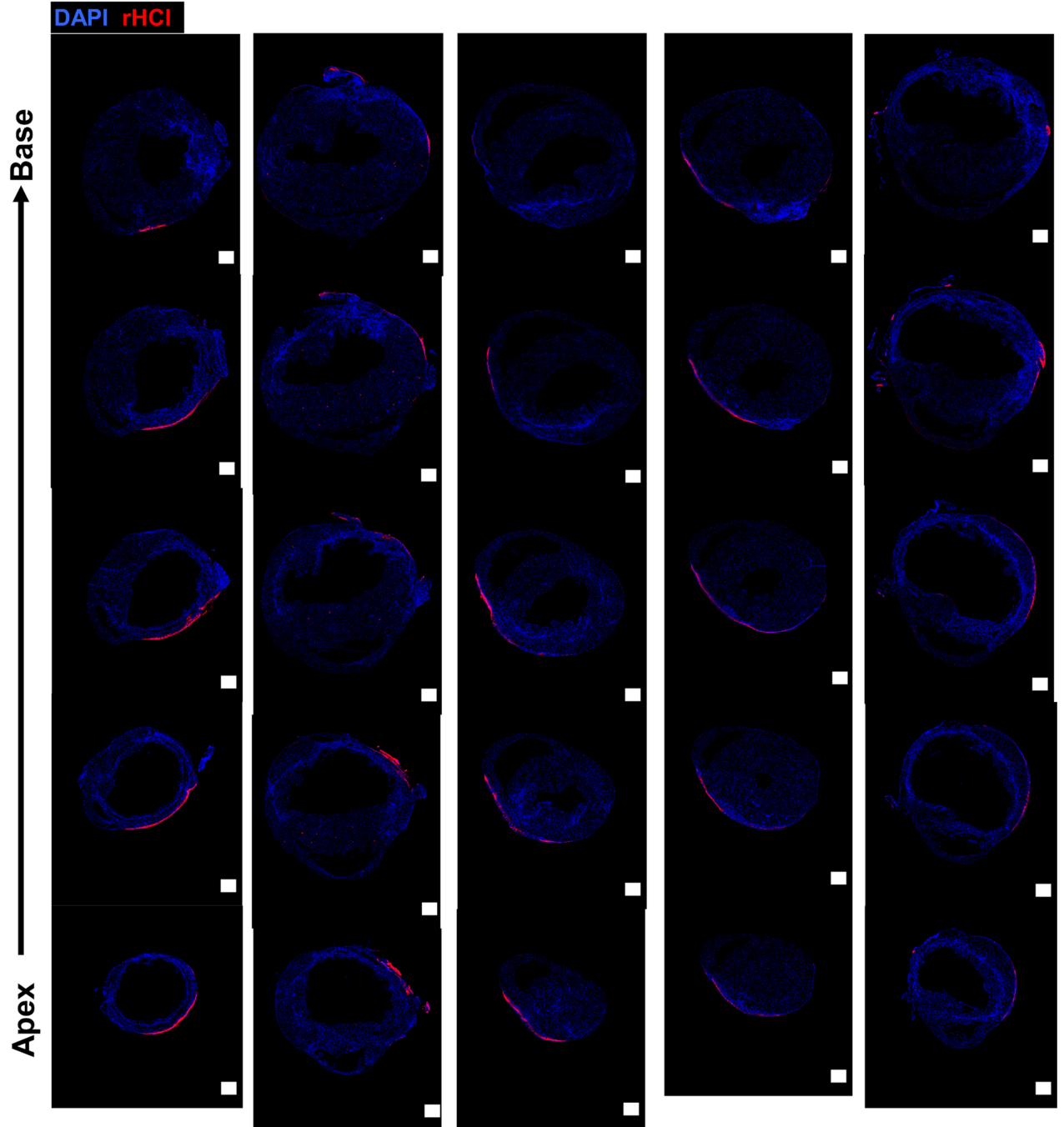


Figure S23. Two days after intramyocardial injection, rHCI hydrogel remains at the epicardial surface of the infarct and borderzone myocardium. Seven days after myocardial infarction mice received an intramyocardial injection of rHCI hydrogel tagged with AlexaFluor 594 dye (red). Two days after injection, hearts were harvested for histology. Heart sections were collected from apex to base, each section was separated by 480 μm with the first section starting 250 μm from the apex. Heart sections were stained with DAPI (blue) to detect the nuclei. Scale bars are 500 μm .

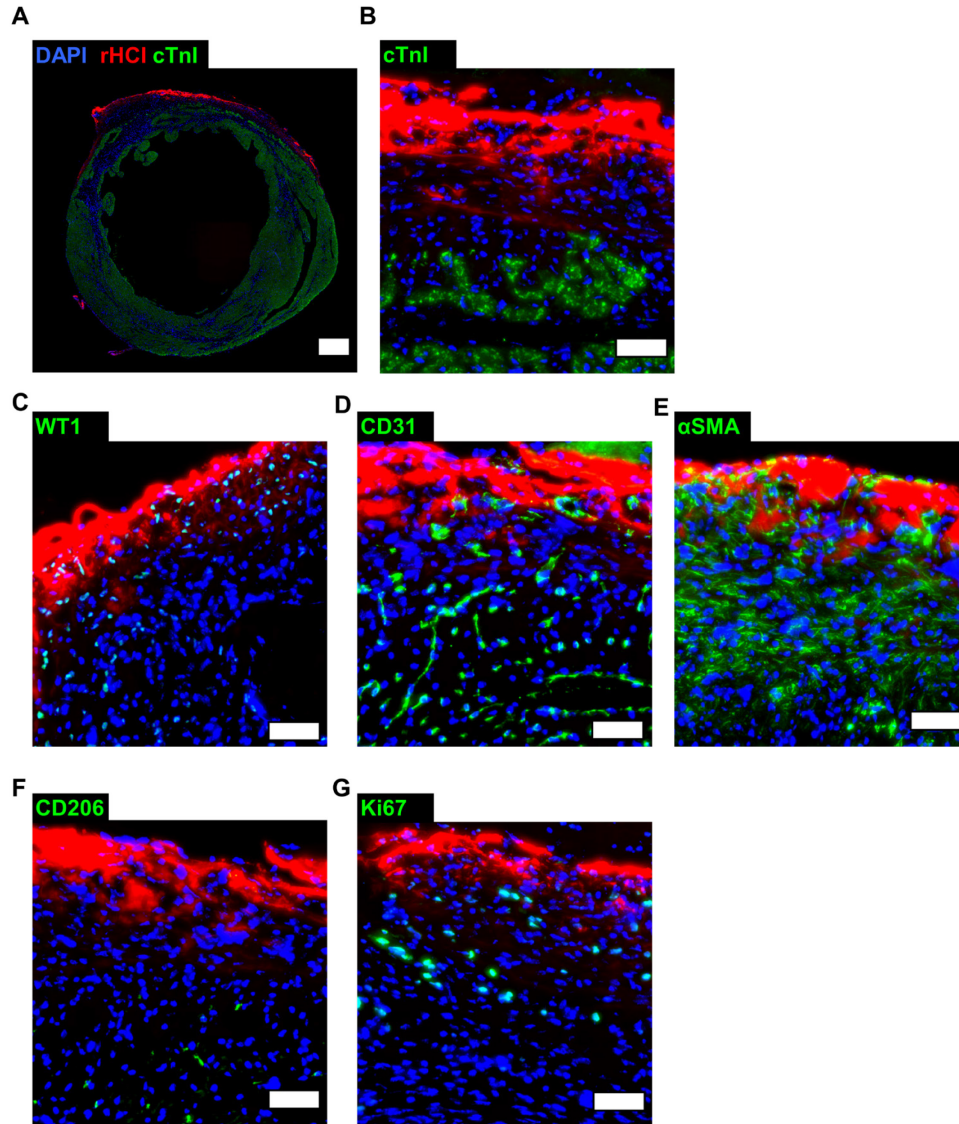


Figure S24. Cells are found within the rHCI hydrogel at the epicardial surface one day after intramyocardial injection in a mouse model of myocardial infarction. Seven days after myocardial infarction, mice received intramyocardial injection of rHCI hydrogel. The rHCI hydrogel was tagged with AlexaFluor 594 prior to injection. One day after treatment injection hearts were harvested for histology. Heart sections were stained with antibodies (green) to detect (A-B) cardiac troponin I (cTnI)⁺cardiomyocytes, (C) Wilms' tumor protein (WT1)⁺ epicardium, (D) CD31⁺ capillaries, (E) alpha smooth muscle actin (αSMA)⁺ myofibroblasts and arterioles, (F) CD206⁺ M2 macrophages, and (G) Ki67⁺ proliferating cells. The rHCI hydrogel appears as red and DAPI (blue) was used as a nuclear stain. Scale bars are 500 μm (A) and 50 μm (B-G).

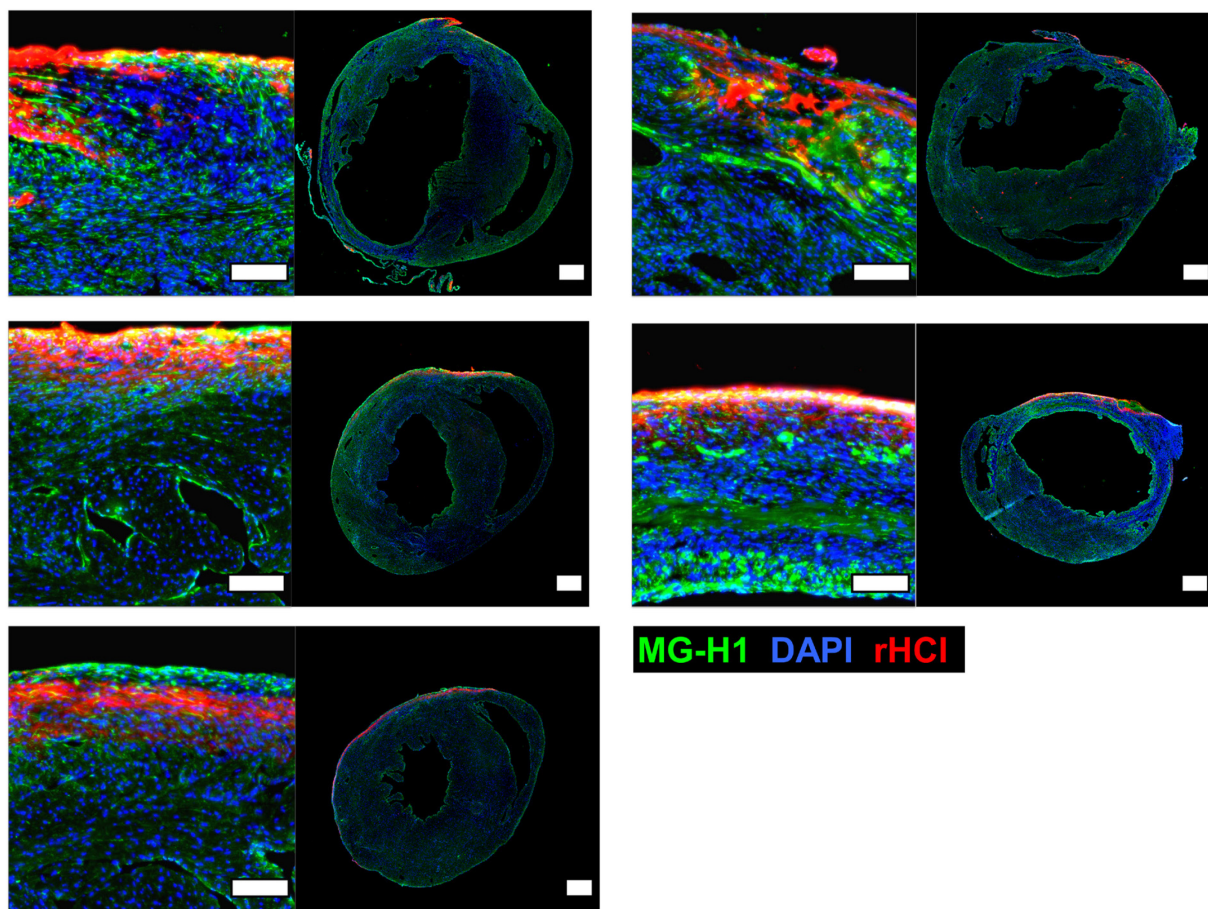


Figure S25. Methylglyoxal modification of rHCI hydrogel two days after injection into the borderzone myocardium of mice with myocardial infarction. Seven days after myocardial infarction, mice received intramyocardial injection of rHCI hydrogel ($n = 5$) that was tagged with AlexFluor 594 dye (red). Two days after injection, hearts were harvested for histology. Heart sections were stained with an antibody that detects methylglyoxal adducts (MG-H1, green) and DAPI. Example images from each mouse are presented for full slide scans (right, scale bar 500 μm) and hydrogel regions (left, scale bar 100 μm).

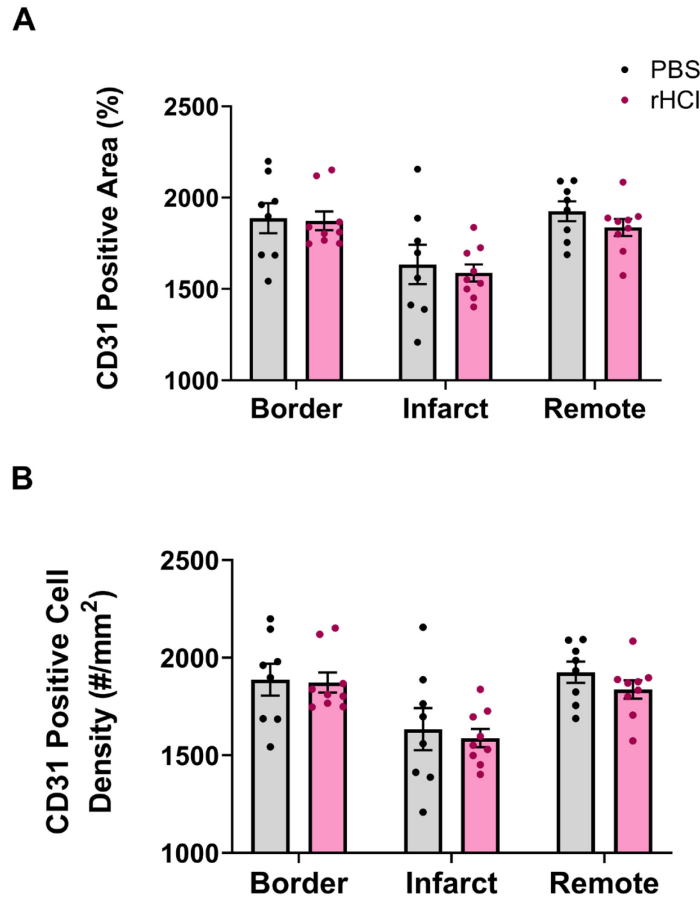


Figure S26. There is no change in capillary density two days after rHCl hydrogel treatment in a mouse model of myocardial infarction. Seven days after myocardial infarction, mice received intramyocardial injection of PBS (n = 8) or rHCl (n = 9). Hearts were harvested 2 days after treatment for histology. A) The percentage of the tissue area that is CD31⁺ in the borderzone (border), infarct and remote myocardium was quantified for PBS and rHCl treatment groups. B) The CD31⁺ cell density in the border, infarct and remote myocardium was also quantified for PBS and rHCl treatment groups. Data are presented as mean $\pm$ SEM. Significance was determined by an unpaired t test between groups in each region. QuPath was used for image analysis. Statistical analysis was done in Prism. Image analysis was run in QuPath software.

