Development of aligned porous structures through directional freezing for osteon regeneration

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Introduction

- **Osteons –** The fundamental unit of compact bone. Concentric layers of bone tissue around the Haversian Canal, which contains the bone's blood supplies. Their dimensions are roughly 200µm in diameter and several millimeters in length.[1]

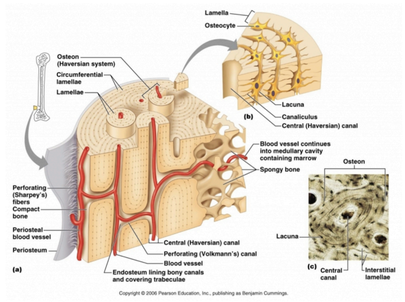


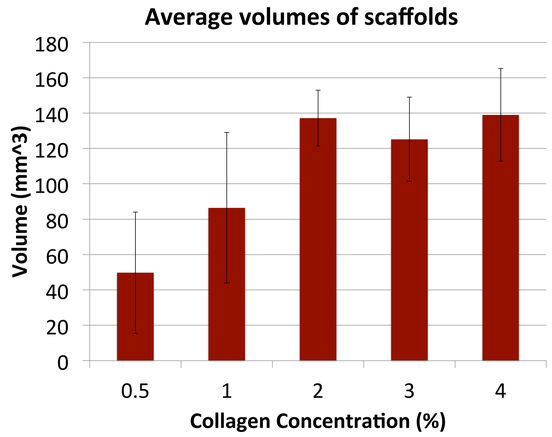
Figure 1 – Diagram showing the structure of compact bone[2]

- **The issue –** Insufficient supplies of bone grafts to repair damaged tissue has stimulated artificial bone tissue engineering. Though a number of technologies have been adapted to clinical treatment, regeneration of large bone defects remains difficult.
- **Past research –** The preparation of aligned porous gelatine scaffolds by using directional freezing has been successful but the pores are not wide enough for appositional cellular growth.[3]
- **Objective –** Scaffolds with large vertical channels should be established using directional freezing, concentrations of collagen, changing temperatures and patterning.

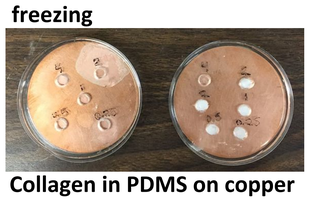
Methodology

1. PDMS – Prepare PDMS on copper plates using silicone elastomer crosslinker and base. Cut holes in surface using hollow hole punch to produce molds.
2. Gelatine – Prepare gelatine solution of 0.5%, 1%, 2%, 3%, 4% concentrations.
3. Directional freezing – Place copper plates with gelatine on metal plate in freezer at -18°C.
4. Freeze dry – Place copper plates in freeze dryer to remove solvent and preserve structures.
5. Data – Measure dimentions of scaffolds and observe with SEM.

Results

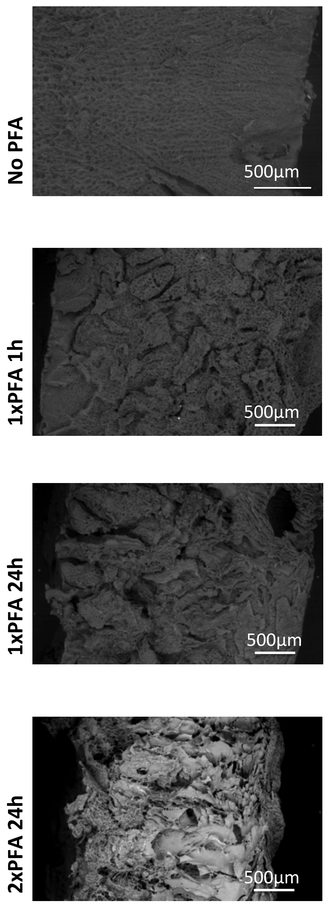


Collagen samples after dry-freezing

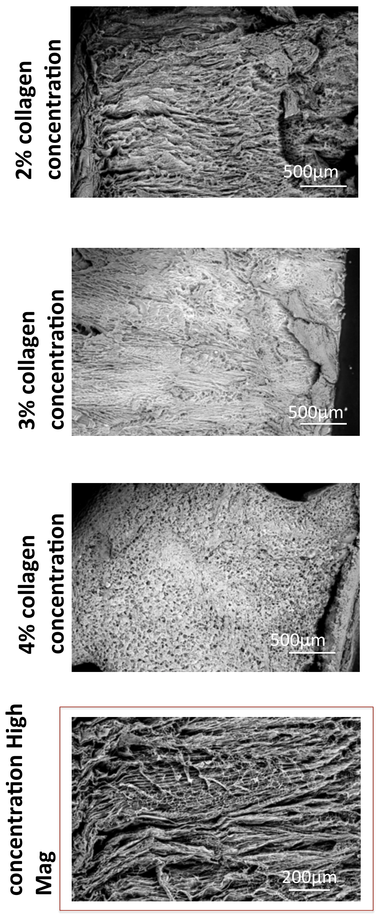


Collagen in PDMS on copper plates

Effect of PFA on scaffolds



Effect of collagen concentration on scaffolds



Discussion

- **Volume analysis –** Lower collagen concentrations produced smaller sample volumes that were not viable for scaffolding and canal formation. Leakage occured with 0.5%, creating a lower average volume.
- **SEM observations –** With PFA, the mixture formed clusters of collagen. Optimal canals were observed with 2% and possibly 3% collagen concentrations. Higher concentrations formed a structure too dense for canal formation. Using PFA created clusters in the scaffolds and did not produce aligned pores.

Conclusion

- **Effect of collagen concentration on volume –** The higher the concentration of collagen, the higher the volume of the scaffolds.
- **Effect of collagen concentration on pores –** The ideal concentration was 2%. Below this concentration, the samples were not strong enough. Above this concentration, the samples were too dense.
- **Effect of PFA on scaffolds –** Contrary to expectations, the use of PFA as a crosslinker produced clusters of collagen. Aligned pores could not be observed in the samples.
- **Future work –** The next step in this research is to test 2% concentrations with various directional freezing temperatures and patterns. The use of nanoparticles will also be introduced to create a scaffold with higher mechanical properties.

References

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[3] Wu, X., Liu, Y., Li, X., Wen, P., Zhang, Y., Long, Y., . . . Gao, J. (2010). Preparation of aligned porous gelatin scaffolds by unidirectional freeze-drying method. *Acta Biomaterialia, 6*(3), 1167-1177. doi:10.1016/j.actbio.2009.08.041

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