Electrospinning Silk Fibroin Scaffolds for Ligament Tissue Engineering Applications

Rachel M. Beard
Virginia Commonwealth University Bioinformatics and Bioengineering Summer Institute 2009

Introduction

Silk fibroin has been shown to be a mechanically superior natural polymer for ligament tissue engineering applications for the anterior cruciate ligament (ACL) found in the knee. A tissue engineered silk-fiber matrix has been successfully designed to mimic the native ACL tissue and match the mechanical properties of the ligament when the matrix was tested using in vitro tissue culturing conditions.[1] Tissue engineering applications for ligament are currently being researched due to the high occurrence of ACL injuries and failures. Failure rates of the ACL are prompting over 200,000 surgeries in the United States alone each year.[2] Surgery is needed when the ACL is torn because the ligament has very poor self-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 to allow for effective self-healing. Current surgery options incorporate grafted ligaments through the use of autografts (from the patient's own patellar tendon, hamstring, or quadriceps), allografts (from cadavers), and xenografts (from another species). All of these surgery options have a significant number of disadvantages such as graft donor site morbidity, limited autograft tissue supply, infectious disease transfer, and severe post operative pain. Synthetic grafts are being researched as an alternative to biological tissue

---

[1] Altman G, Kaplan D, et al. Silk matrix for tissue engineered anterior cruciate ligaments, Biomaterials. 2002, 23: 4131-4141.

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

grafts, but they have also incurred a high number of mechanical failure rates as due to material fatigue.[3]

In response to these clinical findings, silk fibroin should be further explored as an option for designing a tissue engineered scaffold that will promote and support in vivo ligament tissue regeneration. Ultimately, a scaffold would be surgically implanted into the patient to induce ligament regeneration through host tissue ingrowth and constant biological remodeling throughout the scaffold matrix. Silk fibroin is a promising scaffold material for ligament because it is a strong biocompatible protein-based polymer that exhibits slow degradation rates. The slow biodegradation of silk fibroin would allow time for ingrowing host ligament tissue to offer increasing mechanical support for the system as mechanical loading transfered from the scaffold to the newly formed ligament tissue.[4] The goal of clinically implanting scaffolds has not yet occurred in humans, but preliminary findings hold promise for ligament scaffolding applications.

Electrospinning is a method that had been used in various biomedical applications, but one of its most useful applications is the formation of three-dimensional tissue engineered scaffolds. An electrospun scaffold for ligament engineering applications must incorporate mechanically supportive biocompatible polymers that will promote cellular interaction. The scaffold must present a large surface area-to-volume ratio so cells can infiltrate and lay down extracellular matrix. In this study, airgap electrospinning will be used in order to produce sheets of electrospun material, and scaffolds will be punched from these sheets. This type of electrospinning should produce fibers that are significantly smaller than those fibers produced using typical electrospinning methods that utilize rotating drums or collection plates. This airgap electrospinning process utilizes dual collection rings known to produce oriented fibers that are suspended between the two collection targets.[5] For ligament tissue engineering purposes, fibers must exhibit a

---

[3] Wang Y, et al 2006.

[4] Altman G, Kaplan D, et al. 2002.

[5] Dalton, PD, Klee D, Moller M. Electrospinning with dual collection rings, Polymer. 2005, 46: 611-614.

similar diameter range to that of native fibrils found in ligament extracellular matrix. Furthermore, the airgap electrospinning method can create thick scaffolds at faster rates compared to traditional electrospinning methods. Our lab has found that silk fibroin can be spun at rates as fast as 10 ml/hr using this electrospinning method.

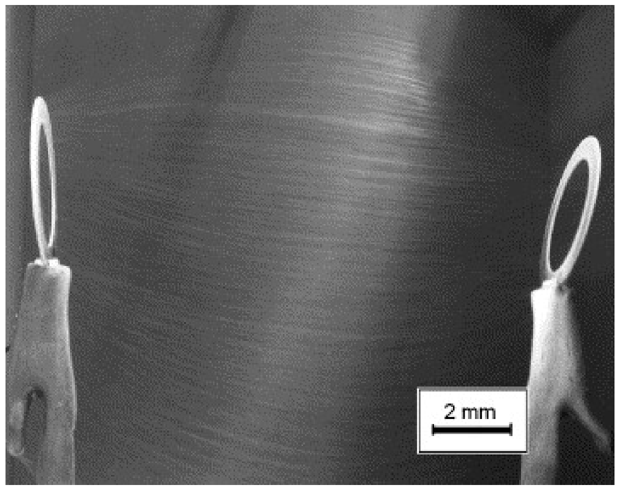

Figure 1: Dual collection rings produce a 3-D formation of electrospun fibers after 60 seconds of applied voltage using airgap electrospinning
[adapted from Dalton, PD, et. al. Polymer, 2005]

