

2012-9

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Recommended Citation

Brougham, C. et al. (2012) Investigation of a New Material for Heart Valve Tissue Engineering. *The 3rd Tissue Engineering and Regenerative Medicine World Congress*, Vienna Austria, September.

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Funder: Irish Heart Foundation and DIT

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INVESTIGATION OF A NEW MATERIAL FOR HEART VALVE TISSUE ENGINEERING

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Introduction

- Approx. 300,000 heart valve replacements are performed annually; however, current treatment options have limited success, especially for paediatric patients.
- Current therapies cannot grow or remodel with the patient. These shortcomings have prompted increased focus on tissue engineering techniques to create fully autologous heart valve replacements.
- Despite significant advances in the field of heart valve tissue engineering, a major problem is the inability of valves to maintain an appropriate seal upon closure as a result of cell-mediated retraction of the leaflets [1].
- We are currently investigating natural biomaterials (collagen, glycosaminoglycans (GAGs) and fibrin) for the development of a scaffold which will have sufficient stiffness to resist the contractile forces of cells acting upon it.
- The novel scaffold proposed is based around the concept of reinforcing a cell-seeded fibrin gel component [2] with a collagen-GAG (CG) matrix.

Aims

- Hypothesis: A CG-fibrin scaffold will provide sufficient structural stiffness to resist the contractile forces of cells.
- Aim of this study: To develop a method of fabricating the CG-fibrin scaffold in a heart valve shape and optimise the resulting material using different concentrations and crosslinking methods for improved mechanical properties.

Materials & Methods

- A 3-D CG scaffold was fabricated through freeze drying in a custom-built mould (Figure 1).