Ensembl ID	Symbol	Gene ID	Log FC	Log CPM	LR	P Value	FDR
ENSMUSG00000040026	Saa3	20210	8.86	3.47	922.84	1.06E-202	2.78E-198
ENSMUSG00000054196	Cthrc1	68588	9.45	5.78	883.22	4.37E-194	5.71E-190
ENSMUSG00000029304	Spp1	20750	7.93	6.28	808.82	6.54E-178	5.70E-174
ENSMUSG00000027750	Postn	50706	5.61	9.91	777.04	5.30E-171	3.47E-167
ENSMUSG00000030074	Gxylt2	232313	4.47	6.17	768.16	4.52E-169	2.36E-165
ENSMUSG00000027996	Sfrp2	20319	6.10	7.61	761.42	1.32E-167	5.76E-164
ENSMUSG00000000290	Itgb2	16414	2.82	4.90	755.12	3.09E-166	1.15E-162
ENSMUSG00000026043	Col3a1	12825	4.25	12.15	740.77	4.07E-163	1.33E-159
ENSMUSG00000001506	Col1a1	12842	4.73	11.51	723.16	2.75E-159	7.99E-156
ENSMUSG00000029661	Col1a2	12843	4.51	11.38	686.02	3.28E-151	8.58E-148
ENSMUSG00000040552	C3ar1	12267	3.37	4.74	671.61	4.47E-148	1.06E-144
ENSMUSG00000028364	Tnc	21923	5.77	6.04	669.94	1.03E-147	2.25E-144
ENSMUSG00000049103	Ccr2	12772	3.75	3.60	642.63	8.95E-142	1.80E-138
ENSMUSG00000049723	Mmp12	17381	7.35	4.55	626.70	2.61E-138	4.88E-135
ENSMUSG00000029838	Ptn	19242	4.30	6.06	617.00	3.36E-136	5.86E-133
ENSMUSG00000073418	C4b	12268	2.82	5.71	611.88	4.35E-135	7.12E-132
ENSMUSG00000026193	Fn1	14268	4.76	10.16	609.25	1.63E-134	2.50E-131
ENSMUSG00000051048	P4ha3	320452	7.58	3.61	602.20	5.56E-133	8.07E-130
ENSMUSG00000028111	Ctsk	13038	3.13	4.78	588.42	5.54E-130	7.62E-127
ENSMUSG00000028197	Col24a1	71355	6.52	2.43	585.97	1.89E-129	2.47E-126
ENSMUSG00000023992	Trem2	83433	4.37	3.39	585.33	2.59E-129	3.23E-126
ENSMUSG00000027239	Mdk	17242	3.32	3.81	576.18	2.54E-127	3.02E-124
ENSMUSG00000020473	Aebp1	11568	3.16	6.73	575.73	3.18E-127	3.61E-124
ENSMUSG00000015340	Cybb	13058	2.58	5.49	570.42	4.56E-126	4.97E-123
ENSMUSG00000001131	Timp1	21857	4.87	4.84	564.37	9.44E-125	9.87E-122
ENSMUSG00000017969	Ptgis	19223	2.69	5.86	559.65	1.00E-123	1.01E-120
ENSMUSG00000060572	Mfap2	17150	3.35	3.91	542.97	4.27E-120	4.13E-117
ENSMUSG00000050335	Lgals3	16854	4.94	5.01	541.93	7.19E-120	6.71E-117
ENSMUSG00000054555	Adam12	11489	4.74	4.04	541.15	1.06E-119	9.54E-117
ENSMUSG00000069516	Lyz2	17105	2.35	8.19	540.80	1.26E-119	1.10E-116
ENSMUSG00000022371	Col14a1	12818	3.27	7.82	537.93	5.31E-119	4.48E-116
ENSMUSG00000047414	Flrt2	399558	2.25	4.53	533.07	6.06E-118	4.96E-115
ENSMUSG00000035279	Ssc5d	269855	2.75	5.43	531.69	1.21E-117	9.61E-115
ENSMUSG00000026786	Apbb1ip	54519	2.29	3.76	531.06	1.66E-117	1.28E-114
ENSMUSG00000040690	Col16a1	107581	4.06	6.90	513.98	8.64E-114	6.46E-111
ENSMUSG00000027995	Tlr2	24088	2.63	3.61	509.35	8.79E-113	6.39E-110
ENSMUSG00000042436	Mfap4	76293	4.57	7.83	503.13	1.98E-111	1.40E-108
ENSMUSG00000036446	Lum	17022	2.34	8.00	502.85	2.28E-111	1.57E-108
ENSMUSG00000027188	Pamr1	210622	4.03	4.51	496.83	4.64E-110	3.11E-107
ENSMUSG00000015947	Fcgr1	14129	2.91	2.82	494.82	1.27E-109	8.31E-107
ENSMUSG00000045312	Lhfpl2	218454	3.23	5.16	491.19	7.85E-109	5.01E-106

ENSMUSG00000040907	Atp1a3	232975	3.88	2.10	486.59	7.87E-108	4.90E-105
ENSMUSG00000038147	Cd84	12523	2.85	3.72	483.56	3.60E-107	2.19E-104
ENSMUSG00000063727	Tnfrsf11b	18383	5.25	1.90	478.58	4.35E-106	2.59E-103
ENSMUSG00000008496	Pou2f2	18987	3.59	2.77	475.31	2.24E-105	1.28E-102
ENSMUSG00000039062	Anpep	16790	1.83	5.26	475.31	2.24E-105	1.28E-102
ENSMUSG00000040254	Sema3d	108151	2.15	4.42	472.51	9.09E-105	5.06E-102
ENSMUSG00000062609	Kcnj15	16516	5.30	5.04	470.96	1.98E-104	1.08E-101
ENSMUSG00000020053	Igf1	16000	2.29	5.68	466.71	1.67E-103	8.90E-101
ENSMUSG00000024529	Lox	16948	5.73	7.86	453.55	1.22E-100	6.38E-98

Table S2. Top 50 genes that are differentially expressed in the left ventricle of PBS injected mice with myocardial infarction compared to sham mice. The 50 genes with the lowest p values out of 12593 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. PBS (n =5) and sham (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	log FC	log CPM	LR	P Value	FDR
ENSMUSG00000040026	Saa3	20210	8.78	3.47	904.99	8.1E-199	2.1E-194
ENSMUSG00000054196	Cthrc1	68588	9.04	5.78	826.30	1.0E-181	1.4E-177
ENSMUSG00000029304	Spp1	20750	7.87	6.28	799.72	6.2E-176	5.4E-172
ENSMUSG00000027750	Postn	50706	5.31	9.91	716.13	9.3E-158	6.1E-154
ENSMUSG00000000290	Itgb2	16414	2.72	4.90	703.52	5.1E-155	2.7E-151
ENSMUSG00000026043	Col3a1	12825	4.03	12.15	681.08	3.9E-150	1.7E-146
ENSMUSG00000030074	Gxylt2	232313	4.09	6.17	665.43	9.9E-147	3.7E-143
ENSMUSG00000001506	Colla1	12842	4.48	11.51	664.45	1.6E-146	5.3E-143
ENSMUSG00000027996	Sfrp2	20319	5.55	7.61	662.09	5.2E-146	1.5E-142
ENSMUSG00000029661	Colla2	12843	4.21	11.38	616.39	4.6E-136	1.2E-132
ENSMUSG00000049723	Mmp12	17381	7.17	4.55	603.10	3.5E-133	8.4E-130
ENSMUSG00000028364	Tnc	21923	5.35	6.04	597.54	5.7E-132	1.3E-128
ENSMUSG00000049103	Ccr2	12772	3.60	3.60	597.16	7.0E-132	1.4E-128
ENSMUSG00000040552	C3ar1	12267	3.09	4.74	576.41	2.3E-127	4.2E-124
ENSMUSG00000001131	Timp1	21857	4.84	4.84	559.93	8.7E-124	1.5E-120
ENSMUSG00000026193	Fn1	14268	4.49	10.16	557.12	3.6E-123	5.8E-120
ENSMUSG00000073418	C4b	12268	2.66	5.71	547.84	3.7E-121	5.7E-118
ENSMUSG00000051048	P4ha3	320452	7.08	3.61	536.44	1.1E-118	1.6E-115
ENSMUSG00000028111	Ctsk	13038	2.91	4.78	516.48	2.5E-114	3.4E-111

ENSMUSG00000029838	Ptn	19242	3.83	6.06	510.01	6.3E-113	8.2E-110
ENSMUSG00000028197	Col24a1	71355	6.01	2.43	499.19	1.4E-110	1.8E-107
ENSMUSG00000023992	Trem2	83433	3.98	3.39	493.75	2.2E-109	2.6E-106
ENSMUSG00000027239	Mdk	17242	3.05	3.81	490.47	1.1E-108	1.3E-105
ENSMUSG00000020473	Aebp1	11568	2.87	6.73	487.68	4.6E-108	5.0E-105
ENSMUSG00000069516	Lyz2	17105	2.22	8.19	483.92	3.0E-107	3.1E-104
ENSMUSG00000050335	Lgals3	16854	4.60	5.01	483.41	3.9E-107	3.9E-104
ENSMUSG00000022371	Col14a1	12818	3.07	7.82	482.08	7.5E-107	7.3E-104
ENSMUSG00000047414	Flrt2	399558	2.13	4.53	477.18	8.8E-106	8.2E-103
ENSMUSG00000015340	Cybb	13058	2.34	5.49	475.88	1.7E-105	1.5E-102
ENSMUSG00000026786	Apbb1ip	54519	2.15	3.76	468.32	7.4E-104	6.5E-101
ENSMUSG00000054555	Adam12	11489	4.35	4.04	467.13	1.3E-103	1.1E-100
ENSMUSG00000017969	Ptgis	19223	2.43	5.86	464.35	5.4E-103	4.4E-100
ENSMUSG00000035279	Ssc5d	269855	2.54	5.43	459.70	5.6E-102	4.4E-99
ENSMUSG00000040907	Atp1a3	232975	3.77	2.10	458.40	1.1E-101	8.2E-99
ENSMUSG00000042436	Mfap4	76293	4.30	7.83	455.67	4.2E-101	3.1E-98
ENSMUSG00000060572	Mfap2	17150	3.03	3.91	452.18	2.4E-100	1.8E-97
ENSMUSG00000027995	Tlr2	24088	2.45	3.61	440.56	8.2E-98	5.8E-95
ENSMUSG00000040690	Col16a1	107581	3.69	6.90	439.64	1.3E-97	8.9E-95
ENSMUSG00000008496	Pou2f2	18987	3.44	2.77	437.99	3.0E-97	2.0E-94
ENSMUSG00000079227	Ccr5	12774	4.00	4.03	425.49	1.6E-94	1.0E-91
ENSMUSG00000027188	Pamr1	210622	3.68	4.51	425.47	1.6E-94	1.0E-91
ENSMUSG00000039062	Anpep	16790	1.72	5.26	420.30	2.1E-93	1.3E-90
ENSMUSG00000024529	Lox	16948	5.41	7.86	416.12	1.7E-92	1.0E-89
ENSMUSG00000036446	Lum	17022	2.11	8.00	415.45	2.4E-92	1.4E-89
ENSMUSG00000062609	Kcnj15	16516	4.88	5.04	413.63	5.9E-92	3.5E-89
ENSMUSG00000038147	Cd84	12523	2.62	3.72	412.66	9.7E-92	5.5E-89
ENSMUSG00000045312	Lhfp12	218454	2.93	5.16	411.54	1.7E-91	9.4E-89
ENSMUSG00000015947	Fcgr1	14129	2.64	2.82	406.19	2.5E-90	1.3E-87
ENSMUSG00000018008	Cyth4	72318	2.41	4.39	405.56	3.4E-90	1.8E-87
ENSMUSG00000030162	Olr1	108078	6.49	0.98	404.27	6.5E-90	3.4E-87

Table S3. Top 50 genes that are differentially expressed in the left ventricle of rHCI injected mice with myocardial infarction compared to sham mice. The 50 genes with the lowest p values out of 11427 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. rHCI (n =5) and sham (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Term	Ont	N	Up	Down	P.Up	P.Down
mitochondrion	CC	1716	329	1011	1	1.1E-190
mitochondrial part	CC	807	126	551	1	2.3E-139
mitochondrial protein complex	CC	258	6	241	1	4.7E-116
intracellular	CC	12394	4236	3942	0.30	1.9E-109
intracellular part	CC	12394	4236	3942	0.30	1.9E-109
intracellular membrane-bounded organelle	CC	9453	3071	3198	1	6.1E-107
cytoplasm	CC	10024	3545	3307	1.9E-05	2.3E-94
intracellular organelle	CC	10931	3687	3536	0.87	1.9E-93
membrane-bounded organelle	CC	10079	3354	3316	0.99	9.3E-93
mitochondrial inner membrane	CC	417	52	309	1	9.7E-92
organelle	CC	11195	3786	3592	0.80	3.2E-90
cytoplasmic part	CC	7759	2709	2682	0.02	9.1E-89
mitochondrial envelope	CC	628	111	403	1	4.8E-88
mitochondrial membrane	CC	585	103	383	1	1.9E-87
response to stimulus	BP	7030	3013	1732	6.4E-87	1
immune system process	BP	2128	1138	408	1.3E-85	1
organelle inner membrane	CC	457	71	317	1	6.4E-82
cell periphery	CC	4361	2011	947	1.9E-80	1
plasma membrane	CC	4228	1950	910	2.2E-77	1
mitochondrial matrix	CC	254	19	209	1	7.1E-77
extracellular region	CC	1858	990	278	2.5E-72	1
extracellular region part	CC	1468	815	214	2.8E-69	1
envelope	CC	1010	242	527	1	5.6E-69
organelle envelope	CC	1010	242	527	1	5.6E-69
organelle part	CC	7046	2266	2411	1	7.5E-69
signal transduction	BP	4372	1974	1055	8.8E-69	1
signaling	BP	4807	2135	1157	2.8E-68	1
intracellular organelle part	CC	6721	2142	2317	1	3.4E-68
cell communication	BP	4868	2152	1173	8.5E-67	1
mitochondrial membrane part	CC	212	18	176	1	5.2E-66

Table S4. Gene ontology enrichment results for genes differentially expressed between PBS injected mice with myocardial infarcts and sham mice. Mice were injected with PBS (n = 5) seven days post-myocardial infarction. RNA was isolated from left ventricles two days after intramyocardial treatment from PBS mice and sham healthy control animals (n = 5). Next generation sequencing of mRNA transcripts at 50 million read depth was performed. Following read trimming and alignment, edgeR pipeline was used for differential expression analysis. Gene ontology (Ont) terms include: CC cellular compartment and BP biological process. N indicates number of genes in the pathway, UP is the number of differentially expressed genes that are upregulated relative to sham, and down is the number of genes in

the pathway that are downregulated relative to sham. P values for gene ontology term upregulation (P.Up) and downregulation (P.Down) are also shown. Significance is $p < 0.05$. Analysis was performed by C3G bioinformatics group.

Term	Ont	N	Up	Dow n	P.Up	P.Down
mitochondrion	CC	1716	298	981	1	7.5E-206
mitochondrial part	CC	807	115	541	1	2.7E-151
mitochondrial protein complex	CC	258	6	240	1	2.2E-124
intracellular	CC	12394	3943	3612	0.17	7.6E-107
intracellular part	CC	12394	3943	3612	0.17	7.6E-107
intracellular membrane-bounded organelle	CC	9453	2831	2942	1	2.6E-105
cytoplasm	CC	10024	3311	3063	2.0E-06	9.3E-101
mitochondrial inner membrane	CC	417	47	306	1	1.7E-100
cytoplasmic part	CC	7759	2506	2504	0.04	1.0E-97
mitochondrial envelope	CC	628	100	396	1	7.6E-97
mitochondrial membrane	CC	585	94	377	1	2.7E-96
membrane-bounded organelle	CC	10079	3105	3046	1	3.3E-91
intracellular organelle	CC	10931	3413	3242	0.90	7.4E-91
immune system process	BP	2128	1093	362	8.0E-91	1
organelle inner membrane	CC	457	64	313	1	5.2E-90
response to stimulus	BP	7030	2832	1592	7.2E-88	1
organelle	CC	11195	3502	3291	0.87	2.4E-87
cell periphery	CC	4361	1912	858	1.4E-85	1
plasma membrane	CC	4228	1856	826	6.4E-83	1
mitochondrial matrix	CC	254	18	206	1	1.4E-81
envelope	CC	1010	217	514	1	7.7E-78
organelle envelope	CC	1010	217	514	1	7.7E-78
extracellular region	CC	1858	952	246	3.2E-77	1
extracellular region part	CC	1468	791	193	3.6E-76	1
mitochondrial membrane part	CC	212	17	175	1	1.0E-71
immune response	BP	1193	664	168	8.7E-71	1
signaling	BP	4807	2016	1066	2.3E-70	1
signal transduction	BP	4372	1862	973	8.6E-70	1
extracellular space	CC	1248	684	152	2.6E-69	1
biological regulation	BP	9758	3630	2369	8.3E-69	0.74

Table S5. Gene ontology enrichment results for genes differentially expressed between rHCI injected mice with myocardial infarcts and sham mice. Mice were injected with rHCI (n = 5) seven days post-myocardial infarction. RNA was isolated from left ventricles two days after intramyocardial treatment from rHCI mice and sham healthy control animals (n = 5). Next generation sequencing of mRNA transcripts at 50 million read depth was performed.

Following read trimming and alignment, edgeR pipeline was used for differential expression analysis. Gene ontology (Ont) terms include: CC cellular compartment and BP biological process. N indicates number of genes in the pathway, UP is the number of differentially expressed genes that are upregulated relative to sham, and down is the number of genes in the pathway that are downregulated relative to sham. P values for gene ontology term upregulation (P.Up) and downregulation (P.Down) are also shown. Significance is $p < 0.05$. Analysis was performed by C3G bioinformatics group.

