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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.
- 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].



Figure 1. This image shows a native aortic valve (taken from the arterial side) [2] where the leaflets form a closed seal. In cases of heart valve disease the shape or elasticity of the leaflets prevents this seal forming which means that there is a leakage of blood through the valve.

- 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 cell-seeded fibrin gel component [3] with a collagen-GAG (CG) matrix.

Aims and objectives

- Hypothesis: A CG-fibrin scaffold will provide sufficient structural stiffness to resist the contractile forces of cells.
- Aim of this study: Develop a method of fabricating the CG-fibrin scaffold and analyse the resulting material.

Materials and methods

- A CG scaffold will be fabricated through freeze drying in a 3-D mould (Figure 2).

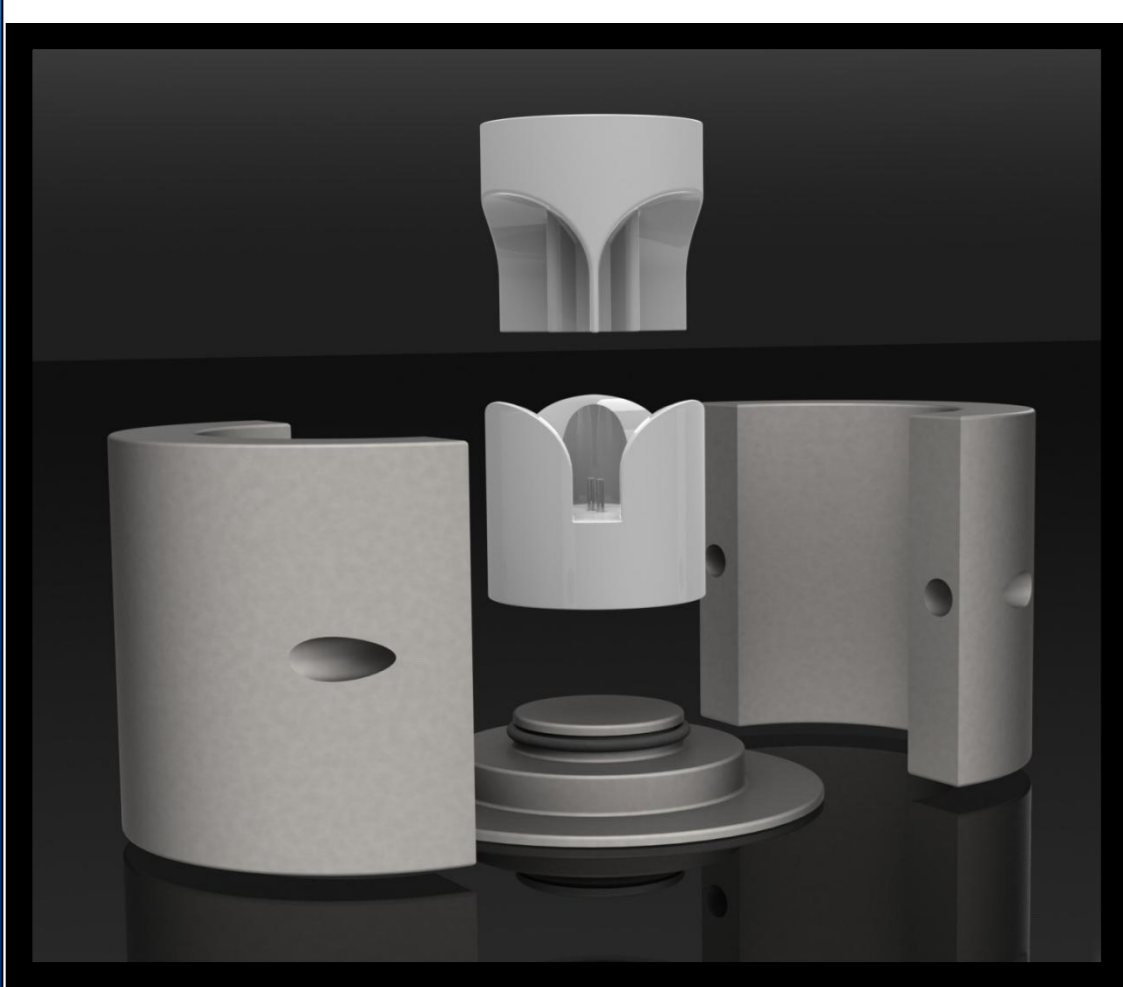


Figure 2. We have previously developed a mould for the construction of tri-leaflet heart valve conduits [1,4,5] This mould was modified for producing freeze-dried CG scaffolds.

- 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 [6].
- The freeze drying cycle optimisation was assessed using SEM, pore size and porosity analysis.