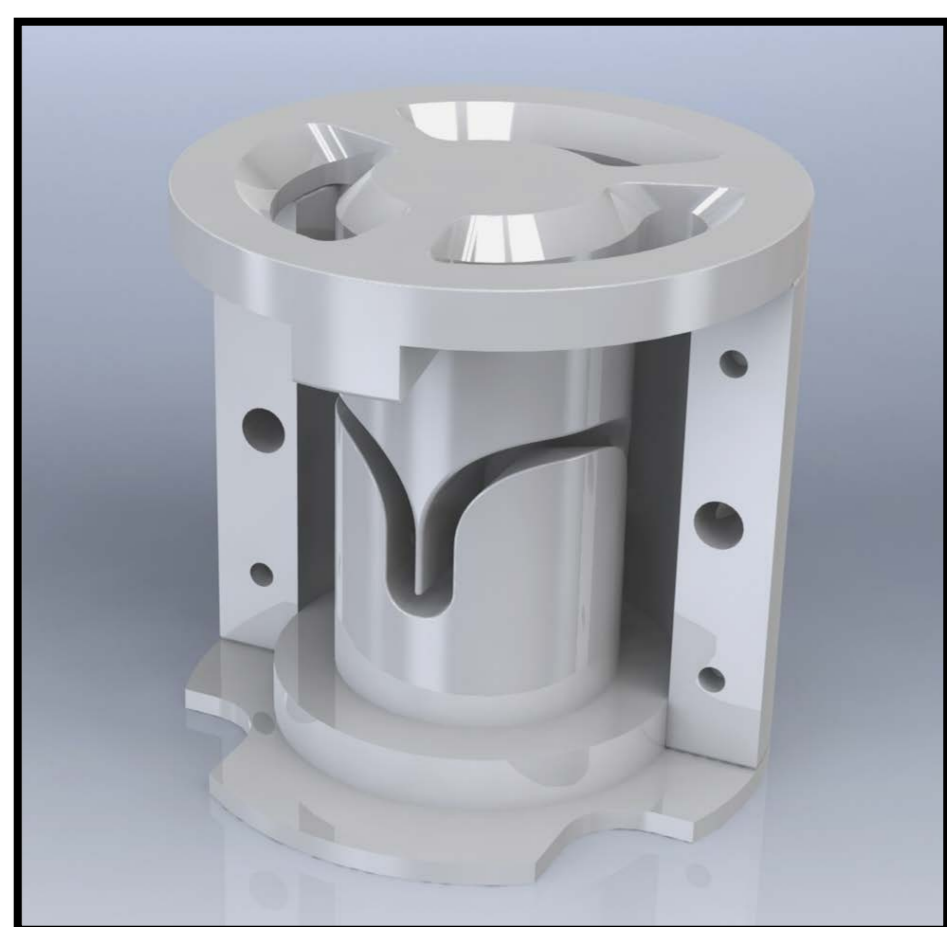


Figure 1. We have previously developed a mould for the construction of tri-leaflet heart valve conduits [1,3,4] This mould was modified for producing freeze-dried CG scaffolds.

- Freeze drying parameters such as final freezing temperature, cooling rate and drying times were optimised to produce a CG scaffold with a homogenous pore size structure [5].
- The freeze drying cycle optimisation was assessed using SEM, pore size and porosity analysis.
- Scaffolds were crosslinked physically by dehydrothermal (DHT) cross-linking at 105°C for 24 hours.
- A protocol was developed for chemical crosslinking using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC) in the presence of N-hydroxysuccinimide (NHS), solution which stiffens the scaffold while maintaining elasticity [6]. The difference to previous work was that the material was crosslinked in the presence of ethanol.
