Design, Development and Biomechanical Analysis of Scaffolds for Augmentation of Rotator Cuff Repairs

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DESIGN, DEVELOPMENT AND BIOMECHANICAL ANALYSIS OF SCAFFOLDS FOR AUGMENTATION OF ROTATOR CUFF REPAIRS

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Dedicated in loving memory of my father

Late Dr. Vijay Kumar Aurora
ACKNOWLEDGEMENTS

“It takes a village to raise a child”, is a famous adage from the Igbo and Yoruba regions of Nigeria. Likewise, it takes the dedication, effort and guidance of a number of people to graduate a doctoral candidate and I would like to thank those people without whose effort this work would have not been accomplished.

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Design, Development and Biomechanical Analysis of Scaffolds for Augmentation of Rotator Cuff Repairs

ABSTRACT

AMIT AURORA

Rotator cuff tears are a source of debilitating pain that commonly affects more than 40% of our aging population. Despite advances in surgical treatment, the failure rate of rotator cuff repairs is as high as 20-90%. Extracellular matrix (ECM) derived scaffolds have recently been investigated as augmentation devices for rotator cuff repairs, but none has yet demonstrated both the appropriate biological and mechanical properties for mitigating re-tears and enhancing healing.

This dissertation proposes to engineer the mechanical properties of allograft fascia lata in a manner that will allow its use as an augmentation device for rotator cuff repairs. This dissertation also aims to develop a simple quasi-linear spring-network model for rotator cuff repairs to elucidate the basic biomechanics of these repairs. The central hypothesis is that engineered fascia lata will have suture retention strength similar to that of human rotator cuff tendon (~250N), even after in vivo implantation. The specific aims are to engineer the mechanical properties of allograft fascia lata ECM and to subsequently evaluate the host response and concomitant mechanical properties of the engineered (reinforced) fascia in a rat model. Further, this dissertation will also develop and validate a spring-network model for simplified rotator cuff repairs.

Studies presented in this dissertation demonstrate stitching as a technology to engineer the suture retention and stiffness of allograft (human derived) fascia lata ECM. Stitching fascia ECM with braided, resorbable, polymer fibers in a unique, controlled
manner increased the suture retention load of reinforced fascia scaffolds by six fold over non-reinforced fascia. Additionally, the suture retention properties of reinforced fascia scaffolds were comparable to that of human rotator cuff tendon (~250N) at time zero and even after *in vivo* implantation for twelve weeks. Except for the increased presence of foreign body giant cells in areas concentrated around the polymer fibers, the host response of the reinforced fascia scaffolds were comparable to the non-reinforced fascia at the time points investigated. The spring-network model predicted that the scaffold component carries ~20-30% of the total load on the repair. Parametric sensitivity analysis predicted that greatest improvements in the force carrying capacity of the repair may be achieved by improving the properties of the tendon-to-bone repair. Parametric simulation studies suggested that in the clinical setting of a weak tendon-to-bone repair, scaffold augmentation could significantly off-load the repair and largely mitigate the poor construct properties. However, engineering a scaffold with supra-physiologic stiffness would not translate into stiffer or stronger repairs.

The results of this dissertation show that reinforced fascia scaffolds may have and possibly maintain mechanical properties comparable to the suture retention properties of human rotator cuff tendon. This suggests that reinforced fascia scaffolds may be able to provide mechanical augmentation to rotator cuff repairs and also modulate tendon retraction in a manner that reduces the incidence of tendon re-tear. The spring-network model provides a starting point to develop more clinically relevant models for rotator cuff repairs.
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Chapter I

BACKGROUND AND SIGNIFICANCE

I have yet to see any problem, however complicated, which, when you looked at it the right way, did not become still more complicated…Paul Anderson, New Scientist

1.1 Rotator Cuff Tears

Rotator cuff tears are a common source of debilitating pain, weakness and reduced shoulder function, which can lead to degenerative changes in the glenohumeral joint over time. An estimated thirty to seventy five thousand rotator cuff surgeries are performed annually in the United States, the treatment, evaluation and management of which puts an annual financial burden of 7 billion US Dollars on the United States economy.

The complex anatomy of the shoulder, contact of the torn rotator cuff with the synovial fluid milieu and the relative hypovascularity of tendons contribute to the impaired healing of these tears and impose significant challenges in the treatment of the disorder. Currently, open and arthroscopic surgical repair is accepted as the “gold” standard for the treatment of tears that fail to improve even after conservative treatment.
Despite improvements in the surgical treatment and our understanding of the etiology of these tears, rotator cuff re-tear rates ranging from 20-90% ³, ¹⁸, ¹⁹, ⁵⁴, ⁵⁶, ⁵⁹, ⁷² have been reported due to factors not restricted to biological, anatomic and mechanical factors, which include inferior tissue quality, tendon retraction, muscle atrophy and fatty infiltration, undue tension at the repair site in the early post-operative period and the synovial fluid environment ¹⁵, ²⁴, ⁴⁹, ⁵⁴, ⁶⁰, ⁸³, ⁸⁶, ⁹⁶, ⁹⁷. Furthermore, recurrent and chronic rotator cuff tears may not be repairable. Treatment of symptomatic irreparable tears is extremely challenging and limited to partial cuff repair ²⁰, surgical debridement with no repair ⁵⁷, ⁷⁷, ⁸⁴ and physical therapy with no surgery ⁵⁶ or major reconstructive procedures such as muscle transfers ³⁷. Hence, there is a need for repair strategies that can augment the repair by mechanically reinforcing it, while at the same time biologically enhancing the intrinsic healing potential of the patient ², ⁸⁵. Tissue engineering offers a viable alternative for the treatment of rotator cuff tears with the aim of restoring tissue and joint function.

![Tissue engineering paradigm](image)

**Figure 1.1:** Tissue engineering paradigm
1.2 Tissue Engineering

Tissue engineering seeks to merge engineering and biology towards the development of biological substitutes that will repair, or replace tissues and/or organs by delivering implanted cells, scaffolds, growth factors or any combination thereof at the time of surgery \(^{22, 50}\) (Figure 1.1). Currently, synthetic and extracellular matrix (ECM) derived scaffolds are extensively being investigated as a tissue engineered strategy for the treatment of rotator cuff tears.

1.3 Scaffolds for Rotator Cuff Repair

The rationale for using a scaffold for rotator cuff repair may include reinforcement of the repair and improvement in the rate and quality of biologic healing \(^{33}\). Thus far, scaffolds derived from synthetic biomaterials, human (allografts) and animal sources (xenografts) have been investigated and developed for clinical use. Table 1.1 and 1.2 gives a list of commercially available scaffolds derived from various natural and synthetic biomaterials that are being marketed as augmentation devices for rotator cuff repairs at the time of surgery, i.e., the scaffold is applied over the primary tendon repair. The US Food and Drug Administration (FDA) has cleared these devices “to support soft tissues where weakness exists” but not “to provide the full mechanical strength for the tendon repair”

1.3.1 Synthetic Scaffolds

Rotator cuff repair with scaffolds derived from poly(urethane urea), polylactic acid \(^{5, 36, 65, 72}\), polytetrafluoroethylene \(^{63}\), chitin \(^{41}\), chitosan-hyaluronan \(^{40}\) and polycarbonate polyurethane polymer \(^{26}\) have been studied in animal models over the past
decade. Of these scaffolds, only biodegradable poly (urethane urea) and polylactic acid scaffolds have been presently cleared by the FDA for the clinical use (Table 1.1). A detailed description of several of these products can be found in recent publications.6, 28, 35, 36, 75, 98.

Synthetic scaffolds can be fabricated into three-dimensional scaffolds of variable structure and porosity with a wide range of mechanical and degradation properties.36, 48, 75 Ideally host neo-matrix tissue deposition would occur at the same rate as the degradation of the synthetic scaffold material thereby maintaining mechanical integrity while functional remodeling occurs. However, the success of these synthetically derived constructs has been limited due to rapid scaffold degradation kinetics as compared to neo-tissue formation/deposition.48 Additionally, synthetic scaffolds are associated with limited tissue formation and incorporation into native tissue as well as a persistent low-level inflammatory response. Thus, while synthetic scaffolds have a distinct advantage of mass production with control over initial strength, rates of degradation and microstructure (e.g. three dimensionality, specified porosity, and shape) one definite drawback is the lack of biological recognition by the host leading to inferior tissue regeneration.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Material</th>
<th>Marketed By</th>
</tr>
</thead>
<tbody>
<tr>
<td>SportMesh™ Soft Tissue Reinforcement</td>
<td>Poly(urethaneurea)</td>
<td>Biomet Sports Medicine</td>
</tr>
<tr>
<td>X-Repair®</td>
<td>Poly-L-Lactide (PLLA)</td>
<td>Synthasome</td>
</tr>
</tbody>
</table>

Table I 1: Synthetic scaffold devices with FDA clearance for rotator cuff repair (Adapted from Table I in Derwin et al., 33)
1.3.2 Extracellular Matrix (ECM) Derived Scaffolds

Extracellular matrix derived scaffolds are natural occurring biomaterials that have a three dimensional architecture with inherent structural and functional proteins, which include but are not restricted to collagen, elastin, proteoglycans and glycosaminoglycans (GAGs). ECM derived biomaterials also contain macromolecules like vascular endothelial growth factor, basic fibroblast growth factor and transforming growth factor β1, which may mediate inflammation as well as direct host functions, such as cell migration and proliferation, collagen deposition and angiogenesis. Further, the degradation products of the molecules that constitute the ECM appear to mediate subsequent remodeling events. In other words, ECM derived scaffolds may possess many of the characteristics desired in an ideal tissue engineered scaffold for regenerative medicine. These features of ECMs have garnered the interest of the orthopedic community, making ECM technology the most commonly utilized tissue engineering strategy to improve the functional outcomes of rotator cuff tears. Presently, ECM scaffolds derived and processed from different origins of human and non-human sources have been cleared by the FDA to reinforce rotator cuff repairs at the time of surgery (Table 1.2). A detailed description of these materials can be found in recent publications.
<table>
<thead>
<tr>
<th>Product Name</th>
<th>ECM Type</th>
<th>ECM Source</th>
<th>Marketed By</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restore®</td>
<td>SIS</td>
<td>Porcine</td>
<td>Depuy Orthopaedics</td>
</tr>
<tr>
<td>CuffPatch™</td>
<td>SIS (crosslinked)</td>
<td>Porcine</td>
<td>Organogenesis</td>
</tr>
<tr>
<td>GraftJacket®</td>
<td>Dermis</td>
<td>Human</td>
<td>Wright Medical</td>
</tr>
<tr>
<td>Conexa™</td>
<td>Dermis</td>
<td>Porcine</td>
<td>Tornier</td>
</tr>
<tr>
<td>TissueMend®</td>
<td>Dermis (fetal)</td>
<td>Bovine</td>
<td>Stryker Orthopaedics</td>
</tr>
<tr>
<td>Zimmer® Collagen</td>
<td>Dermis (crosslinked)</td>
<td>Porcine</td>
<td>Zimmer</td>
</tr>
<tr>
<td>Repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Blanket®</td>
<td>Dermis (crosslinked)</td>
<td>Bovine</td>
<td>Kensey Nash</td>
</tr>
<tr>
<td>OrthADAPT™ Bioimplant</td>
<td>Pericardium</td>
<td>Equine</td>
<td>Pegasus Biologics</td>
</tr>
</tbody>
</table>

Table 1.2: Extracellular Matrix (ECM) derived scaffold devices with FDA clearance for rotator cuff repair. Small Intestinal Submucosa (SIS) (Adapted from Table I in Derwin et al., 33)

Hence, the aim of this chapter is to review the current state of knowledge regarding the use of extracellular matrix derived (ECM) scaffolds for rotator cuff repair including the results of in-vitro biomechanical and biochemical properties, results of pre-clinical models used to evaluate the efficacy of ECM scaffolds as augmentation devices and outcomes of clinical trials using ECM scaffolds for the treatment of rotator cuff tears. The indications for clinical use of these scaffolds will then be presented. The review will conclude with concluding remarks that will provide suggestions for future investigations. Finally, two potential areas of research will be identified and the chapter will conclude with the specific aims of the proposed research.
1.4 *In-vitro* Properties of ECMs

1.4.1 Biomechanical Properties

Both, the mechanical and suture retention properties of a scaffold will influence the extent to which a scaffold can augment the mechanical properties of a tendon repair at the time of implantation (time zero). To serve as an augmentation device, scaffolds must have the necessary mechanical and suture retention properties to withstand the high *in vivo* tensile loads on the repair and resist suture failure at the scaffold-bone and/or scaffold-tendon-suture interface.

The stiffness (approximately 200 N/mm) and ultimate load (approximately 800 N) of human rotator cuff tendon strips have been reported previously. The suture retention properties of human rotator cuff tendon were shown to be approximately 230 N for two mattress sutures subjected to a graduated cyclic load protocol and 224 ± 77 N for one mattress stitch pulled to failure (n = 8, unpublished data from Derwin laboratory). These properties have been suggested to be used as targets that might guide our choice of scaffolds for rotator cuff repair, when mechanical augmentation at time zero is desired.

Previously, Derwin et al., performed uniaxial tension tests (unconstrained) on thin strips of commercially available ECM scaffold materials. These tests demonstrate that small intestinal submucosa (SIS) and dermis derived scaffolds stretch considerably (Figure 1.2) and have material properties (modulus of elasticity) an order of magnitude lower than tendon (Table 1.3). In contrast, fascia lata has material properties comparable to canine infraspinatus tendon and the human supraspinatus tendon (Figure 1.2, Table 1.3). The structural properties (stiffness) of SIS and dermis derived scaffolds are also
more compliant than tendon. In comparison, fascia lata has stiffness similar to that of human infraspinatus tendon (97 - 171 N/mm)\textsuperscript{53}.

Figure 1.2: Representative stress versus grip-to-grip strain curves for 4 mm wide ECM strips as compared to normal canine infraspinatus tendon. The dotted lines were added to demonstrate that the failure point is underrepresented in all curves because all failures occurred at the grip. (Adapted from Figure 1 in Derwin et al.,\textsuperscript{35})
<table>
<thead>
<tr>
<th>Graft Material</th>
<th>Linear Region Strain* (%)</th>
<th>Linear Modulus** (MPa)</th>
<th>Linear Stiffness*** (N/mm)</th>
<th>Suture Failure Load (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infraspinatus tendon (canine)</td>
<td>5-12</td>
<td>405±86</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Allograft Fascia Lata</td>
<td>3-11</td>
<td>304±52</td>
<td>98.2±16.2</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Restore</td>
<td>22-25</td>
<td>35.5±9.1</td>
<td>7.0±1.5</td>
<td>38.2 ± 2.8</td>
</tr>
<tr>
<td>CuffPatch</td>
<td>20-22</td>
<td>40.1±15</td>
<td>6.8±2.3</td>
<td>32 ± 4.1</td>
</tr>
<tr>
<td>GraftJacket</td>
<td>53-93</td>
<td>22.5±5.3</td>
<td>16.4±5.9</td>
<td>229 ± 72</td>
</tr>
<tr>
<td>TissueMend</td>
<td>37-53</td>
<td>15.2±3.5</td>
<td>7.4±1.4</td>
<td>76 ± 21.5</td>
</tr>
<tr>
<td>Zimmer Collagen Patch</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>128 ± 26.3</td>
</tr>
</tbody>
</table>

Table 1. 3: Biomechanical properties of commercially available ECMs (mean ± SD). The table is compiled from Barber et al 14, Derwin et al 34 and Derwin et al 35. The statistical comparisons among groups are given in the respective publications. * Grip-to-grip strain ** Linear modulus was determined from 4 x 30 mm test strips using grip-to-grip strains.*** Linear stiffness was determined from 12 x 30 mm test strips using grip-to-grip strains. # Suture retention loads using GraftJacket Extreme and TissueMend 1.2, respectively. Suture retention load of allograft fascia lata (unpublished data from Derwin laboratory). (Adapted from Table II in Aurora et al., 6)

However, modulus and stiffness alone are insufficient to predict the biomechanical utility of an ECM scaffold. The suture retention properties of a scaffold also influence the extent to which a scaffold can augment the mechanical properties of a tendon repair at the time of implantation (time zero). Barber et al., have shown that dermis derived scaffolds have superior suture retention strength (~200 N) compared to SIS scaffolds (~40 N) 14. Our laboratory has shown that fascia lata has poor suture retention properties (~10 N) compared to other commercially available ECM scaffolds (unpublished data).
Together, these mechanical studies help evaluate the extent to which ECM scaffolds have the ability to “off-load” the repair at the time of implantation. These studies suggest that in their present configuration SIS and dermis derived scaffolds may not be capable of providing appreciable mechanical augmentation to rotator cuff repairs at time zero. Further, even though, fascia lata has material properties comparable to tendon, its poor suture retention properties limit its use as an augmentation device for rotator cuff repairs. It is important to note that the in-vitro mechanical properties are only applicable at the time of implantation. A loss of mechanical and suture retention properties is expected when scaffolds are subjected to the in vivo biological milieu. However, the rate and extent of mechanical changes will in part depend on the nature of tear, scaffold material, its remodeling characteristics, rehabilitation protocol (mechanical loading) and in vivo environment.

1.4.2 Biochemical Properties

ECM derived scaffolds are subjected to an acellularization treatment, which is intended to remove water-soluble cellular proteins in order to reduce antigenicity\(^\text{35, 45}\), disrupt cells and reduce the DNA content. However, recent reports\(^\text{34, 35}\) have shown that show that except for fascia lata that has small, but measurable amounts of DNA, dermis derived (GraftJacket and TissueMend) and SIS derived ECM scaffold (Restore, CuffPatch) contain appreciable amounts of DNA. Based on hydroxyproline content, the collagen content (Type I) of the ECM scaffolds is estimated at 60 to 95% of their dry weight\(^\text{35}\), demonstrating that like tendon, the primary matrix constituent of these ECMs is also collagen. Additionally, processed ECMs have similar chondroitin/dermatan sulfate (CSDS) glycosaminoglycan (GAG) content as fresh tendon. Table 1.4 lists the
biochemical properties of commercially available ECM scaffolds. The influence of glycosaminoglycan (GAG) on tissue regeneration has not been studied directly; however, GAGs are known to modulate the healing of soft tissues in several different ways including organizing the deposition of collagen fibers\textsuperscript{64, 70}, stimulating angiogenesis\textsuperscript{68}, inhibiting coagulation\textsuperscript{17, 68} and initiating cell and tissue proliferation\textsuperscript{39} and differentiation\textsuperscript{71}. Hence, the presence of relative amounts of GAG in ECM scaffolds will most likely favorably impact the host response to the scaffold \textit{in vivo}.

<table>
<thead>
<tr>
<th>Graft Material</th>
<th>DNA (ng/mg dry wt)</th>
<th>CSDS GAG (µg/mg dry wt)</th>
<th>Hyaluronan (µg/mg dry wt)</th>
<th>Hydroxyproline (mg/mg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infraspinatus Tendon (Canine)</td>
<td>-----</td>
<td>0.84 ± 0.24</td>
<td>----</td>
<td>0.110 ± 0.004</td>
</tr>
<tr>
<td>Allograft Fascia Lata</td>
<td>66 ± 43</td>
<td>0.61 ± 0.30</td>
<td>0.31 ± 0.19</td>
<td>0.114 ± 0.014</td>
</tr>
<tr>
<td>Restore</td>
<td>526.8 ± 125.6</td>
<td>0.96 ± 0.22</td>
<td>0.78 ± 0.22</td>
<td>0.102 ± 0.008</td>
</tr>
<tr>
<td>CuffPatch</td>
<td>0.6 ± 0.6</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.112 ± 0.015</td>
</tr>
<tr>
<td>GraftJacket</td>
<td>134.3 ± 44.0</td>
<td>0.27 ± 0.15</td>
<td>0.40 ± 0.08</td>
<td>0.078 ± 0.013</td>
</tr>
<tr>
<td>TissueMend</td>
<td>794.6 ± 97.8</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.122 ± 0.009</td>
</tr>
<tr>
<td>Zimmer Collagen Repair Patch</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

\textit{Table 1.4: Biochemical properties of commercially available ECMs (mean ± SD).} The table is compiled from Derwin et al\textsuperscript{34} and Derwin et al\textsuperscript{35}. The statistical comparisons among groups are given in the respective publications. (Adapted from Table III in Aurora et al., \textsuperscript{6})
In summary, these studies demonstrate that processing does not completely acellularize the ECM scaffolds. The clinical implications of incomplete acellularization of these ECM scaffolds are not known, but it is believed that the cellular remnants may increase antigenicity to the scaffolds \textit{in vivo}. Amongst the available ECM scaffolds, only allograft fascia lata seems to have a low DNA content and a biochemical make-up that is similar to that found in canine flexor tendon.

1.5 Pre-clinical Studies of ECMs

1.5.1 Host Response (Biocompatibility)

The \textit{in vivo} remodeling characteristics of dermis and SIS derived ECM scaffolds were compared in a rat abdominal wall model at various time points out to 16 weeks \textsuperscript{98}. All ECM scaffolds were associated with an early intense cellular response. However, each scaffold elicited a distinct morphological response that depended on the scaffold origin and processing technique. SIS-derived, non-cross-linked (Restore) showed rapid and intense cell-infiltration, angiogenesis and tissue remodeling at early time points. By 16 weeks, these scaffolds were largely degraded and replaced with a mixture of organized muscle cells, collagenous connective tissue, and small islands of adipose tissue. Non-crosslinked dermis derived scaffold, GraftJacket, initially (out to 8 weeks) degraded slowly and was associated with chronic inflammation. By 16 weeks, the scaffold showed partial material degradation with the replacement by moderately organized dense collagenous tissue. The host response of ECM scaffolds that were crosslinked (CuffPatch, TissueMend and Permacol) was consistent with the classical response to non-resorbable foreign materials, namely, low-grade chronic inflammation, minimal scaffold
degradation, fibrous encapsulation, presence of foreign body giant cells and/or accumulation of dense, poorly organized fibrous tissue.

Recent studies using primate body wall model have shown that non-crosslinked porcine and human dermis derived ECM demonstrate a good remodeling response and robust cellular infiltration over time \(^{87,100}\). More recently, a biopsy specimen of human derived scaffold (GraftJacket) retrieved from a patient three months after rotator cuff repair augmentation showed that graft material was intact and filled with numerous elastic fibers and blood vessels \(^{94}\). There was little to no inflammatory response along with extensive host cellular infiltration that was evident along the margins of the graft. The orientation of the collagen fibers indicated early organization of new tissue.

Together these studies demonstrate that ECM scaffolds elicit a distinct histologic and morphologic response, which most likely depends on the processing technique, tissue origin and species, method of terminal sterilization and mechanical loading environment. Further, the temporal sequence of remodeling events of extracellular matrix devices, including the rate and extent of scaffold degradation, incorporation, and/or host tissue deposition is likely to be predictive of the clinical course and may dictate the optimal rehabilitation protocol and functional outcome of the clinical repair procedure \(^{33}\).

1.5.2 Animal Models for Rotator Cuff Repair

Animal models have played an essential role part in the preclinical evaluation of ECM scaffolds for musculoskeletal soft tissue repairs that include Achilles tendon, flexor and rotator cuff tendons, knee joint ligaments and meniscus to name a few \(^{4,8,11,12,27,32,44,66,78,99}\). It is important to point out that the actual commercial ECM scaffolds are not always identified in animal models, but rather a generic device is prepared for research
purposes. Thus far, most studies using animal models have investigated the use of ECM scaffolds as interpositional devices (scaffold bridging tendon and bone) \(^3, 23, 30, 31, 74, 80, 88, 101\). This section only discusses studies that have investigated the use of ECM scaffolds as augmentation devices (scaffold placed over tendon repair), the indication for which they have approved by the FDA. Table 1.5 list the animal models done using ECM scaffolds either as interpositional (i.e., scaffold forms a bridge between torn tendon and bone) or augmentation devices (i.e., scaffold is applied over primary tendon repair).

<table>
<thead>
<tr>
<th>Product</th>
<th>Author</th>
<th>Indication</th>
<th>Model</th>
<th>Tendon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restore</td>
<td>Chen et al.</td>
<td>Interpositional</td>
<td>Rabbit</td>
<td>Supraspinatus</td>
</tr>
<tr>
<td></td>
<td>Dejardin et al.</td>
<td>Interpositional</td>
<td>Canine</td>
<td>Infraspinatus</td>
</tr>
<tr>
<td></td>
<td>Nicholson et al.</td>
<td>Augmentation</td>
<td>Ovine</td>
<td>Infraspinatus</td>
</tr>
<tr>
<td></td>
<td>Perry et al.</td>
<td>Interpositional</td>
<td>Rat</td>
<td>Supraspinatus</td>
</tr>
<tr>
<td></td>
<td>Schlegel et al.</td>
<td>Augmentation</td>
<td>Ovine</td>
<td>Infraspinatus</td>
</tr>
<tr>
<td></td>
<td>Zheng et al.</td>
<td>Interpositional</td>
<td>Rabbit</td>
<td>Supraspinatus</td>
</tr>
<tr>
<td></td>
<td>Zalvaras et al.</td>
<td>Interpositional</td>
<td>Rat</td>
<td>Supraspinatus</td>
</tr>
<tr>
<td>GraftJacket</td>
<td>Adams et al.</td>
<td>Interpositional</td>
<td>Canine</td>
<td>Infraspinatus</td>
</tr>
<tr>
<td></td>
<td>Ide et al.</td>
<td>Interpositional</td>
<td>Rat</td>
<td>Supraspinatus</td>
</tr>
<tr>
<td><em>Zimmer Collagen Repair</em></td>
<td>Nicholson et al.</td>
<td>Augmentation</td>
<td>Ovine</td>
<td>Infraspinatus</td>
</tr>
<tr>
<td><em>Patch (Permacol)</em></td>
<td>Fresh autograft fascia lata</td>
<td>Interpositional</td>
<td>Rabbit</td>
<td>Supraspinatus</td>
</tr>
</tbody>
</table>

**Table 1.5:** Pre-clinical studies using ECM derived scaffolds for rotator cuff repair. (Adapted from Table II in Longo et al., \(^69\))

Schlegel et al., used a SIS patch (10 by 20 mm) to augment a full thickness infraspinatus tendon repair in an ovine shoulder model \(^90\). The control was tendon repair without scaffold. The improvements in the biomechanical outcomes were not
investigated in this study. However, the authors reported that none of the repairs (with or without SIS augmentation) remained intact, demonstrating that SIS augmentation was insufficient to prevent anatomic failure of rotator cuff tendon repair in this animal model.

More recently, Nicholson et al., investigated the effect of repair augmentation with SIS derived (Restore) and cross-linked porcine dermis derived scaffold in a partial width infraspinatus tendon injury model in sheep \(^{79}\). The authors report an improvement in the biomechanical force of the augmented repairs compared to control (no scaffold) repairs at 9 weeks. The dermis derived scaffold showed signs of chronic inflammation, minimal scaffold degradation and integration with the surrounding tissue, while SIS derived scaffolds showed evidence of resorption and remodeling. At 24 weeks, no differences in the biomechanical force were reported between groups. The tendon-to-bone insertion site of the augmented repairs showed signs of maturation at 24 weeks. These findings seem to be in contradiction to a recent host response study in a rat model wherein the authors found the cross-linked porcine dermis derived scaffold to be associated with a classical response to non-resorbable foreign materials at 16 weeks. These differences may be due to differences in the animal species (ovine vs. rat), application of use (tendon repair vs. body wall) and also duration of the study (24 weeks vs. 16 weeks).

These studies though significant use different models of tendon injury which restricts our ability to compare the \textit{in vivo} performance of the two scaffolds. Various researchers have recommended the standardization of injury model and surgical technique; study design and outcome measures that will help provide meaningful comparison and interpretation of animal studies \(^{1,33}\)
1.5.3 Human Shoulder Repair Studies

The performance of the scaffold as an augmentation device is in part also dependent on the surgical method of scaffold application, which includes the number, type and location of fixation sutures and pre-tensioning of scaffolds at the time of repair. Hence, human cadaver models are being used to evaluate the biomechanical efficacy of scaffolds as augmentation devices of primary tendon repair at time zero \(^{13, 75}\). Barber et al., report the only human cadaver model to date that investigates the biomechanical performance of dermis derived, GraftJacket, as augmentation device for rotator cuff repairs. The study demonstrated a ~ 27% increase in the failure load and fewer failures at the tendon-suture interface for full thickness supraspinatus repairs augmented with GraftJacket \(^{13}\).

1.6. Clinical Studies

Restore (porcine SIS), GraftJacket (non-crosslinked human dermis) and Zimmer Collagen Repair (cross-linked porcine dermis) are the only ECM products that have been have been clinically investigated as interpositional \(^{38, 95}\) and augmentation devices for the treatment of rotator cuff tears in the peer-review literature. This section will briefly only discuss the clinical studies that have used scaffolds as augmentation devices.

