Electrospinning for Ligament Tissue Engineering

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Introduction

A new approach for ligament regeneration of the anterior cruciate ligament (ACL) is being researched in response to an overwhelming need. Failure rates of the ACL are prompting over 200,000 surgeries in the United States each year [1]. Surgery is needed when the ACL is torn because the ligament itself has very poor healing capabilities as a result of being surrounded by synovium, a fluid like membrane that cushions joints during periods of impact. The synovium prevents an adequate supply of blood from circulating throughout the injured ACL so effective self-healing can occur. Current surgery options create new ligaments and involve autografts (from the patellar tendon, hamstring, or quadriceps), allografts, and xenografts. All of these surgery options have a significant number disadvantages such as graft donor site morbidity, limited autograft tissue supply, and infectious disease transfer. Synthetic grafts are being researched as an alternative to biological grafts, but they have also incurred a high number of mechanical failure rates as due to material fatigue [1]. Tissue Engineering could ultimately provide a surgery option for synthetic grafts that could be placed in the knee immediately after the ACL fails [2].

This proposal has been prompted due to a need for a crucial step in the current research of ligament tissue engineering. We currently know that silk fibroin is a very promising fiber for creating ligament engineering scaffolds. The best way to create a silk scaffold matrix is being evaluated. Extracted silk fibroin from the cocoon of the Bombyx mori silkworm has been used as a biomaterial in humans for decades, namely sutures. Only recently has it been used as a scaffold for cell culture and tissue engineering. Silk has excellent mechanical properties, proven biocompatibility, and slow degradability which make it a promising candidate for ligament tissue engineering. Silk fibroin will support stem cell adhesion, proliferation, and differentiation in vitro, as

well as promote tissue repair in vivo [1]. It is important for the silk scaffold to have proper mechanical properties to support ACL's linear stiffness and tensile strength. It is crucial for mechanical strength to be incorporated into the extracellular matrix (ECM) that is created on the scaffold in an equally distributed load so the regenerated ligament will be just as strong as the original ACL. A normal human ACL can withstand and ultimate tensile strength of 2160 N, and a linear stiffness of 242 N; these are large forces when compared to the length of a normal ACL, ranging from 28-31mm. This mechanical stabilization through ECM formation can be achieved with silk due to its slow degradation rate in vivo [2].

One newer method for creating a scaffold with high mechanical strength is a textile manufacturing process known as electrospinning. Electrospun pure silk fibroin scaffolds can be easily modified for cell adhesion and proliferation; this property makes electrospinning an obvious choice for scaffold creation. Furthermore, blending silk fibroin with other synthetic polymers may produce scaffolds with better healing properties. Some polymer blends have been found to promote in vivo healing when used as a wound dressing [1]. Polymer blended scaffolds could also produce more promising mechanical properties needed for ligament regeneration.

On a successful scaffold, stem cells will produce collagen, and the scaffold will properly support the cells' regeneration of ECM. Research shows that silk fibroin scaffolds support the attachment, spreading, proliferation, and differentiation of human mesenchymal stem cells (MSCs). After MSCs were seeded onto silk fibroin scaffolds and placed in static culture for 21 days, significantly high amounts of tenascin-C, collagen type I and collagen type III, all of which are needed for ligament regeneration, had formed on scaffold. Bone and cartilage related genes were not found on the scaffold, and this suggests that silk scaffolds are and excellent choice for reproducing cells needed for ligament regeneration [1]. However, it should be noted that these scaffolds were tested in static culture which does not entirely mimic biological conditions. This proposal would involve placing scaffolds in a rotating vessel bioreactor.

Methods

Silk fibroin will be extracted from Bombyx mori silkworm cocoons. The remaining pure silk fibers and silk fibers blended with bioresorbable polymers will be electrospun to produce aligned scaffolds. Adipose stem cells (ASCs) will be placed on each of the electrospun scaffolds.

Mechanical, histological, and collagen assay testing will be performed on the scaffolds at day 1, 7, 14, and 21. When scaffolds are not being tested they will be kept in a rotating bioreactor that will simulate biological conditions. For mechanical testing, a uniaxial tensile test will be performed to test the scaffolds until failure occurs. For histological testing, stain will be applied to the scaffolds that will decipher cell proliferation. For collagen assay testing, scaffolds will be dissolved and tested for newly generated collagen.

Possible Results and Implications

Mechanical testing will determine whether pure electrospun silk scaffolds or blended silk composites yield higher mechanical strength for further use in ligament tissue engineering. Histological and collagen assay testing will help determine if the proliferation and matrix production of stem cells occurs. These results will provide further insight into the optimal scaffold for ligament tissue engineering.

References

[1] Wang Y, et al 2006 Stem cell-based tissue engineering with silk biomaterials Biomaterials 27 6064-6082.

[2] Altman G, Kaplan D, et al 2002 Silk matrix for tissue engineered anterior cruciate ligaments Biomaterials 23 4131