Pathway	N	Up	Down	P.Up	P.Down
Oxidative phosphorylation	127	8	103	1	2.8E-37
Thermogenesis	224	36	151	1	5.1E-37
Parkinson disease	138	14	108	1	2.4E-36
Metabolic pathways	1406	423	560	1	3.8E-28
Alzheimer disease	167	42	106	1	3.5E-23
Cytokine-cytokine receptor interaction	239	156	17	4.1E-23	1
Non-alcoholic fatty liver disease (NAFLD)	144	32	93	1	2.7E-21
Huntington disease	184	38	110	1	5.4E-21
Cell adhesion molecules (CAMs)	144	101	10	8.4E-19	1
Carbon metabolism	112	18	70	1	2.8E-15
Pathways in cancer	510	258	125	5.0E-15	0.90
PI3K-Akt signaling pathway	323	176	76	2.5E-14	0.93
Pertussis	72	56	8	3.4E-14	1
Human papillomavirus infection	324	176	62	3.7E-14	1
ECM-receptor interaction	87	64	10	5.4E-14	1
Focal adhesion	198	118	38	1.4E-13	1
Phagosome	158	99	17	1.7E-13	1
Epstein-Barr virus infection	197	116	44	7.1E-13	1
Proteasome	45	4	35	1	1.6E-12
Hematopoietic cell lineage	89	62	4	7.1E-12	1
Lysosome	119	77	19	7.7E-12	1
NF-kappa B signaling pathway	97	66	16	8.1E-12	1
Viral protein interaction with cytokine and cytokine receptor	84	59	4	1.3E-11	1
Staphylococcus aureus infection	62	47	1	2.0E-11	1
Chemokine signaling pathway	178	104	31	2.1E-11	1
Human cytomegalovirus infection	221	123	44	3.0E-11	1
Valine, leucine and isoleucine degradation	54	6	38	1	3.3E-11
NOD-like receptor signaling pathway	153	92	38	3.3E-11	0.75
Cardiac muscle contraction	83	16	51	1	4.1E-11
Osteoclast differentiation	124	78	21	4.6E-11	1

Table .S6. KEGG pathway enrichment results for genes differentially expressed between PBS injected mice with myocardial infarcts and sham mice. Mice were injected with PBS (n = 5) seven days post-myocardial infarction. RNA was isolated from left ventricles two days after intramyocardial treatment from PBS mice and sham healthy control animals (n = 5). Next generation sequencing of mRNA transcripts at 50 million read depth was performed. Following read trimming and alignment, edgeR pipeline was used for differential expression analysis. KEGG analysis determines enrichment of differentially expressed genes involved in known molecular pathways. N indicates number of genes in the pathway, UP is the number of differentially expressed genes that are upregulated relative to sham, and down is the number of genes in the pathway that are downregulated relative to sham. P values for gene ontology term upregulation (P.Up) and downregulation (P.Down) are also shown. Significance is $p < 0.05$.

Pathway	N	Up	Down	P.Up	P.Down
Oxidative phosphorylation	127	7	103	1	3.1E-41
Thermogenesis	224	32	147	1	4.1E-39
Parkinson disease	138	14	106	1	2.3E-38
Metabolic pathways	1406	408	527	1	7.7E-30
Alzheimer disease	167	40	102	1	7.0E-24
Huntington disease	184	36	109	1	7.1E-24
Non-alcoholic fatty liver disease (NAFLD)	144	29	92	1	1.1E-23
Cytokine-cytokine receptor interaction	239	149	15	8.1E-23	1
Cell adhesion molecules (CAMs)	144	97	10	1.0E-18	1
Pathways in cancer	510	251	108	3.5E-17	1
ECM-receptor interaction	87	64	8	9.7E-16	1
Carbon metabolism	112	18	66	1	7.4E-15
Phagosome	158	96	12	3.5E-14	1
Pertussis	72	54	6	4.4E-14	1
PI3K-Akt signaling pathway	323	166	68	7.5E-14	0.94
Human papillomavirus infection	324	166	52	1.1E-13	1
Staphylococcus aureus infection	62	48	1	1.4E-13	1
Focal adhesion	198	112	31	2.7E-13	1
Viral protein interaction with cytokine and cytokine receptor	84	59	2	3.7E-13	1
Tuberculosis	160	94	30	1.1E-12	1
Leishmaniasis	68	50	10	1.4E-12	1
Osteoclast differentiation	124	77	22	2.4E-12	1
NF-kappa B signaling pathway	97	64	15	3.3E-12	1
Proteasome	45	4	33	1	6.9E-12
Chemokine signaling pathway	178	100	26	8.8E-12	1
NOD-like receptor signaling pathway	153	89	35	9.2E-12	0.71
Valine, leucine and isoleucine degradation	54	5	37	1	1.0E-11
Epstein-Barr virus infection	197	108	39	1.1E-11	1

Hematopoietic cell lineage	89	59	4	1.7E-11	1
Cardiac muscle contraction	83	15	49	1	2.0E-11

Table S7. KEGG pathway enrichment results for genes differentially expressed between rHCI injected mice with myocardial infarcts and sham mice. Mice were injected with rHCI (n = 5) seven days post-myocardial infarction. RNA was isolated from left ventricles two days after intramyocardial treatment from rHCI mice and sham healthy control animals (n = 5). Next generation sequencing of mRNA transcripts at 50 million read depth was performed. Following read trimming and alignment, edgeR pipeline was used for differential expression analysis. KEGG analysis determines enrichment of differentially expressed genes involved in known molecular pathways. N indicates number of genes in the pathway, UP is the number of differentially expressed genes that are upregulated relative to sham, and down is the number of genes in the pathway that are downregulated relative to sham. P values for gene ontology term upregulation (P.Up) and downregulation (P.Down) are also shown. Significance is $p < 0.05$.

Ensembl ID	Symbol	Gene ID	Log FC	Log CPM	LR	P Value	FDR
ENSMUSG00000072844	G530011O06Rik	654820	-2.12	0.29	21.49	3.57E-06	0.021
ENSMUSG00000096768	Erdr1	170942	-1.59	2.14	20.27	6.71E-06	0.029
ENSMUSG00000076577	Igkv8-30	NA	2.32	-1.89	26.39	2.79E-07	0.004
ENSMUSG00000020524	Gria1	14799	-1.17	0.57	28.13	1.14E-07	0.003
ENSMUSG00000072849	Serpina1e	20704	2.25	-3.42	24.45	7.63E-07	0.007
ENSMUSG00000084806	Gm15232	627268	-2.98	-3.40	21.29	3.94E-06	0.021
ENSMUSG00000031779	Ccl22	20299	0.88	-0.59	19.62	9.43E-06	0.035

Table S8. Genes that are differentially expressed between rHCI and PBS treatment groups. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. PBS (n = 5) and rHCI (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to PBS, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Appendix B: Supplemental tables from differential expression analysis of RNAseq data

Ensembl ID	Symbol	Gene ID	Log FC	Log CPM	LR	P Value	FDR
ENSMUSG00000049538	Adamts16	271127	11.42	1.55	371.06	1.1E-82	2.5E-80
ENSMUSG00000022097	Sftpc	20389	10.84	1.77	12.38	4.3E-04	1.3E-03
ENSMUSG00000004885	Crabp2	12904	10.46	0.98	187.99	8.7E-43	4.1E-41
ENSMUSG00000056370	Sftpb	20388	10.24	0.09	19.86	8.3E-06	3.1E-05
ENSMUSG00000058914	C1qtnf3	81799	10.04	4.67	336.05	4.6E-75	8.2E-73
ENSMUSG00000029188	Slc34a2	20531	9.80	-0.29	14.33	1.5E-04	4.8E-04
ENSMUSG00000054196	Cthrc1	68588	9.45	5.78	883.22	4.4E-194	5.7E-190
ENSMUSG00000062345	Serpib2	18788	9.21	1.34	207.11	5.9E-47	3.3E-45
ENSMUSG00000029379	Cxcl3	330122	9.14	-0.04	93.86	3.4E-22	5.6E-21
ENSMUSG00000050711	Scg2	20254	9.13	-0.57	131.14	2.3E-30	5.9E-29
ENSMUSG00000052353	Cemip	80982	8.87	3.18	306.89	1.0E-68	1.4E-66
ENSMUSG00000040026	Saa3	20210	8.86	3.47	922.84	1.1E-202	2.8E-198
ENSMUSG00000039462	Col10a1	12813	8.74	-0.87	41.27	1.3E-10	8.6E-10
ENSMUSG00000021789	Sftpa1	20387	8.71	-0.18	11.47	7.1E-04	2.0E-03
ENSMUSG00000029375	Cxcl15	20309	8.64	-1.29	13.19	2.8E-04	8.4E-04
ENSMUSG00000050359	Spr1a	20753	8.35	3.10	270.94	7.1E-61	6.9E-59
ENSMUSG00000029304	Spp1	20750	7.93	6.28	808.82	6.5E-178	5.7E-174
ENSMUSG00000021795	Sftpd	20390	7.90	-1.89	10.69	1.1E-03	2.9E-03
ENSMUSG00000032473	Cldn18	56492	7.85	-1.93	12.63	3.8E-04	1.1E-03
ENSMUSG00000054764	Mtnr1a	17773	7.81	-1.85	92.73	6.0E-22	9.8E-21
ENSMUSG00000076548	Igkv4-69	NA	7.60	-3.17	8.96	2.8E-03	7.0E-03
ENSMUSG00000051048	P4ha3	320452	7.58	3.61	602.20	5.6E-133	8.1E-130
ENSMUSG00000092627	D130058E05Rik	NA	7.58	-1.82	117.70	2.0E-27	4.4E-26
ENSMUSG00000027966	Col11a1	12814	7.47	5.46	381.11	7.1E-85	1.9E-82
ENSMUSG00000026725	Tnn	329278	7.45	2.76	257.70	5.4E-58	4.7E-56

Table S9. Top 25 genes that are upregulated in left ventricles of PBS injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest log fold change out of 12593 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. PBS (n=5), sham (n=5) and sham (n=5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	Log FC	Log CPM	LR	P Value	FDR
ENSMUSG00000093906	Igkv9-129	NA	-4.88	-4.29	5.41	2.0E-02	4.3E-02
ENSMUSG00000105431	Gm42640	NA	-4.06	-4.39	11.52	6.9E-04	1.9E-03
ENSMUSG00000031471	Defb8	244334	-3.99	-4.56	8.51	3.5E-03	8.8E-03
ENSMUSG00000057223	Gm6578	625347	-3.98	-4.42	11.36	7.5E-04	2.1E-03
ENSMUSG00000044664	Prss42	235628	-3.95	-4.60	13.16	2.9E-04	8.6E-04
ENSMUSG00000079507	H2-Q1	15006	-3.94	-4.53	12.56	3.9E-04	1.2E-03
ENSMUSG00000077360	Gm23482	NA	-3.90	-4.60	7.82	5.2E-03	1.2E-02
ENSMUSG00000084869	Gm13063	NA	-3.90	-4.53	11.40	7.3E-04	2.1E-03
ENSMUSG00000081889	Gm13188	NA	-3.89	-4.64	8.11	4.4E-03	1.1E-02
ENSMUSG00000033770	Clcnka	12733	-3.84	-4.49	7.54	6.0E-03	1.4E-02
ENSMUSG00000031682	1700011L22Rik	67687	-3.80	-4.64	11.00	9.1E-04	2.5E-03
ENSMUSG00000083076	Gm11408	NA	-3.78	-4.46	10.88	9.7E-04	2.7E-03
ENSMUSG00000073375	Lrrc30	240131	-3.71	-1.94	54.96	1.2E-13	1.1E-12
ENSMUSG00000025216	Lbx1	16814	-3.64	-3.80	22.15	2.5E-06	1.0E-05
ENSMUSG00000090843	Heatr4	101055670	-3.61	-4.56	10.09	1.5E-03	4.0E-03
ENSMUSG00000073424	Cyp4f15	106648	-3.59	-4.22	12.00	5.3E-04	1.5E-03
ENSMUSG00000097639	Platr4	78706	-3.57	-4.67	7.09	7.7E-03	1.8E-02
ENSMUSG00000070837	Aurkc	20871	-3.53	-4.10	16.43	5.1E-05	1.7E-04
ENSMUSG00000048978	Nrsn1	22360	-3.50	-4.64	6.05	1.4E-02	3.1E-02
ENSMUSG00000061742	Slc22a12	20521	-3.46	-3.74	16.44	5.0E-05	1.7E-04
ENSMUSG00000107435	RP24-398M13.2	NA	-3.46	-4.64	8.42	3.7E-03	9.2E-03
ENSMUSG00000096463	Gm21750	NA	-3.45	-4.60	8.26	4.1E-03	1.0E-02
ENSMUSG00000036216	Leap2	259301	-3.45	-4.72	7.33	6.8E-03	1.6E-02
ENSMUSG00000098741	Obox4-ps2	NA	-3.43	-4.68	8.13	4.4E-03	1.1E-02
ENSMUSG00000106822	Gm43533	NA	-3.43	-4.68	7.42	6.4E-03	1.5E-02

Table S10. Top 25 genes that are downregulated in left ventricles of PBS injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest negative log fold change out of 12593 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. PBS (n=5), sham (n= 5) and sham (n= 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	Log FC	Log CPM	LR	P Value	FDR
ENSMUSG00000064351	mt-Co1	17708	-0.78	15.55	49.19	2.3E-12	1.8E-11
ENSMUSG00000064370	mt-Cytb	17711	-0.77	14.44	47.56	5.3E-12	4.0E-11
ENSMUSG00000040752	Myh6	17888	-1.01	14.33	51.83	6.0E-13	5.0E-12
ENSMUSG00000064367	mt-Nd5	17721	-0.84	14.26	44.70	2.3E-11	1.6E-10
ENSMUSG00000029467	Atp2a2	11938	-0.95	13.91	54.87	1.3E-13	1.1E-12
ENSMUSG00000064341	mt-Nd1	17716	-0.82	13.85	39.90	2.7E-10	1.7E-09
ENSMUSG00000064345	mt-Nd2	17717	-0.84	13.55	47.05	6.9E-12	5.1E-11
ENSMUSG00000064363	mt-Nd4	17719	-0.82	13.34	49.77	1.7E-12	1.4E-11
ENSMUSG00000018893	Mb	17189	-0.64	13.10	36.77	1.3E-09	7.7E-09
ENSMUSG00000032366	Tpm1	22003	-0.52	12.80	32.84	1.0E-08	5.3E-08
ENSMUSG00000026414	Tnnt2	21956	-0.70	12.58	44.01	3.3E-11	2.3E-10
ENSMUSG00000064339	mt-Rnr2	NA	-1.25	12.56	61.58	4.2E-15	4.3E-14
ENSMUSG00000015568	Lpl	16956	-0.85	12.49	66.25	4.0E-16	4.4E-15
ENSMUSG00000038583	Pln	18821	-0.95	12.43	55.59	8.9E-14	8.0E-13
ENSMUSG00000013936	Myl2	17906	-0.83	12.33	46.62	8.6E-12	6.3E-11
ENSMUSG00000025393	Atp5b	11947	-0.74	12.27	61.92	3.6E-15	3.7E-14
ENSMUSG00000002100	Mybpc3	17868	-0.32	12.18	19.23	1.2E-05	4.2E-05
ENSMUSG00000026043	Col3a1	12825	4.25	12.15	740.77	4.1E-163	1.3E-159
ENSMUSG00000059741	Myl3	17897	-0.66	12.12	39.35	3.5E-10	2.2E-09
ENSMUSG00000031633	Slc25a4	11739	-0.76	12.01	49.98	1.6E-12	1.2E-11
ENSMUSG00000025428	Atp5a1	11946	-0.72	11.93	53.28	2.9E-13	2.5E-12
ENSMUSG00000035458	Tnni3	21954	-0.92	11.52	47.54	5.4E-12	4.0E-11
ENSMUSG00000001506	Col1a1	12842	4.73	11.51	723.16	2.8E-159	8.0E-156
ENSMUSG00000029661	Col1a2	12843	4.51	11.38	686.02	3.3E-151	8.6E-148
ENSMUSG00000024803	Ankrd1	107765	1.24	11.27	28.62	8.8E-08	4.2E-07

Table S11. The 25 most highly expressed genes that are differentially expressed in left ventricles of PBS injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest log cpm out of 12593 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. PBS (n =5), sham (n = 5) and sham (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	log FC	log CPM	LR	P Value	FDR
ENSMUSG00000049538	Adamts16	271127	10.66	1.55	309	4.2E-69	8.7E-67
ENSMUSG00000004885	Crabp2	12904	10.56	0.98	192	1.2E-43	8.3E-42
ENSMUSG00000062345	Serpib2	18788	9.81	1.34	236	2.3E-53	2.4E-51
ENSMUSG00000029379	Cxcl3	330122	9.76	-0.04	108	2.5E-25	6.5E-24
ENSMUSG00000058914	C1qtnf3	81799	9.38	4.67	304	4.2E-68	8.3E-66
ENSMUSG00000054196	Cthrc1	68588	9.04	5.78	826	1.0E-181	1.4E-177
ENSMUSG00000040026	Saa3	20210	8.78	3.47	905	8.1E-199	2.1E-194
ENSMUSG00000050711	Scg2	20254	8.63	-0.57	113	2.3E-26	6.4E-25
ENSMUSG00000076615	Ighg3	NA	8.34	-1.96	32	1.5E-08	1.0E-07
ENSMUSG00000039462	Col10a1	12813	8.31	-0.87	38	8.1E-10	6.6E-09
ENSMUSG00000052353	Cemip	80982	8.29	3.18	275	8.4E-62	1.2E-59
ENSMUSG00000050359	Sprr1a	20753	8.26	3.10	266	7.5E-60	1.1E-57
ENSMUSG00000019987	Arg1	11846	7.98	3.07	376	1.2E-83	4.9E-81
ENSMUSG00000029304	Spp1	20750	7.87	6.28	800	6.2E-176	5.4E-172
ENSMUSG00000092627	D130058E05Rik	NA	7.64	-1.82	120	6.1E-28	1.8E-26
ENSMUSG00000094006	Igkv4-59	NA	7.51	0.07	23	1.9E-06	9.2E-06
ENSMUSG00000024041	Cryaa	12954	7.28	-2.30	103	3.8E-24	9.2E-23
ENSMUSG00000054764	Mtnr1a	17773	7.18	-1.85	71	4.3E-17	6.7E-16
ENSMUSG00000096515	Igkv14-100	NA	7.17	-3.04	12	4.7E-04	1.6E-03
ENSMUSG00000049723	Mmp12	17381	7.17	4.55	603	3.5E-133	8.4E-130
ENSMUSG00000028591	Pramef12	77632	7.09	-2.12	90	2.1E-21	4.4E-20
ENSMUSG00000051048	P4ha3	320452	7.08	3.61	536	1.1E-118	1.6E-115
ENSMUSG00000033740	St18	240690	7.07	-2.18	73	1.6E-17	2.6E-16
ENSMUSG00000101581	C430002N11Rik	319707	6.97	-1.14	147	9.8E-34	4.0E-32
ENSMUSG00000059742	Kcnh7	170738	6.92	-2.98	16	8.1E-05	3.1E-04
ENSMUSG00000026390	Marco	17167	6.90	2.59	174	7.9E-40	4.5E-38
ENSMUSG00000045731	Pnoc	18155	6.86	-2.28	50	1.5E-12	1.6E-11