1.6.1 Restore

Metcalf et al., demonstrated improved post operative outcomes for patients treated for massive chronic rotator cuff tears with Restore SIS as an augmentation device compared to their pre-operative condition in a two year follow-up study. However, more recently, clinical studies have reported formation of non-infectious edema, swelling, pain
and increased skin temperature around the wound when using the Restore device. Zheng et al., have postulated these adverse outcomes are due to the existence of porcine cellular elements in the Restore device. The findings of these clinical trials suggest that uncross-linked porcine SIS Restore is not suited for repair augmentation of large to massive rotator cuff tears in the human condition.

1.6.2 GraftJacket and Zimmer Collagen Repair Patch (Permacol)

Burkhead et al., conducted a follow-up study (mean follow-up 1.2 years) of seventeen patients with massive rotator cuff tears, repaired and subsequently augmented with GraftJacket using a standardized open repair technique. They reported an improvement in the post-operative subjective pain and functional scores, as well as in the measured range of motion and strength were noted in the study population. Additionally, no infections, sterile inflammatory reactions or other complications were observed.

Bond et al., followed (mean follow-up 2.3 years) 16 patients with massive, contracted, immobile rotator cuff tears treated by GraftJacket augmentation using an arthroscopic repair technique. Statistically significant improvements were also seen in the post-operative UCLA scores, pain, forward flexion and external rotation strength. Post-operative imaging in thirteen patients demonstrated complete incorporation of the graft into the native tissue.

Badhe et al., conducted a follow-up study (mean follow-up 4.5 years) of ten patients with massive rotator cuff tears, augmented with Zimmer Collagen Repair patch (Permacol), using a standardized open repair technique. An improvement in the post-operative subjective pain and functional scores, as well as in the measured range of
motion and strength were noted in the study population. Additionally, no infections, sterile inflammatory reactions or other complications were observed.

To summarize, dermis derived scaffolds may be appropriate for augmentation of large to massive rotator cuff tears. However, each of these studies is limited by the lack of cohort of control patients who underwent repair but did not receive a scaffold. Further, Zimmer Collagen Repair patch when used an interpositional graft demonstrated synovitis inflammatory exudate, which may be due to cross-linking. Taken together these studies highlight the need of randomized clinical trials that will allow proper interpretation of the results and help evaluate the clinical efficacy of ECM scaffolds for the treatment of rotator cuff tears.

### 1.7 Indications for Clinical Use of ECM Scaffolds

The paucity of controlled, human trials using ECM scaffolds makes it challenging to define the best indications for their use. Based on the geometry and reparability of the tear, Derwin et al., have proposed six grades of rotator cuff pathology that can be assigned clinically (Table 1.6). They have recommended the use of an appropriate ECM scaffold as an augmentation device for Grade III and IV tears and as an interposition device in selected cases of Grade V disease. (At the current time, only ECM scaffolds derived from human sources have been cleared by the FDA as interpositional devices). It is suggested that the use of any scaffold is warranted in cases of chronic, medium, large-to-massive tears that are surgically reparable, but which otherwise have a high risk not to heal. Since primary repair (repair of tendon to bone) of small and
medium-sized acute cuff tears are likely to heal with proper surgical and post operative care in 90% of cases, the use of ECM scaffolds is not justifiable for such cases.

It should be borne in mind, that the proper selection and use of ECM scaffolds do not guarantee the clinical success of rotator cuff repairs. There are myriad of factors that might affect tendon to bone healing, which include size of the tear, degree of muscle atrophy, tendon quality, passive tension, repair tension, use of nicotine, use of non-steroidal anti inflammatory drugs, reduced acromiohumeral distance, post operative rehabilitation protocol and early accelerated patient activity.
<table>
<thead>
<tr>
<th>Grade</th>
<th>Rotator Cuff Indications</th>
<th>Current Treatment(s)</th>
<th>Outcomes</th>
<th>Graft Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>Massive and retracted irreparable tear with intra-articular pathology</td>
<td>Open reverse total shoulder replacement (aggressive)</td>
<td>Adequate but limited function</td>
<td>Not indicated</td>
</tr>
</tbody>
</table>
| V     | Large, massive tear (3-5 cm, 2-3 tendons)  
Irreparable (unable to reappose to tuberosity with low tension) | Open or arthroscopic attempt at repair, muscle transfer, debridement and/or partial repair | High failure rate (50+ % re-tear and/or low outcome scores) | Interposition in selected instances |
| IV    | Large, massive tear (3-5 cm, 2-3 tendons)  
Reparable | Open or arthroscopic repair | Moderate failure rate (30+ % re-tear; 85% pain-free but reduced function) | Augmentation |
| III   | Small to medium tear (< 3 cm, 1 tendon) | Arthroscopic repair | Moderate failure rate (5-10% re-tear; 85% pain-free but >50% reduced function) | Augmentation |
| II    | Partial thickness tear (articular or bursal surface, >50%) | Arthroscopic decompression/debridement -or- repair with acromioplasty | 40% failure within 5 yrs when debrided only; 95% heal when repaired | Not indicated |
| I     | Partial thickness tear (articular or bursal surface, <50%) | Arthroscopic decompression/debridement -or- repair with acromioplasty | 95% heal when repaired | Not indicated |

Table 1.6: Clinical grades of rotator cuff pathology. Adapted from Table II in Derwin et al., 33
1.8. Concluding Remarks

The rationale for using a scaffold for rotator cuff repair may include reinforcement of the repair and improvement in the rate and quality of biologic healing. Synthetic scaffolds are typically characterized by uniform and reproducible mechanical and material properties; however, these scaffolds lack the bioactivity seen in ECM derived scaffolds and often result in fibrous encapsulation following implantation. ECM derived scaffolds provide a supportive medium for constructive remodeling by providing a chemically and structurally instructive environment for host cells via their natural composition, three-dimensional structure and/or remodeling byproducts \(^9,\ 10,\ 82\), which may improve the biology of repair healing. This has garnered the interest of the orthopedic community making it currently the most common tissue engineered strategy utilized to improve the functional outcomes of rotator cuff tears.

For any scaffolds to serve as an augmentation device, they must have the necessary mechanical and suture retention properties to withstand the high \textit{in vivo} tensile loads on the repair and resist suture failure at the scaffold-bone and/or scaffold-tendon-suture interface. When choosing a scaffold for rotator cuff repair augmentation, the mechanical and suture retention properties of the human rotator cuff tendon should be used as targets to guide our choice of scaffolds for rotator cuff repair.

\textit{In vitro} mechanical studies help evaluate the extent to which ECM scaffolds have the ability to “off-load” the repair at the time of implantation. These studies suggest that in their present configuration, SIS and dermis derived scaffolds may not be capable of providing appreciable mechanical augmentation to rotator cuff repairs at the time of implantation (time zero). Further, even though, fascia lata has material properties
comparable to tendon, its poor suture retention properties limit its use as an augmentation device for rotator cuff repairs.

Scaffolds when used as augmentation devices are believed to improve the initial biomechanical properties of the repair and offer some degree of load sharing in a manner that will reduce the incidence of tendon re-tear. While, human cadaver studies demonstrate the potential for scaffold augmentation to improve the initial biomechanical properties of a rotator cuff repair construct, the percent load carried by a scaffold when used for rotator cuff repair augmentation needs to be investigated. Further, the appropriate scaffold material properties and/or surgical application techniques for achieving optimal biomechanical performance in the setting of rotator cuff repairs are unknown.

Animal models for evaluating the efficacy of ECM scaffolds need to be standardized. More specifically, standardization of the injury model, surgical technique, study design and outcome measures will allow meaningful comparison and interpretation of animal studies using different species.

Despite the current clinical use of ECMs for rotator cuff repair, only a limited number of follow-up studies in human patients have been reported in the peer-reviewed literature. These studies highlight the need of randomized clinical trials that will allow proper interpretation of the results and help evaluate the clinical efficacy of ECM scaffolds for the treatment of rotator cuff tears.

In summary, several ECM scaffolds are now available in the armamentarium of surgeons. There is burgeoning need to design and develop basic and clinical science studies that will address questions related to the indications for use, surgical application,
rehabilitation protocols, in vivo remodeling and efficacy of these scaffolds. ECM scaffolds may have enormous therapeutic potential, but it will require a conscientious and orchestrated effort of manufacturers, clinicians and researchers to develop and validate ECM technology as a safe and effective treatment for improving the healing of rotator cuff tears.

1.9. Allograft Fascia Lata ECM

The in vitro mechanical, biochemical and cellular properties of several ECMs were described in Section 1.4. The properties of human fascia lata will be highlighted again here, to provide rationale for its development and investigation for tendon augmentation in this dissertation. Specifically, human fascia lata is a tendinous structure procured from the deep fascia of the thigh at a region that corresponds to the iliotibial band (Figure 1.3). Although fascia ECM has not been previously evaluated as a tendon augmentation scaffold in a formal clinical study, nor is it currently marketed as an ECM device for rotator cuff repair, human fascia lata has a clinical history of use as an autograft or allograft in the reconstruction of various soft tissues, including pectoralis major tendon, hallucis longus tendon, and achilles tendon.

Derwin et al., investigated the mechanical properties of fascia and several commercially-available ECM scaffolds indicated for rotator cuff repair augmentation. When pulled to failure under uniaxial tension, fascia exhibited an elastic modulus (532 ± 106 MPa) similar to canine infraspinatus tendon (405 ± 86 MPa) and stretched only ~3% before stiffening and bearing any significant load. Additionally, fascia ECM has been shown to have a biochemical composition and histological structure similar to tendon.
The hydroxyproline and chondroitin sulfate/dermatin sulfate GAG concentrations in fascia are similar to canine infraspinatus tendon (Table 1.4)\(^\text{34}\).

Hence, based on similarity with tendon scaffolds derived from human fascia lata, may be appropriate for use as an augmentation device for tendon repair.

![Figure 1.3](image)

**Figure 1.3:** A) Allograft fascia lata ECM procured from the deep fascia of the thigh at a region that corresponds to the iliotibial band B) Pictorial depiction of the anatomical location of fascia lata

![Figure 1.4](image)

**Figure 1.4:** (A) Image of H&E stained section of native fascia ECM, demonstrating the bilayer of collagen fascicles that are orthogonally-oriented and (B) stereomicrograph of the deep layer of fascia, which resembles a flat sheet of tendon
1.10 Summary

Currently, no commercially available ECM scaffold has the appropriate mechanical properties that would allow mechanical augmentation of the repair at the time of implantation. Hence, there is a critical need for an ECM scaffold that provides adequate strength as well as stimulates and enhances healing potential. Allograft fascia lata, a tendinous structure procured from the deep fascia of the thigh at a region that corresponds to the iliotibial band (Figure 1.3), has structural, material and biochemical properties comparable to tendon. Hence, fascia lata may be an attractive biomaterial for use as an augmentation device for rotator cuff repairs. However, fascia lata has poor suture retention properties (~ 10 N) compared to the suture retention of human rotator cuff tendon (200 - 240 N). Hence, there is a need to improve the suture retention strength of fascia in a manner that that will make it comparable to the suture retention properties of human rotator cuff tendon (200 - 240 N), which may then allow its use as an augmentation device for rotator cuff repairs.

Scaffolds when used as augmentation devices are believed to improve the initial biomechanical properties of the repair and offer some degree of load sharing in a manner that will reduce the incidence of tendon re-tear. While the biomechanical benefit achieved by using scaffolds as augmentation devices has recently been reported using cadaver models, no studies have investigated the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation. Furthermore, the manner in which loads on an augmented rotator cuff repair are distributed amongst the various components of the repair is not known, nor is the relative biomechanical importance of the various components of the augmented rotator cuff repair. Hence, there also remains an allied
need to develop an improved understanding of the basic biomechanics of scaffold augmented rotator cuff repairs.

1.11 Specific Aims

The long term goal of the research is to develop effective strategies for successful repair of large, chronic rotator repairs. The objective of this dissertation is to engineer the mechanical properties (suture retention strength and stiffness) of allograft fascia lata in order to develop an extracellular matrix (ECM) derived scaffold with robust mechanical properties for use in musculoskeletal soft tissue repair. An allied objective will also be to develop and validate a simple quasi-static spring-network model for rotator cuff repairs that will help elucidate the basic biomechanics of scaffold augmented rotator cuff repairs. The central hypothesis is that engineered fascia lata will have suture retention strength and stiffness similar to the human rotator cuff tendon, even after in vivo implantation. The rationale for this work is that engineering the mechanical properties of allograft fascia lata will support the translation of a tendon-like, strong, robust scaffold for augmenting tendon repairs in humans. And, the development of a spring-network model will help design and develop effective scaffolds and surgical strategies for the treatment of rotator cuff repairs. We will test the central hypothesis and achieve the objectives of this proposal via the following specific aims:

Specific Aim 1: Engineer the suture retention strength and stiffness of allograft fascia lata ECM at time zero using stitching as a method of reinforcement (Chapters 2 and 3)
Polymer braids with two different compositions, 100% Poly L- Lactic Acid (PLLA) and mix of PLLA and Poly Glycolic Acid (PGA) (6PLLA/2PGA) was used to reinforce fascia patches. Mechanical testing was performed to assess the suture retention strength, stiffness and fatigue behavior of the reinforced fascia scaffolds at time zero. The reinforced fascia scaffolds were designed to have time zero mechanical properties (suture retention load) comparable to the suture retention load of human rotator cuff tendon ($\geq 250N$) and higher than non-reinforced fascia ECM.

**Specific Aim 2:** Investigate the host response and time-dependent changes in mechanical properties of reinforced fascia scaffolds after implantation in a rat model (Chapter 3)

Polymer braids with two different compositions, 100% Poly L- Lactic Acid (PLLA) or 6PLLA/2Poly Glycolic Acid (PGA) was used to reinforce fascia patches. Mechanical testing was performed to assess suture retention and stiffness and of reinforced fascia scaffolds after 4 and 12 weeks implantation in a rat subcutaneous model. The biocompatibility of the constructs was also verified using histological analysis at the same time points. The hypotheses were:

**Hypothesis 2a:** The mechanical properties of reinforced fascia scaffolds will decrease after *in vivo* implantation, and the decrease will be more predominant in scaffolds reinforced with 6PLLA/2PGA, but the suture retention load of reinforced fascia scaffolds will remain $\geq 250N$.

**Hypothesis 2b:** The host response to reinforced fascia scaffolds will be similar to non-reinforced fascia characterized by the presence of lymphocytes and macrophages but with
an increased presence of foreign body giant cells due to the presence of the polymer braid.

**Specific Aim 3:** Develop and validate a quasi-static spring-network model for simplified rotator cuff repairs (Chapters 4, 5 and 6)

The approach was to model the individual components of the rotator cuff repair as non-linear springs and estimate parameters of the individual springs by non-linear least-squares analysis of the load-displacement data determined from isolated mechanical tests of each individual component. The model was developed based on the physics of springs in series and parallel and validated by comparing the predicted model force to experimental results. The validated model was then used to predict the degree of load sharing provided by the scaffold, conduct a parametric sensitivity analysis and parametrically simulate the model for different clinical scenarios.

The proposed research is *innovative* because it combines a tendon-like, allograft ECM with biocompatible polymer fibers in braided form using stitching as a method reinforcement, to achieve a scaffold that is mechanically suitable for rotator cuff repair. Through this research we expect to identify an appropriate reinforcing braid and establish the mechanical efficacy of polymer reinforced fascia scaffold as an augmentation device for rotator cuff repair. The development of the spring-network model of rotator cuff repair will provide for the first time, information about the load-sharing ability of augmentation scaffolds used for rotator cuff repair, and offer unique insight into how changes to various components of the repair may influence the biomechanical performance of the repair construct. These results are expected to have a *positive impact*
because they will support translation of polymer reinforced fascia scaffold for tendon repair in humans, offering the orthopedic surgeon with a robust allograft scaffold for increasing the likelihood of clinical success of large, debilitating, and chronic rotator cuff tears frequently encountered by the aging population. Additionally, the development of a spring-network model is expected to direct and inform the design of new repair strategies and may have broader implications for understanding the basic mechanics of scaffold augmentation of other soft tissue repairs as well.

1.12 Brief Outline of the Dissertation

Chapter 2 - Pilot Studies: This chapter describes preliminary studies that establish stitching as a method for engineering the mechanical properties of fascia lata ECM.

Chapter 3 - Development of Allograft Fascia Lata as an Extracellular Matrix Derived Scaffold for Musculoskeletal Soft Tissue Repairs: This chapter will characterize the mechanical properties, namely, suture retention load and stiffness, and fatigue behavior of reinforced fascia scaffolds at time zero and investigate the host response and time dependent changes in mechanical properties of reinforced fascia scaffolds after implantation in a rat model. The contents of this chapter will be submitted as a manuscript to the Journal of Biomedical Materials Research.

Chapter 4 - Formulation and Development of Spring-Network Model for Rotator Cuff Repairs: This chapter will focus on the formulation and development of quasi-static spring-network models for non-augmented and augmented human and canine rotator cuff repairs.
Chapter 5 - Validation of Spring-Network Model for Rotator Cuff Repairs: This chapter will present studies to validate the developed model in Chapter 4, demonstrate the calculation of confidence intervals for the model predictions using error propagation analysis, predict the degree of load sharing provided by the scaffold, and present an approximate parametric sensitivity analysis to identify which component(s)/parameter(s) most influences the mechanical behavior predicted by the augmented repair model. Various parts of this chapter have been previously published in Clinical Biomechanics. 2010 25(8); 751-8

Chapter 6 – The Biomechanical Role of Scaffolds in Augmented Rotator Cuff Repairs: This chapter will present parametric simulation studies that use the developed spring-network model for human rotator cuff repairs to predict the manner in which simulated changes to components of the tendon repair, such as reduced tendon quality, altered surgical technique and different scaffold designs, influence the biomechanical performance (yield load and stiffness) of the repair construct and also predict the percent load carried by the scaffold augmentation component of the repair construct in each of these simulated clinical scenarios. The contents of this chapter has been submitted as a manuscript titled “The Biomechanical Role of Scaffolds in Augmented Rotator Cuff Repairs to the Journal of Bone and Joint Surgery and is currently under review.

Chapter 7 - Summary and Future Directions: This chapter will summarize the key findings of this dissertation and suggest possible studies for future investigation.
BIBLIOGRAPHY


Chapter II

PILOT STUDIES

You can see a lot by just looking..........Yogi Berra

2.1 Introduction

Fascia lata has been shown to have structural, material and biochemical properties comparable to tendon, which makes it an attractive biomaterial for use as an augmentation device for rotator cuff repairs. However, compared to suture retention of human rotator cuff tendon (200-240 N)\textsuperscript{6,15}; fascia lata has poor suture retention properties (~10 N) (un-published data, Derwin laboratory), which precludes its use for such an application. An objective of this dissertation is to engineer the mechanical properties (suture retention strength and stiffness) of acellular allograft (human derived) fascia lata in order to develop an extracellular matrix (ECM) derived scaffold with robust mechanical properties for use in musculoskeletal soft tissue repair. More specifically, Specific Aim 1 of this study is to engineer the suture retention strength and stiffness of allograft fascia lata ECM at time zero using stitching as a method of reinforcement. To engineer the suture retention load of fascia lata, a suture retention load of \( \geq 250 \) N was
selected as the design criteria as it exceeds the suture retention properties of human rotator cuff tendon (200 - 240 N). A secondary criterion was also to engineer fascia lata without significantly altering its native three-dimensional architecture.

Currently, there are no technologies (for example, electrospinning and cross linking) that allow engineering the mechanical properties of an ECM scaffold without altering its native three-dimensional architecture.\textsuperscript{10, 12, 17-19, 21} Stitching, an existing textile procedure is one such technology that would allow engineering the mechanical properties of fascia lata without disrupting its native architecture. The concept of stitch reinforcement is not new, and it has long been used to develop composites for industrial applications\textsuperscript{3, 8}. However, its use for the reinforcement of an ECM derived biomaterial has never been explored, which demonstrates the novel nature of the proposed work. Stitching is a very simple process, which involves penetrating a needle with a stitching thread through a layer(s) of a material. Since, the objective of the proposed research was to develop a scaffold for musculoskeletal soft tissue repairs, braids made from FDA approved bioresorbable polymeric biomaterials (for example, polylactic acid and polyglycolic acid) were selected as the stitching thread. Resorbable (\textit{that which can be broken down and assimilated back into the body}) braids were selected to avoid a persistent long-term inflammatory response in vivo and thereby facilitate a favorable host-tissue response to the polymer reinforced scaffold.

Together, stitch type, stitch configuration (pattern), stitch density (stitch length) and stitching thread influence the mechanical properties of stitched constructs\textsuperscript{14, 20}. The mechanical properties of the braid (stitching thread) are further affected by the braid processing steps and braiding parameters. This chapter describes preliminary studies that
establish stitching as a method for engineering the mechanical properties of fascia lata ECM. Figure 2.1 gives the brief overview of the chapter. Briefly, the studies include 1) identification of a stitch configuration (pattern) that will give a suture retention load $\geq 250$N, 2) investigating the effect of standard braid processing steps on the suture retention load of reinforced fascia scaffolds, 3) investigating the effect of braiding parameters on the suture retention load of reinforced fascia scaffolds, 4) modification (refinement) of existing stitch pattern to make it suitable for full thickness rotator cuff repairs and 5) investigating effect of stitch length on suture retention load of reinforced fascia scaffolds.

![Figure 2.1: Overview of pilot studies](image-url)
2.2 Stitch Type

While there are different stitch types, namely, loop stitch, chain stitch and lock stitch, the lock stitch is the most widely used stitch and was considered more apt for this application since it will minimize damage to the fascia matrix. More importantly, it was the only stitch possible with the available sewing machine. Figure 2.2 explains the formation of a lock stitch.

**Figure 2.2:** Lock stitch formation: 1) this step is generally known as needle rise. At this stage of stitch formation the thread is not loaded, 2) the needle begins to penetrate the material and the thread on the needle side begins to form a loop, 3) the needle completely penetrates the material and loop widens. At this stage, the needle begins its upward travel, 4) as the needle travels up the widened loop loops the bobbin thread and 5) the needle is completely out of the material and the lock stitch is formed. Adapted from Weimer et al., 20
The human fascia lata used in the pilot studies described in this chapter was procured and processed by the Musculoskeletal Transplant Foundation (MTF, Edison, NJ) from donors aged 18 - 55 years old. More specifically, fascia lata was harvested aseptically from cadavers, cleaned of superficial connective tissue and underlying muscle. Fascia was then subjected to an antibiotic/antifungal soak treatment in a phosphate buffered saline (PBS) containing 1.6 µg/ml of amphotericin B, 30 µg/ml of imipenem cilastatin, and 6 µg/ml gentamicin sulfate for 4 hours at 37° C on a shaker.

The packaged, frozen fascia pieces sent to the Derwin laboratory were again washed in an antibiotic cocktail (ABX) at 37° C with stirring for 20 hours. The antibiotic cocktail consisted of 3.2 mg/ml Amphotericin B (Sigma-Aldrich, Sr. Louis, MO) and 6 mg/ml Gentamicin Sulphate (Lonza, Walkersville, MD, USA) in 1X PBS. Subsequently, fascia was rinsed twice for 20 minutes each in 1X PBS at room temperature with constant stirring. The ABX cleaned fascia pieces were then lyophilized using a freeze dry system maintained at - 40° C and 300 mBar for 24 hours (Model No. 7522800, Labconco, Kansas City, MO, USA). All experiments were done using lyophilized fascia pieces.
2.3 Pilot Study 1: Identification of Stitch Configuration (Pattern)

2.3.1 Objective

The objective of this study was to identify a stitch pattern that has a suture retention load $\geq 250$ N at time zero.

2.3.2 Experimental Design

Reinforced fascia scaffolds were prepared by stitching fascia patches (4 x 4 cm) with black braided silk suture [USP (The United States Pharmacopoeia Convention) Size 2 - 0, Harvard Apparatus, USA] in a controlled manner with five unique, two-dimensional patterns. The suture retention load of reinforced scaffolds were determined at time zero (n = 1 per pattern). Details for preparation of the fascia, scaffold fabrication and experimental method are described below.

2.3.3 Methods

Scaffold Fabrication

Reinforced fascia patches were prepared by stitching the lyophilized fascia patches (4 x 4 cm) with black braided silk suture (USP Size 2 - 0, Harvard Apparatus, USA) in five stitch patterns using a commercial sewing machine (Alphasew, Mini-Walker Zig Zag, Model: PW 400 - ZZ, USA). The five stitch patterns included: (A) two rectangle double stitched; (B) three rectangle double stitched; (C) four rectangle single stitched; (D) peripheral stitched and (E) perpendicular stitched (Figure 2.3). All patterns were stitched with a 2 mm stitch length.
Figure 2.3: Stitch patterns for 4 x 4cm reinforced fascia scaffold (A): Two Rectangle Double Stitched; (B) Three Rectangle Double Stitched; (C) Four Rectangle Single Stitched; (D) Peripheral Stitched and (E) Perpendicular Stitched

Failure Testing

The suture retention load of reinforced fascia scaffolds fabricated in the five stitch patterns (n = 1 per pattern) were evaluated by attaching the sides of the scaffold to a stainless steel ring via peripheral simple sutures [Fiberwire, USP Size 2, Arthrex, Naples, FL] in order to simulate the side tensioning seen when scaffolds are used clinically (Figure 2.4). The other edge of the scaffold was then preloaded to 2 N and subsequently loaded to failure in displacement control at the rate of 30 mm/min. All testing was performed in ambient air at 22° C. The failure data were zeroed at 2 N. The suture retention load was defined as the ultimate tensile load attained by the scaffold. The failure mechanism was recorded for each specimen.
Figure 2.4: Side-tension test: The sides of the scaffold were attached to a stainless steel ring with simple suture configuration to simulate the side tensioning when scaffolds are used clinically.

**Statistical Analysis**

Because of the single sample size of this pilot study, statistical analysis was not possible.

**2.3.4 Results and Discussion**

The suture retention load of fascia patches reinforced with any design showed at least a fourfold increase in the suture retention load of non-reinforced fascia (Figure 2.5). The suture retention load of the perpendicular stitch design was able to achieve the desired suture retention load of $\geq 250$ N and was also relatively higher than that obtained using other stitch patterns (Table 2.1). The mode of failure for all samples occurred by the breaking of the reinforcing suture secondary to slipping of the reinforcing suture through the fascia matrix. Based on the results of this pilot study the perpendicular stitch pattern was selected for future investigations.
Figure 2.5: Load-displacement curve of fascia patches reinforced in the five stitch patterns. The perpendicular stitch pattern was able to achieve the desired suture retention load of ≥ 250 N and was also relatively higher than suture retention load of other patterns.

<table>
<thead>
<tr>
<th>Stitch Pattern</th>
<th>Suture Retention Load (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Reinforced</td>
<td>54</td>
</tr>
<tr>
<td>Two Rectangle Double Stitched</td>
<td>189</td>
</tr>
<tr>
<td>Three Rectangle Double Stitched</td>
<td>203</td>
</tr>
<tr>
<td>Four Rectangle Single Stitched</td>
<td>175</td>
</tr>
<tr>
<td>Peripheral Stitched</td>
<td>155</td>
</tr>
<tr>
<td>Perpendicular Stitched</td>
<td>270</td>
</tr>
</tbody>
</table>

Table 2.1: Suture retention load of fascia patches reinforced in the five stitch patterns (n=1 per pattern).

2.4 Braids as Stitching Threads

Black braided silk sutures are known to elicit an acute inflammatory response in vivo and in some cases albeit few, an allergic response were also noted \(^1,5,9,11,16\). Hence, for further development studies braids made from resorbable polymer biomaterials were used. It is believed that the use of resorbable biomaterials will most likely avoid a long-
term inflammatory response in vivo and thereby facilitate a favorable host-tissue response to reinforced fascia scaffold.

A braid is essentially a tubular structure that is produced by intertwining or weaving three or more parallel strands in such a way that each group of strands (strand is a group of filaments) pass alternately over and under a group of strands laid up in the opposite direction as shown in Figure 2.6 A.

![Figure 2.6: Different types of braids (A) typical braid; (B) typical braid with core and (C) typical braid without core](image)

Braids can be broadly classified as braids with core (Figure 2.6 B) and without core (Figure 2.6 C). Figure 2.7 depicts the typical construction of a braid with a core. Such a braid may be referred to as nXX with mYY core (where n and m are the number of strands used for the sheath and core respectively and XX, YY are the type of materials with which the braid is constructed). The braid in Figure 2.7 is an 8XX with a 2YY core.
Braids with a core were selected for reinforcing fascia as it allowed developing a braid having braid diameter (braid diameter ~ 300 - 400 microns) and a tensile strength of $\geq 40$ N.