Materials and methods

- Scaffolds were crosslinked physically by dehydrothermal (DHT) cross-linking at 105°C for 24 hours. They were subsequently chemically crosslinked using 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDAC) in the presence of N-hydroxysuccinimide (NHS), solution which stiffens the scaffold while maintaining elasticity [7].
- To infiltrate the CG material with fibrin (a four stage solution which polymerises once the final ingredient is added), both drop loading and injection methods were assessed.
- Masson's Trichrome staining and SEM were used to assess the distribution of the fibrin throughout the CG material.
- Mechanical properties were tested using a Zwick/Roell Z050 testing machine.

Results

- Successful development of a freeze dried CG material with a homogenous structure in a tri-leaflet valve conduit shape.



Figure 3. The image above shows the CG freeze dried construct. Through optimisation of the freeze drying process, a tri-leaflet valve was fabricated.

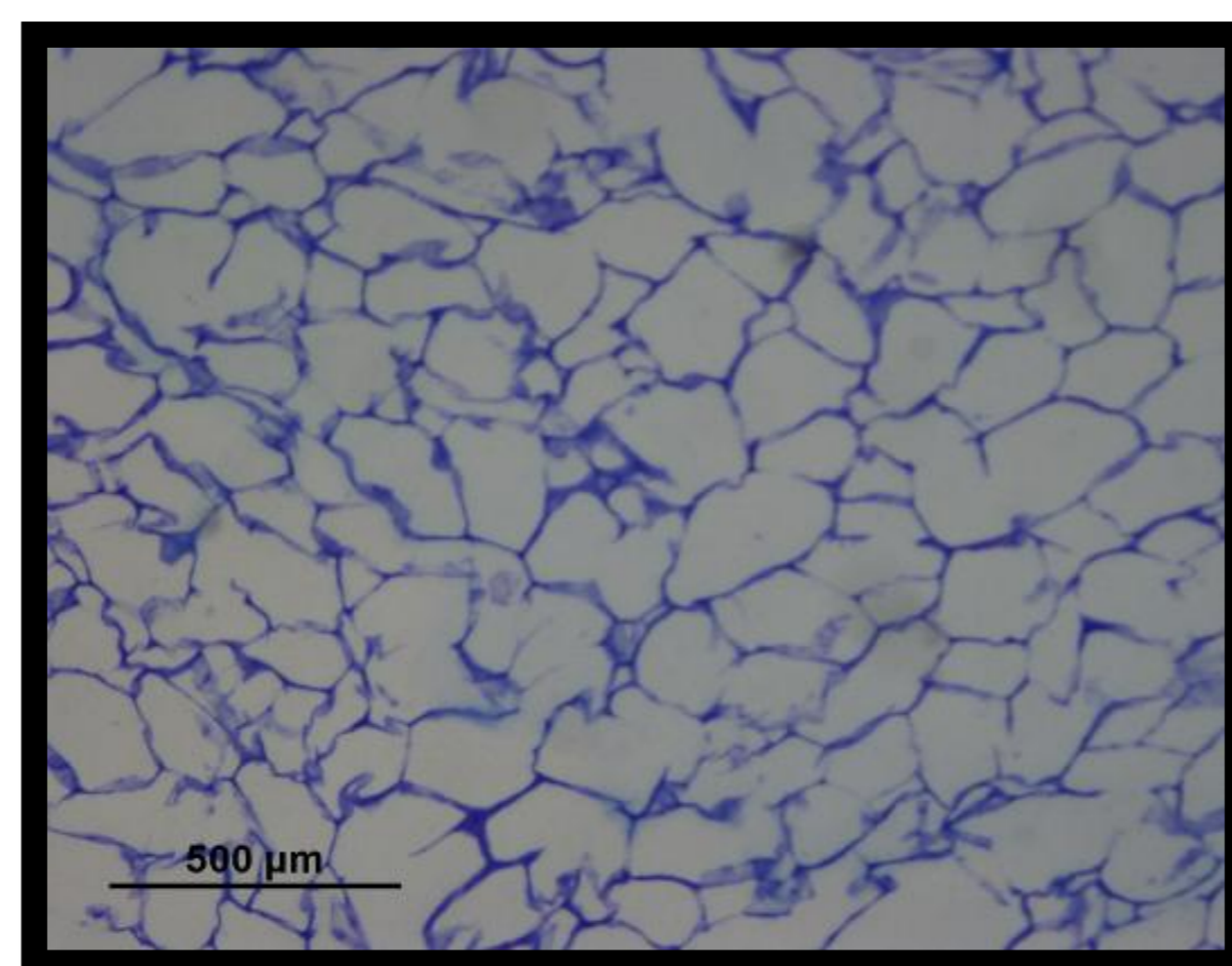


Figure 4. Masson's Trichrome staining was used to assess the pore size and porosity of the scaffolds produced using a variety of freeze drying parameters. This image shown is from the optimised set of parameters. It shows a homogenous pore structure with an average pore size of 128.9 39.3µm and a porosity of 99.2% Analysis was completed at the top, in the middle leaflet section and at the lower section of the scaffold.

