The Influence of Biologics in Regenerative Medicine and Tissue Healing

By

Benoy (Ben) Abraham George

A Dissertation Submitted to

Rutgers University

School of Health Related Professions

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Biomedical Informatics

Department of Health Informatics

Spring 2017

RUTGERS
School of Health Professions

Final Dissertation Defense Approval Form

The Influence of Biologics in Regenerative Medicine and Tissue Healing

BY

Benoy (Ben) Abraham George

Dissertation Committee:

Shankar Srinivasan, Ph.D.

Frederick Coffman, Ph.D.

Neil Riordan, Ph.D.

Approved by the Dissertation Committee:

_____ Date: _____

_____ Date: _____

_____ Date: _____

_____ Date: _____

_____ Date: _____

TABLE OF CONTENTS

ABSTRACT

BACKGROUND: Autologous bone marrow concentrate (BMAC) plus amniotic tissue products contain concentrated growth factors, CD34+ stem cells, and mesenchymal stem cells (MSCs) which yield angiogenic, trophic, and anti-inflammatory effects on tissues and may be useful in the regeneration of connective tissue.

AIM: The author presents a study of 21 patients (20 ACL reconstruction, 1 ultrasound-guided injection) who received bio-enhanced STEMNEXA (BMAC plus amniotic AlphaGEMS) in conjunction with traditional treatment.

METHOD: Patient data on ACL reconstruction and AT tendonitis were collected from Riordan-McKenna Institute. Post-treatment MRI images and impressions were obtained from Dr. Paul Marsh (Monticello Diagnostic Imaging).

Non-surgical method (AT BMAC injection at the clinic) - In an office setting, bone marrow was aspirated from the metaphyseal area of the medial tibia and concentrated with a closed system. The resulting BMAC was injected intralesionally into the affected tendinotic mid-substance area under ultrasound guidance.

Surgical method (ACL reconstruction) – In an operation room setting, bone marrow was aspirated from the iliac crest and was then placed in the Magellan centrifuge to spin down the cells to help harvest the stem cell and plasma platelet rich areas of the blood. The resulting BMAC was then injected at the time of surgery into the base of the graft to aid in healing.

Searches via PubMed and Google Scholar were performed to identify both scientific investigations and review articles to ensure inclusion of pertinent data.

RESULTS: The study included a sample size of 20 ACL reconstruction surgical patients between the ages of 19-57. Post-surgery MRI results with Relative Strength Intensity (RSI) measurements of ACL reconstruction graft treated with BMAC were compared against RSI of intact PCL. Between 16 and 24 weeks, the researcher noticed not only a decrease in the RSI, but also a level of saturation (plateau) indicating incorporation of the graft. The RSI for ACL and PCL closely converged between 16 and 24 weeks, clearly indicating homogeneity between the healing ACL graft and intact PCL.

Research Question (RQ1): Is there a relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods?

Research Question (RQ2): Is there a relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods?

In the present study, recovery time decreased for patients who were treated with biologics (p <.05). The resulting RSI was compatible with the RSI of the uninjured, intact tendon/ligament (p<.05), thereby proving that homogeneity increased with the use of biologics. No correlation was observed between independent factors such as age and gender vs recovery time (p=1).

CONCLUSION: Use of biologics (BMAC) in both the injection for AT tendinopathy and surgical ACL graft reconstruction has shown to decrease time

to recovery, decrease echogenicity, and increase homogeneity of injured connective tissues toward complete healing when compared to traditional nonsurgical and surgical interventions.

ACKNOWLEDGMENTS

First and foremost, I thank Yahweh - God Almighty for His providence and grace in helping me accomplish this momentous milestone. I can do all things through Him who strengthens me.

I express sincere gratitude to my dissertation advisers, Dr. Shankar Srinivasan, Dr. Frederick Coffman, and Dr. Neil Riordan for their kind support, valuable insight, and guidance throughout my doctoral training. I also thank
Dr. McKenna and Dr. Riordan for generous access to their valuable time, knowledge, and resources at Riordan-McKenna Institute. Plaudits go to Dr. Paul Marsh, radiologist at Monticello Diagnostic Imaging, for his invaluable consulting and impressions on the post-treatment MRI's.

I extend heartfelt thanks and eternal indebtedness to my beloved wife, Reeba, and my amazingly wonderful daughters, Hannah, Eden, and Charis. Words cannot express how grateful I am for the countless sacrifices that you have made on my behalf.

Last but not least, I dedicate this dissertation to my parents, siblings, family members, and to all those who have positively impacted my life in one way or the other.

CHAPTER 1 INTRODUCTION

1.1 Statement of the Problem

Over 100 million musculoskeletal (tendon/muscle/bone) injuries occur annually worldwide (Goldspink and Yang 2004). Of these, 30-50% constitute tendon and ligament injures which cause significant loss of performance in sports, decreased functional capacity in the workplace, and decreased overall quality of life and activities of daily living (Lian et al. 2007). A significant proportion of these injures remain difficult to treat, with many individuals exhibiting long-term pain and discomfort.