The mechanical properties of the braid are affected by the braid processing steps and braiding parameters, which in turn may impact the mechanical properties of reinforced fascia scaffolds. Hence, the next two studies (Pilot Study 2 & 3) will investigate the effect of standard braid processing steps and braiding parameters on the suture retention load of reinforced fascia scaffolds.
2.5 Pilot Study 2: Effect of Standard Braid Processing Steps

2.5.1 Background

The reinforced fascia scaffold may be likened to a composite lamina with fascia extracellular matrix (ECM) as the matrix and the braid as the reinforcing fibers. Hence, the selection of an appropriate braid with good handling characteristics and mechanical properties is critical to the performance of the reinforced fascia scaffolds. Braids routinely undergo post braiding processing techniques, which include but are not restricted to hot stretching, scouring, annealing, tipping, packaging and sterilization. Hot stretching reduces the braid diameter and prevents unraveling of the braid after cutting, often called “brooming” effect. Hot stretching also affects the elongation of the braid. Scouring eliminates spin finishes that are applied to yarns during braiding and also helps in the removal of dust particles and oil droplets that may be deposited during braiding. Annealing increases the crystallinity (in case of polymers) and tipping is needed for good needle attachment. Annealing and tipping are optional treatments that are included depending on the intended application of the braid. Sterilization is necessary if the intended application is for in vivo use. These processing techniques may alter the mechanics of the braid and subsequently affect the performance of the reinforced fascia scaffolds.

2.5.2 Objective

The objective of this study was to investigate the effect of standard braid processing steps, namely, hot stretching, scouring and sterilizing on the suture retention load of reinforced fascia scaffolds.
2.5.3 Experimental Design

Polymer braid having a configuration of 6PLLA/2PGA with 2PGA core (6 strands Poly L-Lactic Acid (PLLA) and 2 strands Poly Glycolic Acid (PGA) in sheath, and 2 strands PGA in the core; 1 strand = 30 filaments) was used for this investigation (Concordia Medical, Warwick, Rhode Island, USA). Hereafter, 6PLLA/2PGA with 2PGA core braid will be referred to as 6PLLA/2PGA. Four braid processing steps were investigated, namely, “no” hot stretch, hot stretch (HS), hot stretch scoured/ dried (HSS), and hot stretch/scoured/dried/ sterilized (HSSS) (Table 2.2). Sterilization was by ethylene oxide. Reinforced fascia lata scaffolds were prepared by stitching lyophilized fascia strips (2 x 5 cm) and patches (4 x 4 cm) with each braid. The suture retention load of the reinforced scaffolds were determined (n= 2 - 5 per treatment). Details of scaffold fabrication and experimental method are described below.

2.5.4 Methods

Scaffold Fabrication

Table 2.2 gives the mechanical properties of each braid investigated. Fascia strips (2 x 5 cm) and patches (4 x 4 cm) were reinforced with these braids using a commercial sewing machine (Alphasew, Mini-Walker Zig Zag, Model: PW 400-ZZ, USA). A peripheral stitch pattern was used to prepare the reinforced fascia strips (Figure 2.3 D). The reinforced fascia patches were stitched in the perpendicular pattern (Figure 2.3 E) determined from Pilot Study 1. All scaffolds were fabricated with a 2 mm stitch length.
<table>
<thead>
<tr>
<th>Braid type</th>
<th>Tensile load (N)</th>
<th>Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“No” Hot stretch</td>
<td>31.7</td>
<td>25.4</td>
</tr>
<tr>
<td>Hot stretched, un-scoured (HS)</td>
<td>30.9</td>
<td>17.1</td>
</tr>
<tr>
<td>Hot stretched, scoured/dried (HSS)</td>
<td>30.7</td>
<td>19.4</td>
</tr>
<tr>
<td>Hot stretched, scoured/dried sterilized (HSSS)</td>
<td>28.7</td>
<td>21.5</td>
</tr>
</tbody>
</table>

Table 2.2: Specifications of the 6PLLA/2PGA braids subjected to conventional braid processing steps. Braids were manufactured and characterized by Concordia Medical, Warwick, Rhode Island, USA.

**Failure Testing**

The suture retention load of reinforced fascia strips was evaluated using a suture retention test with simple suture configuration (USP Size 2 Fiberwire, Arthrex, Naples, FL) (n=3 - 5/treatment) (Figure 2.8 A). Briefly, scaffolds were preloaded to 2 N followed by constant rate distraction to failure at 12.5 mm/min. The suture retention load of reinforced fascia patches was evaluated using a tension-with-side-constraint test (n = 2/treatment) (Figure 2.8 B). Briefly, scaffolds were attached via 12 peripheral simple sutures (USP Size 2 Fiberwire, Arthrex, Naples, FL) to a jig that allowed 10 N of static tension to be applied to the sides of the patch in order to simulate the pre-tensioning that occurs when these patches are used clinically. The other edges were then preloaded to 2 N, pre-conditioned for 10 cycles from 5 - 15 N at 0.25 Hz and subsequently loaded to failure in displacement control at the rate of 30 mm/min. For both types of tests, the failure data was zeroed at 2 N. The suture retention load was defined as the ultimate tensile load attained by the scaffold. The failure mechanism was recorded for each specimen.
Statistical Analysis

A one-factor analysis of variance (ANOVA) was used to test for differences in the suture retention load between groups of reinforced fascia strips. A two-sample t-test was used to test for differences in the suture retention load between reinforced fascia patch groups. For all statistical analysis, a p value of ≤ 0.05 was considered significant.

2.5.5 Results and Discussion

The mode of failure for all samples occurred by the breaking of the braid secondary to slipping of the braid through the fascia matrix. The standard fiber processing steps of hot stretching, scouring and sterilizing did not significantly affect the suture retention load of reinforced fascia strips (Figure 2.9, p = 0.585).
Figure 2.9: Suture retention load of strip fascia reinforced with 6PLLA/2PGA braids processed with four braid processing steps (1) “no” Hot Stretch (HS); (2) Hot Stretch (HS); (3) Hot Stretch Scoured (HSS) and (4) Hot Stretched Scoured Sterilized (HSSS). No significant differences (p > 0.05) were found in the suture retention load of strip fascia reinforced with the polymer braids.

However, the suture retention load of patches reinforced with hot stretched/scoured ethylene oxide sterilized polymer braid (HSSS) was significantly lower (244 ± 19N) than scaffolds reinforced with hot stretched/scoured (HSS) (330 ± 10N) polymer braids (p < 0.05) (Figure 2.10).

Figure 2.10: Suture retention load of patch fascia reinforced with Hot Stretch/Scoured (HS) and Hot Stretched Scoured Sterilized (HSSS) 6PLLA/2PGA braids. Ethylene oxide sterilization appeared to negatively impact the suture retention load of patches reinforced with HSSS braids (p < 0.05).
There seems to be no explanation at this point of time for the apparent drop in the suture retention of scaffolds reinforced with hot stretched/scoured ethylene oxide sterilized polymer braid (HSSS) seen in the patch configuration and not in the strip configuration. However, given the fact that the mechanical properties of the braids did not decrease after sterilization (Table 2.2), it may be reasonable to assume that these disparate outcomes may be due to low sample size and not due to a real negative effect of sterilization. Hot stretching reduces the braid diameter and prevents unraveling of the braid after cutting, scouring eliminates spin finishes and oil droplets that may be deposited during braiding and sterilization is necessary if intended application is for *in vivo* use. Because these processing steps are desirable for scaffold development and were shown not to negatively impact the performance of reinforced fascia scaffolds, they were included for future braid processing.

### 2.6. Pilot Study 3: Effect of Braiding Parameters

#### 2.6.1 Background

A braid consists of an intersection repeat of groups of twisted and entangled fibers called yarn. Pick ‘P’ is the repeat of the yarn groups on the braid and pick count is the number of ‘P’ per unit length in a single line parallel to the braid axis (Figure 2.11). The pick count, braid pattern, braid design and denier of the fibers, to name a few, are braiding parameters that are known to affect the mechanics of the braid, which in turn may influence the mechanical performance of the reinforced fascia scaffold \(^{13}\). This study aims to investigate the effect of a subset of these parameters, namely, pick count,
presence of core, and denier of the fibers on the performance of the reinforced fascia scaffolds. Such a study will help identify braid parameter(s) to be used for future studies.

Figure 2.11: Schematic representation of pick ‘P’ of a braid. Adapted from Omeroglu S et al, 2006 13

2.6.2 Objective
The objective of the study was to test for differences in the suture retention load of scaffolds reinforced with 6PLLA/2PGA polymer braids (Pilot Study 2) having a lower pick count, lower pick count with extra PGA core, double denier with no core and extra hot stretch (HS) and scaffolds reinforced with 100% PLLA braid (8 strands Poly L-Lactic Acid (PLLA) and 2 strands Poly L-Lactic Acid (PLLA) in the core; 1 strand = 30 filaments) (Concordia Medical, Warwick, Rhode Island, USA). Hereafter, 100% PLLA braid will be referred to as 100% PLLA. All braids were hot stretched and scoured, but were not sterilized.

2.6.3 Experimental Design
Fascia patches (4 x 4 cm) were reinforced with various 6PLLA x 2PGA and 100% PLLA braids (mentioned above) (Concordia Medical, Warwick, Rhode Island, USA) in a perpendicular stitch pattern (Figure 2.3 E). The suture retention load and stiffness of the
reinforced fascia scaffolds were determined at time zero (n= 2 - 3 per configuration).

Details of scaffold fabrication and all experimental methods are described below.

2.6.4 Methods

Scaffold Fabrication

Fascia patches (4 x 4 cm) were reinforced with hot stretched/scoured 6PLLA/2PGA polymer braids having a lower pick count, lower pick count with extra PGA core, double denier with no core and extra hot stretch (HS) and 100% PLLA braid in a perpendicular stitch pattern using a commercial sewing machine (Figure 2.3 E) (Alphasew, Mini-Walker Zig Zag, Model: PW 400 - ZZ, USA). All scaffolds were fabricated with a 2 mm stitch length.

Failure Testing

The suture retention load of reinforced fascia patches were evaluated using a tension-with-side-constraint test (n = 2 - 3/configuration) (Figure 2.8 B) described in Pilot Study 2. The suture retention load was defined as the ultimate tensile load attained by the scaffold. The failure mechanism was recorded for each specimen.

Statistical Analysis

Because of the small sample size of this pilot study, statistical analysis was not conducted.

2.6.5 Results and Discussion

The mode of failure for all samples occurred by the breaking of the braid secondary to slipping of the braid through the fascia matrix. Figure 2.12 gives the average suture retention load for patch fascia reinforced with the various braids investigated.
Figure 2.12: Suture retention load of fascia patches reinforced with hot stretched/scoured 6PLLA/2PGA polymer braids having a lower pick count, lower pick count with extra PGA core, double denier with no core and extra hot stretch (HS) and 100% PLLA braid. Fascia patches reinforced with hot stretched/scoured (HSS) polymer braid was used as a reference (see Figure 2.10).

The data suggest that fascia scaffolds reinforced with 6PLLA/2PGA braid having a lower pick count with extra PGA core had the highest suture retention load (416 ± 37 N). While, the suture retention load of scaffolds reinforced with the 100% PLLA braid was 353 ± 54 N. Since, fascia reinforced with 6PLLA/2PGA braid demonstrated superior mechanical performance it was selected for future investigations. A 100% PLLA braid was also chosen for future studies (Table 2.3) based on the similarity in construction, diameter and texture to the 6PLLA/PGA braid choice, and the precedent of an FDA-approved 100% PLLA suture (Orthodek, USP Size 2 - 0, Teleflex Medical, CT, USA).
Table 2.3: Specifications of the custom 100% PLLA and 6PLLA/2PGA polymer braids to be used for future studies. Braids were manufactured and characterized by Concordia Medical, Warwick, Rhode Island, USA. (PLLA: Poly L-Lactic Acid and PGA: Poly Glycolic Acid)

2.7. Pilot Study 4: Stitch Pattern Modification (Refinement)

2.7.1 Background

Pilot Study 1 demonstrated that the perpendicular stitch pattern (Figure 2.3 E) was able to achieve the desired suture retention load of \( \geq 250 \text{N} \) at time zero. However, the fabrication of this pattern is cumbersome and involves significant damage to the fascia matrix. The size of the patch (4 x 4 cm) is also not optimal for full thickness rotator cuff repairs seen clinically in human patients.

2.7.2 Objective

Hence, the objective of this study was to modify the perpendicular stitch pattern (Pilot Study 1) to make it suitable for full thickness rotator cuff tendon repairs. The design criteria/objectives were: 1) the patch should have a suture retention load of \( \geq 250 \text{N} \) in the tension-with-side-constraint test at time zero, 2) the stitch pattern must include at least two reinforcing lines between the surgical sutures and the edge of the patch, and must allow the surgeon to readily locate the lines of reinforcement and 3) the pattern must easily be fabricated minimizing time of fabrication, mass of polymer fibers and the number of stop/starts.
2.7.3 Experimental Design

Reinforced fascia lata scaffolds were prepared by stitching fascia patches (5 x 5 cm) with 100% PLLA braid (Teleflex Medical, CT, USA) in a controlled manner with unique, two-dimensional patterns. The suture retention load of reinforced scaffolds were determined at time zero (n = 1 per pattern). Details of scaffold fabrication and experimental method are described below.

2.7.4 Methods

Scaffold Fabrication

Fascia patches (5 x 5 cm) were reinforced with hot stretched 100% PLLA polymer braid (Teleflex Medical, CT, USA) in five stitch patterns using a commercial sewing machine (Alphasew, Mini-Walker Zig Zag, Model: PW 400-ZZ, USA). The patterns are not being presented herein due to proprietary and confidentiality concerns. All scaffolds were fabricated with a 2 mm stitch length.

Failure Testing

The suture retention load of patch reinforced scaffolds were evaluated using a tension-with-side-constraint test (n = 2/configuration) (Figure 2.8 B) described in Pilot Study 2. The suture retention load was defined as the ultimate tensile load attained by the scaffold. The failure mechanism was recorded for each specimen.

2.7.5 Results and Discussions

The stitch pattern that met the desired design objectives had the following dimensions: Overall dimensions of the patch: 5 x 5 cm; Inside footprint of braid reinforcement: 2.8 x 3.4 cm and outside footprint of braid reinforcement: 3.6 x 4.2 cm.
This pattern was selected for future investigations and will now on be referred as the preferred pattern. Compared to the perpendicular pattern that took 30 minutes for scaffold fabrication (Pilot Study 1), scaffold fabrication with the preferred pattern was accomplished in 10 - 15 minutes per patch, with a maximum of two start/stops.

2.8. Pilot Study 5: Effect of Stitch Length

2.8.1 Objective

The effect of the stitch length on the mechanical performance of reinforced scaffolds was then investigated using the preferred pattern. The study also quantified the amount of braid used for stitching the fascia patches (5 x 5 cm) in the preferred pattern with two stitch lengths, 2 mm and 4 mm, respectively.

2.8.2 Methods

Scaffold Fabrication

Fascia patches (5 x 5 cm) were reinforced with hot stretched 100% PLLA polymer braid (Teleflex Medical, CT, USA) in the preferred pattern using a commercial sewing machine (Alphasew, Mini-Walker Zig Zag, Model: PW 400 - ZZ, USA). All scaffolds were fabricated with a 2 mm and 4 mm stitch length.

Failure Testing

The suture retention load of patch reinforced scaffolds were evaluated using a tension-with-side-constraint test (n = 2/stitch length) (Figure 2.8 B) described in Pilot Study 2. The suture retention load was defined as the ultimate tensile load attained by the scaffold. The failure mechanism was recorded for each specimen.
2.8.3 Results and Discussions

Approximately 2.5 yards of braided fibers are used for a 4mm stitch length (Table 2.4). Decreasing the stitch length provides no mechanical benefit (Figure 2.13) and increases both the number of perforations of the fascia and the amount of braid used (Table III), e.g., a 2 mm stitch length uses approximately 3.5 yards of braid. Hence, future studies will use a 4 mm stitch length in order to minimize the number of perforations of the fascia and the amount of braid used.

<table>
<thead>
<tr>
<th>Braid Amount</th>
<th>2mm Stitch Length</th>
<th>4mm Stitch Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spool (yards)</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Bobbin (yards)</td>
<td>0.85</td>
<td>0.9</td>
</tr>
<tr>
<td>Total (yards)</td>
<td>3.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Table 2.4: Amount of braid used per scaffold stitched in the preferred pattern with different stitch lengths (n = 2 per group).

Figure 2.13: Suture retention load of patch fascia reinforced with 100% PLLA braids with 2 mm and 4 mm stitch lengths. There is no significant improvement in the mechanical performance of scaffold stitched with a 2 mm and 4 mm stitch length.
2.9 Summary

In summary, hot stretching, scouring and ethylene oxide sterilization, which are desirable braid processing steps for scaffold development, were shown not to negatively impact the mechanical performance of reinforced fascia scaffolds; hence they were included for future braid processing. Fascia reinforced with 6PLLA/2PGA braid (6 strands Poly L-Lactic Acid (PLLA) and 2 strands Poly Glycolic Acid (PGA) in sheath, and 2 strands PGA in the core; 1 strand = 30 filaments) demonstrated superior mechanical performance and hence was selected for future investigations. A 100% PLLA braid (8 strands Poly L-Lactic Acid (PLLA) and 2 strands Poly L-Lactic Acid (PLLA) in the core; 1 strand = 30 filaments) was also chosen for future studies (Table 2.3) based on the similarity in construction, diameter and texture to the 6PLLA/PGA braid choice, and the precedent of an FDA-approved 100% PLLA suture (Orthodek, USP Size 2-0, Teleflex Medical, CT, USA). A preferred stitch pattern with a 4 mm stitch length was also identified, which allowed scaffold fabrication in 10-15 minutes per patch with a maximum of two start/stops, minimized the amount of braid per patch (~2.5 yards) and most importantly achieved a suture retention load of ≥ 250N at time zero. These pilot investigations resulted in a patent application, Reinforced Tissue Graft, US Patent Application PCT/US 2009/038570, filed in March 2009. The next chapter describes studies that characterize the in vitro mechanical properties and fatigue behavior of scaffolds reinforced with the two selected polymer braids. As well, the study will investigate the host response and time-dependent changes in mechanical properties of reinforced fascia scaffolds with either braid after implantation in a rat model.


Chapter III

DEVELOPMENT OF ALLOGRAFT FASCIA LATA AS AN EXTRACELLULAR MATRIX DERIVED SCAFFOLD FOR MUSCULOSKELETAL SOFT TISSUE REPAIRS

The silly question is the first intimation of some totally new development.....

Alfred North Whitehead

3.1 Introduction

Although surgical treatment and rehabilitation strategies continue to evolve, high repair failure rate (20 - 90 %) of rotator cuff tears continue to pose a significant challenge to the clinicians researchers, at large. Currently, extracellular matrix (ECM) materials are being investigated as a tissue engineered strategy for the treatment of these tears. ECM derived scaffolds are attractive as they have a natural three-dimensional architecture and provide a chemically and structurally instructive environment, which may improve the biology of repair healing.

To also serve an augmentation device, scaffolds must have robust mechanical and adequate suture retention properties that will allow some degree of load sharing with the tendon repair at the time of implantation and for some period of post-operative healing.
In vitro uniaxial mechanical tests (unconstrained) have shown that presently, only fascia lata has material properties comparable to tendon tissue. Furthermore, fascia lata also has biochemical and structural properties similar to tendon. Hence, fascia lata seems to be an attractive biomaterial for use as an augmentation device for rotator cuff repairs. However, fascia lata has poor suture retention properties (~10 N) (unpublished data from Derwin laboratory) compared to the suture retention of human rotator cuff tendon (200 - 240 N). Hence, the suture retention strength of fascia needs to be improved in a manner that will make it comparable to the suture retention properties of human rotator cuff tendon (200 - 240 N), which may then allow it to be used as an augmentation device for rotator cuff repair.

Hence, the overall goal of this work was to engineer the suture retention strength and stiffness of allograft fascia lata in order to develop an extracellular matrix (ECM) derived scaffold with robust mechanical properties for use in musculoskeletal soft tissue repair. Specifically, braided, resorbable, polymer fibers were stitched into fascia ECM in a unique, controlled manner. Fascia scaffolds were reinforced with the pattern and polymer braids chosen based on pilot studies (Chapter 2), namely, the preferred stitch pattern and two braid compositions, 100% Poly L- Lactic Acid (PLLA) or 6PLLA/2Poly Glycolic Acid (PGA).

The specific aims of this study were 1) to characterize the mechanical properties, namely, suture retention load and stiffness, and fatigue behavior of reinforced fascia scaffolds at time zero, and 2) to investigate the host response and time-dependent changes in mechanical properties of reinforced fascia scaffolds after implantation in a rat model. The hypotheses were that 1) The time zero mechanical properties (suture retention load)
of the reinforced fascia scaffolds will be ≥ 250N and higher than non-reinforced fascia ECM, but not different between the braid types, 2) The mechanical properties of reinforced fascia scaffolds will decrease after in vivo implantation, and the decrease will be more predominant in scaffolds reinforced with 6PLLA/2PGA, but the suture retention load of reinforced fascia scaffolds will remain ≥ 250N, and 3) The host response to reinforced fascia scaffolds will be similar to non-reinforced fascia characterized by the presence of lymphocytes and macrophages but with an increased presence of foreign body giant cells due to the presence of the polymer braid.

3.2 Experimental Design

The experimental design for the study is shown in Figure 3.1 Briefly, reinforced fascia lata scaffolds were prepared by stitching fascia patches (5 x 5 cm) with 100% PLLA or 6PLLA/2PGA polymer braids in a controlled manner with a unique, two-dimensional pattern. The suture retention load, stiffness and fatigue behavior of reinforced scaffolds were determined at time zero (n = 8 - 11 per braid group). The suture retention load and stiffness of reinforced scaffolds were also determined following four and twelve weeks dorsal subcutaneous implantation in a rat model (n = 11 per braid group per time point). To evaluate host response at four and twelve weeks, 1x1 cm pieces of non-reinforced and reinforced fascia scaffold were implanted into an abdominal wall defect in a subset of the same rats (n= 4 per braid group per time point). Details for preparation of the reinforced fascia scaffolds and all experimental methods are described below.
3.3 Methods

3.3.1 Scaffold Fabrication

The human fascia lata used in this study was procured and processed by the Musculoskeletal Transplant Foundation (MTF, Edison, NJ) from donors aged 18-55 years old. More specifically, fascia lata was harvested aseptically from cadavers, cleaned of superficial connective tissue and underlying muscle and then subjected to an antibiotic/antifungal soak treatment in a phosphate buffered saline (PBS) containing 1.6 µg/ml of amphotericin B, 30 µg/ml of imipenem cilastatin, and 6 µg/ml gentamicin sulfate for 24 hours at 37°C on a shaker. This soak has also been shown to remove most of the cellular material from the fascia matrix (Table 1.4) and is considered an
“acellularization” treatment; although fragments of double stranded DNA do remain (Figure 3.2). After 24 hours, fascia was rinsed two times in PBS for 20 minutes each at room temperature on a shaker and then lyophilized. Subsequently, individual lyophilized fascia pieces were packaged and sent to the Derwin laboratory.

Reinforced fascia scaffolds were prepared by stitching lyophilized fascia patches (5 x 5 cm) with custom made 100% PLLA or 6PLLA/2PGA polymer braids (Concordia Medical, Warwick, Rhode Island, USA) using a commercial sewing machine (Alphasisew, Mini-Walker Zig Zag, Model: PW 400 - ZZ, USA) (Figure 3.3). The stitch pattern (preferred) and stitch length (4 mm) was the same for all scaffolds and chosen based on pilot studies (Chapter 2). Table 3.1 provides the specific details of the polymer braids used. The polymer braids were hot stretched, scoured, ethylene oxide sterilized and vacuum dried by the manufacturer. Scaffolds intended for in vivo implantation were stitch reinforced under aseptic conditions in a clean room.

**Figure 3.2:** Representative (A) Hematoxylin-Eosin (H&E) and (B) DAPI stained sections of processed allograft fascia lata. Fragments of double stranded DNA (blue strands) do remain after processing (100X)
A. Deep  
B. Superficial

**Figure 3.** Representative sections of stitched fascia reinforced with 100% PLLA A): Lock stitch formation on deep side of fascia ECM and B) Lock stitch formation on superficial side of fascia ECM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>100% PLLA</th>
<th>6PLLA/2PGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheath</td>
<td>8 ends PLLA</td>
<td>6 ends PLLA and 2 ends PGA</td>
</tr>
<tr>
<td>Core</td>
<td>2 ends PLLA</td>
<td>3 ends PGA</td>
</tr>
<tr>
<td>Diameter (microns)</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Tensile Strength (N)</td>
<td>43.4</td>
<td>43</td>
</tr>
<tr>
<td>Percent Elongation (%)</td>
<td>35.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Picks per inch</td>
<td>34.3</td>
<td>32.4</td>
</tr>
<tr>
<td>Denier</td>
<td>120</td>
<td>75</td>
</tr>
<tr>
<td>Filament</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Denier per filament (dpf)</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>PLLA content (% weight)</td>
<td>100</td>
<td>66</td>
</tr>
<tr>
<td>PGA content (% weight)</td>
<td>--</td>
<td>34</td>
</tr>
</tbody>
</table>

**Table 3.1:** Technical specifications of custom 100% PLLA and 6PLLA/2PGA braids used to reinforce the fascia scaffolds. Braids were manufactured and characterized by Concordia Medical, Warwick, Rhode Island, USA.
3.3.2 Time Zero Mechanical Properties

Failure Testing

The suture retention load and stiffness of non-reinforced (n=4) and reinforced fascia scaffolds (n = 11/12 braid type) were evaluated using a tension-with-side-constraint test (Figure 3.4). Briefly, scaffolds were attached via 12 peripheral simple sutures (USP 2 Fiberwire, Arthrex, Naples, FL) to a jig that allowed 10N of static tension to be applied to two opposing sides of the patch in order to simulate the pre-tensioning that occurs when these patches are used clinically for rotator cuff repair. The other sides were then preloaded to 2 N, pre-conditioned for 10 cycles from 5-15 N at 0.25 Hz and subsequently loaded to failure in displacement control at the rate of 30 mm/min. All testing was performed in a saline bath at 37° C. The suture retention load was defined as the ultimate tensile load attained by the scaffold. Stiffness was defined as the slope of the load-displacement curve between 50-150 N. The failure mechanism was recorded for each specimen.

Figure 3.4: Tension-with-side constraint test shown with a 4 x 4 cm non-reinforced fascia patch
Cyclic Fatigue

A fatigue test was performed to evaluate the performance of reinforced fascia scaffolds under cyclic loading conditions (n = 8/braid type). The scaffolds were attached via 12 peripheral simple sutures (USP Size 2 Fiberwire, Arthrex, Naples, FL) to a jig that allowed 10N of static tension to be applied to 2 opposing sides of the patch while the other sides were loaded in dynamic tension (Figure 3.4). A 1-N preload was applied to pretension the specimen. The specimen was then cyclically loaded under force control from 5 to 150 N for 5000 cycles at 0.25 Hz with the use of a half sinusoidal waveform. The cyclic data were zeroed at 10 N to match the static tension that had been applied to the sides of the scaffolds at the onset of testing. Stroke elongation of the nth cycle was defined as the difference between the peak of the nth cycle and the valley at the beginning of the nth cycle. Cyclic elongation of the nth cycle was defined as the difference in the displacement between the valley at the end of the nth cycle and the valley at the beginning of the first cycle. Cyclic stiffness of the nth cycle was calculated as the change in force of the nth cycle (nominally 145 N) divided by the change in displacement from the valley at the beginning of the nth cycle to the peak of the nth cycle.

3.3.3 In Vivo Implantation

Twelve simple suture loops (USP Size 2 Fiberwire, Arthrex, Naples, FL) were added around the periphery of reinforced fascia patches (n = 22 patches/braid type) to simulate the clinical presence of suture with the use of a scaffold. A sterile polypropylene screen was sutured to one side of the scaffold constructs in four locations using 5 - 0 Prolene (Figure 3.5). The purpose of the screen was to prevent folding of the
scaffold onto itself during the implantation period, and to cover the Fiberwire knots so as to minimize subcutaneous irritation. All preparatory procedures were performed under aseptic conditions in a clean room.

Figure 3.5: Scaffold attached to screen for in vivo implantation

**Surgical Procedure**

All surgical procedures were conducted in compliance with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adheres to principles set forth in the Guide for Care and Use of Laboratory Animals, National Research Council, 1996. All surgical procedures were performed by an orthopedic resident, Mena Mesiha, MD at the Cleveland Clinic.
Forty-four adult, male retired breeders Sprague Dawley rats were used for this study (450 - 550 gm, Harlan, Indianapolis, IN). Each rat was anesthetized with isoflurane (maintained at 2 - 3%) and the dorsum was prepared for aseptic surgery. A 4 cm dorsal midline incision was made to expose the underlying areolar layer. Flaps were developed on either side of the midline by sharply detaching the areolar layer insertion from the spinal processes to expose the underlying lumbar fascia and muscle. A 5 x 5 cm reinforced fascia construct (scaffold plus sutures) was hydrated in sterile saline for 20 minutes and then placed in the dorsal subcutaneous pouch with the polypropylene screen oriented superficially (Figure 3.6 A) (n = 22 rats/braid type). The deep side of the construct was laid against the lumbar fascia and muscle but not attached to the animal. The areolar layer was then closed in a running stitch configuration using 4 - 0 Vicryl
suture (Figure 3.6 B & 3.6 C) and the overlying dermis was then closed using surgical staples (Figure 3.6 D).