Table S12. Top 25 genes that are upregulated in left ventricles of rHCI injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest log fold change out of 11427 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. rHCI (n=5), sham (n=5) and sham (n=5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	log FC	log CPM	LR	P Value	FDR
ENSMUSG00000061742	Slc22a12	20521	-5.92	-3.74	26.52	2.6E-07	1.5E-06
ENSMUSG00000104745	Gm43073	NA	-5.05	-4.16	25.67	4.1E-07	2.2E-06
ENSMUSG00000105622	Gm42615	NA	-4.21	-2.21	37.64	8.5E-10	6.9E-09
ENSMUSG00000082706	Gm11663	NA	-4.20	-4.24	12.56	3.9E-04	1.3E-03
ENSMUSG00000049246	Hspe1-ps2	NA	-4.17	-4.43	12.25	4.7E-04	1.5E-03
ENSMUSG00000092097	Gm5819	NA	-4.12	-4.42	9.77	1.8E-03	5.2E-03
ENSMUSG00000077569	Gm24608	NA	-4.09	-4.49	12.47	4.1E-04	1.4E-03
ENSMUSG00000044664	Prss42	235628	-3.95	-4.60	11.37	7.5E-04	2.4E-03
ENSMUSG00000097862	Gm26859	NA	-3.95	-4.56	11.55	6.8E-04	2.2E-03
ENSMUSG00000098588	Mir6340	102465173	-3.92	-4.46	10.24	1.4E-03	4.1E-03
ENSMUSG00000081889	Gm13188	NA	-3.89	-4.64	7.29	6.9E-03	1.8E-02
ENSMUSG00000105762	Gm43605	NA	-3.82	-4.49	9.63	1.9E-03	5.6E-03
ENSMUSG00000031682	1700011L22Rik	67687	-3.80	-4.64	9.57	2.0E-03	5.8E-03
ENSMUSG00000104664	Gm35570	NA	-3.80	-4.56	9.32	2.3E-03	6.6E-03
ENSMUSG00000064637	Snora20	NA	-3.74	-4.56	7.29	7.0E-03	1.8E-02
ENSMUSG00000085377	4930551L18Rik	NA	-3.69	-4.64	7.26	7.0E-03	1.8E-02
ENSMUSG00000085838	Chn1os1	NA	-3.64	-4.49	6.81	9.1E-03	2.3E-02
ENSMUSG00000090079	Gm15849	NA	-3.62	-4.53	7.72	5.5E-03	1.5E-02
ENSMUSG00000093400	Gm20647	NA	-3.61	-4.60	8.57	3.4E-03	9.5E-03
ENSMUSG00000082108	Gm13777	NA	-3.61	-4.43	8.93	2.8E-03	7.9E-03
ENSMUSG00000097811	2810425M01Rik	NA	-3.60	-4.27	7.42	6.5E-03	1.7E-02
ENSMUSG00000024211	Grm8	14823	-3.58	-4.08	15.34	9.0E-05	3.4E-04
ENSMUSG00000082023	Gm14407	NA	-3.57	-4.33	7.44	6.4E-03	1.7E-02
ENSMUSG00000101951	Gm28721	NA	-3.55	-4.46	5.40	2.0E-02	4.7E-02
ENSMUSG00000087632	Gm6058	NA	-3.45	-4.43	7.41	6.5E-03	1.7E-02
ENSMUSG00000036216	Leap2	259301	-3.45	-4.72	6.47	1.1E-02	2.7E-02
ENSMUSG00000088208	Gm23751	NA	-3.43	-4.50	7.52	6.1E-03	1.6E-02
ENSMUSG00000100763	Gm28777	NA	-3.43	-4.71	7.38	6.6E-03	1.7E-02

Table S13. Top 25 genes that are downregulated in left ventricles of rHCI injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest negative log fold change out of 11427 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. rHCI (n =5), sham (n = 5) and sham (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Ensembl ID	Symbol	Gene ID	log FC	log CPM	LR	P Value	FDR
ENSMUSG00000064351	mt-Co1	17708	-0.62	15.55	30.84	2.8E-08	1.8E-07
ENSMUSG00000064370	mt-Cytb	17711	-0.61	14.44	29.71	5.0E-08	3.2E-07
ENSMUSG00000040752	Myh6	17888	-0.77	14.33	30.59	3.2E-08	2.1E-07
ENSMUSG00000064367	mt-Nd5	17721	-0.66	14.26	27.89	1.3E-07	7.7E-07
ENSMUSG00000029467	Atp2a2	11938	-0.80	13.91	38.96	4.3E-10	3.6E-09
ENSMUSG00000064341	mt-Nd1	17716	-0.64	13.85	24.52	7.3E-07	3.9E-06
ENSMUSG00000064345	mt-Nd2	17717	-0.67	13.55	30.03	4.2E-08	2.7E-07
ENSMUSG00000064363	mt-Nd4	17719	-0.65	13.34	31.83	1.7E-08	1.1E-07
ENSMUSG00000018893	Mb	17189	-0.52	13.10	24.47	7.6E-07	4.0E-06
ENSMUSG00000032366	Tpm1	22003	-0.41	12.80	21.06	4.5E-06	2.1E-05
ENSMUSG00000026414	Tnnt2	21956	-0.56	12.58	28.59	8.9E-08	5.5E-07
ENSMUSG00000064339	mt-Rnr2	NA	-0.99	12.56	38.77	4.8E-10	4.0E-09
ENSMUSG00000015568	Lpl	16956	-0.74	12.49	50.93	9.6E-13	1.1E-11
ENSMUSG00000038583	Pln	18821	-0.76	12.43	35.93	2.0E-09	1.6E-08
ENSMUSG00000013936	My12	17906	-0.64	12.33	27.86	1.3E-07	7.8E-07
ENSMUSG00000025393	Atp5b	11947	-0.62	12.27	42.76	6.2E-11	5.8E-10
ENSMUSG00000002100	Mybpc3	17868	-0.27	12.18	13.90	1.9E-04	6.8E-04
ENSMUSG00000026043	Col3a1	12825	4.03	12.15	681.08	3.9E-150	1.7E-146
ENSMUSG00000059741	My13	17897	-0.54	12.12	26.75	2.3E-07	1.3E-06
ENSMUSG00000031633	Slc25a4	11739	-0.65	12.01	36.61	1.4E-09	1.1E-08
ENSMUSG00000025428	Atp5a1	11946	-0.58	11.93	34.77	3.7E-09	2.7E-08
ENSMUSG00000035458	Tnni3	21954	-0.72	11.52	29.70	5.0E-08	3.2E-07
ENSMUSG00000001506	Col1a1	12842	4.48	11.51	664.45	1.6E-146	5.3E-143
ENSMUSG00000029661	Col1a2	12843	4.21	11.38	616.39	4.6E-136	1.2E-132
ENSMUSG00000024803	Ankrd1	107765	1.15	11.27	24.48	7.5E-07	4.0E-06

Table S14. The 25 most highly expressed genes that are differentially expressed in left ventricles of rHCI injected mice with myocardial infarction compared to sham mice. The 25 genes with the largest log cpm out of 11427 total differentially expressed genes are included in this table. RNA was isolated from left ventricles two days after intramyocardial treatment injection in mice that had an infarct induced seven days prior. rHCI (n=5), sham (n = 5) and sham (n = 5) RNA samples were analyzed by next generation RNA sequencing at 50 million read depth for protein coding genes. Quality control, read trimming, alignment, and edgeR differential expression analysis of reads was completed by C3G bioinformatics group. FC: fold change relative to sham, CPM: gene read counts per million, LR: fold change likelihood ratio, and FDR: false discovery rate.

Appendix C: Permissions

Text from Chapter 2: 2.0.1-2.1.20, Chapter 3: 3.1.1-3.1.7, and Chapter 4: 4.2.1-4.2.4 along with Figures from Chapter 2: 2.1-2.3, Chapter 3: 3.1-3.7, and Appendix A: S1-S9 with Table S1 are reproduced with modification from: McLaughlin, S., and McNeill, B. et al. (2019) Injectable human recombinant collagen matrices limit adverse remodeling and improve cardiac function after myocardial infarction. *Nature Communications* 10, 4866.

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Author: Sarah McLaughlin et al
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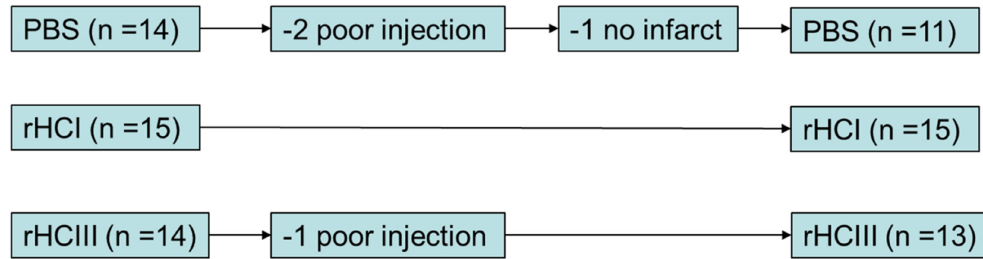
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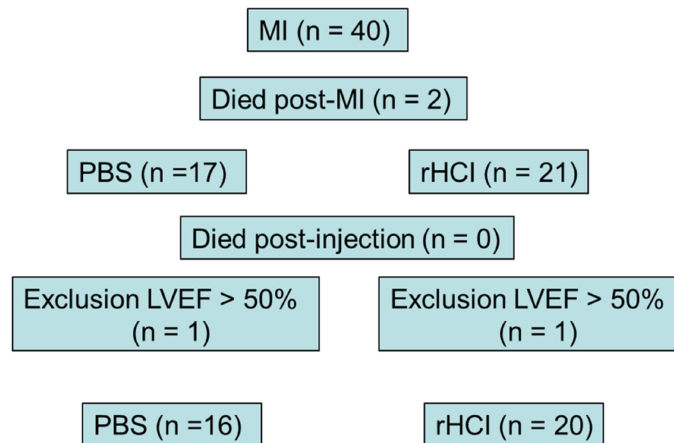
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Appendix D: Endpoint Schematics for MI Mouse Trials

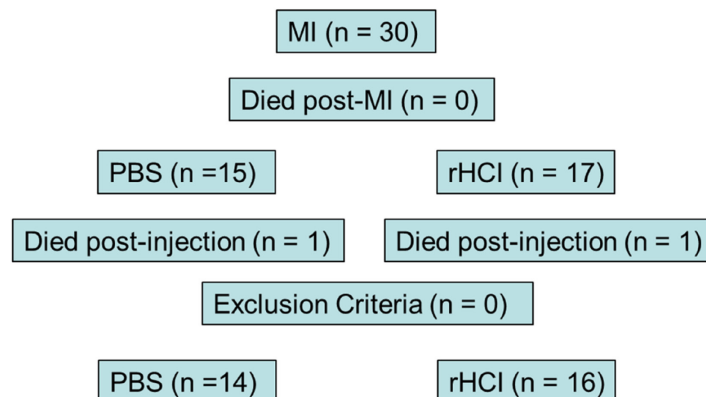
Aim 1 and 2



Aim 3 Day 2 Endpoint Trial



Aim 3 Day 28 Endpoint Trial



References

1. Public Health Agency of Canada, Report from the Canadian Chronic Disease Surveillance System: Heart Disease in Canada, 2018. <https://www.canada.ca/en/public-health/services/publications/diseases-conditions/report-heart-disease-Canada-2018.html>.
2. Anderson, J. L. & Morrow, D. A. Acute Myocardial Infarction. *N Engl J Med* **376**, 2053–2064 (2017).
3. Shanmugasagaram, S., Dai, S. & Waters, C. INCIDENCE AND PREVALENCE OF ACUTE MYOCARDIAL INFARCTION IN CANADIAN PROVINCES AND TERRITORIES. *Canadian Journal of Cardiology* **31**, S44 (2015).
4. Ko, D. T. *et al.* Readmission and Mortality After Hospitalization for Myocardial Infarction and Heart Failure. *Journal of the American College of Cardiology* **75**, 736–746 (2020).
5. Bergmann, O. *et al.* Evidence for cardiomyocyte renewal in humans. *Science* **324**, 98–102 (2009).
6. Bahit, M. C., Kochar, A. & Granger, C. B. Post-Myocardial Infarction Heart Failure. *JACC: Heart Failure* **6**, 179–186 (2018).
7. Hellermann, J. P. *et al.* Heart failure after myocardial infarction: clinical presentation and survival. *European Journal of Heart Failure* **7**, 119–125 (2005).
8. McMurray, J. J. V. Systolic Heart Failure. *The New England Journal of Medicine* **11** (2010).
9. Roger, V. L. Epidemiology of Heart Failure. *Circ Res* **113**, 646–659 (2013).
10. Virani, S. S. *et al.* Heart Disease and Stroke Statistics—2021 Update: A Report From the American Heart Association. *Circulation* **143**, (2021).
11. Yeung, D. F. *et al.* Trends in the incidence and outcomes of heart failure in Ontario, Canada: 1997 to 2007. *CMAJ* **184**, E765–E773 (2012).
12. Ambrosy, A. P. *et al.* The Global Health and Economic Burden of Hospitalizations for Heart Failure. *Journal of the American College of Cardiology* **63**, 1123–1133 (2014).
13. Tran, D. T. *et al.* The current and future financial burden of hospital admissions for heart failure in Canada: a cost analysis. *CMAJ Open* **4**, E365–E370 (2016).
14. Frangogiannis, N. G. The Extracellular Matrix in Ischemic and Nonischemic Heart Failure. *Circ Res* **125**, 117–146 (2019).
15. Fishbein, M. C., Maclean, D. & Maroko, P. R. The Histopathologic Evolution of Myocardial Infarction. *Chest* **73**, 843–849 (1978).
16. Lerman, R. H. *et al.* Myocardial healing and repair after experimental infarction in the rabbit. *Circ Res* **53**, 378–388 (1983).
17. Jugdutt, B. I. Prevention of ventricular remodeling after myocardial infarction and in congestive heart failure. *Heart Failure Rev* **1**, 115–129 (1996).
18. Prabhu, S. D. & Frangogiannis, N. G. The Biological Basis for Cardiac Repair After Myocardial Infarction: From Inflammation to Fibrosis. *Circulation Research* **119**, 91–112 (2016).
19. Holmes, J. W., Borg, T. K. & Covell, J. W. Structure and Mechanics of Healing Myocardial Infarcts. *Annu. Rev. Biomed. Eng.* **7**, 223–253 (2005).
20. Weber, Karl. T., Sun, Y. & Katwa, L. C. Wound healing following myocardial infarction. *Clin Cardiol* **19**, 447–455 (1996).