Methods

Silk fibroin will be extracted from the cocoons of Bombyx mori silkworms. Sericin, the gumming material found in natural silk fibers, will be removed because it can initiate an adverse immune response at the implantation site. Silk fibroin will then be dissolved in solvents of hexafluoroisopropanol (HFIP), water, and a combination of 50/50 HFIP/water ratio in order to determine variance between solvent properties used to create the scaffolds. Currently organic solvents and combinations of organic solvents are being used to dissolve polymers in solution before electrospinning. These organic solvents evaporate away from the electrospinning polymer solution as it is ejected from the needle tip. However, organic solvents are highly toxic and will cause problems to the central nervous system if present in large amounts. The effects of spinning from organic solvents may present some unwanted effects on cells or tissues that come in contact with the scaffold. Therefore electrospinning from an organic solvent, water, and a 50/50 ratio of the organic solvent and water will create scaffolds that can be tested for average porosity, average permeability, average fiber diameter, and mechanical properties to determine if there are any statistical differences resulting from the solvents used to dissolve the silk fibroin. Average fiber diameter will be measured using software and scanning electron microscopy images. Mechanical properties will be measured using an MTS uniaxial tensile testing device.

Furthermore, a cell study will be carried out for 3 weeks to determine if there are any mechanical property and cellular interaction differences between various electrospun silk fibroin scaffolds dissolved in HFIP, water, and the 50/50 HFIP to water ratio. Dermal fibroblasts will be used in this study as they are responsible for the formation of the extracellular matrix components and collagen necessary for ligament regeneration. Fibroblasts will be seeded onto scaffolds and placed in fibroblast growth medium. Cells will be incubated at 37°C with levels of 5% CO_2. Fibroblasts will be used to determine if silk fibroin scaffolds spun from 3 different solvents act as an appropriate host for cell infiltration. Scaffolds will be stained using a live/dead assay kit to determine if fibroblasts are alive and penetrating the scaffold material. Confocal microscopy will produce three-dimensional imaging of the scaffolds to show fibroblasts at day 1, 7, 14, and 21. Mechanical testing will also be performed on the scaffolds at day 7, 14, and 21 to determine which scaffolds are experiencing in vitro biodegradation that will decrease tensile strength or if they are experiencing ECM collagen production that could increase the tensile properties of the scaffold matrix.

Possible Results and Implications

The effects of solvents on electrospun materials has been investigated to determine that both the dimensions and secondary structure of silk fibroin nanofibers were affected by the organic solvent chosen to make the solvent solution prior to electrospinning.[6] However,

---

[6] Jeong L, Lee KY, Park WH. Effect of solvent on the characteristics of electrospun regenerated silk fibroin nanofibers. Key Engineering Materials, 2007, 342-343: 813-816.

elimination of toxic organic solvents from polymer electrospinning solutions would be the best option for electrospinning silk fibroin if the water based solutions could produce small electrospun nanofiber scaffolds with high surface area-to-volume ratio. Furthermore, the electrospun water solution based scaffolds should equal or exceed the mechanical properties of scaffolds created from HFIP to merit further studies using water as a solvent for electrospinning silk fibroin.

References

Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, Lu H, Richmond J, Kaplan DL. Silk-based biomaterials, Biomaterials. 2003, 24: 401-416.

Altman G, Kaplan D, et al. Silk matrix for tissue engineered anterior cruciate ligaments, Biomaterials. 2002, 23: 4131-4141.

Dalton, PD, Klee D, Moller M. Electrospinning with dual collection rings, Polymer. 2005, 46: 611-614.

Jeong L, Lee KY, Park WH. Effect of solvent on the characteristics of electrospun regenerated silk fibroin nanofibers. Key Engineering Materials, 2007, 342-343: 813-816.

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