**Rat Abdominal Wall Defect Implantation**

In a subset of the same rats used for dorsal subcutaneous implantation (n=8 rats/braid type), the abdomen was also prepared for aseptic surgery. Via a ventral midline incision, a partial-thickness 1x1 cm defect was created in the anterior sheath adjacent to the linea alba. The anterior sheath was removed and the underlying rectus muscle, transversalis fascia, and peritoneum were left intact. A 1 x 1 cm piece of reinforced fascia (n = 8 pieces/braid type) was rehydrated in sterile saline for 20 minutes and secured into the defect at the four corners using 5 - 0 Prolene (Figure 3.7). On the contralateral side of the linea alba, a second 1x1 cm defect was created in the anterior sheath and a replaced with a 1x1 cm piece of non-reinforced fascia as a control. The skin incision was closed using 4 - 0 Vicryl suture and the rat was allowed to recover from anesthesia under a heating lamp.

![Figure 3.7: Rat abdominal wall defect implantation](image)
Post-operative Care

For analgesia, each rat received 0.15 mg/kg buprenorphine post-operatively, 12 hours later, and thereafter as needed for breakthrough pain. Rats were housed individually for the duration of the study and were observed for any signs of dehiscence, redness and inflammation around the incision sites.

Euthanasia and Tissue Harvest

At four and twelve weeks, rats were sacrificed via carbon dioxide asphyxiation (n = 11 per braid type per time point). The reinforced fascia scaffold implanted in the dorsal subcutaneous pouch was explanted, carefully detached from the polypropylene screen, wrapped in saline soaked gauze and stored at 4°C for up to 24 hours prior to mechanical testing. Scaffold samples implanted in the abdominal wall defect sites were harvested (n = 4 per braid per time point), fixed in 10% neutral buffered formalin (NBF) for 24 hr and routinely processed for paraffin embedding.

3.3.4 Post-Implantation Mechanical Properties

The suture retention load and stiffness of the explanted, reinforced fascia scaffolds were tested using the tension-with-side-constraint test described in Section 3.3.2 (n = 11 per braid type per time point). The Fiberwire suture loops were placed into the scaffolds prior to implantation, which were used for affixing the constructs to the testing jig.

3.3.5 Histological Analysis

Five-micron thick longitudinal sections were cut from four samples per group per time point and stained with hematoxylin and eosin (H&E) One representative H & E section per sample was semi-quantitatively scored by a board-certified pathologist
(Carmela Tan, MD, Cleveland Clinic) according to a scoring system adapted from ISO Standard 10993-6 (Table 3.2). The sections were scored for the presence of inflammatory cells, i.e., neutrophils, eosinophils, lymphocytes, plasma cells, macrophages, and foreign body giant cells. Non-inflammatory outcomes namely, fibroblast-like cells, vascularization and amount of cellular infiltrate into the graft from the periphery (cellularity) were also scored.
<table>
<thead>
<tr>
<th>Cell type/response</th>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory cell outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td>0</td>
<td>Rare (1-5/hpf)</td>
<td>5-10/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td>0</td>
<td>Rare (1-5/hpf)</td>
<td>5-10/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Plasma cells</td>
<td></td>
<td>0</td>
<td>Rare (1-5/hpf)</td>
<td>5-10/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Macrophages</td>
<td></td>
<td>0</td>
<td>Rare (1-5/hpf)</td>
<td>5-10/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td>Giant cells</td>
<td></td>
<td>0</td>
<td>Rare (1-5/hpf)</td>
<td>3-5/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td><strong>Total Cellularity</strong></td>
<td>--</td>
<td>&lt; 25%</td>
<td>26-50%</td>
<td>51-75%</td>
<td>&gt; 75%</td>
<td></td>
</tr>
<tr>
<td>Fibroblasts</td>
<td></td>
<td>0</td>
<td>Rare (100/hpf)</td>
<td>100-1000/hpf</td>
<td>Heavy infiltrate</td>
<td>Packed</td>
</tr>
<tr>
<td><strong>Non-inflammatory outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascularization</td>
<td></td>
<td>0</td>
<td>Minimal capillary proliferation, focal, 1-3 buds</td>
<td>Groups of 4-7 capillaries with supporting fibroblastic structures</td>
<td>Broad band of capillaries with supporting structures</td>
<td>Extensive band of capillaries with supporting fibroblastic structures</td>
</tr>
</tbody>
</table>

Table 3.2: Histologic scoring system adapted from ISO 10996-6 standard, hpf = high powered field (40x)

### 3.3.6 Statistical Analysis

A two-sample t-test was used to test for differences in the suture retention load, stiffness, and stroke and cyclic elongation at the first cycle of loading, between reinforced fascia groups at time zero. The cyclic elongation and stiffness profiles were compared between reinforced groups at time zero, first for cycles 1 - 2500 and then for cycles 2500 - 5000. Cycle 2500 was chosen as the delimiter because it was the last data point acquired before at least one sample failed. To perform the profile comparisons, the cyclic data were log transformed, and quadratic mixed models were used to compare the profiles
between the two groups. For the post-implantation mechanical properties, a two-factor analysis of variance (ANOVA) with post hoc Tukey test was used to test for differences in suture retention load and stiffness between groups across time. A p-value of ≤ 0.05 was considered significant. For histologic analysis, we were primarily interested in detecting differences in foreign body giant cells between groups at each time point, and not in the interaction between group and time. Hence, Kruskal-Wallis ANOVA on ranks was used for histologic outcomes at each time point. For histologic analysis, no adjustment was made for multiple comparisons, and a p-value of ≤ 0.05 was considered significant.

3.3.7 Power Analysis and Sample Size Justification

The study was powered primarily to detect significant differences between groups as a function of implantation time. A power analysis was performed to detect a minimum difference of 50% between the means with an expected standard deviation of 35%. A sample size of eleven per braid type per time was estimated, which would allow us to detect differences with alpha = 0.05 and power = 0.8.
3.4 Results

3.4.1 Time Zero Mechanical Properties

Failure Testing

The suture retention load of fascia patches reinforced with either braid exceeded 300 N, which is a six fold increase over non-reinforced fascia (Figure 3.8). Fascia reinforced with 6PLLA/2PGA braid has a significantly higher suture retention load (p < 0.05) but no difference in stiffness than fascia reinforced with 100% PLLA braid (Table 3.3). The mode of failure for all samples occurred by the breaking of the braid secondary to slipping of the braid through the fascia matrix.

**Figure 3.8:** Average time zero suture retention load of fascia reinforced with 100% PLLA or 6PLLA/2PGA braid. The suture retention load of fascia patches reinforced with either braid exceeded 300N, which is a six fold increase over non-reinforced fascia (54 ± 14 N) and also greater than the suture retention of human rotator cuff tendon (200 - 240 N) Like letters indicate statistical significance between groups (p < 0.05).
### Table 3.3: Mechanical properties of fascia reinforced with 100% PLLA or 6PLLA/2PGA at time zero and after in vivo implantation: Average values (standard deviation). Statistical differences between groups are noted in the text and related figures.

<table>
<thead>
<tr>
<th>Property</th>
<th>100% PLLA</th>
<th>6PLLA/2PGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time Zero</td>
<td>4 wks</td>
</tr>
<tr>
<td><strong>Suture Retention Load (N)</strong> §</td>
<td>315(23)</td>
<td>315(56)</td>
</tr>
<tr>
<td><strong>Stiffness (N/mm)</strong> §</td>
<td>21(2)</td>
<td>17(3)</td>
</tr>
<tr>
<td><strong>First Cycle Stroke Elongation (mm)</strong> *</td>
<td>7.3(0.2)</td>
<td>----</td>
</tr>
<tr>
<td><strong>First Cycle Cyclic Elongation (mm)</strong> *</td>
<td>5.6(0.3)</td>
<td>----</td>
</tr>
<tr>
<td><strong>Cycles to failure</strong> *</td>
<td>3 of 8 did not fail after 5000 cycles</td>
<td>----</td>
</tr>
</tbody>
</table>

§ Data obtained from failure testing, n = 10 - 12 per group

* Data obtained from cyclic testing, n = 8 except where noted
Cyclic Fatigue

At the first cycle of loading, scaffolds reinforced with 100% PLLA had an average stroke elongation of 7.3 ± 0.2 mm and cyclic elongation of 5.6 ± 0.3 mm, which was significantly less than the average stroke elongation (8.1 ± 0.8 mm) and cyclic elongation (7.4 ± 0.8 mm) of scaffolds reinforced with 6PLLA/2PGA (p ≤ 0.025, Table 3.3).

There were no significant differences in the cyclic elongation (p = 0.75) or stiffness (p = 0.56) profiles between groups over the first 2500 cycles. However, the cyclic elongation (p = 0.0003) and stiffness (p = 0.0002) profiles were significantly different between groups for cycles > 2500 (Figures 3.9 A & 3.9 B).

Three of eight reinforced scaffolds in the 100% PLLA group and eight of eight reinforced scaffolds in the 6PLLA/2PGA group did not fail after 5000 cycles of cyclic loading (Table 3.3). The remaining five of eight reinforced fascia scaffolds in the 100% PLLA group failed at an average of 3250 ± 650 cycles, with the mode of failure being breaking of the braid secondary to slipping of the reinforcing braid through the fascia matrix.
Figure 3.9: Average time zero (A) cyclic elongation and (B) cyclic stiffness of fascia scaffolds reinforced with 100% PLLA or 6PLLA/2PGA braid. There were no significant differences in the cyclic elongation or stiffness profiles between groups over the first 2500 cycles. However, the cyclic elongation and stiffness profiles were significantly different between groups for cycles > 2500.
3.4.2 Gross Observations

The fiber reinforcing braids were clearly visible within the fascia scaffolds at four and twelve weeks. There were no obvious changes in the size of the scaffold, and the fascia matrix did not seem to be grossly resorbed at either time points (Figure 3.10 A). Scaffolds implanted in the abdominal wall defect were clearly distinguishable from the underlying muscle at both time points (Figure 3.10 B).

![Figure 3.10](image)

**Figure 3.10:** Gross observations at tissue harvest, in this case 12 weeks: (A) 5x5 cm reinforced fascia ECM scaffold attached to screen at dorsal implantation site and (B) 1x1 cm reinforced (left) and non-reinforced control (right) fascia ECM scaffold at abdominal wall defect site.

3.4.3 Descriptive Histology

All scaffolds elicited a chronic inflammatory response composed of lymphocytes, plasma cells and macrophages. Eosinophils were conspicuous in and around the scaffolds of all experimental groups at the four week time point but these had mostly disappeared by twelve weeks. Neutrophils were absent. A foreign-body giant cell response was evident and concentrated to the area of polymer reinforcement at four weeks and persisted at twelve weeks. In the non-reinforced fascia scaffolds, multinucleated giant cells were few and were found only around possible foreign bodies. The fascia matrix in all groups exhibited variable regions of cellularity, and most of the cellular infiltrates in the central region of the scaffolds by twelve weeks were accounted for by spindle-shaped fibroblasts, lymphocytes and macrophages interspersed between the collagen bundles (Figures 3.11 & 3.12).
Figure 3.11: Histologic images of fascia scaffolds at 4 weeks stained with hematoxylin-eosin. Representative images of non-reinforced fascia (A, C, E, G) and 100% PLLA reinforced fascia (B, D, F, H).

A, B (Magnification 15x): The scaffolds appeared intact and composed of longitudinal collagenous bands without (A) and with polymers (B, asterisks).

C, D (Magnification 100x): The cellular infiltrates were more pronounced at the periphery of the scaffold, though sparse infiltrates were seen throughout the fascia matrix. Minimal disorganization of the collagenous scaffold was observed.

E, F (Magnification 400x): The chronic inflammatory cellular infiltrates were composed predominantly of lymphocytes and plasma cells with scattered eosinophils (arrows) in both groups.

G, H (Magnification 600x): Foreign-body giant cell reaction in the non-reinforced fascia was minimal and superficial in location (G). In the reinforced scaffolds, multinucleated giant cells are concentrated around the polymer reinforcing braids (H, asterisks), which appear as birefringent material in cross-section and longitudinal orientation.
Figure 3.12: Histologic images of fascia scaffolds at 12 weeks stained with hematoxylin-eosin. Representative images of non-reinforced fascia (A, C, E) and 100% PLLA reinforced fascia (B, D, F).

A, B (Magnification 15x): The fascia scaffolds appeared intact with more pronounced cellular infiltrates than at four weeks.

C, D (Magnification 100x): Cellular infiltrates were observed in the peripheral and central regions of the scaffold.

E, F (Magnification 200x): The scaffolds were infiltrated by spindle-shaped fibroblast-like cells, macrophages and lymphocytes in variable distribution. Vascularization (arrows) of the grafts was mild and comparable between groups.
3.4.4 Histological Outcomes

The individual and average scores of the non-inflammatory and inflammatory outcomes from each experimental group at both time points are presented in Table 3.4 & 3.5, respectively. There was an increase in the number of foreign body giant cells (FBGC) in the reinforced fascia groups compared to non-reinforced fascia (p = 0.01) at both time points. However, there were no significant differences in the amount of other inflammatory cellular infiltrates, i.e., neutrophils, lymphocytes, plasma cells and macrophages between groups at either time point. There were no differences in the cellularity, density of fibroblasts-like cells or vascularity between groups at either time point.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Experimental Group</th>
<th>Non-Inflammatory Cell Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vascularization</td>
</tr>
<tr>
<td>4 weeks</td>
<td>Native</td>
<td>1,1,1,1</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>100% PLLA Reinforced</td>
<td>1,1,1,1</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>6PLLA/2PGA Reinforced</td>
<td>1,1,1,1</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1.75)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>Native</td>
<td>1,1,1,1</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1.25)</td>
</tr>
<tr>
<td></td>
<td>100% PLLA Reinforced</td>
<td>1,1,1,1</td>
</tr>
<tr>
<td></td>
<td>(1)</td>
<td>(1.5)</td>
</tr>
<tr>
<td></td>
<td>6PLLA/2PGA Reinforced</td>
<td>1,1,1,2</td>
</tr>
<tr>
<td></td>
<td>(1.25)</td>
<td>(2.5)</td>
</tr>
</tbody>
</table>

Table 3.4: Histological scores (mean) for non-inflammatory outcomes
n = 4 per group per time point
<table>
<thead>
<tr>
<th>Time Point</th>
<th>Experimental Group</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Plasma Cells</th>
<th>Macrophages</th>
<th>Giant Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>Native</td>
<td>1,1,2,2</td>
<td>2,2,3,3</td>
<td>1,3,3,3</td>
<td>1,2,2,2</td>
<td>1,1,1,1&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100% PLLA</td>
<td>1,2,2,2</td>
<td>1,1,2,2</td>
<td>2,3,3,3</td>
<td>2,2,2,2</td>
<td>3,3,3,3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Reinforced</td>
<td>(1.5)</td>
<td>(1.5)</td>
<td>(2.5)</td>
<td>(2.75)</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>6PLLA/2PGA</td>
<td>1,1,2,2</td>
<td>2,2,2,3</td>
<td>1,1,2,2</td>
<td>2,3,3,4</td>
<td>2,3,3,3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Reinforced</td>
<td>(1.5)</td>
<td>(2.25)</td>
<td>(1.5)</td>
<td>(3)</td>
<td>(2.75)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>Native</td>
<td>0,0,0,0</td>
<td>2,3,3,3</td>
<td>1,1,2,2</td>
<td>1,2,2,3</td>
<td>1,1,1,1&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>100% PLLA</td>
<td>0,0,1,1</td>
<td>2,2,2,4</td>
<td>1,2,2,2</td>
<td>1,2,2,3</td>
<td>2,3,3,3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Reinforced</td>
<td>(0)</td>
<td>(2.5)</td>
<td>(1.5)</td>
<td>(2)</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>6PLLA/2PGA</td>
<td>0,0,0,0</td>
<td>2,2,2,3</td>
<td>0,1,2,2</td>
<td>0,1,2,2</td>
<td>2,3,3,3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Reinforced</td>
<td>(0)</td>
<td>(2.25)</td>
<td>(1.25)</td>
<td>(1.25)</td>
<td>(2.75)</td>
</tr>
</tbody>
</table>

Table 3.5: Histological scores (mean) for inflammatory cell outcomes
Like letters indicate significant differences for giant cells between groups at each time point (p = 0.01)
n = 4 per group per time point
3.4.5 Post-Implantation Mechanical Properties

The mode of failure for all samples occurred by breaking of the reinforcing braid secondary to slipping of the braid through the fascia matrix (Figure 3.13).

![Figure 3.13](image)

**Figure 3.13:** Mechanism of slipping of braid through fascia matrix: With application of force (F), the braids in the matrix begin slipping through the fascia matrix ultimately leading to breaking of braid.

The suture retention load of fascia reinforced with 100% PLLA did not change after 12 weeks of implantation, whereas the suture retention load of fascia reinforced with 6PLLA/2PGA significantly decreased (~40%) after four and twelve weeks (Figure 3.14 A, Table 3.3). The stiffness of fascia reinforced with either braid significantly decreased after implantation (Figure 3.13 B, p ≤ 0.0001), but the decrease was significantly greater at 12 weeks in scaffolds reinforced with 6PLLA/2PGA (~35%) than scaffolds reinforced with 100% PLLA (~15%) (Figure 3.14 B, Table 3.3) (p ≤ 0.0001). Hence by twelve weeks, scaffolds reinforced with 6PLLA/2PGA had significantly less suture retention strength and stiffness than scaffolds reinforced with 100% PLLA.
Figure 3.14: (A) Suture retention load and (B) stiffness of fascia reinforced with 100% PLLA or 6PLLA/2PGA braid after in vivo implantation. Fascia reinforced with 100% PLLA demonstrates no significant change in suture retention load after 12 weeks of implantation, whereas the suture retention load of fascia reinforced with 6PLLA/2PGA significantly decreased (~40%) after in vivo implantation. There is a significant decrease in the stiffness of fascia reinforced with either braid; however, the decrease is more pronounced in scaffolds reinforced with 6PLLA/2PGA (~35%) than scaffolds reinforced with 100% PLLA (~15%).
3.5 Discussion

The goal of this study was to develop a novel extracellular matrix (ECM) derived scaffold with robust mechanical properties for use in musculoskeletal soft tissue repair. Specifically, the suture retention strength and stiffness of allograft fascia lata was engineered by stitching braided, resorbable polymer fibers into fascia extracellular matrix (ECM) in a unique, controlled manner. Two types of polymer reinforcing braids (100% PLLA or 6PLLA/2PGA braid) were investigated. At time zero, reinforced fascia scaffolds had a six-fold increase in suture retention strength compared to non-reinforced fascia, and the suture retention of fascia reinforced with 6PLLA/2PGA was significantly greater than fascia reinforced with 100% PLLA braid. Reinforced fascia scaffolds from both braid groups had similar cyclic loading profiles up to 2500 cycles, however, only scaffolds reinforced with 6PLLA/2PGA consistently survived loading to 5000 cycles. The mechanical properties of reinforced fascia scaffolds changed over time following in vivo implantation, and by twelve weeks scaffolds reinforced with 6PLLA/2PGA had significantly less suture retention strength and stiffness than scaffolds reinforced with 100% PLLA. The host response to all fascia scaffolds was characterized by the infiltration of inflammatory and non-inflammatory fibroblast-like cells. Giant cells were more predominant in the reinforced fascia scaffolds and concentrated around the polymer braids.

At time zero, reinforced fascia ECM had a six-fold increase in suture retention strength compared to non-reinforced fascia and exceeds the suture retention properties of human rotator cuff tendon (~250 N) 20. Contrary to our hypothesis, however, the suture retention of fascia reinforced with 6PLLA/2PGA was significantly greater than fascia
reinforced with 100% PLLA braid despite both braids having similar ultimate tensile strength (Table 3.1). One possible explanation for this outcome was that the 6PLLA/2PGA braid was noted to be qualitatively smoother than the 100% PLLA braid, which may have allowed for tighter stitching and more efficient load-sharing by the 6PLLA/2PGA braid across the whole range of displacement. The similarity in stiffness between both reinforced fascia groups at lower-displacements despite 6PLLA/2PGA being a stiffer braid (Table 3.1), suggests that the initial mechanism of scaffold loading is not primarily dictated by the mechanics of the braids, but more likely a function of friction/slipping at the interface between the braids and the ECM.

Reinforced fascia scaffolds from both braid groups had similar cyclic loading profiles up to 2500 cycles, though scaffolds reinforced with 100% PLLA experienced significantly less stroke and cyclic elongation on the first cycle of pull. Between groups differences in stroke elongation at cycle one seem to contradict the results from failure testing which showed similarity in stiffness between reinforced fascia groups at lower-displacements. Given the relatively low sample size for both types of tests (n = 8 to 12), this discrepancy could simply be a statistical anomaly. Since the groups otherwise had similar loading profiles up to 2500 cycles, we conclude that any differences that may exist between groups at cycle one are small and not clinically meaningful. Regardless, it is important to emphasize that when reinforced scaffolds were subjected to 5 - 150 N of cyclic load, they stretch 7 - 8 mm the first time they are pulled, and have 6 - 7 mm of cyclic (presumably permanent) elongation when they are unloaded. (This elongation is not likely to be eliminated simply by pre-tensioning the scaffold in the operating room, because it occurred as a result of loading to 150 N). After the first cycle of loading, the
fibers have presumably “set”, the scaffold stiffens, and cyclic elongation progresses at a slower rate. For example, reinforced scaffolds demonstrate ~12 mm of cyclic elongation after 1000 cycles to 150 N. Only scaffolds reinforced with 6PLLA/2PGA consistently survived loading to 5000 cycles, which may again be due a tighter stitch afforded by the 6PLLA/2PGA braid allowing for a more efficient load-sharing and better resistance to fatigue failure.

After four and twelve weeks of implantation, the suture retention load of fascia reinforced with 6PLLA/2PGA significantly decreased (~40%). Because the suture retention load of fascia reinforced with 100% PLLA did not change in the same time frame, we hypothesize that the loss of suture retention with the 6PLLA/2PGA braid (contains 34% PGA by weight) was primarily due to degradation of PGA and subsequent weakening of the overall braid construct. PLLA and PGA are aliphatic polyesters that degrade by hydrolytic chain scission of the esters linkage $^{16}$. PGA is a hydrophilic and degrades on the order of days to weeks, whereas PLLA is hydrophobic and degrades on the order of months $^{19,26}$. The absence of any further loss of suture retention in scaffolds reinforced with 6PLLA/2PGA braid between four and twelve weeks suggests that the majority of the PGA in the 6PLLA/2PGA braid was degraded by four weeks post-implantation.

The stiffness of both groups of reinforced scaffolds decreased following four weeks of in vivo implantation. The reduction in scaffold stiffness is likely, at least in part, a consequence of the influx of macrophages and giant cells, which are known to secrete matrix metalloproteinases (MMP) -2, -3, -9, -12 $^{2,14}$. Further it has also been shown that ECM collagen degradation occurs by action of matrix metalloproteinases (MMP) -1,- 8,
-13, -14 and through phagocytosis by macrophages and fibroblasts\textsuperscript{1, 8, 9, 15, 21, 25}. Together, these could have reduced the mechanical properties of fascia ECM following \textit{in vivo} implantation as seen by other investigators\textsuperscript{12, 24}. In reinforced fascia scaffolds degradative enzyme activity would likely be particularly pronounced around the braids where giant cells were predominantly localized, which could result in dramatically reduced stiffness of the fascia matrix locally, leading to reduced interface friction between the fascia and the braids. The greater loss of stiffness of fascia reinforced with 6PLL/2PGA braid (35\%) compared to fascia reinforced with 100\% PLLA braid (15\%) may be explained by a possible concomitant decrease in mechanical properties of the 6PLL/2PGA braid as a consequence of PGA hydrolysis. That the stiffness of the scaffolds did not further decrease from four to twelve weeks of implantation suggests the degradative (inflammatory) phase may be resolving or at least balanced by the presence of non-inflammatory cells involved in new tissue deposition. This conclusion is supported by studies which have shown that soft tissue repairs are weakest at 3 - 4 weeks after surgery as the inflammatory phase is transitioning to the remodeling phase of wound repair\textsuperscript{4, 27}.

The host response to all fascia scaffolds was characterized by the infiltration of macrophages, giant cells, lymphocytes, plasma cells and non inflammatory fibroblast-like cells. In particular, giant cells were more predominant in the reinforced fascia scaffolds and concentrated around the polymer braids. The presence of giant cells around PLLA braids has been shown previously\textsuperscript{11} and is expected as the macrophages attempt to phagocytose the “foreign” polymeric materials. We speculate that the giant cell response would persist until the braids are fully hydrolyzed. Although eosinophils are known to be
present as an inflammatory infiltrate associated with cutaneous wounds \textsuperscript{5, 6}, and as a response to allergic conditions or during parasitic infections \textsuperscript{18}, their presence at four weeks and resolution at twelve weeks in scaffolds from all groups remains unclear. Their presence could be related to processing steps, as they were not seen in a previous study from our laboratory where the fascia scaffolds were rinsed in water for 24 hours prior to implantation \textsuperscript{17}. However, their transient presence did not seem to have any deleterious effect on scaffold integrity. The chronic lymphocytic infiltrate in all fascia scaffolds may represent an immune reaction to the xenograft, possibly due to remnant cellular elements that remain in the fascia even after processing. It is hypothesized that fascia ECM would elicit less lymphocyte infiltrate than seen here, if used as an allograft in human patients. Finally, the presence of fibroblast-like cells in all fascia scaffolds is suggestive of host remodeling and new tissue deposition.

The current study is not without limitation. First, the dorsum of the rat does not fully mimic the intra-articular environment of the human shoulder, nor were the scaffolds mechanically loaded. Synovial fluid and/or mechanical loading could affect the rate and extent of braid degradation and scaffold remodeling. Second, the host response of the reinforced fascia scaffolds was not evaluated in the context of a rotator cuff injury. However, the rat body wall defect model is an established preclinical model for evaluating the host response to biomaterials \textsuperscript{23} and is useful for comparative studies. Third, the change in the surface morphology, molecular weight, crystallinity and mechanical properties of the braids themselves after \textit{in vivo} implantation were not investigated but would be useful to know in order to design braids that have desired mechanical properties and degradation profiles. Lastly, twelve weeks was the latest time
point investigated. A longer time would allow us to evaluate the effect of braid degradation and fascia matrix remodeling on the mechanical properties of the reinforced fascia scaffolds.

The overall goal of this work was to engineer the suture retention strength and stiffness of allograft fascia lata in order to develop an extracellular matrix (ECM) derived scaffold with robust mechanical properties for general use in musculoskeletal soft tissue repair, and more specifically rotator cuff repairs. The precise design criteria of scaffolds for such applications are difficult to specify because the mechanical demands in the clinical scenario are variable and unknown. As a guidance, however, one might consider the suture retention properties of human rotator cuff tendon (~250 N)\(^{20}\), the maximum expected loads on a rotator cuff repair post-operatively (~180 N)\(^{13,22}\), the number of duty cycles that a rotator cuff repair may be exposed to in the early post-operative period (it is estimated that during the first six weeks of post-operative rehabilitation, a human rotator cuff repair might experience ~60 cycles/day = ~2500 cycles), and the amount of medial tendon retraction that might increase the likelihood of repair failure (5 - 10 mm) as possible design criteria\(^{7,22}\). Here, it has been demonstrated that the suture retention load of reinforced fascia scaffolds remained \(\geq 250\) N, even after twelve weeks of \textit{in vivo} transplantation. Furthermore, the scaffold augmentation component is estimated to carry only 20 - 30% of the load on a tendon repair (i.e., ~50 - 60 N) (Chapter 4)\(^3\). Although reinforced fascia scaffolds in this study were not cycled to these lower levels of load, the data support the possibility that the cyclic elongation of reinforced fascia scaffolds at 50 N cyclic loads would remain on the order of 5 mm or less, even after 2500 cycles. Together these results suggest that fascia scaffolds reinforced with either braid have
robust load-deformation properties that are possibly sufficient to provide mechanical augmentation to rotator cuff repairs and modulate tendon retraction in a manner that reduces the incidence of tendon re-tear.

In summary, this study presents fiber reinforcement by stitching as a novel and versatile method for engineering the suture retention strength and stiffness of allograft fascia lata, which may be extended to other extracellular matrix derived materials as well. Conceptually, this approach retains the biologic advantages of a naturally-derived extracellular matrix, while improving its mechanical performance for demanding musculoskeletal applications. The *in vitro* and *in vivo* studies performed herein suggest the potential of the reinforced fascia scaffolds to provide and sustain mechanical augmentation of tendon repairs. The extent to which reinforced fascia scaffolds can in fact limit tendon retraction in the context of rotator cuff repair is the subject of an ongoing work in a human shoulder cadaver model in our laboratory. However, a full understanding of the clinical efficacy of these novel materials will require pre-clinical and clinical studies.

The next chapter will present the formulation and development of a spring-network model for rotator cuff repairs.