21. Münzel, T. *et al.* Impact of Oxidative Stress on the Heart and Vasculature. *Journal of the American College of Cardiology* **70**, 212–229 (2017).
22. Hartupee, J. & Mann, D. L. Neurohormonal activation in heart failure with reduced ejection fraction. *Nat Rev Cardiol* **14**, 30–38 (2017).
23. Zhou, T., Chuang, C.-C. & Zuo, L. Molecular Characterization of Reactive Oxygen Species in Myocardial Ischemia-Reperfusion Injury. *BioMed Research International* **2015**, e864946 (2015).
24. Sack, M. N., Fyhrquist, F. Y., Saijonmaa, O. J., Fuster, V. & Kovacic, J. C. Basic Biology of Oxidative Stress and the Cardiovascular System. *Journal of the American College of Cardiology* **70**, 196–211 (2017).
25. Panth, N., Paudel, K. R. & Parajuli, K. Reactive Oxygen Species: A Key Hallmark of Cardiovascular Disease. *Adv Med* **2016**, (2016).
26. Rababa'h, A. M., Guillory, A. N., Mustafa, R. & Hijawi, T. Oxidative Stress and Cardiac Remodeling: An Updated Edge. *Curr Cardiol Rev* **14**, 53–59 (2018).
27. Arslan, F., de Kleijn, D. P. & Pasterkamp, G. Innate immune signaling in cardiac ischemia. *Nat Rev Cardiol* **8**, 292–300 (2011).
28. Mann, D. L. The Emerging Role of Innate Immunity in the Heart and Vascular System: For Whom the Cell Tolls. *Circ Res* **108**, 1133–1145 (2011).
29. Mouton, A. J., Rivera, O. J. & Lindsey, M. L. Myocardial infarction remodeling that progresses to heart failure: a signaling misunderstanding. *American Journal of Physiology-Heart and Circulatory Physiology* **315**, H71–H79 (2018).
30. Kobayashi, Y. Neutrophil infiltration and chemokines. *Crit Rev Immunol* **26**, 307–316 (2006).
31. White, G. E., Iqbal, A. J. & Greaves, D. R. CC chemokine receptors and chronic inflammation--therapeutic opportunities and pharmacological challenges. *Pharmacol Rev* **65**, 47–89 (2013).
32. Frangogiannis, N. G. & Kovacic, J. C. Extracellular Matrix in Ischemic Heart Disease, Part 4/4. *Journal of the American College of Cardiology* **75**, 2219–2235 (2020).
33. Yan, X. *et al.* Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *Journal of Molecular and Cellular Cardiology* **62**, 24–35 (2013).
34. Nahrendorf, M. *et al.* The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *Journal of Experimental Medicine* **204**, 3037–3047 (2007).
35. Heidt, T. *et al.* Differential Contribution of Monocytes to Heart Macrophages in Steady-State and After Myocardial Infarction Novelty and Significance. *Circulation Research* **115**, 284–295 (2014).
36. Horekmans, M. *et al.* Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. *Eur Heart J* ehw002 (2016) doi:10.1093/eurheartj/ehw002.
37. Hilgendorf, I. *et al.* Ly-6Chigh Monocytes Depend on Nr4a1 to Balance Both Inflammatory and Reparative Phases in the Infarcted Myocardium. *Circulation Research* **114**, 1611–1622 (2014).
38. Shiraishi, M. *et al.* Alternatively activated macrophages determine repair of the infarcted adult murine heart. *Journal of Clinical Investigation* **126**, 2151–2166 (2016).
39. Weirather, J. *et al.* Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res* **115**, 55–67 (2014).

40. Sun, Y. & Weber, K. T. Infarct scar: a dynamic tissue. *Cardiovascular Research* **7** (2000).
41. Kobayashi, K. *et al.* Dynamics of angiogenesis in ischemic areas of the infarcted heart. *Sci Rep* **7**, 7156 (2017).
42. Virag, J. I. & Murry, C. E. Myofibroblast and Endothelial Cell Proliferation during Murine Myocardial Infarct Repair. *The American Journal of Pathology* **163**, 2433–2440 (2003).
43. Ruiz-Villalba Adrián *et al.* Interacting Resident Epicardium-Derived Fibroblasts and Recruited Bone Marrow Cells Form Myocardial Infarction Scar. *Journal of the American College of Cardiology* **65**, 2057–2066 (2015).
44. Ma, Y. *et al.* Myofibroblasts and the extracellular matrix network in post-myocardial infarction cardiac remodeling. *Pflugers Arch - Eur J Physiol* **466**, 1113–1127 (2014).
45. Yang, F. *et al.* Myocardial Infarction and Cardiac Remodelling in Mice. *Experimental Physiology* **87**, 547–555 (2002).
46. Frangogiannis, N. G., Michael, L. H. & Entman, M. L. Myofibroblasts in reperfused myocardial infarcts express the embryonic form of smooth muscle myosin heavy chain (SMemb). *Cardiovasc Res* **48**, 89–100 (2000).
47. Sutton, M. G. St. J. & Sharpe, N. Left Ventricular Remodeling After Myocardial Infarction. *Circulation* **101**, 2981–2988 (2000).
48. Yokota, T. *et al.* Type V Collagen in Scar Tissue Regulates the Size of Scar after Heart Injury. *Cell* **182**, 545–562.e23 (2020).
49. Takimoto, E. & Kass, D. A. Role of Oxidative Stress in Cardiac Hypertrophy and Remodeling. *Hypertension* **49**, 241–248 (2007).
50. Cohn, J. N., Ferrari, R. & Sharpe, N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* **35**, 569–582 (2000).
51. Fu, X. *et al.* Specialized fibroblast differentiated states underlie scar formation in the infarcted mouse heart. *Journal of Clinical Investigation* **128**, 2127–2143 (2018).
52. González-Santamaría, J. *et al.* Matrix cross-linking lysyl oxidases are induced in response to myocardial infarction and promote cardiac dysfunction. *Cardiovasc Res* **109**, 67–78 (2016).
53. Blackburn, N. J. R. *et al.* Methylglyoxal-derived advanced glycation end products contribute to negative cardiac remodeling and dysfunction post-myocardial infarction. *Basic Res Cardiol* **112**, 57 (2017).
54. Konstam, M. A., Kramer, D. G., Patel, A. R., Maron, M. S. & Udelson, J. E. Left Ventricular Remodeling in Heart Failure. *JACC: Cardiovascular Imaging* **4**, 98–108 (2011).
55. Talman, V. & Ruskoaho, H. Cardiac fibrosis in myocardial infarction—from repair and remodeling to regeneration. *Cell Tissue Res* **365**, 563–581 (2016).
56. Pfeffer, M. A. & Braunwald, E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation* **81**, 1161–1172 (1990).
57. Sharpe, N. Ventricular remodeling following myocardial infarction. *Am J Cardiol* **70**, 20C–26C (1992).
58. Pfeffer, M. A. *et al.* Myocardial infarct size and ventricular function in rats. *Circ Res* **44**, 503–512 (1979).

59. Bogen, D. K., Rabinowitz, S. A., Needleman, A., McMahon, T. A. & Abelmann, W. H. An analysis of the mechanical disadvantage of myocardial infarction in the canine left ventricle. *Circ Res* **47**, 728–741 (1980).
60. Voorhees, A. P. & Han, H.-C. Biomechanics of Cardiac Function. in *Comprehensive Physiology* (ed. Terjung, R.) 1623–1644 (John Wiley & Sons, Inc., 2015). doi:10.1002/cphy.c140070.
61. Halliday, B. P., Senior, R. & Pennell, D. J. Assessing left ventricular systolic function: from ejection fraction to strain analysis. *European Heart Journal* **42**, 789–797 (2021).
62. McKay, R. G. *et al.* Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation* **74**, 693–702 (1986).
63. Solomon, S. D. *et al.* Changes in Ventricular Size and Function in Patients Treated With Valsartan, Captopril, or Both After Myocardial Infarction. *Circulation* **111**, 3411–3419 (2005).
64. Solomon, S. D. *et al.* Influence of Ejection Fraction on Cardiovascular Outcomes in a Broad Spectrum of Heart Failure Patients. *Circulation* **112**, 3738–3744 (2005).
65. Hutchins, G. M. & Bulkley, B. H. Infarct expansion versus extension: Two different complications of acute myocardial infarction. *The American Journal of Cardiology* **41**, 1127–1132 (1978).
66. Frangogiannis, N. The inflammatory response in myocardial infarction. *Cardiovascular Research* **53**, 31–47 (2002).
67. Gaudron, P., Eilles, C., Kugler, I. & Ertl, G. Progressive left ventricular dysfunction and remodeling after myocardial infarction. Potential mechanisms and early predictors. *Circulation* **87**, 755–763 (1993).
68. Jugdutt, B. J. Identification of patients prone to infarct expansion by the degree of regional shape distortion on an early two-dimensional echocardiogram after myocardial infarction. *Clin Cardiol* **13**, 28–40 (1990).
69. Rohde, L. E. *et al.* Echocardiography-derived left ventricular end-systolic regional wall stress and matrix remodeling after experimental myocardial infarction. *Journal of the American College of Cardiology* **33**, 835–842 (1999).
70. Lew, W. Y., Chen, Z. Y., Guth, B. & Covell, J. W. Mechanisms of augmented segment shortening in nonischemic areas during acute ischemia of the canine left ventricle. *Circ Res* **56**, 351–358 (1985).
71. Jackson, B. M. *et al.* Extension of borderzone myocardium in postinfarction dilated cardiomyopathy. *Journal of the American College of Cardiology* **40**, 1160–1167 (2002).
72. Blair, C. A. *et al.* Heart Failure in Humans Reduces Contractile Force in Myocardium From Both Ventricles. *JACC: Basic to Translational Science* **5**, 786–798 (2020).
73. Kohlhaas, M. *et al.* Elevated Cytosolic Na⁺ Increases Mitochondrial Formation of Reactive Oxygen Species in Failing Cardiac Myocytes. *Circulation* **121**, 1606–1613 (2010).
74. Wijnker, P. J. M., Murphy, A. M., Stienen, G. J. M. & van der Velden, J. Troponin I phosphorylation in human myocardium in health and disease. *Neth Heart J* **22**, 463–469 (2014).
75. Papadaki, M. *et al.* Diabetes with heart failure increases methylglyoxal modifications in the sarcomere, which inhibit function. *JCI Insight* **3**, e121264 (2018).
76. Hori, M. & Nishida, K. Oxidative stress and left ventricular remodelling after myocardial infarction. *Cardiovascular Research* **81**, 457–464 (2008).

77. Moris, D. *et al.* The role of reactive oxygen species in the pathophysiology of cardiovascular diseases and the clinical significance of myocardial redox. *Ann. Transl. Med.* **5**, 326–326 (2017).
78. Gomes, K. M. S. *et al.* Aldehyde dehydrogenase 2 activation in heart failure restores mitochondrial function and improves ventricular function and remodelling. *Cardiovascular Research* **103**, 498–508 (2014).
79. Santin, Y. *et al.* Mitochondrial 4-HNE derived from MAO-A promotes mitoCa²⁺ overload in chronic postischemic cardiac remodeling. *Cell Death Differ* **27**, 1907–1923 (2020).
80. Wencker, D. *et al.* A mechanistic role for cardiac myocyte apoptosis in heart failure. *J. Clin. Invest.* **111**, 1497–1504 (2003).
81. Beltrami, A. P., Finato, N. & Leri, A. Evidence That Human Cardiac Myocytes Divide after Myocardial Infarction. *The New England Journal of Medicine* **8** (2001).
82. Rajabi, M., Kassiotis, C., Razeghi, P. & Taegtmeyer, H. Return to the fetal gene program protects the stressed heart: a strong hypothesis. *Heart Fail Rev* **12**, 331–343 (2007).
83. Taegtmeyer, H., Sen, S. & Vela, D. Return to the fetal gene program. *Ann N Y Acad Sci* **1188**, 191–198 (2010).
84. Westman, P. C. *et al.* Inflammation as a Driver of Adverse Left Ventricular Remodeling After Acute Myocardial Infarction. *Journal of the American College of Cardiology* **67**, 2050–2060 (2016).
85. Bansal, S. S. *et al.* Dysfunctional and Proinflammatory Regulatory T-Lymphocytes Are Essential for Adverse Cardiac Remodeling in Ischemic Cardiomyopathy. *Circulation* **139**, 206–221 (2019).
86. Jessup, M. & Brozena, S. Heart failure. *N Engl J Med* **348**, 2007–2018 (2003).
87. McMurray, J. J. V. Systolic Heart Failure. *New England Journal of Medicine* **362**, 228–238 (2010).
88. The Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). Guidelines on myocardial revascularization. *European Journal of Cardio-Thoracic Surgery* **38**, S1–S52 (2010).
89. Nallamothu, B. K., Bradley, E. H. & Krumholz, H. M. Time to Treatment in Primary Percutaneous Coronary Intervention. *N Engl J Med* **357**, 1631–1638 (2007).
90. Alkhalil, M. & Choudhury, R. P. Reperfusion Treatment in Late Presentation Acute Myocardial Infarction. *Circulation: Cardiovascular Interventions* **11**, e007287 (2018).
91. Ki, Y.-J. *et al.* Immediate Compared With Delayed Percutaneous Coronary Intervention for Patients With ST-Segment–Elevation Myocardial Infarction Presenting ≥ 12 Hours After Symptom Onset Is Not Associated With Improved Clinical Outcome. *Circulation: Cardiovascular Interventions* **14**, e009863 (2021).
92. Yellon, D. M. & Hausenloy, D. J. Myocardial Reperfusion Injury. *The New England Journal of Medicine* **15** (2007).
93. von Lueder, T. G., Kotecha, D., Atar, D. & Hopper, I. Neurohormonal Blockade in Heart Failure. *Card Fail Rev* **3**, 19–24 (2017).
94. Pfeffer, J. M., Pfeffer, M. A. & Braunwald, E. Influence of chronic captopril therapy on the infarcted left ventricle of the rat. *Circ Res* **57**, 84–95 (1985).
95. Pfeffer, M. A. *et al.* Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and

- ventricular enlargement trial. The SAVE Investigators. *N Engl J Med* **327**, 669–677 (1992).
96. Wilcox, J. E., Fang, J. C., Margulies, K. B. & Mann, D. L. Heart Failure With Recovered Left Ventricular Ejection Fraction. *Journal of the American College of Cardiology* **76**, 719–734 (2020).
 97. Aimo, A., Gaggin, H. K., Barison, A., Emdin, M. & Januzzi, J. L. Imaging, Biomarker, and Clinical Predictors of Cardiac Remodeling in Heart Failure With Reduced Ejection Fraction. *JACC: Heart Failure* **7**, 782–794 (2019).
 98. Lupón, J. *et al.* Dynamic Trajectories of Left Ventricular Ejection Fraction in Heart Failure. *Journal of the American College of Cardiology* **72**, 591–601 (2018).
 99. Kim, G. H., Uriel, N. & Burkhoff, D. Reverse remodelling and myocardial recovery in heart failure. *Nat Rev Cardiol* **15**, 83–96 (2018).
 100. Rane, A. A. & Christman, K. L. Biomaterials for the Treatment of Myocardial Infarction. *Journal of the American College of Cardiology* **58**, 2615–2629 (2011).
 101. Ott, H. C. *et al.* Perfusion-decellularized matrix: using nature’s platform to engineer a bioartificial heart. *Nat Med* **14**, 213–221 (2008).
 102. Sewanan, L. R. *et al.* Extracellular Matrix From Hypertrophic Myocardium Provokes Impaired Twitch Dynamics in Healthy Cardiomyocytes. *JACC: Basic to Translational Science* **4**, 495–505 (2019).
 103. Wu, C.-C., Jeratsch, S., Graumann, J. & Stainier, D. Y. R. Modulation of Mammalian Cardiomyocyte Cytokinesis by the Extracellular Matrix. *Circ Res* **127**, 896–907 (2020).
 104. Jacot, J. G., Martin, J. C. & Hunt, D. L. Mechanobiology of cardiomyocyte development. *Journal of Biomechanics* **43**, 93–98 (2010).
 105. del Monte-Nieto, G., Fischer, J. W., Gorski, D. J., Harvey, R. P. & Kovacic, J. C. Basic Biology of Extracellular Matrix in the Cardiovascular System, Part 1/4. *Journal of the American College of Cardiology* **75**, 2169–2188 (2020).
 106. Janicki, J. S. & Brower, G. L. The role of myocardial fibrillar collagen in ventricular remodeling and function. *Journal of Cardiac Failure* **8**, S319–S325 (2002).
 107. Weber, K. T. Cardiac interstitium in health and disease: The fibrillar collagen network. *Journal of the American College of Cardiology* **13**, 1637–1652 (1989).
 108. Frangogiannis, N. G. The extracellular matrix in myocardial injury, repair, and remodeling. *J Clin Invest* **127**, 1600–1612 (2017).
 109. Zeugolis, D. I. & Raghunath, M. Collagen: Materials Analysis and Implant Uses. in *Comprehensive Biomaterials* 261–278 (Elsevier, 2011). doi:10.1016/B978-0-08-055294-1.00074-X.
 110. Jugdutt, B. I. Ventricular Remodeling After Infarction and the Extracellular Collagen Matrix: When Is Enough Enough? *Circulation* **108**, 1395–1403 (2003).
 111. Fomovsky, G. M. & Holmes, J. W. Evolution of scar structure, mechanics, and ventricular function after myocardial infarction in the rat. *American Journal of Physiology-Heart and Circulatory Physiology* **298**, H221–H228 (2010).
 112. Knight, C. G. *et al.* The collagen-binding A-domains of integrins alpha(1)beta(1) and alpha(2)beta(1) recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. *J Biol Chem* **275**, 35–40 (2000).
 113. Kim, J. K. *et al.* A novel binding site in collagen type III for integrins alpha1beta1 and alpha2beta1. *J Biol Chem* **280**, 32512–32520 (2005).

