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).
- 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-hydroxy succinimide (NHS), solution which stiffens the scaffold while maintaining elasticity [8]. 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.

Results

- We successfully developed a freeze-dried CG material with a homogenous structure in a tri-leaflet valve conduit shape.
- The freeze drying cycle optimisation was assessed using different concentrations and crosslinking methods for improved mechanical properties.
- We successfully developed a method of infiltrating 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.

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 homogenous 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 exerced by the cells.

References