5. Baker JR BESP. Eosinophils in healing dermal wounds In 1976, p 401


4.1 Introduction

Rotator cuff tears affect 40% or more of those over age 60 \(^{26, 33, 38}\) and are a common cause of debilitating pain, reduced shoulder function and weakness. Despite advances in the surgical treatment of these tears, high surgical failure rates that range from 20 to 90% have been reported \(^{1, 6, 7, 15-17, 20}\) due to various factors \(^{5, 8, 18, 19, 21, 27, 28, 34, 35}\).

Currently, natural and synthetic scaffolds are being used as devices to augment soft tissues repaired by sutures or suture anchors during the repair of large to massive rotator cuff tears \(^{4, 9, 10}\). While significant advances have been made in the development of scaffolds for use as augmentation devices, no studies have investigated the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation. Furthermore, the manner in which loads are distributed amongst the various components on an augmented
rotator cuff repair is not yet known, nor is the relative biomechanical importance of the various components of the augmented rotator cuff. Hence, there is a need to develop a model that will help answer the aforementioned questions. This dissertation aims to develop quasi-static spring - network models of simplified rotator cuff repairs, (2) validate the models by comparing the predicted model force to experimental measurements of force for human and canine rotator cuff repairs, and (3) use the models to predict the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation.

This chapter will focus on the formulation and development of the quasi-static spring - network models. The model structure for non-augmented and augmented human and canine rotator cuff repairs will be discussed. The description, mechanical testing, modeling and parameter estimation of the individual components of the repair will also be presented. Finally, model equations for the aggregate models will be formulated based on the physics of “springs” connected in series and parallel. Since, the models are developed from in-vitro mechanical testing of repair constructs carried out at a slow loading rate, the models were assumed to have a quasi-static behavior.

4.2 Current Models for Rotator Cuff Tears

Finite element models (FE) have been employed to determine the general stress field in intact, torn and surgically repaired rotator cuff tendon. Luo and co-workers were the first to use a two-dimensional (2D) finite element model to demonstrate the presence of stress concentration on the articular side of the supraspinatus tendon. Wakabayashi et al., also established a 2D finite model, which included the tendon insertion and showed
high stress distributions towards the insertion site as the arm was abducted. Sano et al.,
have reported the stress distribution patterns in the human supraspinatus tendon with
partial thickness tears. Others have used a FE model to compare the stress distribution
pattern in the supraspinatus tendon after repair using a transosseous and suture anchor
fixation technique. More recently, Seki et al., using a three-dimensional (3D) finite
element have reported the stress distribution pattern in the intact supraspinatus tendon.
Further, Adams and co-workers investigated the changes in the rotator cuff tendon
excursion and muscle moment arms using an explicit 3D finite element model of the
glenohumeral joint.

Although these studies allow investigating the stress distribution patterns of the
rotator cuff tendons under complex loading conditions, they do not allow predicting the
biomechanics (stiffness and yield load) of repair constructs during different loading
scenarios and surgical repair techniques. More importantly, the response of the repair
construct to the change in a parameter of interest (e.g., stiffness) cannot be examined
using these models. In contrast, a parametric simulation study is possible with simpler
spring-network models in an efficient and less computationally expensive manner than
sophisticated finite element models. Additionally, spring-network models do not require
explicitly stating the boundary conditions, which is a critical step in the finite element
approach, as these conditions are implicitly included in the mechanical behavior of the
individual springs.

Additionally, spring-network models have routinely been used to investigate the
biomechanics of flexor tendon repairs and load transfer from prosthetic stem to
surrounding bone. Hence, a spring-network model was selected to examine the
biomechanics of rotator cuff repairs. Such a model will also allow prediction of the biomechanics (stiffness and yield load) of repair constructs during different loading scenarios and/or in the context of surgical repair techniques as will be discussed in Chapter 5.

4.3. Approach

From the physical observation of non-augmented and augmented rotator cuff repairs, the assembly of the individual spring components into an aggregate model was based on the analogy of springs in series and parallel. The individual components of the repair constructs representing the tendon attachment to bone, the tendon itself, the scaffold along with its attachment to bone, and the scaffold attachment to the tendon were modeled using a single phase non-linear equation or biphasic non-linear equation. The parameters of the non-linear equations representing the individual components were then estimated by non-linear least-squares analysis of the component-specific load-displacement data. Finally, the model equations were formulated using the physics of springs in series and parallel. The detailed methods are described below.

4.4 Model Structure

4.4.1 Non-Augmented Rotator Cuff Repair (Repairs sans Scaffolds)

A non-augmented rotator cuff repair is a repair wherein the tendon is re-apposed to the bone using a surgical technique without the use of a scaffold as an augmentation
device. Such a repair is shown in Figure 4.1 for human (Figure 4.1 A) and canine repairs (Figure 4.1 B), respectively.

The non-augmented repairs were modeled as two springs in series, namely, the bone-suture-tendon interface (spring#1) and the tendon itself (spring#2) (Fig 4.1 C).

**Figure 4.1:** Schematics of non-augmented (A) human, (B) canine and augmented (D) human, (E) canine rotator cuff repairs and their corresponding analogies with the spring models (C, F). The dotted lines represent suture markers that were placed on the tendon, and the black dot represents the optical marker that was placed on the bone, for optical displacement measurements during mechanical testing. Adapted from Figure 1 in Aurora et al, 3

### 4.4.2 Augmented Rotator Cuff Repair (Repairs with Scaffolds)

Augmented rotator cuff repairs are repairs wherein the scaffolds placed over the tendon repair and sutured to bone at one end and to the tendon at the other. Such a repair is shown in Figure 4.1 for human (Figure 4.1 D) and canine repairs (Figure 4.1 E), respectively.

The augmented rotator cuff repairs were modeled as five springs connected in series and parallel as shown in Figure 4.1 F. The tendon (spring#2) was split into two half
springs, spring#2’ and spring#2”. The bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4) were in series with each other and together in parallel with the primary tendon repair (spring 1 and 2’). This four spring construct was then placed in series with the other half tendon spring#2”, to conform the model for augmented rotator cuff repairs.

4.5 Experimental Mechanical Testing of the Individual Components

The experimental displacement data for spring#1 (bone-suture-tendon) and spring#2 (tendon only) were gathered from mechanical tests carried out on human and canine non-augmented repairs, respectively. The experimental displacement data for spring#3 (bone-screw-scaffold-suture) and spring#4 (medial suture-tendon) was collected from independent (isolated) mechanical tests. The detailed methods and testing protocol are described as follows:

4.5.1 Spring#1 (Bone-Suture-Tendon)

For the human repairs, a strip of the superior infraspinatus tendon (12 mm wide) was released and repaired to the greater tuberosity using a double row transosseous technique with two Mason Allen sutures per row (Figure 4.1 A) 25. Similarly, repair of the canine shoulders involved release and repair of the infraspinatus tendon to the greater tuberosity using two transosseous Mason Allen sutures. All repairs were performed by an orthopedic surgeon, Jesse A. McCarron, MD, Cleveland Clinic. For mechanical testing of all repairs, the muscle belly was freeze-clamped and the repair was cycled between 5-100 N at 0.25 Hz and subsequently loaded to failure at 30 mm/min 11, 25. The distance between an optical marker placed on the humeral head and in the tendon just medial to
the repair sutures provided local displacements across the bone-suture-tendon interface (Figures 4.1 A & 4.1 B).

4.5.2 Spring#2 (Tendon only)

The distance between two optical markers placed on the tendon provided local displacements in the tendon. These displacement data were obtained from mechanical testing of non-augmented human and canine repairs (n = 5, respectively) (Figures 4.1 A & 4.1 B). The displacements of spring#2 were divided in half to obtain the displacements of spring#2’ and spring#2” used in the augmented repair models.

4.5.3 Spring#3 (Bone-Screw-Scaffold-Suture)

A prototypic polymer scaffold (12 mm wide, by 40 mm long, by 0.8 mm thick) (X-Repair, Synthesome Inc., San Diego, CA) was screwed to a wood block on one end and sutured with 3 simple stitches to a rod on the other end (Figure 4.2 A). The construct was preloaded to 5 N and subsequently loading to failure at 30 mm/min. The displacements of spring#3 were obtained using actuator position from these isolated mechanical tests (n = 5).

Figure 4.2: Schematic of the experimental load-displacement testing for (A) spring#3 (bone-screw-scaffold-suture) and (B) spring#4 (medial suture-tendon interface).
4.5.4 Spring#4 (Medial Suture-Tendon)

Three modified Mason Allen sutures were placed in either isolated human superior infraspinatus tendon or canine infraspinatus tendon and secured over a rod using a double half-hitch suture configuration (six throws) (Figure 4.2 B). All repairs were performed by Andrew Baker, Principal Research Engineer, Derwin Laboratory at the Cleveland Clinic. The associated muscle was freeze-clamped and the suture interface was cyclically loaded (5-30 N) for 20 cycles at 0.25 Hz and subsequently loaded to failure at 30 mm/min. The displacements of spring#4 were obtained using actuator position from these isolated mechanical tests (n = 5, respectively).

4.6 Modeling the Individual Components of the Repair

The mechanical behavior of the individual components of the repair constructs was examined by plotting the component-specific load-displacement (L-D) data obtained from in vitro mechanical tests (Section 4.5). Noting the non-linearity of the individual components of the repair construct, a non-linear equation was selected that would reliably approximate the observed non-linear behavior. The form of the non-linear equation was selected by plotting component specific L-D data on a log-log scale and finding the exponential correlation. Based on these results, a power-law equation (of the form $F = F^0 + Ax^b$) was selected to model the individual components of the repair constructs. The appropriateness of this power-law equation to reliably approximate the non-linear behavior of the individual components was then verified by finding the exponential correlation [along with 95% Confidence Intervals (CI)] of the linearized form of the power-law equation, using a linear least-squares analysis in MATLAB (MathWorks,
Natick, MA, USA). If the correlation was not acceptable (experimental data were not within the 95% CI), the mechanical behavior of the individual components was modeled using an alternate (biphasic) non-linear equation. For simplicity, only the modeling of the individual components of canine repairs is being presented here. However, a similar approach was adopted for modeling the individual components of the human repair constructs, and results for both types of repairs will be presented in Chapter 5.

A representative load-displacement (L-D) plot, typical of all the individual components of canine repairs is shown in Figure 4.3. The plot exhibits a non-linear behavior observed in soft tissues including tendon and ligaments. The mechanical behavior is characterized by an initial toe region and a subsequent linear region where the mechanical behavior of the component gradually evolves into a region of quasi-linear behavior with the gradual application of load.

![Figure 4.3: Representative load-displacement plot, typical of all individual components of canine repairs](image)

This shape of the L-D curves verifies the need to model the individual components of repairs using a non-linear equation. An appropriate non-linear equation
was determined by plotting component specific L-D data on a log-log scale. Figure 4.4 is a representative log-log plot of the load-displacement data of the bone-suture-tendon component (spring#1) of canine repairs.

Figure 4.4: Representative log-log plot of the bone-suture-tendon component (spring#1) of canine repairs.

The log-log plot (Figure 4.4) indicates a strong linear correlation ($R^2 = 0.9$) between log (load-displacement) data of the bone-suture-tendon component (spring#1) of canine repairs. Similarly, linear correlations were obtained for from the log-log plots for all the individual components of the canine repair constructs (not shown). This suggests that the individual components could be modeled by a non-linear equation that can be linearized to verify its appropriateness to model the individual components. A power-law equation [Eqn. (1)] is one such equation that can easily be linearized and has been used to model non-linear behavior for various applications[^22][^32]. Hence, a power-law equation was selected to model the non-linear behavior of the individual components of the canine repairs.

\[ F = F^0 + A x^b \] \hspace{1cm} (1)
Where, $F^0$, $A$, $b$, $B$, $c$ are parameters of the model equation.

The linearized formulation of Eqn. (1) is

$$\log(F-F^0) = \log(b) + \log(A) \hspace{1cm} (2)$$

The ability of the power-law equation Eqn. (1) to reliably model the individual components of the canine repairs constructs was then determined by plotting $\log(F-F^0)$ vs. $\log(x)$ and performing a linear least-squares regression of Eqn. (2) in MATLAB (Mathworks, Natick, MA, USA). The results of the linear least-squares regression for the individual components of canine repairs is presented in the next section.
4.7 Linear Least-Squares Regression of the Individual Components

Figure 4.5: Plot of load-displacement experimental data for (A) the bone-suture-tendon component (spring#1), (B) the tendon only component (spring#2), (C) the bone-screw-scaffold-suture component and (D) the medial suture-tendon interface of canine repairs. The exponential correlation [Eqn. (1)] results (dotted blue line) and the 95% confidence intervals (dotted red lines) are also shown.

Figure 4.5 shows the linear least-squares regression of the linearized power-law formulation Eqn. (2) for the individual components of canine repairs. These results suggest that the power-law equation [Eqn. (1)] is able to reliably model the non-linear mechanical behavior of the tendon only component (spring#2) (Figure 4.5 B) and the bone-screw-scaffold-suture component (spring#3) (Figure 4.5 C) of canine repairs. The experimental data of these components remained within 95% confidence intervals (CI) of the correlation.
However, the bone-suture-tendon component (spring#1) (Figure 4.5 A) and the medial suture-tendon interface (spring#4) (Figure 4.5 D) do not appear to be reliably modeled using the power-law equation [Eqn. (1)], as evidenced by large portion of the experimental data outside the 95% CI for these components. These plots suggest that these components seem to follow a two asymptotic exponential phases that may be best modeled using a two-phase non-linear model.

4.7.1 Biphasic (Two-Phase) Non-Linear Equation

Figure 4.6 shows the linear least-squares regression of the linearized power-law formulation Eqn. (2) performed separately on the two exponential asymptotic phases of the bone-suture-tendon component (spring#1) and the medial suture-tendon interface (spring#4) of canine repairs. These plots confirm the two-phase (biphasic) non-linear behavior of these components. The experimental data of these components in the two asymptotic phases remained within 95% confidence intervals (CI) of the separate correlations.

Figure 4.6: Plot of load-displacement experimental data for (A) the bone-suture-tendon component (spring#1) and (B) the medial suture-tendon interface (spring#4) of canine repairs. The exponential correlation [Eqn. (1)] results (solid blue line) and the 95% confidence intervals (dotted blue lines) for the two asymptotic exponential phases are also shown.
The observed biphasic (two-phase) behavior of these components is commonly found in the kinetic analysis of catalytic mechanisms in chemical reaction engineering. A common approach in reaction engineering analysis is to use the Langmuir isotherm equation to estimate kinetic parameters and elucidate mechanisms following a similar biphasic non-linear as seen in Figure 4.6. Hence, it was believed that the biphasic trend of the bone-suture-tendon component (spring#1) and the medial suture-tendon interface (spring#4) of canine repairs may be reliably modeled using a modified form of the Langmuir equation as in Eqn. (3).

\[ F = F^0 + \frac{Ax^b}{1+Bx^c} \]  

Henceforth, the power-law equation [Eqn. (1)] will be referred to as the single phase non-linear equation and the two-phase non-linear equation [Eqn. (3)] as the biphasic equation. For the human repairs, the tendon only component (spring#2), the bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4) were modeled using the single phases non-linear equations, while the bone-suture-tendon component (spring#1) was modeled using the biphasic non-linear equation.

### 4.8 Non-Linear Parameter Estimation

The parameters of the non-linear equations representing the individual components of the repairs were then estimated using non-linear least-squares analysis of the component specific experimental load-displacement data. While linear least-squares analysis employs a closed-end form solution of equations, non-linear least-squares analysis requires an iterative solution. A robust non-linear least-squares approach relies on an optimal selection of the starting guess to minimize convergence problems and
eliminate the possibility of obtaining local minima (sub-optimal solution). To minimize convergence problems, parameters of the individual spring components were first estimated through the linear least-squares regression method described in Section 4.6 (Table 4.1) and were used as the initial (starting) guesses for parameter estimation using a non-linear least-squares approach.

<table>
<thead>
<tr>
<th>Spring</th>
<th>F0</th>
<th>A</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5E+01 (4.3E+00)</td>
<td>1.3E+02 (6.4E+00)</td>
<td>8E-01 (5E-02)</td>
</tr>
<tr>
<td>2'</td>
<td>NS</td>
<td>5E+02 (2E+01)</td>
<td>1.4E+00 (1.0E-01)</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>1E+02 (3E+00)</td>
<td>7.4E-01 (3.0E-02)</td>
</tr>
<tr>
<td>4</td>
<td>NS</td>
<td>6.4E+01 (4.8E-01)</td>
<td>5.3E-01 (6.0E-03)</td>
</tr>
<tr>
<td>2''</td>
<td>NS</td>
<td>5E+02 (2E+01)</td>
<td>1.4E+00 (1.0E-01)</td>
</tr>
</tbody>
</table>

Table 4.1: Linear Least-Squares Parameter Estimation: Estimated parameters and standard errors (for a 95% confidence-level) of the individual component of canine repairs. Significant figures were reported based on the magnitude of the standard errors, which represent the 95% confidence level. “NS” implies that the parameter is Not Significantly different from zero at 95% confidence level.

The parameters of the non-linear equations were then estimated using non-linear least-squares analysis of the component specific experimental load-displacement data up to the yield load. The yield load was defined as the first relative maximum load achieved during the experimental mechanical tests. All non-linear least-squares analysis was performed using Sigma Stat 3.5 (SYSTAT, Chicago, USA). Table 4.2 (A) shows the estimated parameters and the standard errors (95% confidence-levels) for the individual components of the canine rotator cuff repairs. Table 4.2 (B) presents the corresponding p-
values for the individual components of the canine rotator cuff repairs. The p-values indicate the probability that a given parameter is not significantly different from zero.

<table>
<thead>
<tr>
<th>Spring</th>
<th>F°</th>
<th>A</th>
<th>b</th>
<th>B</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1E+01 (6E+00)</td>
<td>8E+02 (5E+02)</td>
<td>2E+00 (4E-01)</td>
<td>4E+00 (3E+00)</td>
<td>1.5E+01 (3.6E-01)</td>
</tr>
<tr>
<td>2'</td>
<td>NS</td>
<td>3.5E+02 (4.6E+01)</td>
<td>1E+00 (2E-01)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>-3.5E+01 (2.8E+00)</td>
<td>9.5E+01 (2.6E+00)</td>
<td>7.0E-01 (1E-02)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>4E+00 (2E+00)</td>
<td>1.7E+02 (1.1E+01)</td>
<td>1.3E+00 (1E-02)</td>
<td>1.3E+00 (1.4E-01)</td>
<td>1.5E+00 (1E-01)</td>
</tr>
<tr>
<td>2''</td>
<td>NS</td>
<td>3.5E+02 (4.6E+01)</td>
<td>1E+00 (2E-01)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

| Spring |  |  |  |  |  |
|--------|---|---|---|---|
| 1      | 0.012 | 0.095 | 0.0001 | 0.18 | <0.001 |
| 2'     | 0.74 | <0.0001 | <0.0001 | -- | -- |
| 3      | <0.001 | <0.001 | <0.001 | -- | -- |
| 4      | 0.045 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| 2''    | 0.74 | <0.0001 | <0.0001 | -- | -- |

Table 4.2: Non-linear least-squares estimates for (A) parameters standard errors (95% confidence-levels) and (B) corresponding p-values of the individual components for canine repairs. Significant figures were reported based on the magnitude of the standard errors, which represent 95% confidence levels. “NS” implies that the parameter is Not Significantly different from zero at the 95% confidence level. N/A implies parameter does not exist for the individual component because of the form of the equation used to fit the data.
4.9 Model Formulation

Single equations were formulated for human and canine non-augmented and augmented rotator cuff repairs based on the physics of springs connected in series and parallel. Single equations were formulated as is robust numerical approach that ensures convergence to a unique solution (See Appendix A for details for formulation of the model equations). The estimated parameters for the individual components (Section 4.8) were used to solve the fully assembled model equations. Since the model structure and the formulation of single equations for the rotator cuff repairs were the same for human and canine repairs, only the formulation of the model equations for non-augmented and augmented canine repairs is presented here.

4.9.1 Modeling the Mechanical Behavior of Non-Augmented Rotator Cuff Repairs

Figure 4.7: Schematics of canine (A) non-augmented and (B) augmented rotator cuff repairs and their corresponding analogies with the spring models (C, D). This figure is an excerpt of Figure 4.1
As described in Section 4.4, canine non-augmented rotator cuff repairs (Figure 4.7 A) were modeled as two non-linear springs connected in series, namely, the bone-suture-tendon interface (spring#1) and the tendon itself (spring#2) (Figure 4.7 B). The load-displacement behavior of this arrangement of springs is governed by the following equations:

\[ F_1(x_1) - F_2(x_2) = 0 \] \hspace{2cm} (4)
\[ (x_1) + (x_2) = d \] \hspace{2cm} (5)

In other words, there are two equations (4) and (5) with two unknowns, where:

- \( F_1 \) = Force in the bone-suture-tendon interface (spring#1)
- \( F_2 \) = Force in the tendon only component (spring#2)
- \( x_1 \) = Displacement of the bone-suture-tendon interface (spring#1),
- \( x_2 \) = Displacement of the tendon only component (spring#2), and
- \( d \) = Displacement of the non-augmented repair construct (from experimental investigations)

As described in Section 4.6, the mechanical behavior of the bone-suture-tendon interface (spring#1) for canine repairs was modeled using the biphasic non-linear equation and the tendon only component (spring#2) using the single phase non-linear equation, respectively:

\[ F_1 = F_{10} + \frac{A_1 x_1^{b_1}}{1 + B_1 x_1^{c_1}} \] \hspace{2cm} (6)
\[ F_2 = F_{20} + A_2 x_2^{b_2} \] \hspace{2cm} (7)

Where \( F_{10}, A_k, b_k, B_k, c_k \) are component specific parameters estimated by non-linear-least squares analysis of component specific data (Table 4.2)
This problem can be efficiently rendered into a single-equation problem as described in Appendix A, which greatly simplifies its numerical solution and validation analysis.

4.9.2 Modeling the Mechanical Behavior of Augmented Rotator Cuff Repair

As described in Section 4.4, canine augmented rotator cuff repairs (Figure 4.7 C) were modeled as five springs connected in series and parallel. The tendon (spring#2) was split into two half springs, spring#2’ and spring#2”. The bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4) were in series with each other and together in parallel with the primary tendon repair (spring 1 and 2’). This four spring construct was then placed in series with the other half tendon spring#2”, to conform the model for augmented rotator cuff repairs (Figure 4.7 D).

The load-displacement behavior of this arrangement of springs is governed by the following equations:

\[ F_1(x_1) - F_2(x_2') = 0 \] …………………………………………(8)

\[ F_3(x_3) - F_4(x_4) = 0 \] …………………………………………. (9)

\[ F_1(x_1) - F_2'(x_2') + F_3(x_3) - F_4(x_4) - F_2''(x_2'') = 0 \] …………………………………………..(10)

\[ (x_1 + x_2') - (x_3 + x_4) = 0 \] …………………………………………..(11)

\[ (x_1 + x_2') + x_2'' = (x_3 + x_4) + x_2'' = d \] …………………………………………..(12)

In other words, there are five equations (8-12) with five unknowns, with \( F_i \) represents the force in spring “i” and \( x_i \) the corresponding displacement.

\( F_i \) = Force in the spring ‘i’

\( x_i \) = Displacement of spring ‘i’

\( d \) = Displacement of the augmented repair construct
As described previously, the mechanical behavior of the bone-suture-tendon interface (spring#1) and the tendon-only component (spring#2) of canine repairs were modeled as a biphasic [Eqn. (6)] and single phase non-linear equations [Eqn. (7)], respectively. Furthermore, the bone-screw-scaffold-suture component (spring#3) was modeled using a single-phase non-linear equation and the medial suture-tendon interface (spring#4) using a biphasic non-linear equation:

\[ F_3 = F_3^0 + A_3x_3^{b_3} \]  \hspace{1cm} (13)

\[ F_4 = F_4^0 + \frac{A_4x_4^{b_4}}{1 + B_4x_4^{c_4}} \]  \hspace{1cm} (14)

Where:

\( F_3 \) = Force in the bone-screw-scaffold-suture component (spring#3)

\( F_4 \) = Force in the medial suture-tendon interface (spring#4)

\( x_3 \) = Displacement of the bone-screw-scaffold-suture component (spring#3)

\( x_4 \) = Displacement of the medial suture-tendon interface (spring#4) and

\( F^0_k, A_k, b_k, B_k, c_k \) are component specific parameters estimated by non-linear-least squares analysis of component specific data (Table 4.3).

As demonstrated in Appendix A, the equations for both non-augmented and augmented repairs can be efficiently re-arranged to render a compact reduced system of non-linear equations. The resulting system of equations can be solved numerically under static equilibrium conditions using a robust (i.e., will always converge to a unique solution) non-linear solver for a system of non-linear equations available in MATLAB (MathWorks Inc, Natick, MA, USA). Similar equations were formulated for human
augmented rotator cuff repairs and efficiently re-arranged to render a compact reduced system of non-linear equations. The scripts employed for the numerical solutions can be found in Appendix B.

4.10 Summary

In summary, this chapter establishes the model structure of non-augmented and augmented rotator cuff repairs, which are applicable to both human and canine models. The non-linear equations that reliably approximate the mechanical behavior of the individual components of rotator cuff repair constructs were determined. The parameters of the non-linear equations for each of the individual components were then estimated using the non-linear least-squares method. Finally the model equations were formulated based on the physics of springs in series and in parallel. The next chapter will validate the model, demonstrate the calculation of the 95% confidence intervals for the model predictions using error propagation analysis, predict the degree of load sharing provided by the scaffold, and present an approximate parametric sensitivity analysis to identify which component(s)/parameter(s) most influences the mechanical behavior predicted by the augmented repair model.


Chapter V

VALIDATION OF SPRING-NETWORK MODEL FOR ROTATOR CUFF REPAIRS

The logic of validation allows us to move between the two limits of dogmatism and skepticism...Paul Ricoeur

5.1 Introduction

As mentioned in the previous chapter, there remains a need to develop a model that will help elucidate the basic biomechanics of rotator cuff repairs. Hence, the Specific Aim 3 of this dissertation was to develop quasi-static spring-network models of simplified rotator cuff repairs. More specifically, the objectives of this work is to (1) develop quasi-static spring-network models of simplified rotator cuff repairs, (2) validate the models by comparing the predicted model force to experimental measurements of force for human and canine rotator cuff repairs, and (3) use the models to predict the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation.

Chapter 4 focused on the formulation and development of the quasi-static spring-network model. This chapter will present studies to validate the model, demonstrate the calculation of confidence intervals for the model predictions using error propagation
analysis, predict the degree of load sharing provided by the scaffold, and present an approximate parametric sensitivity analysis to identify which component(s)/parameter(s) most influences the mechanical behavior predicted by the augmented repair model.

5.2 Experimental Mechanical Testing of the Repairs

Figure 5.1: Schematics of human and canine non-augmented and augmented cuff repairs. The dotted lines represent suture markers that were placed on the tendon, and the black dot represents the optical marker that was placed on the bone, for optical displacement measurements.

All human and canine rotator cuff repairs described below were done using USP Size 0 and USP Size 2 Fiberwire sutures respectively (Arthrex Corporation, Naples, FL, USA). All repairs were performed by an orthopedic surgeon, Jesse A. McCarron, MD, at the Cleveland Clinic.
5.2.1 Non-Augmented Rotator Cuff Repairs

Human (n = 5) and canine (n = 5) cadaveric shoulders were used to perform non-augmented rotator cuff repairs. For the human repairs, a strip of the superior infraspinatus tendon (12 mm wide) was released and repaired to the greater tuberosity using a double row transosseous technique with two Mason Allen sutures per row (Figure 5.1 A). Similarly, repair of the canine shoulders involved release and repair of the infraspinatus tendon to the greater tuberosity using two transosseous Mason Allen sutures (Figure 5.1 B).

5.2.2 Augmented Rotator Cuff Repairs

The contra lateral human (n = 5) and canine (n = 5) cadaveric shoulders were used to perform augmented rotator cuff repairs. For both human (Figure 5.1 C) and canine (Figure 1 D) specimens, a primary rotator cuff repair was performed as described above. The repairs were augmented with a 12 mm x 35 mm prototypical polymer scaffold (X-Repair, Synthasome Inc., San Diego, CA), fixed to the bone laterally with a screw and sutured medially to the tendon using three modified Mason Allen sutures.

For mechanical testing of all repairs, the muscle belly was freeze-clamped and the repair was cycled between 5 – 100 N at 0.25 Hz and subsequently loaded to failure at 30 mm/min. Optical markers placed in the bone and on the tendon were used to determine the displacement of the repair constructs. Experimental data for the non-augmented and augmented human rotator cuff repairs have been published previously.
5.3 Parameter Estimation

The parameters of the individual spring components were estimated by non-linear least square analysis of the component specific load-displacement data of the individual spring components up to the yield load either to a single phase or biphasic non-linear equation [Chapter 4 (Section 4.6)]. The yield load was defined as the first relative maximum load achieved during the experimental test. The non-linear least-squares analysis was performed using Sigma Stat 3.5 (SYSTAT, Chicago, USA).