114. Bugg, D. *et al.* Infarct Collagen Topography Regulates Fibroblast Fate via p38-Yes-Associated Protein Transcriptional Enhanced Associate Domain Signals. *Circ Res* **127**, 1306–1322 (2020).
115. Reinhardt, D. *et al.* Cardiac remodelling in end stage heart failure: upregulation of matrix metalloproteinase (MMP) irrespective of the underlying disease, and evidence for a direct inhibitory effect of ACE inhibitors on MMP. *Heart* **88**, 525–530 (2002).
116. Perestrelo, A. R. *et al.* Multiscale Analysis of Extracellular Matrix Remodeling in the Failing Heart. *Circ Res* **128**, 24–38 (2021).
117. Hartog, J. W. L., Voors, A. A., Bakker, S. J. L., Smit, A. J. & Veldhuisen, D. J. van. Advanced glycation end-products (AGEs) and heart failure: Pathophysiology and clinical implications. *European Journal of Heart Failure* **9**, 1146–1155 (2007).
118. Jyothirmayi, G. N., Soni, B. J., Masurekar, M., Lyons, M. & Regan, T. J. Effects of Metformin on Collagen Glycation and Diastolic Dysfunction in Diabetic Myocardium. *J Cardiovasc Pharmacol Ther* **3**, 319–326 (1998).
119. Goldin, A., Beckman, J. A., Schmidt, A. M. & Creager, M. A. Advanced Glycation End Products: Sparking the Development of Diabetic Vascular Injury. *Circulation* **114**, 597–605 (2006).
120. Hartog, J. W. L. *et al.* Clinical and prognostic value of advanced glycation end-products in chronic heart failure. *Eur Heart J* **28**, 2879–2885 (2007).
121. Raposeiras-Roubín, S. *et al.* Predictive value of advanced glycation end products for the development of post-infarction heart failure: a preliminary report. *Cardiovasc Diabetol* **11**, 102 (2012).
122. Brings, S. *et al.* Dicarbonyls and Advanced Glycation End-Products in the Development of Diabetic Complications and Targets for Intervention. *IJMS* **18**, 984 (2017).
123. Weber, K. T. Cardiac interstitium in health and disease: The fibrillar collagen network. *Journal of the American College of Cardiology* **13**, 1637–1652 (1989).
124. Tchaikovski, V., Olieslagers, S., Böhmer, F.-D. & Waltenberger, J. Diabetes Mellitus Activates Signal Transduction Pathways Resulting in Vascular Endothelial Growth Factor Resistance of Human Monocytes. *Circulation* **120**, 150–159 (2009).
125. Zhang, M. *et al.* Glycated Proteins Stimulate Reactive Oxygen Species Production in Cardiac Myocytes: Involvement of Nox2 (gp91phox)-Containing NADPH Oxidase. *Circulation* **113**, 1235–1243 (2006).
126. Yan, S. F., Ramasamy, R. & Schmidt, A. M. The RAGE Axis: A Fundamental Mechanism Signaling Danger to the Vulnerable Vasculature. *Circulation Research* **106**, 842–853 (2010).
127. Vulesevic, B. *et al.* Glyoxalase-1 overexpression in bone marrow cells reverses defective neovascularization in STZ-induced diabetic mice. *Cardiovascular Research* **101**, 306–316 (2014).
128. Molgat, A. S. D. *et al.* Hyperglycemia Inhibits Cardiac Stem Cell-Mediated Cardiac Repair and Angiogenic Capacity. *Circulation* **130**, S70–S76 (2014).
129. Paulus, W. J. & Dal Canto, E. Distinct Myocardial Targets for Diabetes Therapy in Heart Failure With Preserved or Reduced Ejection Fraction. *JACC: Heart Failure* **6**, 1–7 (2018).
130. Langer, R. & Vacanti, J. P. Tissue Engineering. 8 (2021).
131. Christman, K. L. & Lee, R. J. Biomaterials for the Treatment of Myocardial Infarction. *Journal of the American College of Cardiology* **48**, 907–913 (2006).

132. Zhang, J., Zhu, W., Radisic, M. & Vunjak-Novakovic, G. Can We Engineer a Human Cardiac Patch for Therapy? *Circ Res* **123**, 244–265 (2018).
133. Gao, L. *et al.* Large Cardiac Muscle Patches Engineered From Human Induced-Pluripotent Stem Cell-Derived Cardiac Cells Improve Recovery From Myocardial Infarction in Swine. *Circulation* **137**, 1712–1730 (2018).
134. Chong, J. J. H. *et al.* Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. *Nature* **510**, 273–277 (2014).
135. Lunde, K. *et al.* Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction. *N Engl J Med* **355**, 1199–1209 (2006).
136. Schächinger, V., Haberbosch, W., Yu, J., Hamm, C. W. & Tonn, T. Intracoronary Bone Marrow-Derived Progenitor Cells in Acute Myocardial Infarction. *n engl j med* **12** (2006).
137. Zwetsloot, P. P. *et al.* Cardiac Stem Cell Treatment in Myocardial Infarction: A Systematic Review and Meta-Analysis of Preclinical Studies. *Circ Res* **118**, 1223–1232 (2016).
138. Yanamandala, M. *et al.* Overcoming the Roadblocks to Cardiac Cell Therapy Using Tissue Engineering. *Journal of the American College of Cardiology* **70**, 766–775 (2017).
139. Lipinski, M. J. *et al.* Impact of Intracoronary Cell Therapy on Left Ventricular Function in the Setting of Acute Myocardial Infarction. *Journal of the American College of Cardiology* **50**, 1761–1767 (2007).
140. Fisher, S. A., Doree, C., Mathur, A. & Martin-Rendon, E. Meta-Analysis of Cell Therapy Trials for Patients With Heart Failure. *Circ Res* **116**, 1361–1377 (2015).
141. Carbone, R. G., Monselise, A., Bottino, G., Negrini, S. & Puppo, F. Stem cells therapy in acute myocardial infarction: a new era? *Clin Exp Med* **21**, 231–237 (2021).
142. Makkar, R. R. *et al.* Intracoronary ALLogeneic heart STem cells to Achieve myocardial Regeneration (ALLSTAR): a randomized, placebo-controlled, double-blinded trial. *European Heart Journal* **41**, 3451–3458 (2020).
143. Mathur, A. *et al.* The effect of intracoronary infusion of bone marrow-derived mononuclear cells on all-cause mortality in acute myocardial infarction: the BAMI trial. *European Heart Journal* **41**, 3702–3710 (2020).
144. He, L., Nguyen, N. B., Ardehali, R. & Zhou, B. Heart Regeneration by Endogenous Stem Cells and Cardiomyocyte Proliferation: Controversy, Fallacy, and Progress. *Circulation* **142**, 275–291 (2020).
145. Menasché, P. *et al.* The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* **117**, 1189–1200 (2008).
146. Teng, C. J., Luo, J., Chiu, R. C. J. & Shum-Tim, D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: Implications for cellular cardiomyoplasty. *The Journal of Thoracic and Cardiovascular Surgery* **132**, 628–632 (2006).
147. Kanda, P. & Davis, D. R. Cellular mechanisms underlying cardiac engraftment of stem cells. *Expert Opinion on Biological Therapy* **17**, 1127–1143 (2017).
148. Hodgkinson, C. P., Bareja, A., Gomez, J. A. & Dzau, V. J. Emerging Concepts in Paracrine Mechanisms in Regenerative Cardiovascular Medicine and Biology. *Circulation Research* **118**, 95–107 (2016).

149. Gneccchi, M. *et al.* Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. *Nat Med* **11**, 367–368 (2005).
150. Gallet, R. *et al.* Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. *European Heart Journal* ehw240 (2016) doi:10.1093/eurheartj/ehw240.
151. Vagnozzi, R. J. *et al.* An acute immune response underlies the benefit of cardiac stem cell therapy. *Nature* **577**, 405–409 (2020).
152. Saha, P. *et al.* Circulating exosomes derived from transplanted progenitor cells aid the functional recovery of ischemic myocardium. *Sci. Transl. Med.* **11**, eaau1168 (2019).
153. Gao, L. *et al.* Exosomes secreted by hiPSC-derived cardiac cells improve recovery from myocardial infarction in swine. *Sci. Transl. Med.* **12**, eaay1318 (2020).
154. Griffith, L. G. Polymeric biomaterials. *Acta Materialia* **48**, 263–277 (2000).
155. Cheng, K. *et al.* Intramyocardial Injection of Platelet Gel Promotes Endogenous Repair and Augments Cardiac Function in Rats With Myocardial Infarction. *Journal of the American College of Cardiology* **59**, 256–264 (2012).
156. Giordano, C. *et al.* Preclinical Evaluation of Biopolymer-Delivered Circulating Angiogenic Cells in a Swine Model of Hibernating Myocardium. *Circ Cardiovasc Imaging* **6**, 982–991 (2013).
157. Godier-Furnémont, A. F. G. *et al.* Composite scaffold provides a cell delivery platform for cardiovascular repair. *PNAS* **108**, 7974–7979 (2011).
158. Ceccaldi, C. *et al.* Alginate scaffolds for mesenchymal stem cell cardiac therapy: influence of alginate composition. *Cell Transplant* **21**, 1969–1984 (2012).
159. Mayfield, A. E. *et al.* The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. *Biomaterials* **35**, 133–142 (2014).
160. Lin, Y.-D. *et al.* Intramyocardial Peptide Nanofiber Injection Improves Postinfarction Ventricular Remodeling and Efficacy of Bone Marrow Cell Therapy in Pigs. *Circulation* **122**, S132–S141 (2010).
161. Wang, T. *et al.* Bone marrow stem cells implantation with alpha-cyclodextrin/MPEG-PCL-MPEG hydrogel improves cardiac function after myocardial infarction. *Acta Biomater* **5**, 2939–2944 (2009).
162. Tang, J. *et al.* Heart Repair Using Nanogel-Encapsulated Human Cardiac Stem Cells in Mice and Pigs with Myocardial Infarction. *ACS Nano* **11**, 9738–9749 (2017).
163. Ban, K. *et al.* Cell Therapy with Embryonic Stem Cell-Derived Cardiomyocytes Encapsulated in Injectable Nanomatrix Gel Enhances Cell Engraftment and Promotes Cardiac Repair. *ACS Nano* **8**, 10815–10825 (2014).
164. Piao, H. *et al.* Effects of cardiac patches engineered with bone marrow-derived mononuclear cells and PGCL scaffolds in a rat myocardial infarction model. *Biomaterials* **28**, 641–649 (2007).
165. Kutschka, I. Collagen Matrices Enhance Survival of Transplanted Cardiomyoblasts and Contribute to Functional Improvement of Ischemic Rat Hearts. *Circulation* **114**, I-167–I-173 (2006).
166. Chachques, J. C. *et al.* Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM Clinical Trial): One Year Follow-Up. *Cell Transplant* **16**, 927–934 (2007).

167. Chachques, J. C. *et al.* Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM Trial): Clinical Feasibility Study. *The Annals of Thoracic Surgery* **85**, 901–908 (2008).
168. Christman, K. L., Fok, H. H., Sievers, R. E., Fang, Q. & Lee, R. J. Fibrin Glue Alone and Skeletal Myoblasts in a Fibrin Scaffold Preserve Cardiac Function after Myocardial Infarction. *Tissue Engineering* **10**, 403–409 (2004).
169. Christman, K. L. *et al.* Injectable Fibrin Scaffold Improves Cell Transplant Survival, Reduces Infarct Expansion, and Induces Neovasculature Formation in Ischemic Myocardium. *Journal of the American College of Cardiology* **44**, 654–660 (2004).
170. Tous, E. *et al.* Influence of Injectable Hyaluronic Acid Hydrogel Degradation Behavior on Infarction-Induced Ventricular Remodeling. *Biomacromolecules* **12**, 4127–4135 (2011).
171. Zhu, Y., Matsumura, Y. & Wagner, W. R. Ventricular wall biomaterial injection therapy after myocardial infarction: Advances in material design, mechanistic insight and early clinical experiences. *Biomaterials* **129**, 37–53 (2017).
172. Kadner, K. *et al.* The beneficial effects of deferred delivery on the efficiency of hydrogel therapy post myocardial infarction. *Biomaterials* **33**, 2060–2066 (2012).
173. Fujimoto, K. L. *et al.* Synthesis, characterization and therapeutic efficacy of a biodegradable, thermoresponsive hydrogel designed for application in chronic infarcted myocardium. *Biomaterials* **30**, 4357–4368 (2009).
174. Jiang, X.-J. *et al.* Injection of a novel synthetic hydrogel preserves left ventricle function after myocardial infarction. *J. Biomed. Mater. Res.* **90A**, 472–477 (2009).
175. Blackburn, N. J. R. *et al.* Timing underpins the benefits associated with injectable collagen biomaterial therapy for the treatment of myocardial infarction. *Biomaterials* **39**, 182–192 (2015).
176. Sabbah, H. N. *et al.* Augmentation of Left Ventricular Wall Thickness With Alginate Hydrogel Implants Improves Left Ventricular Function and Prevents Progressive Remodeling in Dogs With Chronic Heart Failure. *JACC: Heart Failure* **1**, 252–258 (2013).
177. Dorsey, S. M. *et al.* MRI evaluation of injectable hyaluronic acid-based hydrogel therapy to limit ventricular remodeling after myocardial infarction. *Biomaterials* **69**, 65–75 (2015).
178. Seif-Naraghi, S. B. *et al.* Safety and Efficacy of an Injectable Extracellular Matrix Hydrogel for Treating Myocardial Infarction. *Science Translational Medicine* **5**, 173ra25-173ra25 (2013).
179. Mewhort, H. E. M. *et al.* Epicardial infarct repair with bioinductive extracellular matrix promotes vasculogenesis and myocardial recovery. *The Journal of Heart and Lung Transplantation* **35**, 661–670 (2016).
180. Lin, Y.-D. *et al.* Instructive Nanofiber Scaffolds with VEGF Create a Microenvironment for Arteriogenesis and Cardiac Repair. *Science Translational Medicine* **4**, 146ra109-146ra109 (2012).
181. Hsieh, P. C. H. Controlled delivery of PDGF-BB for myocardial protection using injectable self-assembling peptide nanofibers. *Journal of Clinical Investigation* **116**, 237–248 (2005).
182. Davis, M. E. *et al.* Local Myocardial Insulin-Like Growth Factor 1 (IGF-1) Delivery with Biotinylated Peptide Nanofibers Improves Cell Therapy for Myocardial

- Infarction. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 8155–8160 (2006).
183. Johnson, N. R. *et al.* Coacervate Delivery of Growth Factors Combined with a Degradable Hydrogel Preserves Heart Function after Myocardial Infarction. *ACS Biomater. Sci. Eng.* **1**, 753–759 (2015).
 184. Eckhouse, S. R. *et al.* Local Hydrogel Release of Recombinant TIMP-3 Attenuates Adverse Left Ventricular Remodeling After Experimental Myocardial Infarction. *Science Translational Medicine* **6**, 223ra21-223ra21 (2014).
 185. Prestwich, G. D. *et al.* What Is the Greatest Regulatory Challenge in the Translation of Biomaterials to the Clinic? *Science Translational Medicine* **4**, 160cm14-160cm14 (2012).
 186. Burdick, J. A., Mauck, R. L., Gorman, J. H. & Gorman, R. C. Acellular biomaterials: an evolving alternative to cell-based therapies. *Sci Transl Med* **5**, 176ps4 (2013).
 187. Brown, B. N. & Badylak, S. F. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. *Translational Research* **163**, 268–285 (2014).
 188. Christman, K. L. Biomaterials for tissue repair. *Science* **363**, 340–341 (2019).
 189. Wall, S. T., Walker, J. C., Healy, K. E., Ratcliffe, M. B. & Guccione, J. M. Theoretical Impact of the Injection of Material Into the Myocardium: A Finite Element Model Simulation. *Circulation* **114**, 2627–2635 (2006).
 190. Heerdt, P. M. *et al.* Chronic Unloading by Left Ventricular Assist Device Reverses Contractile Dysfunction and Alters Gene Expression in End-Stage Heart Failure. *Circulation* **102**, 2713–2719 (2000).
 191. Kortsmit, J. *et al.* The effect of hydrogel injection on cardiac function and myocardial mechanics in a computational post-infarction model. *Comput Methods Biomech Biomed Engin* **16**, 1185–1195 (2013).
 192. Kichula, E. T. *et al.* Experimental and Computational Investigation of Altered Mechanical Properties in Myocardium after Hydrogel Injection. *Ann Biomed Eng* **42**, 1546–1556 (2014).
 193. Miller, R., Davies, N. H., Kortsmit, J., Zilla, P. & Franz, T. Outcomes of myocardial infarction hydrogel injection therapy in the human left ventricle dependent on injectate distribution: DISTRIBUTION-SPECIFIC EFFICACY OF GEL INJECTATE IN INFARCTED HUMAN LV. *Int. J. Numer. Meth. Biomed. Engng.* **29**, 870–884 (2013).
 194. Wang, H. *et al.* Effects of hydrogel injection on borderzone contractility post-myocardial infarction. *Biomech Model Mechanobiol* **17**, 1533–1542 (2018).
 195. Wenk, J. F. *et al.* A Method for Automatically Optimizing Medical Devices for Treating Heart Failure: Designing Polymeric Injection Patterns. *Journal of Biomechanical Engineering* **131**, (2009).
 196. Ilisar, I. *et al.* Acute Left Ventricular Reconstruction With Circumferential Mid-Ventricular Intramyocardial Injections of Alginate Hydrogel in Dogs with Chronic Heart Failure. *Journal of Cardiac Failure* **16**, S42–S43 (2010).
 197. Ahmadi, A. *et al.* The role of integrin $\alpha 2$ in cell and matrix therapy that improves perfusion, viability and function of infarcted myocardium. *Biomaterials* **35**, 4749–4758 (2014).