ACL tear represents one of the most common and detrimental injuries in athletes today. In fact, some researchers estimate that between 100,000 to 200,000 ACL injuries occur per year in the United States alone (Gordon and Steiner 2004; Albright et al. 1999). ACL injuries are commonly classified into two categories: contact-injuries and non-contact-injuries (ncACL); seventy percent of injuries are classified as non-contact and thirty percent as direct contact with another player and/or object (Griffin et al. 2000). Furthermore, ACL injuries are also four to six times more frequent in female athletes than male athletes at the same level for the same sport (Hewett et al. 2005; Arendt and Dick 1995). ACL injury occurs most commonly when athletes suffer from arthrokinetic dysfunction during

vigorous activity. Most ncACL injuries occur when an athlete attempts to decelerate from a jump or forward running while the knee is in a shallow flexion angle (Shimokochi and Shultz 2008; Ferretti et al. 1992) and worsened if its combined with dynamic knee valgus and/or knee internal-external axis rotation (Hutchings 2014).

With the cost of these injuries approaching a billion dollars annually, the prudent identification of risk factors and development of prevention strategies will have widespread health and fiscal significance (Griffin et al. 2000).

The ACL, with proven poor healing capacity, has demonstrated a significantly high rate of failure (40% - 100%) in the ACL primary repair surgical method using suture. This substandard outcome of ACL primary repair technique has led to its abandonment and subsequent global adoption of ACL reconstruction surgery. ACL reconstruction, as a result, has remained the gold standard of care for ACL ruptures or tears, especially for young individuals and athletes who aim to return to high-level sports. Reconstructive surgical treatment of ACL injury, however, remains costly with variable outcomes. Furthermore, the reconstructive surgical technique has yielded a high risk of post-traumatic osteoarthritis (OA) within two decades of injury. Here remains the conundrum: although few athletes are able to resume sports at pre-injury levels without surgery, surgical reconstruction has not demonstrated consistent success in returning patients to their pre-injury activity levels either. Moreover, these athletes who successfully return to activity present a high risk of recurrent or

secondary knee injury with substantially less favorable outcomes (Hutchings 2014).

The Achilles tendon, on the other hand, represents the most frequently ruptured tendon in humans. The incidence of rupture has steadily escalated since the 1980s, from a reported 4.7/100,000 in 1981 to 6/100,000 in 1994 from a Scottish cohort study; the rupture rate found in a Danish cohort study revealed 22.1/100,000 in 1991 to 32.6/100,000 in 2002. The incidence rate climbed the highest among males aged 30 to 39, with badminton appearing to be the most hazardous sport. The mean age of presentation is 35 years with a male to female ratio of 20 to 1 (Gulati et al. 2015).

The most common site of Achilles tendon rupture, a region 3 to 6 cm above the os calcis, corresponds to a watershed region of poor perfusion. Stretching and contraction further compromise blood flow into this region. Maffulli et al and Järvinen et al histologically observed significant collagen degeneration in patients with Achilles tendon rupture, noting the following: decreased collagen-crosslinking (with a greater content of collagen III than collagen I) and weakening of tendon tensile strength with increasing patient age (Gulati et al. 2015).

Risk factors for Achilles tendon rupture include but are not limited to: oral steroid therapy; intra-tendinous steroid injection; hypercholesterolemia; gout;

rheumatoid arthritis; long-term dialysis; and renal transplantation (Gulati et al. 2015).

Non-surgical management of acute Achilles tendon ruptures involves either cast immobilization for 6—8 weeks or functional bracing. Clinically, conservative treatment has shown a re-rupture rate of 10-30% – considerably higher than that reported after surgical intervention. Furthermore, patients treated with immobilization have presented decreased plantar flexion strength and endurance when compared to those repaired surgically (Shimokochi and Shultz 2008; McKenna and Riordan 2014).

Reports in literature have, however, suggested that the results of non-operative treatment are equivalent to those of surgical repair. Recent reports have favored operative treatment of acute rupture of the Achilles tendon in physically active patients, either via open or percutaneous techniques. Although surgical repair appears to yield superior functional results and a lower rate of re-rupture relative to conservative management, surgical intervention unfortunately has shown to increase incidence of post-operative complications like delayed wound healing, skin necrosis, infection, re-rupture, and sensory loss in in 7—42% of cases (Shimokochi and Shultz 2008; McKenna and Riordan 2014).

1.2 Background

1.2.1 Overview of Connective Tissue

Composed of extracellular fibers, an amorphous matrix, and stationary and migrating cells, connective tissue functions primarily by supporting and binding tissues in the body. Ligaments represent tough fibrous bands of connective tissue that serve to support internal organs and hold bones together in proper articulation at the joints. Tendons, on the other hand, contain dense collagenous fibrous connective tissue that attach muscle to bone and have the highest tensile strength among tissues (Brittberg et al. 1994; Hidaka et al. 1999).