- Different concentrations of both collagen and GAG were freeze dried in the 3-D mould to find the most stable concentration post-crosslinking for infiltration with fibrin.

Materials & Methods

- To infiltrate the 3-D CG material with fibrin (a four-component solution which polymerises once the final ingredient is added), both drop loading and injection methods were assessed and a support structure was designed and built.
- Masson's trichrome staining and scanning electron microscopy (SEM) were used to assess the distribution of the fibrin throughout the CG material. Cryosectioning was used to preserve the morphology of the materials.
- Mechanical properties were tested using a Zwick/Roell Z050 testing machine.

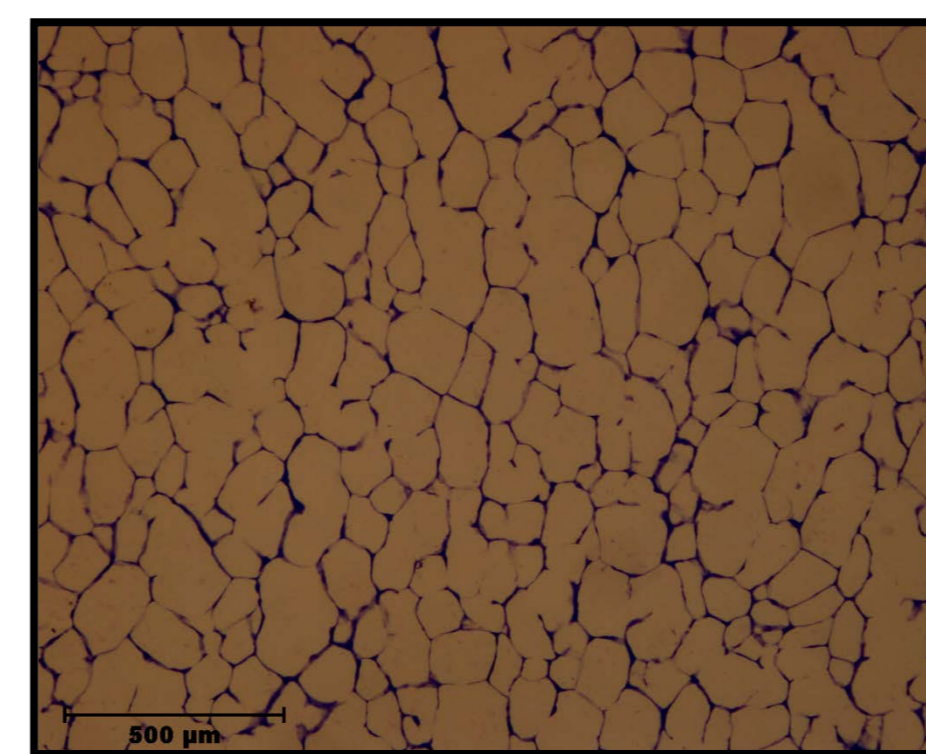
Results

- We successfully developed a freeze-dried CG material with a homogenous structure in a tri-leaflet valve conduit shape.