198. Wassenaar, J. W. *et al.* Evidence for Mechanisms Underlying the Functional Benefits of a Myocardial Matrix Hydrogel for Post-MI Treatment. *Journal of the American College of Cardiology* **67**, 1074–1086 (2016).
199. Lister, Z., Rayner, K. J. & Suuronen, E. J. How Biomaterials Can Influence Various Cell Types in the Repair and Regeneration of the Heart after Myocardial Infarction. *Front Bioeng Biotechnol* **4**, (2016).
200. Mewhort, H. E. M. *et al.* Bioactive Extracellular Matrix Scaffold Promotes Adaptive Cardiac Remodeling and Repair. *JACC: Basic to Translational Science* **2**, 450–464 (2017).
201. Lee, R. J. *et al.* The feasibility and safety of Algisyl-LVR™ as a method of left ventricular augmentation in patients with dilated cardiomyopathy: Initial first in man clinical results. *International Journal of Cardiology* **199**, 18–24 (2015).
202. Johnson, T. D. & Christman, K. L. Injectable hydrogel therapies and their delivery strategies for treating myocardial infarction. *Expert Opinion on Drug Delivery* **10**, 59–72 (2013).
203. Gyöngyösi, M. & Dib, N. Diagnostic and prognostic value of 3D NOGA mapping in ischemic heart disease. *Nat Rev Cardiol* **8**, 393–404 (2011).
204. Traverse, J. H. *et al.* First-in-Man Study of a Cardiac Extracellular Matrix Hydrogel in Early and Late Myocardial Infarction Patients. *JACC: Basic to Translational Science* **4**, 659–669 (2019).
205. Laakmann, S. *et al.* Minimally invasive closed-chest ultrasound-guided substance delivery into the pericardial space in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology* **386**, 227–238 (2013).
206. Garcia, J. R. *et al.* A Minimally Invasive, Translational Method to Deliver Hydrogels to the Heart Through the Pericardial Space. *JACC: Basic to Translational Science* **2**, 601–609 (2017).
207. Lee, K. Y. & Mooney, D. J. Hydrogels for Tissue Engineering. *Chem. Rev.* **101**, 1869–1880 (2001).
208. Radisic, M. & Christman, K. L. Materials Science and Tissue Engineering: Repairing the Heart. *Mayo Clinic Proceedings* **88**, 884–898 (2013).
209. Koetting, M. C., Peters, J. T., Steichen, S. D. & Peppas, N. A. Stimulus-responsive hydrogels: Theory, modern advances, and applications. *Materials Science and Engineering: R: Reports* **93**, 1–49 (2015).
210. Rane, A. A. *et al.* Increased Infarct Wall Thickness by a Bio-Inert Material Is Insufficient to Prevent Negative Left Ventricular Remodeling after Myocardial Infarction. *PLOS ONE* **6**, e21571 (2011).
211. Dobner, S., Bezuidenhout, D., Govender, P., Zilla, P. & Davies, N. A Synthetic Non-degradable Polyethylene Glycol Hydrogel Retards Adverse Post-infarct Left Ventricular Remodeling. *Journal of Cardiac Failure* **15**, 629–636 (2009).
212. Ratner, B. D. A paradigm shift: biomaterials that heal. *Polym. Int.* **56**, 1183–1185 (2007).
213. Yoshizumi, T. *et al.* Timing effect of intramyocardial hydrogel injection for positively impacting left ventricular remodeling after myocardial infarction. *Biomaterials* **83**, 182–193 (2016).
214. Ren, S. *et al.* Physical properties of poly(N-isopropylacrylamide) hydrogel promote its effects on cardiac protection after myocardial infarction. *J Int Med Res* **40**, 2167–2182 (2012).

215. Yu, J. *et al.* Restoration of left ventricular geometry and improvement of left ventricular function in a rodent model of chronic ischemic cardiomyopathy. *The Journal of Thoracic and Cardiovascular Surgery* **137**, 180–187 (2009).
216. Ifkovits, J. L. *et al.* Injectable hydrogel properties influence infarct expansion and extent of postinfarction left ventricular remodeling in an ovine model. *Proceedings of the National Academy of Sciences* **107**, 11507–11512 (2010).
217. Lee, R. J., Hinson, A., Helgersson, S., Bauernschmitt, R. & Sabbah, H. N. Polymer-Based Restoration of Left Ventricular Mechanics. *Cell Transplant* **22**, 529–533 (2013).
218. Stuckey, D. J. *et al.* Magnetic resonance imaging evaluation of remodeling by cardiac elastomeric tissue scaffold biomaterials in a rat model of myocardial infarction. *Tissue Eng Part A* **16**, 3395–3402 (2010).
219. Hetzer, R., Javier, M. F. D. M., Wagner, F., Loebe, M. & Javier Delmo, E. M. Organ-saving surgical alternatives to treatment of heart failure. *Cardiovasc Diagn Ther* **11**, 213–225 (2021).
220. Ruvinov, E. & Cohen, S. Alginate biomaterial for the treatment of myocardial infarction: Progress, translational strategies, and clinical outlook. *Advanced Drug Delivery Reviews* **96**, 54–76 (2016).
221. Rao, S. V. *et al.* A randomized, double-blind, placebo-controlled trial to evaluate the safety and effectiveness of intracoronary application of a novel bioabsorbable cardiac matrix for the prevention of ventricular remodeling after large ST-segment elevation myocardial infarction: Rationale and design of the PRESERVATION I trial. *American Heart Journal* **170**, 929–937 (2015).
222. Rao, S. V. *et al.* Bioabsorbable Intracoronary Matrix for Prevention of Ventricular Remodeling After Myocardial Infarction. *Journal of the American College of Cardiology* **68**, 715–723 (2016).
223. Leor, J. *et al.* Intracoronary Injection of In Situ Forming Alginate Hydrogel Reverses Left Ventricular Remodeling After Myocardial Infarction in Swine. *Journal of the American College of Cardiology* **54**, 1014–1023 (2009).
224. Anker, S. D. *et al.* A prospective comparison of alginate-hydrogel with standard medical therapy to determine impact on functional capacity and clinical outcomes in patients with advanced heart failure (AUGMENT-HF trial). *Eur Heart J* **36**, 2297–2309 (2015).
225. Mann, D. L. *et al.* One-year follow-up results from AUGMENT-HF: a multicentre randomized controlled clinical trial of the efficacy of left ventricular augmentation with Algisyl in the treatment of heart failure: One-year follow-up results from AUGMENT-HF. *Eur J Heart Fail* **18**, 314–325 (2016).
226. Gerdisch, M. W., Shea, R. J. & Barron, M. D. Clinical experience with CorMatrix extracellular matrix in the surgical treatment of mitral valve disease. *The Journal of Thoracic and Cardiovascular Surgery* **148**, 1370–1378 (2014).
227. McCready, R. A. *et al.* Long-term Results With CorMatrix Extracellular Matrix Patches After Carotid Endarterectomy. *Journal of Surgical Research* **262**, 21–26 (2021).
228. Mosala Nezhad, Z. *et al.* Small intestinal submucosa extracellular matrix (CorMatrix®) in cardiovascular surgery: a systematic review. *Interact CardioVasc Thorac Surg* **22**, 839–850 (2016).
229. Badylak, S. F. & Gilbert, T. W. Immune response to biologic scaffold materials. *Semin Immunol* **20**, 109–116 (2008).

230. Brown, B. N., Valentin, J. E., Stewart-Akers, A. M., McCabe, G. P. & Badylak, S. F. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials* **30**, 1482–1491 (2009).
231. Padalino, M. A. *et al.* Porcine Intestinal Submucosa (CorMatrix) for Semilunar Valve Repair in Children: A Word of Caution After Midterm Results. *Seminars in Thoracic and Cardiovascular Surgery* **28**, 436–445 (2016).
232. van Rijswijk, J. W. *et al.* Failure of decellularized porcine small intestinal submucosa as a heart valved conduit. *The Journal of Thoracic and Cardiovascular Surgery* **160**, e201–e215 (2020).
233. Nelson, J. S., Heider, A., Si, M.-S. & Ohye, R. G. Evaluation of Explanted CorMatrix Intracardiac Patches in Children With Congenital Heart Disease. *The Annals of Thoracic Surgery* **102**, 1329–1335 (2016).
234. Sood, V., Heider, A., Rabah, R., Si, M.-S. & Ohye, R. G. Evaluation of Explanted CorMatrix Tyke Extracardiac Patches in Infants With Congenital Heart Disease. *The Annals of Thoracic Surgery* S0003497520317318 (2020) doi:10.1016/j.athoracsur.2020.06.151.
235. Singelyn, J. M. *et al.* Catheter-Deliverable Hydrogel Derived From Decellularized Ventricular Extracellular Matrix Increases Endogenous Cardiomyocytes and Preserves Cardiac Function Post-Myocardial Infarction. *Journal of the American College of Cardiology* **59**, 751–763 (2012).
236. Singelyn, J. M. *et al.* Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. *Biomaterials* **30**, 5409–5416 (2009).
237. Cascalho, M. & Platt, J. L. The Immunological Barrier to Xenotransplantation. *Immunity* **14**, 437–446 (2001).
238. Hernandez, M. J. *et al.* Manufacturing considerations for producing and assessing decellularized extracellular matrix hydrogels. *Methods* **171**, 20–27 (2020).
239. Johnson, T. D. *et al.* Human versus porcine tissue sourcing for an injectable myocardial matrix hydrogel. *Biomater. Sci.* **2**, 735–744 (2014).
240. Browne, S., Zeugolis, D. I. & Pandit, A. Collagen: Finding a Solution for the Source. *Tissue Engineering Part A* **19**, 1491–1494 (2013).
241. Dai, W., Wold, L. E., Dow, J. S. & Kloner, R. A. Thickening of the Infarcted Wall by Collagen Injection Improves Left Ventricular Function in Rats. *Journal of the American College of Cardiology* **46**, 714–719 (2005).
242. Caliarì, S. R. & Harley, B. A. C. Collagen–GAG Materials. in *Comprehensive Biomaterials* 279–302 (Elsevier, 2011). doi:10.1016/B978-0-08-055294-1.00075-1.
243. Harley, B., Leung, J., Silva, E. & Gibson, L. Mechanical characterization of collagen–glycosaminoglycan scaffolds. *Acta Biomaterialia* **3**, 463–474 (2007).
244. Yannas, I. V., Lee, E., Orgill, D. P., Skrabut, E. M. & Murphy, G. F. Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proc Natl Acad Sci U S A* **86**, 933–937 (1989).
245. Wangenstein, K. J. & Kallianen, L. K. Collagen Tube Conduits in Peripheral Nerve Repair: A Retrospective Analysis. *Hand (N Y)* **5**, 273–277 (2010).
246. Chamberlain, L. J., Yannas, I. V., Hsu, H.-P., Strichartz, G. & Spector, M. Collagen-GAG Substrate Enhances the Quality of Nerve Regeneration through Collagen Tubes up to Level of Autograft. *Experimental Neurology* **154**, 315–329 (1998).

247. Suuronen, E. J. *et al.* Innervated human corneal equivalents as in vitro models for nerve-target cell interactions. *FASEB j.* **18**, 170–172 (2004).
248. Ahmadi, A. *et al.* PET imaging of a collagen matrix reveals its effective injection and targeted retention in a mouse model of myocardial infarction. *Biomaterials* **49**, 18–26 (2015).
249. Suuronen, E. J. Tissue-Engineered Injectable Collagen-Based Matrices for Improved Cell Delivery and Vascularization of Ischemic Tissue Using CD133+ Progenitors Expanded From the Peripheral Blood. *Circulation* **114**, I-138–I-144 (2006).
250. Kuraitis, D. *et al.* Ex vivo generation of a highly potent population of circulating angiogenic cells using a collagen matrix. *J Mol Cell Cardiol* **51**, 187–197 (2011).
251. Everaerts, F., Torrianni, M., Hendriks, M. & Feijen, J. Biomechanical properties of carbodiimide crosslinked collagen: Influence of the formation of ester crosslinks. *J. Biomed. Mater. Res.* **85A**, 547–555 (2008).
252. Olde Damink, L. H. *et al.* Cross-linking of dermal sheep collagen using a water-soluble carbodiimide. *Biomaterials* **17**, 765–773 (1996).
253. Huang-Lee, L. L. H., Cheung, D. T. & Nimni, M. E. Biochemical changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived crosslinks. *J. Biomed. Mater. Res.* **24**, 1185–1201 (1990).
254. Kuraitis, D., Giordano, C., Ruel, M., Musarò, A. & Suuronen, E. J. Exploiting extracellular matrix-stem cell interactions: a review of natural materials for therapeutic muscle regeneration. *Biomaterials* **33**, 428–443 (2012).
255. Suuronen, E. J. *et al.* An acellular matrix-bound ligand enhances the mobilization, recruitment and therapeutic effects of circulating progenitor cells in a hindlimb ischemia model. *FASEB j.* **23**, 1447–1458 (2009).
256. Kuraitis, D., Berardinelli, M. G., Suuronen, E. J. & Musarò, A. A necrotic stimulus is required to maximize matrix-mediated myogenesis in mice. *Disease Models & Mechanisms* dmm.011072 (2013) doi:10.1242/dmm.011072.
257. Ahmadi, A., Vulesevic, B., Ruel, M. & Suuronen, E. J. A Collagen-Chitosan Injectable Hydrogel Improves Vascularization and Cardiac Remodeling in a Mouse Model of Chronic Myocardial Infarction. *Canadian Journal of Cardiology* **29**, S203–S204 (2013).
258. Yang, C. *et al.* The Application of Recombinant Human Collagen in Tissue Engineering: *BioDrugs* **18**, 103–119 (2004).
259. Merrett, K. *et al.* Tissue-Engineered Recombinant Human Collagen-Based Corneal Substitutes for Implantation: Performance of Type I versus Type III Collagen. *Invest. Ophthalmol. Vis. Sci.* **49**, 3887 (2008).
260. Fagerholm, P. *et al.* Stable corneal regeneration four years after implantation of a cell-free recombinant human collagen scaffold. *Biomaterials* **35**, 2420–2427 (2014).
261. Fagerholm, P. *et al.* A biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24-month follow-up of a phase 1 clinical study. *Sci Transl Med* **2**, 46ra61 (2010).
262. Islam, M. M., Griffith, M. & Merrett, K. Fabrication of a Human Recombinant Collagen-Based Corneal Substitute Using Carbodiimide Chemistry. in *Corneal Regenerative Medicine* (eds. Wright, B. & Connon, C. J.) vol. 1014 157–164 (Humana Press, 2013).

263. Bhan, A. *et al.* High-frequency speckle tracking echocardiography in the assessment of left ventricular function and remodeling after murine myocardial infarction. *Am J Physiol Heart Circ Physiol* **306**, H1371–H1383 (2014).
264. Ravichandran, R. *et al.* Functionalised type-I collagen as a hydrogel building block for bio-orthogonal tissue engineering applications. *J. Mater. Chem. B* **4**, 318–326 (2015).
265. Cleutjens, J. P., Blankesteijn, W. M., Daemen, M. J. & Smits, J. F. The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. *Cardiovasc Res* **44**, 232–241 (1999).
266. Nascimento, D. S. *et al.* MIQuant – Semi-Automation of Infarct Size Assessment in Models of Cardiac Ischemic Injury. *PLOS ONE* **6**, e25045 (2011).
267. Liang, W., Cho, H. C. & Marbán, E. Wnt signalling suppresses voltage-dependent Na⁺ channel expression in postnatal rat cardiomyocytes. *J Physiol* **593**, 1147–1157 (2015).
268. Weischenfeldt, J. & Porse, B. Bone Marrow-Derived Macrophages (BMM): Isolation and Applications. *Cold Spring Harb Protoc* **2008**, pdb.prot5080 (2008).
269. McLaughlin, S. *et al.* Injectable human recombinant collagen matrices limit adverse remodeling and improve cardiac function after myocardial infarction. *Nat Commun* **10**, 4866 (2019).
270. Bourgey, M. *et al.* GenPipes: an open-source framework for distributed and scalable genomic analyses. *GigaScience* **8**, (2019).
271. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).
272. Dobin, A. *et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
273. Roberts, A., Trapnell, C., Donaghey, J., Rinn, J. L. & Pachter, L. Improving RNA-Seq expression estimates by correcting for fragment bias. *Genome Biology* **12**, R22 (2011).
274. McCarthy, D. J., Chen, Y. & Smyth, G. K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research* **40**, 4288–4297 (2012).
275. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).
276. Chen, E. Y. *et al.* Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14**, 128 (2013).
277. Xie, Z. *et al.* Gene Set Knowledge Discovery with Enrichr. *Current Protocols* **1**, e90 (2021).
278. Kuleshov, M. V. *et al.* Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res* **44**, W90-97 (2016).
279. Kim, H. J. *et al.* Erythroid differentiation regulator 1 (Erdr1) is a proapoptotic factor in human keratinocytes: Erdr1 is a proapoptotic factor in human keratinocytes. *Experimental Dermatology* **20**, 920–925 (2011).
280. Jung, M. K. *et al.* Erythroid Differentiation Regulator 1, an Interleukin 18-Regulated Gene, Acts as a Metastasis Suppressor in Melanoma. *Journal of Investigative Dermatology* **131**, 2096–2104 (2011).
281. Wang, F. *et al.* RNAscope. *J Mol Diagn* **14**, 22–29 (2012).

