Biomimetic Scaffold for Anterior Cruciate Ligament Tissue Engineering

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INTRODUCTION

The anterior cruciate ligament (ACL) is the most commonly injured ligament of the knee, with an estimated annual incidence of 1 per 1000 Americans1. It is estimated that 100,000 – 200,000 Americans require ligament replacement surgery annually, at a total cost of five billion USD. There are three options to replace the damaged tissue: autografts, allografts, and xenografts; with autografts of the patella or hamstring tendons considered the "gold standard"2. The use of these tissues is limited by a number of factors including availability of the tissue and risk of donor site morbidity3. The lack of a suitable ACL replacement has been a motivating force in the fields of tissue engineering.

Ligaments possess a crimp pattern, which is thought to explain the characteristic toe-region on a stress-strain curve during uniaxial extension. An engineered scaffold must have mechanical and architectural properties similar to native ACL tissue. Crimped polymer fibres have previously been fabricated by the Amsden research group, using a simple electrospinning technique and UV crosslinking4. Previous work from the group has shown that, while cells can attach and proliferate on the polymer scaffolds5, the degree of cellular attachment is insufficient for adequate transmission of mechanical signalling. A peptide sequence containing the RGD cell-binding domain has been reported to promote cell attachment and spreading of human ACL fibroblasts when used to modify scaffolds6. The GFOGER peptide sequence for collagen-binding, can be compared as a scaffold modification. While ACL fibroblasts (ACFs) are required for regeneration of the extra cellular matrix, their availability is limited and they are slow to proliferate. Studies have shown that co-culturing with stem cells increases the proliferation of the ACLFs8. Additionally, mechanical stimulation of the cells during proliferation has been shown to improve the resulting structure. By combining the successes of previous research, an improved scaffold may be produced.

The goal is to develop a scaffold which mimics the biological structure and surface properties of native ACL, co-seeded with ACFs and adipose-derived stem cells (ASCs), and cultured under dynamic conditions, to generate tissue resembling native ACL in structure and function.

RESULTS & DISCUSSION

Polymer Synthesis: Previous research from the Amsden group has demonstrated the potential of using a co-polymer made with L-lactic acid (LLA) and an acryloyl carbonate (AC), referred to as P(LLLAC)4. This polymer can be photo-crosslinked to stabilize the crimp structure, as well as increase the Young’s modulus of the scaffold. The monomer is first prepared using a four-step process, described by Chen et al. Using the AC monomer, a ring-opening polymerization was performed with L-lactide, resulting in P(LLLAC) (Figure 1).

**Figure 1: Ring-opening polymerization of LLA with the AC monomer.**

The polymerization results were confirmed using 1H NMR, shown in Figure 2. The target AC content was 25%, and the actual value was shown to be 15%. Results significantly lower than the target were consistent with previous work.

**Figure 2: 1H NMR spectrum showing P(LLLAC) product.**

Peptide Conjugation: In order to attach the desired peptide (GPDGGRGGYGGCG) to the fiber mats, a block polymer of PLLA or P(LLLAC) and polyethylene glycol (PEG) with a maleimide functional group was prepared. The AC group allows the polymer to attach to the scaffold matrix via crosslinking, while the maleimide group enables attachment of the cysteine on the peptide via Michael-type addition (Figure 3).

**Figure 3: Schematic description for polymerization of MAL-PEG-PDLLA, and its conjugation with the cysteine-containing peptide.**

The peptide was conjugated to both the MAL-PEG-PDLLA and MAL-PEG-P(LLLAC) polymers. 1H-NMR spectroscopy was used to confirm the conjugation (Figure 4).

**Figure 4: 1H-NMR spectra demonstrating conjugation of RGD peptide to MAL-PEG-PDLLA.** Conjugation was done in DMF, using 2 equiv. of peptide and 0.7 equiv. TCEP.

Electrospun Scaffolds: To produce polymer fiber scaffolds, the P(LLLAC) is electrospun from a mixture of DCM and DMF onto a rotating wire mandrel (Figure 5). For the polymer scaffolds to mimic native ACL tissue, the fibers must be uniaxially aligned. Collecting the fibers onto the rotating mandrel aids in this goal, as does manipulating the process variables (voltage, collector speed, weight % of polymer in solution, distance to collector), which must be optimized for different polymer formulations. The polymer fiber mats are removed from the wire mandrels, and then viewed under optical or scanning electron microscopy to determine fiber properties such as diameter and alignment.

**Figure 5: Schematic representation of electrospinning set-up and fiber crimping.**

Various polymer formulations have been tested to form electrospun fiber scaffolds, altering the process variables to align the fibers. For the polymer shown in Figure 4, a solution of 35 wt% in 3:1 DCM: DMF was used. The conditions were 15 kV, 15 cm from the needle to the collector, and a collector speed of 1000 rpm. Under these conditions, good fiber alignment was observed (Figure 6A). Attempts to electrospin polymer formulations including the peptide-conjugated MAL-PEG-P(LLLAC) have been performed; however alignment is not achieved (Figure 6B). The process parameters will be altered to improve fiber alignment.

**Figure 6A: SEM image demonstrating alignment of electrospun P(LLLAC) fibers, at a magnification of 1000X.** Figure 6B: SEM image of electrospun P(LLLAC) and RGD-PEG-PDLLA Scale bars: 50 μm.

CONCLUSION

A co-polymer of L-lactide and acryloyl carbonate (P(LLLAC)) can be prepared with a relatively high degree of acrylation. This polymer can then be electrosprun onto a rotating wire mandrel to produce fiber scaffolds. Through the manipulation of various process parameters, satisfactory fiber alignment can be achieved. This process will be particularly important with the introduction of the MAL-PEG-P(LLLAC), as it will also influence the properties of the electrospinning solution, and consequently the fiber alignment.

MAL-PEG-OH can be used to initiate the polymerization of lactide, resulting in a polymer capable of conjugating a cysteine-containing RGD peptide to the polymer scaffold. When the acrylated monomer is included in the polymerization, DBU cannot be used as a catalyst, but Ti(II) 2-ethylhexanoate is functional. The cysteine of the peptide can be conjugated to the maleimide functional group in DMF, with the presence of TCEP in 24 hours. The conjugated polymer can be co-electrospun with P(LLLAC) onto the rotating mandrel. With the inclusion of the peptide conjugated polymer, fiber alignment has not yet been achieved.

**REFERENCES**


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