- We successfully developed of a method of infiltrating the CG scaffold with fibrin using an injection technique. Through optimisation of fibrin infiltration, a final volume of 0.7µl of fibrin gel per mm³ of CG was established as the most stable ratio.

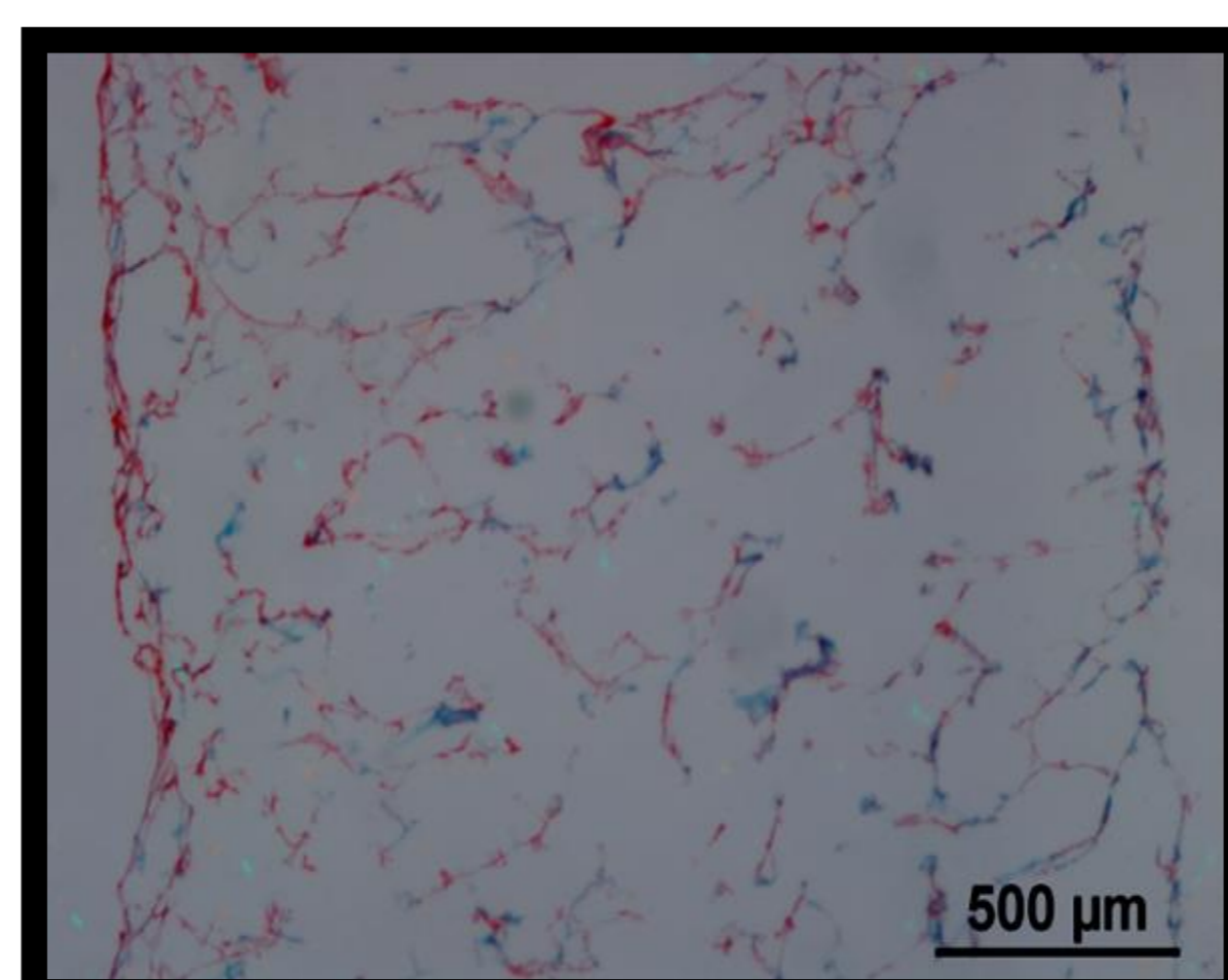


Figure 5. Masson's Trichrome staining of a section of CG (blue) with fibrin (red) showing that the fibrin has completely permeated the CG scaffold.

Results

- Drop loading resulted in a layer of fibrin being established on the edges of the material due to the rapid polymerisation time of fibrin. Injection of the fibrin solution allowed for an even distribution of polymerised fibrin throughout the material as demonstrated in the SEM image shown.