282. Bankhead, P. *et al.* QuPath: Open source software for digital pathology image analysis. *Scientific Reports* **7**, 16878 (2017).
283. Patten, R. D. & Hall-Porter, M. R. Small animal models of heart failure: development of novel therapies, past and present. *Circ Heart Fail* **2**, 138–144 (2009).
284. De Luca, G., Suryapranata, H., Ottervanger, J. P. & Antman, E. M. Time delay to treatment and mortality in primary angioplasty for acute myocardial infarction: every minute of delay counts. *Circulation* **109**, 1223–1225 (2004).
285. Ting, H. H. *et al.* Delay in Presentation and Reperfusion Therapy in ST-Elevation Myocardial Infarction. *Am J Med* **121**, 316–323 (2008).
286. Dobaczewski, M., Gonzalez-Quesada, C. & Frangogiannis, N. G. The extracellular matrix as a modulator of the inflammatory and reparative response following myocardial infarction. *J Mol Cell Cardiol* **48**, 504–511 (2010).
287. Yang, F. *et al.* Myocardial Infarction and Cardiac Remodelling in Mice. *Experimental Physiology* **87**, 547–555 (2002).
288. Christia, P. *et al.* Systematic Characterization of Myocardial Inflammation, Repair, and Remodeling in a Mouse Model of Reperfused Myocardial Infarction. *J Histochem Cytochem* **61**, 555–570 (2013).
289. Gobert, M. *et al.* Regulatory T Cells Recruited through CCL22/CCR4 Are Selectively Activated in Lymphoid Infiltrates Surrounding Primary Breast Tumors and Lead to an Adverse Clinical Outcome. *Cancer Res* **69**, 2000–2009 (2009).
290. Weis, A. M., Soto, R. & Round, J. L. Commensal regulation of T cell survival through Erdr1. *Gut Microbes* 1–7 (2018) doi:10.1080/19490976.2018.1441662.
291. Lee, J., Jung, M., Park, H., Kim, K. & Cho, D. Erdr1 Suppresses Murine Melanoma Growth via Regulation of Apoptosis. *IJMS* **17**, 107 (2016).
292. Thirunavukkarasu, M. *et al.* Novel role of NADPH oxidase in ischemic myocardium: a study with Nox2 knockout mice. *Functional & Integrative Genomics* **12**, 501–514 (2012).
293. Spurney, C. F. *et al.* Dystrophin-deficient cardiomyopathy in mouse: Expression of Nox4 and Lox are associated with fibrosis and altered functional parameters in the heart. *Neuromuscular Disorders* **18**, 371–381 (2008).
294. Sun, A. *et al.* Mitochondrial Aldehyde Dehydrogenase 2 Plays Protective Roles in Heart Failure After Myocardial Infarction via Suppression of the Cytosolic JNK/p53 Pathway in Mice. *JAHA* **3**, (2014).
295. Rabbani, N. & Thornalley, P. J. Methylglyoxal, glyoxalase 1 and the dicarbonyl proteome. *Amino Acids* **42**, 1133–1142 (2012).
296. Han, J. *et al.* Dual Roles of Graphene Oxide To Attenuate Inflammation and Elicit Timely Polarization of Macrophage Phenotypes for Cardiac Repair. *ACS Nano* **12**, 1959–1977 (2018).
297. Perea-Gil, I., Prat-Vidal, C. & Bayes-Genis, A. In vivo experience with natural scaffolds for myocardial infarction: the times they are a-changin'. *Stem Cell Research & Therapy* **6**, 248 (2015).
298. Charriere, G., Bejot, M., Schnitzler, L., Ville, G. & Hartmann, D. J. Reactions to a bovine collagen implant. Clinical and immunologic study in 705 patients. *J Am Acad Dermatol* **21**, 1203–1208 (1989).
299. Bayomy, A. F., Bauer, M., Qiu, Y. & Liao, R. Regeneration in heart disease-Is ECM the key? *Life Sci* **91**, 823–827 (2012).

300. Jourdan-Lesaux, C., Zhang, J. & Lindsey, M. L. Extracellular matrix roles during cardiac repair. *Life Sci* **87**, 391–400 (2010).
301. Badylak, S. F., Freytes, D. O. & Gilbert, T. W. Extracellular matrix as a biological scaffold material: Structure and function. *Acta Biomater* **5**, 1–13 (2009).
302. Huang, N. F., Yu, J., Sievers, R., Li, S. & Lee, R. J. Injectable biopolymers enhance angiogenesis after myocardial infarction. *Tissue Eng* **11**, 1860–1866 (2005).
303. Zhang, P., Ruel, M. & Suuronen, E. J. Collagen biomaterials for cardiac tissue engineering applications. in *Tissue Engineering: roles, materials and applications*. 67–82 (Nova Science Publishers Inc., 2008).
304. Chattopadhyay, S. & Raines, R. T. Review collagen-based biomaterials for wound healing. *Biopolymers* **101**, 821–833 (2014).
305. Mandegar, M. H., Yousefnia, M. A., Roshanali, F., Rayatzadeh, H. & Alaeddini, F. Interaction between two predictors of functional outcome after revascularization in ischemic cardiomyopathy: left ventricular volume and amount of viable myocardium. *J Thorac Cardiovasc Surg* **136**, 930–936 (2008).
306. White, H. D. *et al.* Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* **76**, 44–51 (1987).
307. Cokkinos, D. V. & Belogiannas, C. Left Ventricular Remodelling: A Problem in Search of Solutions. *Eur Cardiol* **11**, 29–35 (2016).
308. Kirkpatrick, J. N. & St. John Sutton, M. Assessment of Ventricular Remodeling in Heart Failure Clinical Trials. *Curr Heart Fail Rep* **9**, 328–336 (2012).
309. Oka, T., Akazawa, H., Naito, A. T. & Komuro, I. Angiogenesis and cardiac hypertrophy: maintenance of cardiac function and causative roles in heart failure. *Circ Res* **114**, 565–571 (2014).
310. Cochain, C., Channon, K. M. & Silvestre, J.-S. Angiogenesis in the infarcted myocardium. *Antioxid Redox Signal* **18**, 1100–1113 (2013).
311. van der Laan, A. M., Piek, J. J. & van Royen, N. Targeting angiogenesis to restore the microcirculation after reperfused MI. *Nat Rev Cardiol* **6**, 515–523 (2009).
312. Ben-Mordechai, T. *et al.* Targeting and modulating infarct macrophages with hemin formulated in designed lipid-based particles improves cardiac remodeling and function. *J Control Release* **257**, 21–31 (2017).
313. Gomboszapova, A. *et al.* Macrophage activation and polarization in post-infarction cardiac remodeling. *J Biomed Sci* **24**, (2017).
314. Newby, A. C. Metalloproteinase Expression in Monocytes and Macrophages and its Relationship to Atherosclerotic Plaque Instability. *ATVB* **28**, 2108–2114 (2008).
315. Pupkaite, J. *et al.* Delivering More of an Injectable Human Recombinant Collagen III Hydrogel Does Not Improve Its Therapeutic Efficacy for Treating Myocardial Infarction. *ACS Biomater. Sci. Eng.* **6**, 4256–4265 (2020).
316. McWhorter, F. Y., Davis, C. T. & Liu, W. F. Physical and mechanical regulation of macrophage phenotype and function. *Cell Mol Life Sci* **72**, 1303–1316 (2015).
317. Heart and Stroke. The burden of Heart Failure. <https://www.heartandstroke.ca/-/media/pdf-files/canada/2017-heart-month/heartandstroke-reportonhealth-2016.ashx?la=en&hash=91708486C1BC014E24AB4E719B47AEEB8C5EB93E> (2016).
318. Jackson, B. M. *et al.* Border zone geometry increases wall stress after myocardial infarction: contrast echocardiographic assessment. *American Journal of Physiology-Heart and Circulatory Physiology* **284**, H475–H479 (2003).

319. Dagres, N. & Hindricks, G. Risk stratification after myocardial infarction: is left ventricular ejection fraction enough to prevent sudden cardiac death? *European Heart Journal* **34**, 1964–1971 (2013).
320. Varela, C. E., Fan, Y. & Roche, E. T. Optimizing Epicardial Restraint and Reinforcement Following Myocardial Infarction: Moving Towards Localized, Biomimetic, and Multitherapeutic Options. *Biomimetics (Basel)* **4**, (2019).
321. Richardson, W., Clarke, S., Quinn, T. & Holmes, J. Physiological Implications of Myocardial Scar Structure. *Compr Physiol* **5**, 1877–1909 (2015).
322. Liu, Y. *et al.* RNA-Seq identifies novel myocardial gene expression signatures of heart failure. *Genomics* **105**, 83–89 (2015).
323. Weinheimer, C. J. *et al.* Load-Dependent Changes in Left Ventricular Structure and Function in a Pathophysiologically Relevant Murine Model of Reversible Heart Failure. *Circ Heart Fail* **11**, (2018).
324. Zimmermann, M. *et al.* Analysis of region specific gene expression patterns in the heart and systemic responses after experimental myocardial ischemia. *Oncotarget* **8**, 60809–60825 (2017).
325. Yamada, S. & Nomura, S. Review of Single-Cell RNA Sequencing in the Heart. *IJMS* **21**, 8345 (2020).
326. Wassenaar, J. W., Braden, R. L., Osborn, K. G. & Christman, K. L. Modulating in vivo degradation rate of injectable extracellular matrix hydrogels. *J. Mater. Chem. B* **4**, 2794–2802 (2016).
327. Bischoff, L. *et al.* Cellular Mechanisms of CCL22-Mediated Attenuation of Autoimmune Diabetes. *The Journal of Immunology* **194**, 3054–3064 (2015).
328. Dörmer, P., Spitzer, E., Frankenberger, M. & Kremmer, E. Erythroid differentiation regulator (EDR), a novel, highly conserved factor. *Cytokine* **26**, 231–242 (2004).
329. Dörmer, P., Spitzer, E. & Möller, W. EDR is a stress-related survival factor from stroma and other tissues acting on early haematopoietic progenitors (E-Mix). *Cytokine* **27**, 47–57 (2004).
330. Soto, R. *et al.* Microbiota promotes systemic T-cell survival through suppression of an apoptotic factor. *Proceedings of the National Academy of Sciences* **114**, 5497–5502 (2017).
331. Kim, M. *et al.* Recombinant erythroid differentiation regulator 1 inhibits both inflammation and angiogenesis in a mouse model of rosacea. *Experimental Dermatology* **24**, 680–685 (2015).
332. Kim, K. E. *et al.* Therapeutic effect of erythroid differentiation regulator 1 (Erdr1) on collagen-induced arthritis in DBA/1J mouse. *Oncotarget* **7**, 76354–76361 (2016).
333. Kim, K. E. *et al.* Therapeutic effect of erythroid differentiation regulator 1 (Erdr1) on collagen-induced arthritis in DBA/1J mouse. *Oncotarget* **7**, 76354–76361 (2016).
334. Mango, R. L. *et al.* C-C Chemokine Receptor 5 on Pulmonary Mesenchymal Cells Promotes Experimental Metastasis via the Induction of Erythroid Differentiation Regulator 1. *Mol Cancer Res* **12**, 274–282 (2014).
335. Abo, H. *et al.* Erythroid differentiation regulator-1 induced by microbiota in early life drives intestinal stem cell proliferation and regeneration. *Nature Communications* **11**, (2020).
336. Wu, J. M. F. *et al.* Prostaglandin E₂ Receptor 2 Modulates Macrophage Activity for Cardiac Repair. *Journal of the American Heart Association* **7**, (2018).

337. Looi, Y. H. *et al.* Involvement of Nox2 NADPH Oxidase in Adverse Cardiac Remodeling After Myocardial Infarction. *Hypertension* **51**, 319–325 (2008).
338. van Duijvenboden, K. *et al.* Conserved *NPPB* + Border Zone Switches From MEF2- to AP-1–Driven Gene Program. *Circulation* **140**, 864–879 (2019).
339. Breitzig, M., Bhimineni, C., Lockey, R. & Kolliputi, N. 4-Hydroxy-2-nonenal: a critical target in oxidative stress? *Am J Physiol Cell Physiol* **311**, C537–C543 (2016).
340. Schaur, R. J., Siems, W., Bresgen, N. & Eckl, P. M. 4-Hydroxy-nonenal—A Bioactive Lipid Peroxidation Product. *Biomolecules* **5**, 2247–2337 (2015).
341. Ide, T. *et al.* Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res* **85**, 357–363 (1999).
342. Gomes, K. M. S. *et al.* Aldehydic load and aldehyde dehydrogenase 2 profile during the progression of post-myocardial infarction cardiomyopathy: Benefits of Alda-1. *International Journal of Cardiology* **179**, 129–138 (2015).
343. Hwang, H. V. *et al.* 4HNE Impairs Myocardial Bioenergetics in Congenital Heart Disease-Induced Right Ventricular Failure. *Circulation* **142**, 1667–1683 (2020).
344. Lucas, D. T. & Szweda, L. I. Cardiac reperfusion injury: aging, lipid peroxidation, and mitochondrial dysfunction. *Proc Natl Acad Sci U S A* **95**, 510–514 (1998).
345. Mak, S. *et al.* Unsaturated aldehydes including 4-OH-nonenal are elevated in patients with congestive heart failure. *J Card Fail* **6**, 108–114 (2000).
346. Baldi, A. *et al.* Apoptosis and Post-infarction Left Ventricular Remodeling. *Journal of Molecular and Cellular Cardiology* **34**, 165–174 (2002).
347. Monceau, V. *et al.* Myocyte apoptosis during acute myocardial infarction in rats is related to early sarcolemmal translocation of annexin A5 in border zone. *American Journal of Physiology-Heart and Circulatory Physiology* **291**, H965–H971 (2006).
348. Tekabe, Y. *et al.* Imaging of Receptors for Advanced Glycation End Products in Experimental Myocardial Ischemia and Reperfusion Injury. *JACC: Cardiovascular Imaging* **5**, 59–67 (2012).
349. Dai, Y. *et al.* Glycated Apolipoprotein A-IV Induces Atherogenesis in Patients With CAD in Type 2 Diabetes. *Journal of the American College of Cardiology* **70**, 2006–2019 (2017).
350. Wang, X.-L. *et al.* Methylglyoxal increases cardiomyocyte ischemia-reperfusion injury via glycative inhibition of thioredoxin activity. *American Journal of Physiology-Endocrinology and Metabolism* **299**, E207–E214 (2010).
351. Shimizu, Y. *et al.* Role of DJ-1 in Modulating Glycative Stress in Heart Failure. *JAHA* **9**, (2020).
352. Ruiz-Meana, M. *et al.* Ryanodine Receptor Glycation Favors Mitochondrial Damage in the Senescent Heart. *Circulation* **139**, 949–964 (2019).
353. Dobler, D., Ahmed, N., Song, L., Eboigbodin, K. E. & Thornalley, P. J. Increased dicarbonyl metabolism in endothelial cells in hyperglycemia induces anoikis and impairs angiogenesis by RGD and GFOGER motif modification. *Diabetes* **55**, 1961–1969 (2006).
354. Mewhort, H. E. M., Turnbull, J. D., Meijndert, H. C., Ngu, J. M. C. & Fedak, P. W. M. Epicardial infarct repair with basic fibroblast growth factor–enhanced CorMatrix-ECM biomaterial attenuates postischemic cardiac remodeling. *The Journal of Thoracic and Cardiovascular Surgery* **147**, 1650–1659 (2014).

355. Yao, Y. *et al.* Reactive oxygen species (ROS)-responsive biomaterials mediate tissue microenvironments and tissue regeneration. *J. Mater. Chem. B* **7**, 5019–5037 (2019).
356. Satthenapalli, V. R., Lamberts, R. R. & Katare, R. G. Concise Review: Challenges in Regenerating the Diabetic Heart: A Comprehensive Review. *STEM CELLS* **35**, 2009–2026 (2017).
357. Ballard, V. L. T. & Edelberg, J. M. Stem Cells and the Regeneration of the Aging Cardiovascular System. *Circulation Research* **100**, 1116–1127 (2007).
358. Hanssen, N. M. J. *et al.* Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease and Mortality in Individuals With Type 2 Diabetes. *Diabetes Care* **41**, 1689–1695 (2018).