5.4 Formulation of the Model

The formulation of the model has been explained in Chapter 4. Briefly, the model was developed by representing the individual components of the repair as non-linear springs. Each non-linear spring was modeled using either a single phase non-linear equation or a biphasic non-linear equation, depending on the equation goodness of fit (Chapter 4). Model equations were then formulated for the non-augmented and augmented rotator cuff repair models using the physics of springs in series and parallel and solved using parameters estimated for the individual components of the repairs.

5.5 Model Validation

To predict the force response of non-augmented and augmented rotator cuff repairs, the fully assembled model equations were solved under static equilibrium conditions using standard non-linear equation solver, (fsolve/fzero) provided with the optimization toolbox in MATLAB (MathWorks, Natick, MA, USA). The model was validated by comparing the model predicted force to experimental measurements of the
force of human and canine rotator cuff repairs for a given displacement. The 95% confidence intervals for the model predictions were calculated using the error propagation formula

\[ E_{F,i} = \sum_{k=1}^{n} \left| \frac{\partial F}{\partial p_k} \right| E_{p,k} \]  

Where:

\( E_{F,i} \) is the total error in the model equation at the displacement \( x_i \) and \( E_{p,k} \) is the standard error of the \( k^{th} \) parameter. \( \left| \frac{\partial F}{\partial p_k} \right| \) is the partial derivative of the model equation with respect to the \( k^{th} \) parameter evaluated at the displacement \( x_i \).

The goodness of fit for each model was assessed using the root mean square error (RMSE) and the root mean square relative error (RMSRE) defined as follows:

\[ RMSE = \sqrt{\frac{\sum_{i=1}^{n} (F_i^e - F_i^m)^2}{n}} \]  

\[ RMSRE = \sqrt{\frac{\sum_{i=1}^{n} \epsilon_i^2}{n}} \]

Where,

\[ \epsilon_i = \frac{F_i^e - F_i^m}{F_i^e} \]
\( F_i^e \) is the experimental measured force value, \( F_i^m \) is the model predicted force for a displacement \( x_i \) and \( n \) is the number of data points. The predictions of the model were considered acceptable if the experimental data fell within the 95% confidence intervals of the predicted force response of the model and the RMSE (% of the average experimental yield load) values of the model predicted force was less than or equal to 5%. Five percent was chosen based on the consideration that, on average, model predictions could be reported with at least two significant figures.

5.6 Parametric Sensitivity Analysis

A parametric sensitivity analysis was used to identify which of the component(s)/parameter(s) most influenced the mechanical behavior of the augmented repair model. The sensitivity of each parameter was investigated by calculating the sensitivity coefficient ‘\( S \)’ defined as

\[
S_{p_k, x_i} = \left. \frac{\partial F}{\partial p_k} \right|_{x_i} \cdot \left. \frac{p_k^o}{F_i^o} \right| \quad \forall \; i = 1 \ldots N \ldots \ldots \ldots(5)
\]

Where \( p_k^o \) and \( F_i^o \) are the baseline values of the \( k \)th parameter and the \( i \)th force measurement respectively. The partial derivatives were estimated numerically by central differences. The clinical interests and application of this model lie in predicting the change in the mechanics of rotator cuff repair with a change in surgical procedure and/or scaffold design. Hence, the parametric sensitivity analysis was carried out only for the parameters A and b of the bone-suture-tendon component (spring#1), bone-screw-
scaffold-suture component (spring#3) and the medical suture-tendon interface (spring#4), which are considered to be associated with such modifications.

The model validation and predictions for non-augmented and augmented rotator cuff repairs, using both human and canine experimental data will be presented. The validated non-augmented and augmented repair models will then be compared to each other to investigate the mechanical role of scaffold augmentation. The distribution of forces in the individual components of the augmented repair will also be assessed through the model. This data will predict the degree of load sharing offered by using a prototype polymeric scaffold as an augmentation device for rotator cuff repairs. Finally, the results of the parametric sensitivity analysis will also be presented.

5.7 Results

5.7.1 Parameter Estimation

Table 5.1 (A) and 5.2 (A) shows the estimated parameters and the standard errors (95% confidence-levels) for the individual components of the human and canine rotator cuff repairs, respectively. Table 5.1 (B) and 5.2 (B) presents the corresponding p-values for the individual components of human and canine rotator cuff repairs, respectively. The p-values indicate the probability that a given parameter is not significantly different from zero.
(A) Human Non-Linear Fit Parameters

<table>
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<tr>
<th>Spring</th>
<th>( F^0 )</th>
<th>A</th>
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<th>B</th>
<th>c</th>
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<td>2''</td>
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(B) Human Non-Linear Fit P-values

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Table 5.1: Non-linear least-squares estimates for (A) parameters and standard errors (95% confidence-levels) and (B) corresponding p-values of the individual components for human repairs. Significant figures were reported based on the magnitude of the standard errors, which represent 95% confidence levels. “NS” implies that the parameter is Not Significantly different from zero at the 95% confidence level. N/A implies parameter does not exist for the individual component because of the form of the equation used to fit the data.
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<th>b</th>
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Table 5.2: Non-linear least-squares estimates for (A) parameters and standard errors (95% confidence-levels) and (B) corresponding p-values of the individual components for canine repairs. Significant figures were reported based on the magnitude of the standard errors, which represent 95% confidence levels. “NS” implies that the parameter is Not Significantly different from zero at the 95% confidence level. N/A implies parameter does not exist for the individual component because of the form of the equation used to fit the data.
5.7.2 Non-Augmented Repairs

Figure 5.2: Experimental load displacement data, model predictions, and model 95% confidence intervals for (A) human non-augmented repairs, (B) human augmented repairs, (C) canine non-augmented repairs and (D) canine augmented repairs.

Figures 5.2 A and 5.2 C show the experimental load displacement data for the human and canine non-augmented repairs respectively, as well as the model predictions and the 95% confidence interval for the model predictions. The two spring non-augmented rotator cuff repair model appears to reliably reproduce the experimental data for both human and canine non-augmented rotator cuff repairs. Except for a small portion of the data corresponding to two human repairs at large displacement values, the experimental data remained within the 95% CI limits, for both the human and canine models. The RMSE for the human non-augmented rotator cuff repair model (14 N) is 8%
of the average experimental yield load (180 N) and the RMSRE is 7%. Ninety-seven percent of the model predictions for the human non-augmented rotator cuff repair can be reported with at least two significant figures. The RMSE for the canine non-augmented rotator cuff repair model (15 N) is 11% of the average experimental yield load (140 N) and the RMSRE is 12% (Table 5.3). Eighty-four percent of the model predictions for the canine non-augmented rotator cuff repair can be reported with at least two significant figures.

<table>
<thead>
<tr>
<th></th>
<th>Non-augmented repairs</th>
<th>Augmented repairs</th>
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</thead>
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<tr>
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<td>Human</td>
<td>Canine</td>
</tr>
<tr>
<td>RMSE (N)</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>RMSE (%)</td>
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<td>11%</td>
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<tr>
<td>RMSRE (%)</td>
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</tr>
<tr>
<td>Percent model</td>
<td>97%</td>
<td>84%</td>
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<tr>
<td>predictions with at least two significant figures</td>
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<td></td>
</tr>
</tbody>
</table>

Table 5.3: The root mean square error (RMSE) as a % of the average experimental yield load, the root mean square relative error (RMSRE) and the percent model predictions with at least two significant figures for human and canine rotator cuff repair models.

5.7.3 Augmented Repairs

Figures 5.2 B and 5.2 D show the experimental load displacement data for the human and canine augmented repairs respectively, as well as the model predictions and the 95% confidence interval for the model predictions. The five spring augmented rotator cuff repair model appears to reliably reproduce the experimental data for both human and canine augmented rotator cuff repairs. Except for a small portion of the data corresponding to two human repairs at large displacement values, the experimental data
remained within the 95% CI limits, for both the human and canine models. The RMSE for the human augmented rotator cuff repair model (8 N) is 3% of the average experimental load (250 N) and the RMSRE is 19%. Ninety-three percent of the model predictions for the human augmented rotator cuff repair can be reported with at least two significant figures. The RMSE for the canine augmented rotator cuff repair model (12 N) is 7% of the average experimental yield load (180 N) and the RMSRE is 14% (Table 5.3). Eighty-five percent of the model predictions for the canine augmented rotator cuff repair can be reported with at least two significant figures.
5.7.4 Comparison of Non-Augmented Vs. Augmented Repair Model

![Graph showing model predictions for non-augmented versus augmented rotator cuff repairs for the human model. The model suggests that scaffold augmentation will stiffen the repair construct, but only after displacements exceed 2 mm. Further the model suggests that scaffold augmentation increases the yield load of the repairs. Similar results were found when comparing model predictions for the canine models (data not shown).]

**Figure 5.3:** Model predictions for non-augmented versus augmented human rotator cuff repair.
5.7.5 Load Sharing

![Figure 5.4: Load distribution in the different components of the augmented human rotator cuff tendon repair.](image)

Figure 5.4 shows the distribution of load between the augmentation components (spring#3 and spring#4) and the underlying tendon repair component (spring#1 and spring#2') as predicted by the model for human augmented rotator cuff repairs. The model suggests for displacements of 2 mm or less, the tendon repair component carries ~80% of the total load acting on the augmented repair construct. Thereafter, the load carried by the tendon repair component is predicted to decrease from 80% to 73% of the total load. In other words, the augmentation component is predicted to carry between 20 - 27% of the total load acting on the augmented rotator cuff repair for the entire range of displacements. Similar results were found when comparing model predictions for canine augmented rotator cuff repairs (data not shown).
5.7.6 Parametric Sensitivity Analysis

The sensitivity analysis was carried out only for parameters A and b of bone-screw-scaffold-suture component (spring#3) and the medical suture-tendon interface (spring#4), which may be considered to represent modifications associated with changes in surgical procedure and/or scaffold design.

Figures 5.5 A and 5.5 B show the parametric sensitivity coefficients for the parameter ‘A’ corresponding to springs#1, #3 and #4 for both human and canine augmented rotator cuff repair models.
Figure 5.5: Parametric sensitivity plots for the parameters ‘A’ corresponding to springs #1, #3 and #4 for both human (A) and canine (B) augmented rotator cuff repair models.

Both models are most sensitive to perturbations in parameter ‘A’ for spring #1. The model appears to be less sensitive to parameter ‘A’ in springs #3 and #4. A value of 0.5 for a given sensitivity coefficient suggests that a 100% change for the corresponding parameter from its baseline value \( P_k^o \) would result in a 50% change in the model response from its baseline value \( F_i^o \) (See Section 5.6).
Figure 5.6 A and 5.6 B shows the parametric sensitivity coefficients for the parameter ‘b’ corresponding to springs#1, #3 and #4 for both human and canine augmented rotator cuff repair models. Similarly to the results observed for parameter ‘A’, both models are more sensitive to perturbations in parameter ‘b’ for spring#1 than for springs#3 and #4.

**Figure 5.6:** Parametric sensitivity plots for the parameters ‘b’ corresponding to springs#1, #3 and #4 for both human (A) and canine (B) augmented rotator cuff repair models.
5.8 Discussion

There has been much interest in developing scaffolds as devices to augment the repair of large to massive rotator cuff tears. However, to date no studies have investigated the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation. Furthermore, the manner in which loads on an augmented rotator cuff repair are distributed amongst the various components of the repair is not yet known. Finally, the relative biomechanical importance of various components of the rotator cuff repair construct is also unknown. Aiming to answer these questions, this study was designed to meet three objectives. The first objective was to develop quasi-static spring-network models of simplified rotator cuff repairs, which was accomplished through formulating non-linear models based on the physics of springs in series and parallel (Chapter 4). The second objective was to validate the models by comparing the model predicted forces to experimental measurements for human and canine rotator cuff repairs.

The results indicate that except for a small portion of the data corresponding to two human repairs at large displacement values, the experimental data remained within the 95% CI limits, for both the human and canine models. The RMSE (% of the average experimental yield load) of both human and canine augmented rotator cuff repair models was less than or equal to 6%. The RMSE of both human and canine non-augmented rotator cuff repairs was slightly higher (8 - 11%) as was the RMSRE of both human and canine rotator cuff repair models (7 - 19%). The generally higher values for RMSRE arise primarily from large relative differences between the model predictions and experimental data at the low displacement measurements. However, more than 90% and 80% of the model predictions for the human and canine rotator cuff repair models
respectively can be reported with at least two significant figures. This suggests that the models can provide a reliable prediction of the expected performance of the rotator cuff repairs. The models also predicted an increase in the yield load but not initial stiffness of repairs augmented with a prototypic polymeric scaffold, which is in agreement with the findings of our experimental repairs with this same scaffold \(^3\,^4\). In summary, these results demonstrate the validity of the formulated models for predicting the biomechanics of these simplified human and canine rotator cuff repairs.

The final objective was to predict the degree of load sharing provided by a scaffold used for rotator cuff repair augmentation. The model predicts that the augmentation component (i.e., the scaffold plus its attachments to tendon and bone) will carry between 20 - 30 % of the total load acting on the repair construct for the entire range of displacement. A corollary to this result is that the underlying tendon repair carries the majority of the total load (70 - 80 %) acting on the augmented rotator cuff repair component for the entire range of displacement. This finding suggests that this particular scaffold, together with its attachments components, is less stiff than the tendon and its repair.

The model appears to be most sensitive to perturbations in the parameters ‘A’ and ‘b’ of spring#1 (the bone-suture-tendon interface). These results highlight the biomechanical importance of the suture attachment site, and suggest that the greatest improvements in the force carrying capacity of a tendon repair may be achieved by improving the bone-suture-tendon interface. At this time we are unable to explain the reversal in trend seen in the sensitivity curve of parameter ‘b’ for spring#1 for both human and canine augmented rotator cuff repair models, but this result may be related
more to an interdependence among the model parameters than to the actual mechanics of the repair. It should be noted that these model parameters do not carry any particular physical significance; rather they are derived from non-linear least squares analysis.

The parameter ‘A’ for spring#1, for both the human and canine models [Table 5.1 and 5.2], has a large standard error compared to those of the remaining model parameters. This result is attributed to the inherent variability associated with performing a surgical repair. Further, spring#1 for both human and canine models and spring#4 for the canine model were most reliably modeled using the biphasic non-linear equation whereas all other spring components in both human and canine rotator cuff repairs were most reliably modeled using the single-phase non-linear equation. The need for a biphasic equation to model the load-displacement behavior of the suture interface components may be due to the combined mechanisms of stretch of the tendon and slip of the suture from the tendon that occur in these components. The fact that spring#4 of the human model was not best modeled by the biphasic non-linear equation as other suture interfaces may be due to the difference in the architecture of the human infraspinatus tendon compared to that of the canine infraspinatus tendon. Unlike the organized nature of collagen fibers of the canine infraspinatus tendon, the collagen fibers of the human infraspinatus tendon are more randomly organized, particularly in the region medial to the insertion site \(^1, 2\). This random organization of the collagen fibers in the human tendon may minimize suture slip and thus explain why a single phase non-linear equation was better for fitting the medial suture-tendon interface (spring#4) of the human model.

The study is not without limitations. First, compared to the clinical scenario, the experimental repairs used to develop our models were greatly simplified and idealized.
An isolated tendon released and repaired acutely, with only one type of surgical technique and one type of scaffold, and tested under only one loading condition was modeled. This was done in order to formulate a conceptual model to predict trends associated with augmentation of rotator cuff, but limits the direct translation of our data to human rotator cuff tendon repairs which involve chronically degenerate tissues and are inherently multidimensional and structurally variable. Secondly, the model was developed from mechanical testing performed under in vitro conditions, which may not reflect the biomechanics of in vivo repairs that are subjected to biological processes. Third, the model parameters for springs#1 and 2 were obtained from failure testing of specimens that were first subjected to a cyclic loading protocol. Hence, the models cannot be used to predict the biomechanics of repairs on the first initiation of load following surgical repair. And finally, the model predictions of the mechanical performance of the repairs is only valid up to and including the point of maximum (yield) load. Hence, the model cannot be used to predict failure loads of the repair.

In summary, the study has developed and validated simple spring-based non-linear models for predicting the trends associated with scaffold augmentation of rotator cuff repairs. The ability of the models to predict the biomechanics of non-augmented and augmented rotator cuff repairs from both human and canine strengthens the interpretation, application and relevance of our observations. For the simplified repairs modeled herein, the total load was distributed ~70 - 80% to the tendon repair component (i.e., tendon plus its suture attachment to bone) and ~20 - 30% to the augmentation component (i.e., the scaffold plus its attachments to tendon and bone). This finding suggests that this particular scaffold, together with its attachments components, is less
stiff than the tendon and its repair. The model results and sensitivity analysis suggests that although the scaffold contributes to the overall mechanical properties of the repair construct, the greatest improvements in the force carrying capacity of a tendon repair may be achieved by improving the properties of the bone-suture-tendon interface. The next chapter will use this model to conduct a parametric simulation study with the aim to predict the manner in which changes to the individual components of the repair, representing different surgical techniques and scaffold devices, may influence the biomechanics of the repair construct.

**BIBLIOGRAPHY**


Chapter VI

THE BIOMECHANICAL ROLE OF SCAFFOLDS IN AUGMENTED ROTATOR CUFF REPAIRS

*It is tough to make predictions, especially about the future.... Yogi Berra*

6.1 Introduction

Scaffolds have been the most common strategy investigated to date for the treatment of rotator cuff tears. Currently, scaffolds derived from various natural and synthetic biomaterials are being marketed as augmentation devices for rotator cuff repairs at the time of surgery \(^2,4,5\). Based on the mechanical connotation of their intended use, it is commonly believed that when applied appropriately, these devices may provide some degree of load sharing of forces across the tendon repair site and thus decrease the likelihood of tendon re-tear. Although significant advances have been made in the development of scaffolds for rotator cuff repair augmentation, there is limited experimental data to support the notion that scaffold augmentation of a tendon repair will actually improve the biomechanical performance of the repair construct \(^3,6\). Even though these studies demonstrate the potential for scaffold augmentation to improve the initial biomechanical properties of a rotator cuff repair construct, the appropriate scaffold material properties and/or surgical application techniques for achieving optimal
biomechanical performance in the setting of rotator cuff repairs are unknown. Furthermore, no studies to date have investigated the percent load carried by a scaffold when used for rotator cuff repair augmentation.

To address these questions and enhance our understanding of the basic mechanics of scaffold augmentation, a spring-network model for non-augmented and augmented human rotator cuff repairs was developed and validated (Chapter 4 & 5) \(^1\). The objectives of the current study are now to use this model to predict: (1) the manner in which simulated changes to components of the tendon repair, such as reduced tendon quality, altered surgical technique and different scaffold designs, influence the biomechanical performance (yield load and stiffness) of the repair construct and (2) the percent load carried by the scaffold augmentation component of the repair construct in each of these simulated clinical scenarios.

### 6.2 Methods

Chapter 4 and 5 explained the development and validation of a spring-network model for rotator cuff repairs. Briefly, the non-augmented repair was modeled as two springs in series (Figure 6.1 A and 6.1 B), while the augmented repair was modeled as a combination of five springs in series and parallel (Figure 6.1 C and 6.1 D). The individual components of the repair were modeled as non-linear springs (Table 6.1).
Figure 6.1: Schematics of (A) non-augmented and (B) augmented human rotator cuff repair along with their corresponding analogies (C & D) with spring models.

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<thead>
<tr>
<th>Spring</th>
<th>Physical Component of Repair Construct</th>
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<tbody>
<tr>
<td>1</td>
<td>Tendon-to-Bone Repair (bone-suture-tendon interface)</td>
</tr>
<tr>
<td>2, 2’, 2”</td>
<td>Tendon (Springs 2’ and 2” are two half springs of Spring 2)</td>
</tr>
<tr>
<td>3</td>
<td>Scaffold Augmentation Component (bone-screw-scaffold-suture)</td>
</tr>
<tr>
<td>4</td>
<td>Scaffold-Tendon Attachment (scaffold-suture-tendon interface)</td>
</tr>
</tbody>
</table>

Table 6.1: Definition of Individual Springs in the Rotator Cuff Repair Model

For the human rotator cuff repairs models the springs representing the tendon (spring#2), scaffold augmentation component (spring#3) and scaffold-tendon attachment (spring#4) were modeled using a single phase non-linear equation [Eqn.(1)]

\[ F = F^0 + A x^b \]
And the spring representing the tendon-to-bone repair (spring#1) was modeled using a biphasic non-linear equation [Eqn. (2)].

\[ F = F^0 + \frac{A x^b}{1 + B x^c} \] ...........................................................(2)

In this chapter the human rotator cuff repair spring-network models will be varied parametrically to simulate clinically relevant scenarios, namely, changes in tendon quality, altered surgical technique(s) and different scaffold designs.

**Figure 6.2:** Schematics of the components of augmented rotator cuff repairs being simulated. (A) tendon-to-bone repair (spring#1) [bracketed] (B) scaffold augmentation component (spring #3) and (C) scaffold-tendon attachment (spring #4). Adapted from Figures 1 and 2 in Aurora et al.,

More specifically, parameter ‘A’ of the tendon-to-bone repair (spring#1) (Figure 6.2 A), the scaffold augmentation component (spring#3) (Figure 6.2 B) and the scaffold-tendon attachment (spring#4) (Figure 6.2 C) of human rotator cuff repairs was varied from its respective baseline value, while keeping other parameters at their respective baseline values. The baseline values are those estimated by non-linear least square analysis of the component specific load-displacement data of the individual spring components up to the yield load either to a single phase (for spring#3 & #4) or biphasic non-linear equation (for spring#1). While, the parameter ‘A’ itself does not have any
particular physical significance; it is a proportionality constant associated with changes in load-displacement characteristics of a given spring component and hence can be varied to simulate different clinical scenarios, such as weak and/or strong tendon-to-bone fixation, degenerative tendon tissue or compliant/stiff scaffolds.

Specifically, to simulate changes in tendon quality and/or surgical repair technique, parameter ‘A’ of the tendon-to-bone repair (spring#1) or the scaffold-tendon attachment (spring#4) was varied ± 50% from baseline (Table 6.2). To simulate a change in the scaffold design, which could include changes to the scaffold mechanical properties and/or its method of fixation, parameter ‘A’ of the scaffold augmentation component (spring#3) was varied ± 25% and ± 50% from baseline (Table 6.2). The biomechanical performance of the repair constructs, i.e., the yield load and stiffness, and the percent load carried by the scaffold augmentation component (spring#3), were evaluated for each of the parametrically simulated model conditions. (The model was fit to the experimental data only up to the point of “yield load”, where “yield load” was defined at the first instantaneous drop in load of at least 10% during the experimental tests. Hence, the maximum load predicted by the model simulations is equivalent to this “yield load”). All results are reported with respect to the non-augmented repair condition in order to estimate value of using scaffold augmentation for the simulated clinical indications.
### Table 6.2: Values of parameter ‘A’ of tendon-to-bone repair (spring#1), scaffold augmentation component (spring#3) and scaffold-tendon attachment (spring#4) varied by ± 25% and ± 50% from baseline values (highlighted)

<table>
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<tr>
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<td>7.1E+01</td>
<td>4.8E+01</td>
<td>9.5E+01</td>
</tr>
<tr>
<td>4</td>
<td>--</td>
<td>6.5E+01</td>
<td>1.3E+02</td>
</tr>
</tbody>
</table>

6.3 Results

6.3.1 Parametric Variation in Parameter ‘A’ of the Tendon-to-Bone Repair (spring#1)

Parameter ‘A’ of the tendon-to-bone repair (spring#1) was varied to simulate changes in tendon quality and/or surgical repair technique. Results are shown in Figure 6.3 and summarized in Table 6.3. For non-augmented repair constructs, the model predicted a yield load of 384 N and stiffness of 105 N/mm. These are the baseline properties to which all simulated conditions are compared.
<table>
<thead>
<tr>
<th>Clinical Scenario</th>
<th>Parameter 'A' Variation</th>
<th>Repair Type</th>
<th>Percent Change from Non-Augmented (Primary Repair)</th>
<th>Percent Load Carried by the Scaffold Augmentation Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tendon-to-Bone Repair Varied (Spring #1)</td>
<td></td>
<td></td>
<td>Yield load</td>
<td>Stiffness</td>
</tr>
<tr>
<td>Reduced tendon quality, i.e., repair of chronic degenerative tendon to bone</td>
<td>50% decrease</td>
<td>Non-augmented</td>
<td>-43%</td>
<td>-62%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Augmented with a prototypical scaffold</td>
<td>-4%</td>
<td>-21%</td>
</tr>
<tr>
<td>Improved repair strategy of tendon attachment to bone</td>
<td>50% increase</td>
<td>Non-augmented</td>
<td>34%</td>
<td>38%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Augmented with a prototypical scaffold</td>
<td>43%</td>
<td>32%</td>
</tr>
<tr>
<td>Scaffold Augmentation Component Varied (Spring #3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in scaffold mechanical properties and/or its method of fixation</td>
<td>Prototypical Polymer</td>
<td>Augmented</td>
<td>25%</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>25% decrease</td>
<td></td>
<td>20%</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>50% decrease</td>
<td></td>
<td>12%</td>
<td>no change</td>
</tr>
<tr>
<td></td>
<td>25% increase</td>
<td></td>
<td>29%</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>50% increase</td>
<td></td>
<td>32%</td>
<td>20%</td>
</tr>
<tr>
<td>Scaffold-Tendon Attachment Varied (Spring #4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced tendon quality and/or reduced repair strategy of scaffold attachment to tendon</td>
<td>50% decrease</td>
<td>Augmented with a prototypical scaffold</td>
<td>14%</td>
<td>no change</td>
</tr>
<tr>
<td>Improved repair strategy of scaffold attachment to tendon</td>
<td>50% increase</td>
<td></td>
<td>31%</td>
<td>19%</td>
</tr>
</tbody>
</table>

Table 6.3: All results are reported with respect to the non-augmented repair condition (Yield load: 384 N, Stiffness: 105 N/mm), in order to estimate value of scaffold augmentation for the simulated clinical scenarios.
Decreasing parameter ‘A’ of the tendon-to-bone repair (spring #1) by 50% decreases the yield load (43%) and stiffness (62%) of non-augmented repairs. When an augmentation scaffold is used, the same decrease in parameter ‘A’ resulted in only a modest decrease in yield load (4%) and stiffness (21%) compared to the non-augmented baseline condition (Figure 6.3 A). In this scenario, the scaffold augmentation component carries approximately 45% of the total load on the construct (Figure 6.3 B) (Table 6.3).

Conversely, increasing parameter ‘A’ of the tendon-to-bone repair (spring #1) by 50% increases the yield load (34%) and stiffness (38%) of non-augmented repairs (Figure 6.3 A). When an augmentation scaffold is used, the same increase in parameter ‘A’ resulted in a similar increase in yield load (43%) and stiffness (32%) as when no scaffold is used (Figure 6.3 A). In this scenario, however, the scaffold component carries approximately 25% of the total load on the construct (Figure 6.3 B) (Table 6.3).
Figure 6.3: Parametric variation in parameter ‘A’ of the tendon-to-bone (TB) repair (spring#1). (A) The biomechanical performance of the non-augmented and augmented repair constructs and (B) The percent load carried by the scaffold augmentation component for simulated tendon-to-bone repair.
6.3.2 Parametric Variation in Parameter ‘A’ of the Scaffold Augmentation Component (spring#3)

Parameter ‘A’ of the scaffold augmentation component (spring#3) was varied to simulate a change in the scaffold design, which could include changes to the scaffold mechanical properties and/or its method of fixation. Results are shown in Figure 6.4 and summarized in Table 6.3. Augmenting the repair with a prototypical polymer scaffold results in a repair construct with higher yield load (25%) and stiffness (16%) than the non-augmented repair (Figure 6.4 A), and the scaffold component carries approximately 31% of the total load on the construct (Figure 6.4 B).

Decreasing parameter ‘A’ of the scaffold augmentation component by 25% and 50% reduces the properties of the augmented construct to similar levels as the non-augmented baseline repair (Figure 6.4 A), and the percent total load carried by the scaffold reduces to 20% (Figure 6.4 B).

Increasing parameter ‘A’ of the scaffold augmentation component by 25% and 50% does not appreciably increase the yield load, stiffness or load sharing capability of the augmented construct over the properties obtained with the prototypical polymer scaffold (Figures 6.4 A & 6.4 B)
Figure 6.4: Parametric variation in parameter ‘A’ of the scaffold augmentation component (spring#3) (A) The biomechanical performance of the augmented repair constructs and (B) The percent load carried by the scaffold augmentation component for simulated scaffold augmentation component.
6.3.2 Parametric Variation in Parameter ‘A’ of the Scaffold-Tendon Attachment (spring#4)

Parameter ‘A’ of the scaffold-tendon attachment (spring#4) was varied to simulate changes in tendon quality and/or scaffold attachment technique. Results are shown in Figure 6.5 and summarized in Table 6.3. For repairs augmented with a prototypical polymer scaffold, decreasing parameter ‘A’ of the scaffold-tendon attachment by 50% reduces the properties of the augmented repair construct to similar levels as the non-augmented baseline repair, and the percent total load carried by the scaffold reduces to 22% (Figure 6.5 A).