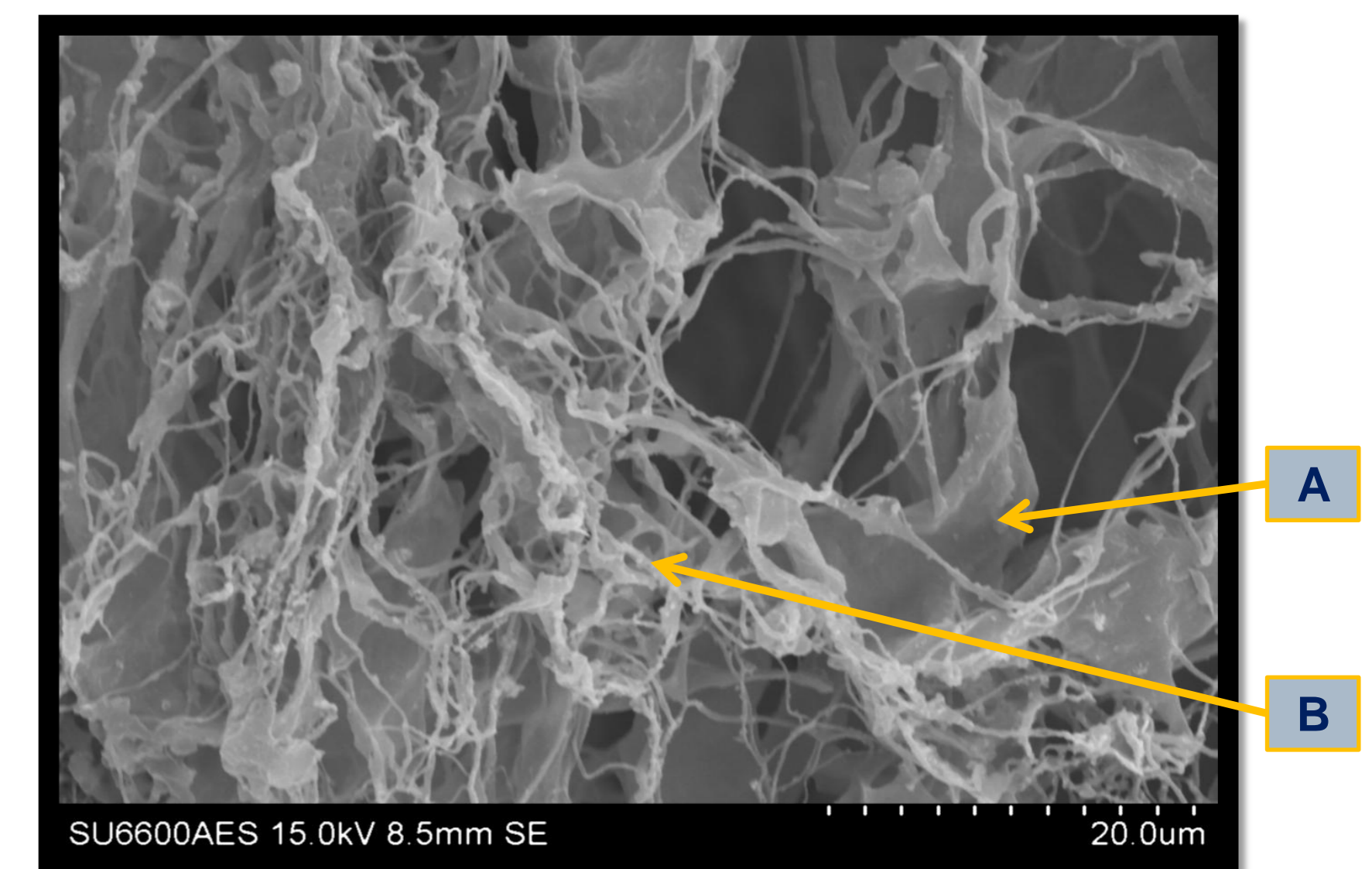


Figure 6. SEM image of freeze-dried CG material (A), infiltrated with fibrin (B) using an injection method.

- The tensile and compressive moduli of the materials were also tested and the crosslinking greatly enhanced the stiffness of the material.

Discussion and conclusions

- This study has led to the development of a freeze-dried CG-fibrin scaffold which will be used for heart valve tissue engineering.
- This CG scaffold has a homogeneous pore structure throughout and can be manufactured in a repeatable, 3-D form.
- The proof of principle that fibrin can be successfully infiltrated into the CG material has been demonstrated.
- The stable ratio of fibrin to CG has been established at 0.7µl per mm³ CG.
- A method of injecting the fibrin into the CG has been developed which has demonstrated full infiltration of the fibrin through the CG.
- The use of crosslinking has been found to increase the compressive and tensile moduli of the material backbone which will improve the ability of the material to withstand the contractile forces of the cells on the material.

On-going Work

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

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