Figure 2. This image shows the 3-D CG freeze-dried material. Through optimisation of the freeze drying process, a tri-leaflet valve was fabricated.

Figure 3. The image here shows the pore structure of a section of the leaflet from the 3-D CG freeze dried material. The average pore size (~100µm) was homogenous between the leaflet and the cuffs of the material. Porosity of approx. 85% was measured in the material.



- We successfully developed a method of infiltrating the 3-D CG material with fibrin using an injection technique and a support structure. Injection of the fibrin solution allowed for an even distribution of polymerised fibrin throughout the material as demonstrated in Figure 5.

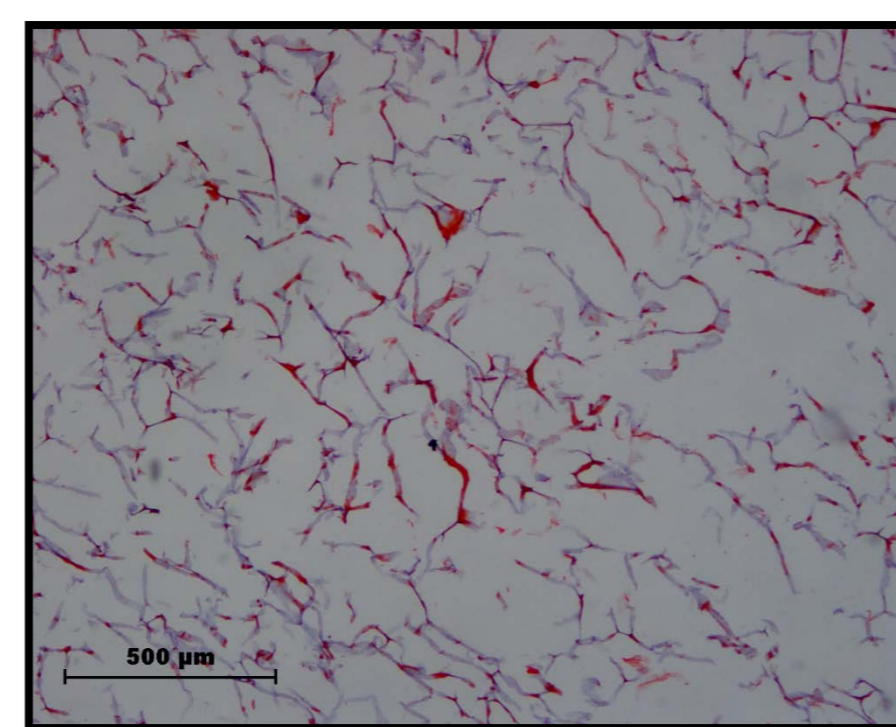
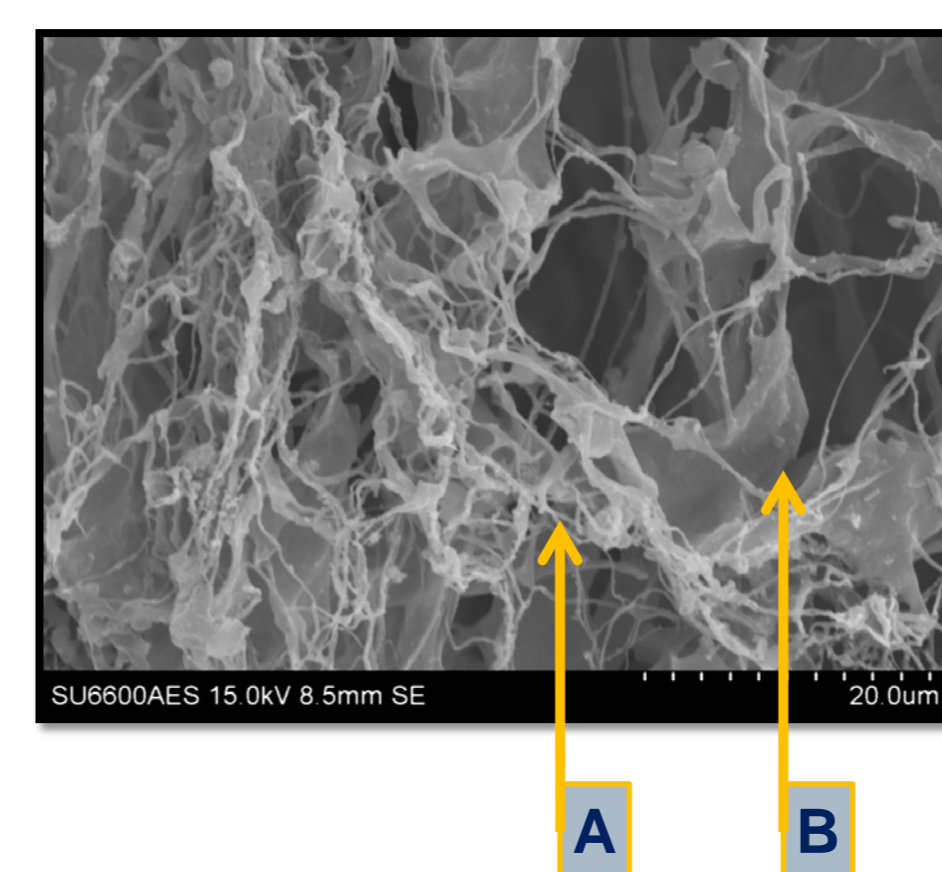