Increasing parameter ‘A’ of the scaffold-tendon attachment by 50% does not appreciably increase the yield load, stiffness or load sharing capability of the augmented construct over the properties obtained with the prototypical polymer scaffold and the baseline condition for spring#4 (Figure 6.5 B). In other words, changes to the properties of the repair construct with variation in the scaffold-tendon attachment properties were essentially the same as when the properties of the scaffold augmentation component (spring#3) were varied to the same degree (Figures 6.5 A & 6.5 B)
Figure 6.5: Parametric variation in parameter ‘A’ of the scaffold-tendon attachment (spring#4). (A) The biomechanical performance of the augmented repair constructs and (B) The percent load carried by the scaffold augmentation component for simulated scaffold-tendon attachment.
6.4 Discussion

Scaffold augmentation may be a viable strategy to improve the initial biomechanical properties of a rotator cuff repair construct and thereby reduce the incidence of repair failure. However, numerous questions remain about the appropriate scaffold properties, surgical application techniques and load-sharing abilities of a scaffold when used in a rotator cuff repair construct. To investigate these questions and enhance our understanding of the basic mechanics of scaffold augmentation, a spring-network model was developed and validated for non-augmented and augmented human rotator cuff repairs \(^1\). The objectives of the current study were to use this model to predict: (1) the manner in which simulated changes to components of the tendon repair, such as reduced tendon quality, altered surgical technique and different scaffold designs, influence the biomechanical performance (yield load and stiffness) of the repair construct and (2) the percent load carried by the scaffold augmentation component of the repair construct in each of these simulated clinical scenarios. The model was developed and validated from our in-vitro experimental study of non-augmented and augmented human rotator cuff repairs, performed using a polymer scaffold designed to have stiffness and ultimate load comparable to human rotator cuff tendon \(^6\). Except for a small portion of the data at large displacement values, the experimental data fell within the 95% confidence interval of the model thus validating the model as a predictive tool for investigating the basic mechanics of scaffold augmentation \(^1\).

The model predicts that augmenting a tendon repair with a polymer scaffold designed to have tendon-like mechanical properties results in a repair construct with modestly higher yield load (25%) and stiffness (16%) than the non-augmented repair condition. The model also predicts that the scaffold component of the repair construct
carries 31% of the total load on the repair. The model predicts only slight further increases in repair construct stiffness or yield load when the mechanical properties of the scaffold augmentation component and/or its attachment to tendon are increased. Decreasing the properties of the scaffold augmentation component itself, and/or its attachment to the repaired tendon, reduces the properties of the overall augmented repair construct to similar levels as the non-augmented repair. Together, these findings suggest that to provide modest improvements to the stiffness and yield load of non-augmented repairs in healthy tendon tissue, the scaffold must have mechanical properties similar to that of tendon tissue. However, the results also suggest that applying a scaffold with supra-physiologic stiffness will not translate into yet stiffer or stronger repairs.

Importantly, the model predicts that in the presence or absence of an augmentation scaffold, the mechanical properties of the overall repair construct are most influenced by the properties of the primary tendon-to-bone repair. The model predicts that decreasing the properties of the tendon-to-bone repair (i.e., repair of a chronic degenerative tendon, fixation in osteopenic bone, or a poorly performed surgical repair technique) will appreciably decrease the yield load (43%) and stiffness (62%) of the construct. The model predicts that scaffold augmentation in this setting can largely mitigate this drop in properties and that the scaffold will carry approximately 45% of the total load on the repair construct. This result suggests that scaffold augmentation would be particularly advantageous when repairing poor quality tendon. Conversely, the model also predicts that increasing the properties of the tendon-to-bone repair (perhaps representing an improved tendon-to-bone repair strategy), will appreciably increase the yield load (34%) and stiffness (38%) of the repair construct even without scaffold
In this case, scaffold augmentation provides minimal further improvement in construct properties, although 25% of the total load on the over-all construct would still be carried by the scaffold component of the repair. It is important to note that because the mechanical properties of the primary tendon-to-bone repair most influence the overall mechanical performance of the repair construct, using a surgical repair technique that maximizes the strength and stability of the direct tendon-to-bone fixation site is essential, even if repair augmentation with a scaffold is anticipated. Surgical repair strategies that compromise the fixation strength at the tendon-to-bone repair site in favor of improved scaffold fixation are unlikely to confer mechanical benefit to the over-all repair construct.

Several limitations should be noted in interpreting the findings of the study. First, the objectives of the study were met by parametrically simulating a simplified rotator cuff repair model that was validated for one surgical repair technique, one type of scaffold and tested under one loading condition. Hence, the results reported herein are dependent on the particular experimental conditions tested. Secondly, the parameter ‘A’ does not have any direct physical corollary. It is a proportionality constant associated with the load-displacement characteristics of a given spring component. While varying parameter ‘A’ allowed us to simulate the model for clinical scenarios that may be representative of change in tendon quality, altered surgical techniques and/or scaffold designs, the absolute translation of our model predictions to clinical practice must be done judiciously. Thirdly, the model does not account for the biological processes of healing and remodeling. Hence, the results of the study are only applicable to the immediate post-operative period. Fourth, our results are limited to quasi-static loading and thus have relevance to activities
involving slow loading rates only. Finally, the model as validated only allows us to predict trends and relative changes as opposed to absolute values for failure loads and stiffness. Hence, there remains a need to experimentally verify the biomechanical efficacy of surgical techniques and scaffold designs regardless of how they perform when simulated in the current spring-network model.

In summary, a previously validated human rotator cuff repair model was used to simulate changes in tendon quality, altered surgical technique(s) and different scaffold designs (Chapter 5)\(^1\). This model allows predictions of the biomechanical performance of non-augmented and augmented repair constructs, as well as the percent load carried by the scaffold augmentation component for various clinically relevant scenarios. The model predicts that the yield load and stiffness of a rotator cuff repair at the time of surgery can be modestly increased by augmenting the repair with a scaffold which has tendon-like properties. However, the model also suggests that engineering a scaffold with supra-physiologic stiffness will not translate into yet stiffer or stronger repairs. Importantly, the model also predicts that the mechanical properties of a repair construct are most influenced by the properties of the tendon-to-bone repair. This result illustrates the need to prioritize the primary tendon-to-bone repair site fixation, even if repair augmentation with a scaffold is anticipated. In the clinical setting of a weak tendon-to-bone repair, scaffold augmentation will significantly off-load the repair and largely mitigate the poor construct properties, based on the current model predictions.

To our knowledge, this work provides for the first time, information about the load-sharing ability of augmentation scaffolds used for rotator cuff repair, and offers unique insight into how changes to various components of the repair may influence the
biomechanical performance of the repair construct. Given the increasing prevalence of scaffold devices being developed and marketed for rotator cuff repair, the information provided by this study is of great clinical relevance as surgeons endeavor to further understand the role of scaffolds for rotator cuff repair augmentation. Importantly, the model simulations can be used to direct and inform the design of new repair strategies aimed at improving the biomechanical performance of rotator cuff repairs and may have broader implications for understanding the basic mechanics of scaffold augmentation of other soft tissue repairs as well. The simulations suggest that future efforts in the field of rotator cuff repair augmentation could be directed toward strategies that strengthen the tendon–to-bone repair or toward engineering scaffolds with tendon-like mechanical properties that also promote rapid or effective biologic healing.

BIBLIOGRAPHY


Chapter VII

SUMMARY AND FUTURE DIRECTIONS

*Work is the greatest thing in the world, so we should always save some of it for tomorrow....Don Herald*

7.1 Summary

Despite advances in the development of extracellular matrix (ECM) scaffolds for rotator cuff repair, no commercially available ECM scaffold has been shown to have appropriate mechanical properties to provide mechanical augmentation to the repair at the time of implantation. Additionally, numerous questions about the appropriate scaffold properties, surgical application techniques and load-sharing abilities of a scaffold when used as an augmentation device for rotator cuff repairs are unknown. To address the *critical need* for an ECM scaffold that provides adequate strength as well as stimulates and enhances the healing potential, the proposed research aims to engineer the mechanical properties (suture retention strength and stiffness) of allograft fascia lata ECM in a manner that will allow it to be used as an augmentation device for rotator cuff repairs. As described in this dissertation, stitching was developed as a method to engineer the mechanical properties of fascia lata ECM (Chapter 2). A rat model was used to
investigate the host response and time-dependent changes in mechanical properties of reinforced fascia scaffolds after implantation (Chapter 3). Further, simple quasi-static spring-network models of human and canine rotator cuff repairs were also developed to elucidate the basic biomechanics of scaffold augmented rotator cuff repairs (Chapter 4). The human augmented repair model was then parametrically simulated for clinically relevant scenarios, namely, changes in tendon quality, altered surgical technique(s) and different scaffold designs, to predict the manner in which these scenarios may influence the biomechanical performance of the repair construct and the load carrying capacity of the scaffold component (Chapter 5).

**Specific Aim 1: Engineer the suture retention strength and stiffness of allograft fascia lata ECM at time zero using stitching as a method of reinforcement**

Studies presented in this dissertation demonstrate stitching as a technology to engineer the suture retention and stiffness of allograft (human derived) fascia lata ECM. The results show that stitching fascia ECM with braided, resorbable, polymer fibers in a unique, controlled manner not only increased the suture retention load of reinforced fascia scaffolds ($\geq 250$N) by six fold over non-reinforced fascia, but also exceeded the suture retention properties of human rotator cuff tendon ($\sim 250$N). The suture retention load of $6\text{PLLA}/2\text{PGA}$ reinforced fascia was significantly greater than $100\% \text{ PLLA}$ reinforced fascia at time zero. However, the stiffness of fascia scaffolds reinforced with either braid was not significantly different, particularly at low displacements. Scaffolds reinforced with either braid were able to sustain at least 2500 cycles of cyclic loading, but
only 6PLLA/2PGA reinforced scaffolds consistently survived 5000 cycles of cyclic loading.

Together, these tests suggest that reinforced fascia scaffolds could be used to mechanically augment the rotator cuff repair and modulate retraction of the tendon repair during the post-operative period. Further, these studies establish stitching as a novel and versatile method for engineering the mechanical properties of fascia lata ECM. Finally, the tension-with-side constraint test, which mimics the surgical application of scaffolds for rotator cuff repair is being advocated as a tool to assess the mechanical properties of scaffolds for such applications.

Specific Aim 2: Investigate the host response and time-dependent changes in mechanical properties of reinforced fascia scaffolds after implantation in a rat model

The host response to all fascia scaffolds after implantation in rat abdominal wall defect model was characterized by infiltration of chronic inflammatory cells and non-inflammatory fibroblasts-like cells, and as hypothesized, reinforced fascia scaffolds had an increased presence of foreign body giant cells due to the presence of the polymer braid. The mechanical properties of the reinforced fascia scaffolds after implantation in a rat dorsal subcutaneous model were compared to the properties at time zero (before implantation) (Specific Aim 1). The suture retention load of 100% PLLA reinforced fascia scaffolds remained essentially unchanged after in vivo implantation (~300N). In contrast, the suture retention load of 6PLLA/2PGA reinforced fascia scaffolds decreased by ~40% after implantation, but still remained at ~250N. The stiffness of the scaffolds reinforced with either braid trended towards a decrease after implantation, with the
decrease being more predominant (~35%) in 6PLLA/2PGA reinforced fascia scaffolds than 100% PLLA reinforced fascia scaffolds (~15%). Together, these studies support the hypothesis that the decrease in the mechanical properties of 6PLLA/2PGA reinforced fascia scaffolds will be more predominant than 100% PLLA reinforced scaffolds.

These studies demonstrate that the suture retention load of reinforced fascia scaffolds after *in vivo* implantation is comparable to the suture retention properties of human rotator cuff tendon (~250N). The change in the mechanical properties of the reinforced fascia scaffolds after *in vivo* implantation, amongst other factors, is dependent on the type of braid used to engineer fascia lata ECM. These results suggest that fascia reinforced with either braid may be able to withstand the mechanical loads on a rotator cuff tendon repair.

**Specific Aim 3: Develop and validate a quasi-static spring-network model for simplified rotator cuff repairs**

The spring-network model was developed by modeling the individual components of the repair constructs as non-linear springs, and the model equations were formulated based on the physics of springs in series and parallel (Chapter 4). The developed model predicted that the scaffold component (i.e., the scaffold plus its attachments to tendon and bone) carries ~20-30% of the total load on the repair. The sensitivity analysis suggested that the greatest improvements in the force carrying capacity of a tendon repair may be achieved by improving the properties of the bone-suture-tendon interface.

The parametric simulation study predicted that the biomechanics (yield load and stiffness) of a rotator cuff repair can modestly be increased by augmenting the repair with a scaffold that has tendon-like properties at the time of surgery. Based on model
predictions, in the clinical setting of a weak tendon-to-bone repair, scaffold augmentation could significantly off-load the repair and largely mitigate the poor construct properties. However, engineering a scaffold with supra-physiologic stiffness is predicted to not translate into stiffer or stronger repairs. Further, the mechanical properties of a repair construct are most influenced by the properties of the tendon-to-bone repair. Finally, this study suggested that future efforts should be directed toward strategies that strengthen the tendon-to-bone repair or towards engineering scaffolds with tendon-like mechanical properties that may promote rapid or effective biologic healing.

7.2 Future Studies

The results of these studies set stage for future studies. The future studies described herein are being broadly classified in four areas, namely, 1) scaffold development 2) *in vitro* studies, 3) *in vivo* studies and 4) spring-network model development.

7.2.1 Scaffold Development

Irrespective of the type of test (failure or fatigue), the mode of failure for all samples (*in vitro* and *in vivo*) occurred by breaking of the braid secondary to slipping of the braid through the fascia matrix. Hence, there is a need for strategies that may strengthen the fascia matrix/braid interface and minimize the observed slipping through the fascia matrix. Potential strategies included are 1) use of tissue adhesives, 2) modification of the surface roughness of braids, 3) coating braids with polymer coating(s) and 4) use of silk based biomaterials as braids. These potential strategies along with their pros and cons are being explained as follows:
Use of Tissue Adhesives

Tissue adhesives may be applied over the stitching regions after scaffold fabrication. The mechanism for using tissue adhesives is believed to be “gluing” of the braids to the fascia matrix at the points of insertion in the fascia matrix, which may prevent early slippage of the braids through the fascia matrix and result in an efficient load sharing across the interface. Two concerns with the use of this strategy are 1) current tissue adhesives options have been approved by the FDA only for topical use (e.g., wound closure), that is to say, they are not intended for deep tissue application and 2) the hardening of the tissue adhesive is achieved by exothermic polymerization of cyanoacrylate (polymer used to make synthetic adhesives), which may damage both, the fascia matrix and braid and may elicit unfavorable response in vivo at these sites.

Modification of Surface Roughness of Braids

Another strategy is to increase the surface roughness of the braids in a manner that will increase the frictional resistance at the fascia/braid interface and minimize slipping at the interface. The Murthy lab at Rutgers University, NJ attempted to increase the surface roughness of 100% PLLA braids using ultra violet (UV) laser ablation (removal of material). However, the lack of aromatic groups in PLLA did not allow absorption of UV required to achieve ablation. An alternative approach suggested by Dr. Murthy was to coat the braid with microspheres of PLLA. The only immediate concern with the use of this strategy is the possibility of the microspheres detaching from the braid during stitching.
Coating Braids with a Polymer Coating

Needle penetration during scaffold fabrication widens the fascia matrix (on the order of millimeters) to accommodate for braid (B) during stitch formation as shown in Figure 7.1 A. It is hypothesized that filling this so called “gap” may result an efficient bonding between the braid and the fascia matrix. One possible approach is to coat the polymer braid with a polymer coating that swells and polymerizes (hardens) on hydration (blue color in Figure 7.1 B). The advantage of this approach is that the coating may not get disrupted during stitching as it will only polymerize (hardens) on hydration, i.e., during the pre-requisite soak prior to implantation. However, such a polymer will have to be identified and possibly engineered. The University of Akron, Akron, Ohio is one possible source that may provide assistance in such an endeavor.

![Figure 7.1: Pictorial depiction of mechanism of polymer coating for braid (A) “Gap” formation during stitching and (B) Filling of “gap” after coating braid with polymer](image)

Silk Based Biomaterials as Braids

Silk is a natural biomaterial with slow resorption kinetics and a long standing history in biomedical applications. More importantly, its mechanical properties (Ultimate Tensile Strength: 500-1000 MPa) are superior to any biomaterial including tendon (150-200 MPa) \(^2\). Hence, its superior mechanical strength may allow development of a braid
having braid dimensions < 300\(\mu\)m and tensile strength > 100N. It is hypothesized that a smaller diameter braid may minimize the so called “gap” formation during scaffold fabrication, achieve a tighter stitch (due to increased needle and bobbin tension), which together may minimize slipping of the braid through the fascia matrix. Additionally, a high strength braid may allow a stitch configuration (pattern) that will minimize the amount of braid in the fascia matrix. Further, such a braid may also increase the mechanical properties (suture retention strength and stiffness) of the reinforced fascia scaffold. However, eliminating contaminating sericin from silk worm is critical to avoid biocompatibility problems \textit{in vivo}. Although several groups\textsuperscript{2, 3, 15, 20, 24, 25} have reported use of sericin free silk for different biomedical applications, sericin free silk is currently commercially unavailable, which makes it difficult to use it as braid material for scaffold development. One possibility is the use of recombinant spider silk\textsuperscript{13, 14}, but this technology is still in the research phase. Nevertheless, silk as a potential braid material has excellent potential and must actively be pursued.

**Recommendation for Scaffold Development**

As the development of reinforced fascia scaffold enters the second phase of “re”-development in our laboratory, it is believed that collaboration with a School of Textiles may augment this work by providing their expertise in the area of stitching and braid development. In particular, identifying different stitches, braid designs (braiding parameters) and high output commercial sewing machine(s) will be helpful. One such collaboration that may be pursued is with Dr. Martin W. King, Professor of Biotextiles, College of Textiles, North Carolina State University, NC, USA. Dr. King has previously collaborated with the Cleveland Clinic in the area of endovascular devices.
7.2.2 In Vitro Testing - Pseudo In Vivo Tests

Caruso et al., 9 have shown accelerated collagen degradation of ACL grafts that affect their mechanical properties when cyclically loaded in solutions having proteolytic enzyme concentrations similar to the synovial fluid. Development of a similar test for in vitro fatigue testing of ECM scaffolds might predict the fatigue behavior of the scaffold when cyclically loaded in vivo. Such a test could be used as an in vitro screening tool for any collagen based scaffold prior to in vivo animal studies. The validity of such a test to mimic the in vivo environment could be assessed by comparing the post implantation failure tests (Chapter 3) to tests conducted in a proteolytic enzyme solution.

7.2.3. In Vivo Studies

Braid Degradation

In Chapter 3, decrease in the mechanical properties of the reinforced fascia scaffolds after in vivo implantation was hypothesized to be due to a possible concomitant decrease in mechanical properties as a consequence of polymer degradation. Hence, future studies should investigate the change in the surface morphology (using Scanning Electron Microscopy), molecular weight (using Gel Permeation Chromatography), crystallinity (using Differential Scanning Calorimetry) and mechanical properties of the braids after in vivo implantation. Such a study may help characterize the time dependent changes in the properties of polymer braids and their possible influence on the mechanical properties of the scaffold constructs. Further, these studies may also help develop resorbable braids that have slow degradation kinetics and are able to retain their mechanical properties after implantation. It should be noted here that similar studies are also possible using an in vitro degradation tests (ISO 15814:1999) 26 . While these studies
do not truly replicate the *in vivo* environment, they could be considered for the preliminary evaluation of candidate braid materials, particularly polymers.

**Cell Mediated Response Studies**

**Immune Response**

Macrophages and lymphocytes are known to modulate the downstream remodeling outcome of ECM scaffolds. Hence, characterizing the macrophage phenotypes (M1 and M2) and T lymphocytes phenotypes (Th1 and Th2) immune response to the scaffolds would be of great interest.

M1 are pro-inflammatory macrophage phenotypes and M2 are pro-remodeling phenotypes. Th1 lymphocytes produce cytokines (e.g., interleukin (IL)-2, interferon (IFN)-γ, and tumor necrosis factor (TNF)-β) that lead to a pathway associated with implant rejection and Th2 lymphocytes produce cytokines (IL-4, IL-5, IL-6 and IL-10) that lead to a pathway associated with implant acceptance.

Previously, our laboratory has established an immunostaining protocol for CCR7 (M1 macrophage marker) and CD163 (M2 macrophage marker). However, an immunostaining protocol for CD69/CD134 (Th1 lymphocyte marker) and CD30 (Th2 lymphocyte marker) would need to be established for such a study. Alternatively, the cytokine expression of the Th1/Th2 cells can also be determined using reverse transcriptase polymerase chain reaction (RT-PCR).

**Host Tissue Response**

For the *in vivo* study (Chapter 3), twelve weeks was the latest time point investigated. It would also be important to investigate the clearance of lymphocyte infiltrates and foreign body giant cells, as well as scaffold remodeling (incorporation in
host tissue) at later time points. Additionally, longer time points would allow evaluating
the extent of braid degradation and fascia matrix remodeling on the mechanical properties
of the scaffolds.

The host tissue response could also be investigated in athymic mice (laboratory
mouse lacking a thymus gland). The use of such a model will mount no rejection due to
the greatly reduced number of T cells and allow investigating the biocompatibility of the
reinforced fascia scaffolds in a model that may better represent their use as allografts in
human patients.

7.2.4 Alternate Model for Time Dependent Variation in Scaffold Mechanical
Properties

One of the limitations of the *in vivo* study (Chapter 3) was that scaffolds were not
mechanically loaded using the rat subcutaneous model. Mechanical loading may well
affect the rate and extent of scaffold remodeling and braid degradation. Hence, there
remains a need to develop *in vivo* model(s) that will allow dynamic loading of the
scaffolds and investigate the time dependent variation in the scaffold mechanical
properties under mechanical loaded conditions. Further, a full understanding of the
clinical efficacy of these novel materials will require pre-clinical studies using cadaveric
and animal models for rotator cuff repair.

7.2.5 Quantification of MMP Activity

In Chapter 3, it was hypothesized that the secretion of matrix metalloproteinases
(MMP) -2, -3, -9 and -12 by macrophages and giant cells and degradation of fascia
matrix possibly due to secretion of matrix metalloproteinases (MMP) -1, -8, -13 and -14
as possible mechanisms of decrease in the stiffness of reinforced fascia scaffolds after
The presence of these enzymes in the explanted fascia scaffolds could possibly be verified using an enzyme-linked immunosorbent assay (ELISA) and/or Western blotting or Zymography to support the hypothesis.

7.2.6 Model Development

One limitation of the spring-network model is that compared to clinical scenario, the experimental repairs used to develop the models were greatly simplified and idealized. More importantly, the model as validated only allows prediction of trends and relative changes as opposed to absolute values for failure loads and stiffness. Future studies could develop a clinically relevant model that will allow prediction of the failure loads and stiffness for different scaffolds. The mechanical test data for such a model could be obtained from a clinically relevant human cadaver model developed in our laboratory that measures gap-formation during cycling loading in two dimensions. And, also using the data generated from the time zero failure testing of reinforced fascia scaffolds presented in Chapter 3. The approach to the development of the model would be the same as explained in Chapter 4. While the advantage of the development of such a model will be it’s clinically relevance, it applicability as a predictive tool would still be restricted to only one type of surgical technique.

Another limitation of the existing model was that the parameter ‘A’ did not have any direct physical corollary. Future studies could include collecting a library (set) of parameters for different surgical techniques and scaffold designs. This will allow simulation of the model with parameters that have clinically meaningful representations.
7.3 Other Applications of Reinforced Fascia Scaffolds

The concept of stitching may be easily extended to other ECM materials as well. This technology could also be translated for the development of large scaffolds much needed for the treatment of massive tendon and bone defects commonly seen in trauma applications. In particular, these large scaffolds could be developed by stitching two or more pieces of similar or different ECM material(s). However, in such cases in addition to the suture retention strength of the scaffold, the seam strength will be of equal importance as it will dictate the structural integrity of the scaffold in vivo. Reinforced fascia scaffold may also be effective in treatment of repairs other than musculoskeletal repairs including gynecology, urology and abdominal wall repairs.

7.4 Conclusion

The overall goal of this work was to engineer the suture retention strength and stiffness of allograft fascia lata in order to develop an extracellular matrix (ECM) derived scaffold with robust mechanical properties for general use in musculoskeletal soft tissue repair, and more specifically rotator cuff repairs. The precise design criteria of scaffolds for such applications are difficult to specify because the mechanical demands in the clinical scenario are variable and unknown. As a guidance, however, one might consider the suture retention properties of human rotator cuff tendon (~250 N) \(^{21}\), the maximum expected loads on a rotator cuff repair post-operatively (~180 N), the number of duty cycles that a rotator cuff repair may be exposed to in the early post-operative period (it is estimated that during the first six weeks of post-operative rehabilitation, the human rotator cuff repair might experience ~60 cycles/day = 2500 cycles), and the amount of
medial tendon retraction that might increase the likelihood of repair failure (5-10 mm)\(^8,23\) as a possible design criteria.

The studies in this dissertation demonstrate that the suture retention load of fascia reinforced with either braid remain\(≥ 250\) N, even after twelve weeks of implantation. Furthermore, the model predicted that the scaffold augmentation component carries only \(~20-30\%\) of the total load acting on the tendon repair (\(~50 - 60\) N). Although reinforced fascia scaffolds in this study were not cycled to 50 N, the data support the possibility that at cyclic elongation of reinforced fascia scaffolds at 50 N would remain on the order of 5 mm or less, even after 2500 cycles. Together these results suggest that fascia scaffolds reinforced with either braid have robust load-deformation properties that are possibly sufficient to provide mechanical augmentation to rotator cuff repairs, modulating tendon retraction in a manner that reduces the incidence of tendon re-tear.

The spring-network model provides for the first time, estimates of the load-sharing ability of augmentation scaffolds used for rotator cuff repair, and offers unique insight into how changes to various components of the repair may influence the biomechanical performance of the repair construct. The simulation studies suggest that future efforts in the field of rotator cuff repair augmentation should be directed toward strategies that strengthen the tendon-to-bone repair or toward engineering scaffolds with tendon-like mechanical properties that also promote rapid or effective biologic healing.

The results in this dissertation are expected to have a positive impact because they support the further development and translation of polymer reinforced fascia scaffold for tendon repair in humans, offering the orthopedic surgeon with a robust allograft scaffolds for increasing the likelihood of clinical success of large, debilitating, and chronic rotator
cuff tears frequently encountered by the aging population. The spring-network model can be used to direct and inform the design of new repair strategies aimed at improving the biomechanical performance of rotator cuff repairs and may have broader implications for understanding the basic mechanics of scaffold augmentation of other soft tissue repairs as well.
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Conditioning of the Model Equations and Numerical Solution

As described in Chapter 4, the mechanical behavior of the cuff repairs was modeled using a network of springs connected in series and parallel as shown in Figure A.1C and A.1F. The formulation of the model equations for non-augmented and augmented canine repairs is presented here, though the equations for human repairs were similarly derived.

Non-Augmented Rotator Cuff Repair

The model structure has been explained in Chapter 4. Briefly, the non-augmented rotator cuff repairs were modeled as two springs in series, namely, the bone-suture-tendon interface (spring#1) and the tendon itself (spring#2) (Figure A.1C).

![Figure A.1: Schematics of non-augmented (A) human, (B) canine and augmented (D) human, (E) canine rotator cuff repairs and their corresponding analogies with the spring models (C, F). The dotted lines represent suture markers that were placed on the tendon, and the black dot represents the optical marker that was placed on the bone, for optical displacement measurements during mechanical testing.]

The load-displacement behavior of this arrangement of springs is governed by the following equations:
\[ F_1(x_1) - F_2(x_2) = 0 \] (A.1)

\[ (x_1) + (x_2) = d \] (A.2)

In other words, there are two equations Eqns. (A.1) and (A.2) with two unknowns, where:

- \( F_1 \) = Force in the bone-suture-tendon interface (spring#1)
- \( F_2 \) = Force in the tendon only component (spring#2)
- \( x_1 \) = Displacement in the bone-suture-tendon interface (spring#1)
- \( x_2 \) = Displacement in the tendon only component (spring#2)
- \( d \) = Displacement of the non-augmented repair constructs

The displacement, ‘d’, is known from experimental in vitro mechanical testing of non-augmented repair constructs.

As described in Chapter 4 (Section 4.6), the mechanical behavior of the bone-suture-tendon interface (spring#1) and the tendon only component (spring#2) for canine repairs were modeled using a biphasic and single phase non-linear equation, respectively.

Hence:

\[ F_1 = F_1^0 + \frac{A_1 x_1^{b_1}}{1 + B_1 x_1^{c_1}} \] (A.3)

\[ F_2 = F_2^0 + A_2 x_2^{b_2} \] (A.4)

\( F_1^0, A_k, b_k, B_k, c_k \) are component specific parameters estimated by non-linear-least squares analysis of component specific data (See Chapter 4, Section 4.8 for details) From the physics of springs in series, we know that the forces in the two springs will be equal to the force in the non-augmented repair model:

\[ F_1 = F_2 = F_{\text{Non-Aug Repair Model}} \] (A.5)
This implies:

\[ \frac{F_2}{F_1} = 1 \] \hspace{1cm} (A.6)

Substituting equations (A.3) and (A.4) in (A.6):

\[ \frac{F_2^0 + A_2 x_2^{b_2}}{F_1^0 + \frac{A_1 x_1^{b_1}}{1 + B_1 x_1^{c_1}}} = 1 \] \hspace{1cm} (A.7)

Rearranging (A.7):

\[ F_1^0 + \frac{A_1 x_1^{b_1}}{1 + B_1 x_1^{c_1}} = F_2^0 + A_2 x_2^{b_2} \]

\[ F_1^0 (1 + B_1 x_1^{c_1}) + A_1 x_1^{b_1} = F_2^0 (1 + B_1 x_1^{c_1}) + A_2 x_2^{b_2} (1 + B_1 x_1^{c_1}) \]

\[ (F_1^0 - F_2^0) (1 + B_1 x_1^{c_1}) + A_1 x_1^{b_1} = F_2^0 (1 + B_1 x_1^{c_1}) + A_2 x_2^{b_2} (1 + B_1 x_1^{c_1}) \]

\[ A_1 x_1^{b_1} + (F_1^0 - F_2^0) (1 + B_1 x_1^{c_1}) - A_2 x_2^{b_2} (1 + B_1 x_1^{c_1}) = 0 \]

\[ x_1^{b_1} + \frac{F_1^0 - F_2^0}{A_1} (1 + B_1 x_1^{c_1}) - \frac{A_2}{A_1} x_2^{b_2} (1 + B_1 x_1^{c_1}) = 0 \] \hspace{1cm} (A.8)

Where:

\[ A_{1,2} = \frac{A_2}{A_1} \]

Again from the physics of springs in series, we know that the displacement of the two springs will be additive

That implies:

\[ d = x_1 + x_2 \]

\[ d - x_1 + x_2 = 0 \] \hspace{1cm} (A.9)

\[ d = \text{displacement of the non-augmented repair constructs} \]

Hence, a single equation [Eqn. (A.8)] was formulated for the non-augmented rotator cuff repairs, which was then solved numerically under static equilibrium
conditions using a robust (i.e., will always converge to a unique solution) non-linear solver for system of non-linear equations available in MATLAB (MathWorks Inc, Natick, MA, USA). The advantage of formulating a single equation is that it allows finding a solution to the non-augmented repair model (problem) using a combination of open methods (faster, but with convergence constraints, which becomes severe for systems with exponentials) and closed methods (robust, i.e., will always converge to a unique solution).

**Augmented Rotator Cuff Repair**

The non-augmented rotator cuff repairs were modeled as five springs connected in series and parallel. The tendon (spring#2) was split into two half springs, spring#2’ and spring#2”. The bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4) were in series with each other and together in parallel with the non-augmented repair (spring 1 and 2’). The entire augmented rotator cuff repair model was then placed in series with the other half tendon spring#2” to conform the model for augmented rotator cuff repairs (Figure A.1F).

The load-displacement behavior of this arrangement of springs is governed by the following equations:

\[
F_1(x_1) - F_2(x_2') = 0 \quad \ldots \quad (A.10)
\]

\[
F_3(x_3) - F_4(x_4) = 0 \quad \ldots \quad (A.11)
\]

\[
F_1(x_1) - F_2(x_2') + F_3(x_3) - F_4(x_4) - F_2' = 0 \quad \ldots \quad (A.12)
\]

\[
(x_1 + x_2') - (x_3 + x_4) = 0 \quad \ldots \quad (A.13)
\]

\[
(x_1 + x_2') + x_2'' = (x_3 + x_4) + x_2'' = d \quad \ldots \quad (A.14)
\]

In other words, there are five equations Eqns. (A.10 - A.14) with five unknowns:
Where $F_i$ represents the force in spring “i” and $x_i$ the corresponding displacement

$F_i = \text{Force in the spring ‘i’}$

$x_i = \text{Displacement of spring ‘i’}$

$d = \text{Displacement of the augmented repair constructs}$

The displacement, ‘d’, is known from experimental in vitro mechanical testing of augmented repair constructs.

As described in Chapter 4 (Section 4.6), the mechanical behavior of the bone-suture-tendon interface (spring#1) and the medial suture-tendon interface (spring#4) of canine repairs were modeled using a biphasic non-linear equation. And, the mechanical behavior of the tendon only component (spring#2) and the bone-screw-scaffold-suture component (spring#3) using a single phase non-linear equation

Hence:

$$F_3 = F_3^0 + A_3x_3^{b_3}$$ ………………………………………(A.15)

$$F_4 = F_4^0 + \frac{A_4x_4^{b_4}}{1 + B_4x_4}$$ ………………………………………(A.16)

where:

$F_3 = \text{Force in the bone-screw-scaffold-suture component (spring#3)}$

$F_4 = \text{Force in the medial suture-tendon interface (spring#4)}$

$x_3 = \text{Displacement in the bone-screw-scaffold-suture component (spring#3)}$

$x_4 = \text{Displacement in the medial suture-tendon interface (spring#4)}$

$F_k^0, A_k, b_k, B_k, c_k$ are component specific parameters estimated by non-linear-least squares analysis of component specific data (See Chapter 4, Section 4.8 for details)
The bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4) are in series with each other. Hence, from the physics of springs in series, we know that the forces in the two springs will be equal.

\[ F_3 = F_4 \] …………………………………………………(A.17)

Previously, a single equation was formulated for the non-augmented repair model. Using [Eqn. (8)] again,

\[
x_1^{b_1} + F_{1,2}^{0}(1 + B_1x_1^{c_1}) - A_{1,2}x_2^{b_2}(1 + B_1x_1^{c_1}) = 0 \]……………. (A.18)

A similar equation was now formulated for the augmentation component, i.e., the bone-screw-scaffold-suture component (spring#3) and the medial suture-tendon interface (spring#4), which are in series with each other.

\[
x_4^{b_4} + F_{4,3}^{0}(1 + B_4x_4^{c_4}) - A_{4,3}x_3^{b_3}(1 + B_4x_4^{c_4}) = 0 \] ……………. (A.19)

where:

\[
A_{4,3} = \frac{A_3}{A_4}
\]

\[
F_{4,3}^{0} = \frac{F_4^{0} - F_3^{0}}{A_4}
\]

From the physics of springs in series, we know that the displacement of the springs in series will be additive.

That implies:

\[
x_{1,2} = x_1 + x_2
\]

\[
x_{3,4} = x_3 + x_4
\]

\[= z \]………………………………………………………………(A.20)

The displacement of the augmented rotator cuff repair construct is given by:

\[
d - (x_1 + x_2 + x_2^\prime) = 0
\]

\[
d - (x_3 + x_4 + x_2^\prime) = 0
\]

\[d - (z + x_2^\prime) = 0 \]…………………………………. (A.21)
where:

\( d \) = Displacement of the augmented rotator cuff repair construct

\( x_2' \) = Displacement in the tendon half spring component (spring\#2’)

\( x_2'' \) = Displacement in the tendon half spring component (spring\#2’’)

From the model structure, we know that the entire augmented rotator cuff repair model was placed in series with the half tendon spring\#2’’. The force acting on tendon half spring (spring\#2’’) will be:

\[ F_{2''} = F_{\text{Aug Repair Model}} \]

We know that the bone-screw-scaffold-suture component (spring\#3) and the medial suture-tendon interface (spring\#4) were in series with each other and together in parallel with the non-augmented repair (spring 1 and 2’). Hence, from the physics of springs in series we know that:

\[ F_{\text{Aug Repair Model}} = F_2' + F_4 = F_1 + F_3 \]

Therefore:

\[ F_{2''} = F_1 + F_3 \]

\[ F_{2''} = F_2' + F_4 \]

That implies:

\[ F_1 + F_3 - F_{2''} = 0 \]

\[ F_2' + F_4 - F_{2''} = 0 \] \hspace{1cm} (A.22)

We now have a set of four equations A.18, A.19, A.21 and A.22 and four unknowns, \( x_1, x_4, x_2'', z \)

This set of equations can be solved numerically under static equilibrium conditions using a robust (i.e., will always converge to a unique solution) non-linear solver for a system of non-linear equations available in MATLAB (Mathworks Inc, MA, USA). Unlike, the solution of a single equation formulation that uses both an open and
closed method; the solution of a set of non-linear equations only uses an open method whose convergence depends on the starting (initial guess).

Model equations for human repairs were similarly formulated. The scripts employed for the numerical solutions of non-augmented and augmented repairs can be found in Appendix B.
APPENDIX B
MATLAB SCRIPTS FOR ROTATOR CUFF REPAIR MODELS

All Matlab Scripts were developed with the help of Dr. Jorge E. Gatica

B. 1 Single Equation Formulation for Non-Augmented Repair Model

% function single_equation.m
% System of Equations were reduced to a
% has been reduced to a single equation with the help from Dr. Gatica

% The springs’ parameters “A” and “b” are
% found via independent experiments
% and then used to correlate experiments
% from the “augmented rotator cuff repair”

%Lower Arm springs:
% Spring#1: Bone-tendon-suture component
% Spring#2: Tendon only component

function f = single_equation02(x)
% contains equations of the form f(x) = 0
% Single equation, can be solved using fzero
% [and the more robust open-closed methods combination]

% the experimental displacement is
% considered free of error [or negligible compared to F]
global d Fo A B b c

% display ([Fo A B b c]);

f = 0;

% recover the unknown
x1 = x;

x1 = min(max(0, x1), d);
x2 = d - x1;

% As explained in the Model Formulation
% The Original System of Equations
% can be reduced to a single equation
% system equations
% The displacement of the springs
% are additive for springs in series
% x = x1 + x2 => x2 = x - x1

% Force definition / correlated independently
% Fi = Foi + Ai * xi^bi/(1 + Bi * xi^ci)

% Main hypothesis,
% Forces in the two springs will be
% identical for spring in series
% $F_1 = F_2 \Rightarrow F_2/F_1 = 1$

\[
A_{12} = A(2)/A(1);
F_{12} = (F_0(1)-F_0(2))/A(1);
denom1 = 1;
\text{if } B(1) > 0
\hspace{1cm} denom1 = 1 + B(1) \cdot (x_1^{c(1)});
\text{end}
\]

\[
f = (x_1^{b(1)}) + F_{12} \cdot denom1 - A_{12} \cdot denom1 \cdot (x_2^{b(2)});
\]

% display ([x1 x2 f]);

\text{return;}
B.2 Code for Reading the Data for Non-Augmented Repair Model

% This formulation uses a single equation for the 
NON-AUGMENTED REPAIR (TWO SPRING MODEL) was developed with the help of 
Dr. Gatica 

% Program calculates the CI intervals 
% of the model using error propagation analysis

clear;

% the experimental displacement is 
% considered free of error [or negligible compared to F] 
global d Fo A B b c

% Springs Constants: Estimated Parameters 
% define name of data file 
! copy Spring_parameters.dat DataFile.dat

load -ASCII DataFile.dat; 
Fo0 = DataFile(:,2); 
A0  = DataFile(:,3); 
b0  = DataFile(:,4); 
B0  = DataFile(:,5); 
c0  = DataFile(:,6); 

% Springs Constants: Standard Errors 
% define name of data file 
! copy Spring_errors.dat DataFile.dat

load -ASCII DataFile.dat; 
FoE = DataFile(:,2); 
AE  = DataFile(:,3); 
bE  = DataFile(:,4); 
BE  = DataFile(:,5); 
cE  = DataFile(:,6); 

Fo1 = [Fo0, Fo0-FoE, Fo0+FoE]; 
A1  = [A0 , A0-AE , A0+AE]; 
b1  = [b0 , b0-bE , b0+bE]; 
B1  = [B0 , B0 , B0]; 
c1  = [c0 , c0 , c0]; 

% Read Excel Data: Experimental_Data.xls 
DataRead = xlsread('Experimental_Data.xls'); 
% data = DataRead;

% sort data, according to x 
Data_sorted = sort(DataRead(:,1:2), 1, 'ascend'); 
% data = Data_sorted;

% Assign 'data' matrix to
Total displacement, \( x = x_1 + x_2 \)

\[
x = \text{data}(:,1);
\]

The measured force

\[
F = \text{data}( :, 2);
\]

\[
\text{plot}( x, F, 'ro');
\]

\text{title ('Primary Rotator Cuff Repair (Human)');}

\text{legend ('Experiments');}

\text{grid;}

\text{xlabel ('Displacement, x, [mm]');}

\text{ylabel ('Force, F, [N]');}

\text{pause(2);}

set options

\[
\text{nsig} = 3;
\]

\[
\text{TolX} = 0.5*10^{(1-\text{nsig})};
\]

\[
\text{TolF} = 1.e-05;
\]

\[
\text{Maxit} = 20;
\]

\text{options = optimset('TolX', TolX,'TolFun', TolF, 'MaxIter', Maxit);}

\text{options = optimset('MaxFunEvals', 10*Maxit,'Display', 'off');}

\text{model = 'single_equation02';}

\text{for k = 1 % Since we are only interested in using the first column made}

\text{k = 1}

\text{Fo = Fo1(:, k);}

\text{A = A1(:, k);}

\text{b = b1(:, k);}

\text{B = B1(:, k);}

\text{c = c1(:, k);}

\text{% initialize the model force matrix}

\text{F_model = zeros(length(F), 2);}

\text{% Initialize matrix for errors of the two springs}

\text{E = zeros(length(F), 2);}

\text{% Initialize matrix for writing the CI of the two springs}

\text{Bounds = zeros(length(F), 4);}

\text{% the first point has no displacement, skip}

\text{F_model(1, 1) = F(1);}

\text{F_model(1, 2) = F(1);}

\text{% added 06/03/09 (JEG)}

\text{Bounds = zeros(size(F_model));}

\text{N = length(x);}

\text{x1 = 0.5*x(2);}

\text{for i = 2:N}
\(d = x(i);\)
\% initial guess
\%\text{x0} = [0 x1 px2];
x0 = d*0.5;
x0 = x1;

\[xx, fval, Exit\_Flag, Output\] = fzero(model, x0, options);

x1 = xx;
x2 = d - x1;
fnorm = abs(fval);

denom1 = 1;
if B(1) > 0
  denom1 = (1 + B(1) * (x1^c(1)));
end
F\_model(i, 1) = Fo(1) + A(1) * (x1^b(1)) / denom1;

Q = denom1^2;
Term1 = abs(x1^b(1)/denom1);
Term2 = abs((A(1)*x1^b(1)*\log(x1))/denom1);
Term3 = abs(A(1)*x1^b(1)*x1^c(1)/Q);
Term4 = abs(A(1)*B(1)*x1^b(1)*x1^c(1)*\log(x1)/Q);

\%This error term is when using the \(F = F0 + Ax^b/1 + Bx^c\) (Biphasic) for spring\#1
E(i, 1) = FoE(1) + abs(Term1) * AE(1) + abs(Term2) * bE(1) + abs(Term3) * cE(1);

\% added 06/05/09 (JEG)
E(i, 1) = FoE(1) + abs(x1^b(1)) * AE(1) + abs(A(1)*((x1)^b(1))*\log(x1)) * bE(1);

denom2 = 1;
if B(2) > 0
  denom2 = (1 + B(2) * (x2^c(2)));
end
F\_model(i, 2) = Fo(2) + A(2) * (x2^b(2)) / denom2;

\% added 06/05/09 (JEG)
E(i, 2) = FoE(2) + abs(x2^b(2)) * AE(2) + abs(A(2)*((x2)^b(2))*\log(x2)) * bE(2);

\% Bounds(i,1) = abs(E(i,1) + F\_model(i,1));% + CI bound for SPring\#1
\% Bounds(i,2) = abs(E(i,1) - F\_model(i,1));% - CI bound for SPring\#1
\% Bounds(i,3) = abs(E(i,2) + F\_model(i,2));% + CI bound for SPring\#2
\% Bounds(i,4) = abs(E(i,2) - F\_model(i,2));% - CI bound for SPring\#2

\% added 06/03/09 (JEG)
% the first point is not calculated, as it is experimental
if i > 1
    Bounds(i,1) = E(i,1); % CI span for Spring #1
    Bounds(i,2) = E(i,2); % CI span for Spring #2
end

iter = Output.iterations;

fprintf ('\n \t point no. %3i \t x_1 = %12.3g \t x_2 = %12.3g \t ||f|| = %12.3g \%4i', ...
        i, x1, x2, fnorm, iter)

if Exit_Flag < 0
    fprintf ('\n *** Convergence problems for point # %3i ***',i);
    fprintf ('\n \t Exit Flag = %3i ', Exit_Flag);
end

end

% added 06/03/09 (JEG)
display ([x F F_model(:,1) Bounds(:,1) F_model(:,2) Bounds(:,2)]);

% pause;

%plot( x, F, 'ro', x, F_model(:,1), 'b-*', x, F_model(:,2), 'r-s',x,Bounds(:,1), 'k*',x,Bounds(:,2),'k*', x,Bounds(:,3), 'c:',x,Bounds(:,4),'c:');

% added 06/03/09 (JEG)
N = length(x);
plot( x(2:N), F_model(2:N,1) + Bounds(2:N,1), 'b.-', x(2:N), F_model(2:N,2) + Bounds(2:N,2), 'r.-', ... 
     x(2:N), F_model(2:N,1) - Bounds(2:N,1), 'b.-', x(2:N), F_model(2:N,2) - Bounds(2:N,2), 'r.-');

title ('Non-Augmented Rotator Cuff Repair');
legend ('Experiments', 'Model: F_1', 'Model: F_2', ... 
    '95\% CI (F_1 \ pm E_1)', '95\% CI (F_2 \ pm E_2)');
grid;
hold on
xlabel ('Displacement, x, [mm]');
ylabel ('Force, F, [N]');
pause(5);
hold off
end
B.3 Set of Equations for Augmented Repair Model

% function simplified_model.m
% function f = simplified_model01 (x)
% contains equations of the form f(x) = 0
% Single equation, can be solved using fzero
% [and the more robust open-closed methods combination]

% [Experimental] displacement
% considered free of error [or negligible compared to F]
global d Fo A B b c

f = zeros(size(x));

% 4 equations with 4 unknowns
% (x1, x4, x5, and z).
% recover the unknown
x1 = x(1);
x4 = x(2);
x5 = x(3);
z = x(4);

x2 = z - x1;
x3 = z - x4;

% The displacement of the springs
% are additive for springs in series
% x = x1 + x2 => x2 = x - x1

% Force definition / correlated independently
% F1 = Foi + A1 * xi^bi/(1 + Bi xi^ci)
% Main hypothesis,
% Forces in the two springs will be
% identical for spring in series
% F1 = F2 => F2/F1 = 1

% A21 = A(2)/A(1);
% F21 = (Fo(2)-Fo(1))/A(1);
% f(1) = x1^b(1) - F21 - A21 * (z-x1)^b(2);

A12 = A(2)/A(1);
F12 = (Fo(1)-Fo(2))/A(1);
denom1 = 1;
if B(1) > 0
    denom1 = 1 + B(1) * (x1^c(1));
end

f(1) = (x1^b(1)) + F12 * denom1 - A12 * denom1 * (x2^b(2));

% F3 = F4 => F3/F4 = 1
\%
\% A34 = A(3)/A(4);
\% F34 = (Fo(3)-Fo(4))/A(4);
\%
\% \ f(2) = x4^b(4) - F34 - A34 * (z-x4)^b(3);
\%

A43 = A(3)/A(4);
F43 = (Fo(4)-Fo(3))/A(4);

denom4 = 1;
if B(4) > 0
   denom4 = 1 + B(4)* (x4^c(4));
end

f(2) = (x4^b(4)) + F43 * denom4 - A43 * denom4 * (x3^b(3));

f(3) = d - (z + x5);

x2 = z - x1;
x3 = z - x4;

% F1 + F3 - F5 = F2 + F4 - F5 = 0

   denom1 = 1;
   if B(1) > 0
      denom1 = (1 + B(1)*(x1^c(1)));
   end

   F1 = Fo(1) + A(1)*(x1^b(1)) / denom1;
   F3 = Fo(3) + A(3)*(x3^b(3));

   denom4 = 1;
   if B(4) > 0
      denom4 = (1 + B(4)*(x4^c(4)));
   end

   F2 = Fo(2) + A(2)*(x2^b(2));
   F4 = Fo(4) + A(4)*(x4^b(4)) / denom4;

   F5 = Fo(5) + A(5)*(x5^b(5));

f(4) = F1 + F3 - F5;

return;
B.4 Code for Reading the Data for Augmented Repair Model

% Five-Spring - AUGMENTED REPAIR
% This formulation uses a Simplified Model having 4 Equations with 4
unknowns
% The unknowns in this case are the displacements of the individual
springs
% from which the individual force of the springs and the force of the
% repair will be calculated
% Springs No.1 & 4 are represented by
% \( F_i = F_{oi} + A_i x_i^b_i/(1 + B_i x_i^c_i) \) - Biphasic equation
% Springs No. 2 & 3 are represented by
% \( F_i = F_{oi} + A_i x_i^b_i \) - Power law with y intercept

clear;

% the experimental displacement is considered free of error [or
negligible compared to F]
% \( d \) is the system displacement OR measured displacement from
experiments
% assign global to the parameters and the system displacement
global d Fo A B b c

% Springs Constants: Estimated Parameters
% define name of data file
! copy FiveSpring_parameters01.dat DataFile.dat

load -ASCII DataFile.dat;
Fo0 = DataFile(:,2);
A0  = DataFile(:,3);
b0  = DataFile(:,4);
B0  = DataFile(:,5);
c0  = DataFile(:,6);

% Springs Constants: Standard Errors
% define name of data file
! copy FiveSpring_errors01.dat DataFile.dat

load -ASCII DataFile.dat;
FoE = DataFile(:,2);
AE  = DataFile(:,3);
bE  = DataFile(:,4);
BE  = DataFile(:,5);
cE  = DataFile(:,6);

% Define matrix with the parameters and +/- errors to calculate the
% approximate Confidence Intervals of the model

Fo1 = [Fo0, Fo0-FoE, Fo0+FoE];
A1  = [A0 , A0-AE , A0+AE];
b1  = [b0 , b0-bE , b0+bE];
B1  = [B0 , B0 , B0];
c1  = [c0 , c0 , c0];
% Read Excel Data: Experimental_Data05.xls
DataRead = xlsread('Experimental_Data05.xls');

% sort data, according to x
Data_sorted = sort(DataRead(:,1:2), 1, 'ascend');

data = Data_sorted;

% Assign 'data' matrix to
x = data(:,1);

% The measured force
F = data(:,2);

% initialize the model force matrix
F_model = zeros(length(F), 2);

% set options
nsig  = 5;
TolX  = 0.5*10^(1-nsig);
TolF  = 1.e-07;
Maxit = 20;

options = optimset('TolX', TolX,'TolFun', TolF, 'MaxIter', Maxit);
options = optimset('MaxFunEvals', 100*Maxit,'Display', 'off');

% load function file
model = 'simplified_model01';

% for loop for calculating forces using the model generated forces and the
% CI of the model
for k = 1
  % set parameters
  Fo  = Fo(:,k);
  A   = A(:,k);
  b   = b(:,k);
  B   = B(:,k);
  c   = c(:,k);
  N   = length(x);

  % the first point has no displacement, skip
  F_model(1, 1) = F(1);
  F_model(1, 2) = F(1);
% Initialize matrix for errors of the two springs
E = zeros(length(F), 4);
Bounds = zeros(length(F), 4);

% Assign values to alpha and beta that will be used for calculating the
% initial values for fsolve
alpha = 0.3;
beta  = 0.2;
%for loop
for i = 2:N

    d = x(i); % Assign experimental displacement to d

    % initial guess 4 equations with 4 unknowns (x1, x4, x5, and z)
x0 = [alpha*d; beta*d; (1-1.1*(alpha+beta))*d; (alpha+beta)*0.9*d];

    % [X, FVAL, EXITFLAG, OUTPUT] = FSOLVE(FUN, X0, OPTIONS)
    [xx, fval, Exit_Flag, Output] = fsolve(model, x0, options);
    x1 = xx(1);
    x4 = xx(2);
    x5 = xx(3);
    z  = xx(4);

    fnorm = norm(fval);
    iter = Output.iterations;

    %if statement for ignoring points for which there is no convergence.
    % Good programming practice as suggested by Dr. Gatica
    if Exit_Flag < 0
        fprintf ('
 *** Convergence problems for point # %3i ***',i);
        fprintf ('
 \t Exit Flag = %3i ', Exit_Flag);
        fprintf ('
 \t %12.3g %12.3g %12.3g %12.3g', ...
             x1, x4, x5, z);
        fprintf ('
 ** point no. %3i (%7.3f %7.2f) \t ||f|| = %12.3g
             %4i', ...
             i, x(i), F(i), fnorm, iter);
        x1 = min(d, max(0,x1));
        x4 = min(d, max(0,x4));
        x5 = min(d, max(0,x5));
        z  = min(d, max(0,z));

        F_model(i, 1) = 0;
        F_model(i, 2) = 0;
        pause(2);
    else
        x2 = z - x1;

    end
\[ x_3 = z - x_4; \]

denom1 = 1;
if B(1) > 0
denom1 = (1 + B(1)*(x1^c(1)));
end

F1 = Fo(1) + A(1)*(x1^b(1)) / denom1;
F3 = Fo(3) + A(3)*(x3^b(3));

denom4 = 1;
if B(4) > 0
denom4 = (1 + B(4)*(x4^c(4)));
end

F2 = Fo(2) + A(2)*(x2^b(2));
F4 = Fo(4) + A(4)*(x4^b(4)) / denom4;

F_model(i, 1) = F1 + F3;
F_model(i, 2) = F2 + F4;

% Error term for spring\#1 for biphasic equation
Q = denom1^2;
Term1 = x1^b(1)/denom1;
Term2 = (A(1)*x1^b(1)*log(x1))/denom1;
Term3 = A(1)*x1^b(1)*x1^c(1)/Q;
Term4 = A(1)*B(1)*x1^b(1)*x1^c(1)*log(x1)/Q;

E(i,1) = abs(FoE(1)) + abs(Term1*AE(1)) + abs(Term2*bE(1)) +
abs(Term3*BE(1)) + abs(Term4*cE(1));

% This is the error term when spring\#1 is of the form \( F = F_0 + Ax^b \)

%E(i,1) = abs(FoE(1)) + abs((x1^b(1))*AE(1)) +
abs((A(1)*((x1)^b(1))*log(x1))*bE(1));

% Error term for spring\#4
Term5 = x4^b(4)/denom4;
Term6 = (A(4)*x4^b(4)*log(x4))/denom4;
Term7 = A(4)*x4^b(4)*x4^c(4)/denom4^2;
Term8 = A(4)*B(4)*x4^b(4)*x4^c(4)*log(x4)/denom4^2;

E(i,4) = abs(FoE(4)) + abs(Term5*AE(4)) + abs(Term6*bE(4)) +
abs(Term7*BE(4)) + abs(Term8*cE(4));

%Error in spring\#2 (spring\#2 is of the form \( F = F_0 + Ax^b \))

E(i,2) = abs(FoE(2)) + abs((x2^b(2))*AE(2)) +
abs((A(2)*((x2)^b(2))*log(x2))*bE(2));

%Error in spring\#3 (spring\#3 is of the form \( F = F_0 + Ax^b \))

E(i,3) = abs(FoE(3)) + abs((x3^b(3))*AE(3)) +
abs((A(3)*((x3)^b(3))*log(x3))*bE(3));
alpha = x1/d;
beta  = x4/d;

% the first point is not calculated, as it is experimental
if i > 1
    Bounds(i,1) = E(i,1); % CI span for Spring #1
    Bounds(i,2) = E(i,2); % CI span for Spring #2
    Bounds(i,3) = E(i,3); % CI span for Spring #3
    Bounds(i,4) = E(i,4); % CI span for Spring #4
end

end %end statement for the 'if exitflag'

end % end statement for 'for i = 2:N'

N = length(x);

Upper = [F_model(2:N,1) + Bounds(2:N,1), F_model(2:N,1) - Bounds(2:N,1)];

%display ([x F F_model]);

plot( x, F, 'ro', x, F_model(:,1), 'k-*', x, F_model(:,2), 'm-s', x(2:N), F_model(2:N,1) + Bounds(2:N,1), 'b.-', x(2:N), F_model(2:N,1) - Bounds(2:N,1), 'b.-', x(2:N), F_model(2:N,2) + Bounds(2:N,2), 'r.-', x(2:N), F_model(2:N,2) - Bounds(2:N,2), 'r.-');

title ('Augmented Rotator Cuff Repair');
legend ('Experiments', 'Model: F1+F3', 'Model: F2+F4');
grid;
hold on
xlabel ('Displacement, x, [mm]');
ylabel ('Force, F, [N]');
pause(5);
hold off

end % end statement for 'for k = 1:3'