Figure 4. Masson's trichrome staining of a leaflet section of 3-D CG (blue) with fibrin (red) showing that the fibrin has completely permeated the 3-D CG material.

Figure 5. SEM image of freeze-dried CG (B), infiltrated with fibrin (A) using an injection method. Image shows infiltration of fibrin through the CG structure.



- Freeze drying using a concentration of 0.75% collagen and 0.05% GAG demonstrated the best infiltration of fibrin and had the optimum handling characteristics post-crosslinking (see Figure 6). These concentrations also exhibited the highest compressive modulus from all samples.

Results



Figure 6. This image shows a sample of the 3-D CG material, post crosslinking and post infiltration with fibrin. The material stands up under its own weight and is easy to handle.

- There were no statistically significant differences in the compressive moduli between the 3-D CG-fibrin cuff and leaflet, as shown in Figure 7. This suggests we have produced a material with homogenous stiffness characteristics.

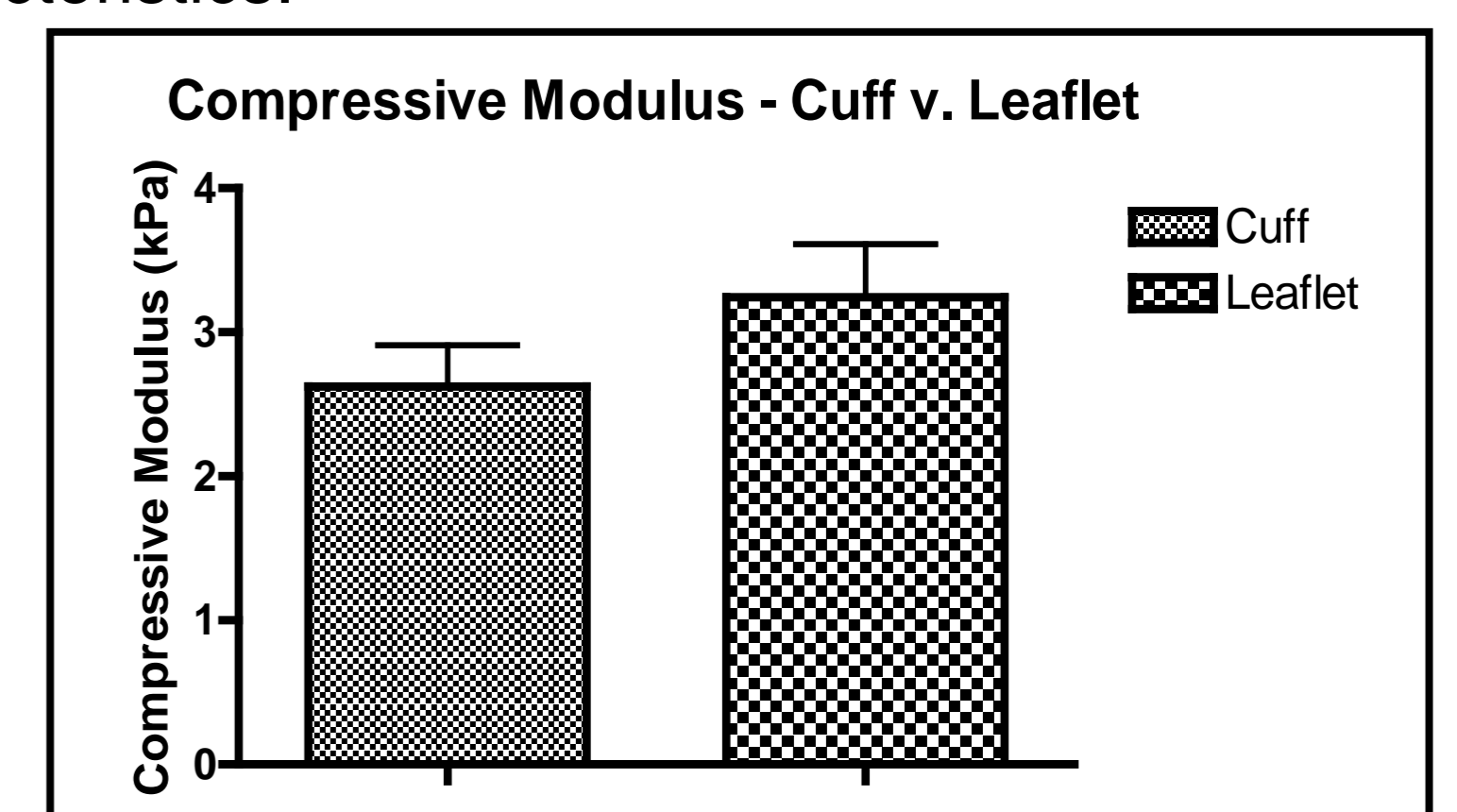


Figure 7. With no statistical difference between the compressive moduli of the leaflet versus the cuff, the material appears homogenous and ready to be examined when cells are introduced with the fibrin acting as the cell delivery matrix.

Discussion & Conclusions

- This study has led to the development of a freeze-dried 3-D CG-fibrin scaffold which will be further investigated for heart valve tissue engineering.
- This freeze-dried 3-D CG scaffold has a homogeneous pore structure throughout and can be manufactured in a repeatable, 3-D form.
- A 0.75% collagen and 0.05% GAG concentration ratio, has been identified as the most stable crosslinked material to work with.
- Crosslinking CG using DHT and an EDAC:NHS solution in ethanol has been found to produce a repeatable material with excellent handling characteristics and a stiffness both in tension and compression greater than un-crosslinked CG (data not shown).
- Fibrin has been shown to have been infiltrated successfully through the 3-D CG material using an injection method together with a support structure.
- Mechanical testing of the 3-D CG-fibrin material demonstrated a compressive modulus of approx. 3kPa for both the cuff and the leaflet areas.

On-going Investigation

- The next phase of this study is to introduce cells to the material in order to assess its biological performance. This will also demonstrate the resistance of the material to the contractile forces exerted by the cells.

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Acknowledgements

Acknowledgements to RCSI Tissue Engineering Research Group for their expertise and the FOCUS institute, DIT for SEM work.

Funding for this research was provided by Irish Heart Foundation and the European Research Council. Funding for this conference was provided by DIT.