1.2.2 Overview of Anterior Cruciate Ligament (ACL)

The Anterior Cruciate ligament, one of four major ligaments of the knee joint, helps coordinate function and promote rotational and anterior-posterior stability of the knee joint. With an annual incidence of more than 200,000 ACL injuries in the USA, approximately half of these cases require reconstruction surgery. The majority of ACL injuries occur while playing agility sports, with high prevalence (1 in 1,750 persons) in active 15-45 year-olds and in females who are two to eight times more likely to get injured than males (Vaishya et al. 2015).

ACL reconstruction is performed by surgical intervention, wherein, the tissue graft to replace the damaged ACL comes from either the patient's body (autograft) or from a donor (allograft) (Vaishya et al. 2015).

Allografts:

Allografts carry the following benefits: lack of harvest morbidity; less traumatic surgical technique; decreased postoperative pain; and easier rehabilitation. On the contrary, allografts transfer the following drawbacks: higher failure rates than autografts; more recovery time (1-2 years) than autografts (6 months – 1 year); and higher incidence of infections than autografts (Vaishya et al. 2015).

Autografts:

Autografts present the following advantages: lower failure rates than allografts (Vaishya et al. 2015); less recovery time (6 months – 1 year) than allografts (1-2 years); and lower incidence of infections than allografts (Vaishya et al. 2015; Hootman et al. 2007). The following disadvantages, however, surround autografts: increased pain post-operatively (6-12 months) (Wittenberg et al. 1998); increased chance of patellar facture if bone-patellar-tendon-bone (B-PT-B) autografts are used; and increased incidence of hamstring weakness if hamstring autografts are used (Vaishya et al. 2015; Demirag et al. 2011).

Table 1. Different types of grafts used in ACL reconstruction (Raju Vaishya et al. 2014; SJ Shultz et al. 2012).

Tissue	**Ultimate Tensile Load (N)**	**Stiffness (N/mm)**	**Cross-sectional area (mm2)**	**Advantages**	**Disadvantages**
Intact ACL	2160	242	44		
Bone-Patellar-Tendon-Bone (10 mm)	2977	620	35	Bone-to-Bone healing	Anterior knee pain; larger incision
Quadrupled Hamstring	4090	776	53	Small incision; less anterior knee pain	Hamstring weakness; soft-tissue healing; bone tunnel widening
Quadriceps Tendon (10mm)	2352	463	62	Bone-to-Bone healing; thick, and can be made into two bundles	Larger incision; patella fracture if take bone plug; soft-tissue healing
Patellar Tendon allograft	1403	224		Bone-to-Bone healing	Longer incorporation
Achilles allograft	1189	741	105		Longer incorporation; soft-tissue healing
Tibialis Anterior allograft	3012	343			Longer incorporation; soft-tissue healing

1.2.3 Overview of Achilles Tendon (AT)

The Achilles tendon, a dense and richly vascularized connective tissue, binds the gastrocnemius and soleus muscles to the calcaneus, or heel bone, and helps bear heavy loads during physical exertion (Stein et al. 2015).

Achilles tendinopathy and tears usually occur due to sports and other high-impact recreational activities. Injury management typically consists of conservative treatment like ice, stretching exercise, brace, shockwave, and physical therapy. While these treatments may reduce swelling, they unfortunately *do not improve the tendon structure* (September et al. 2007; Woo et al. 2007).

1.2.4 Overview of Biologics

1.2.4.1 Autologous Bone Marrow Aspirate Concentrate (BMAC)

This study will focus on Autologous Bone Marrow Aspirate Concentrate (BMAC), produced by density gradient centrifugation of bone marrow that is usually aspirated from the iliac crest. Mesenchymal stem cells (MSCs), CD34+ stem cells, and concentrated growth factors comprise the BMAC concoction (STEMNEXA) used in the study (RMI 2016).

1.2.4.2 Mesenchymal stem cells (MSCs)

MSCs have angiogenic (ability to enhance blood vessel formation), trophic, and anti-inflammatory qualities that aid in the regeneration of injured connective tissue. CD34+ stem cells, also known as hematopoietic progenitor cell antigen CD34 or CD34 antigen, is a protein that is encoded by the CD34 gene in humans. CD34+ stem cells enhance angiogenesis, promote cell to cell adhesion, mediate the attachment of stem cells to extracellular matrix or directly to stromal cells, and facilitate cell migration (Pochampally 2005; RMI 2016).

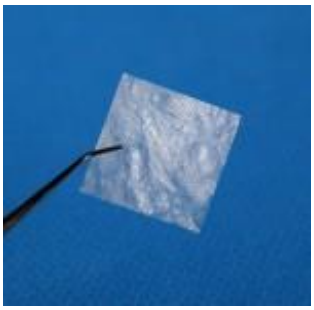
1.2.4.3 Amniotic Tissue Products

Amniotic tissue product, a pliable allograft (transplant) derived from human placental amnion, functions as a biologic structural matrix (scaffold) to facilitate and enhance tissue healing and repair while maintaining immuno-privileged qualities. Used since 1910 in the USA, amniotic tissue is now FDA-exempt for treating wounds and other conditions. First used for treating eye problems (primarily cornea pathology), amniotic tissue is still widely used in ophthalmology today (Thomas et al. 2008).

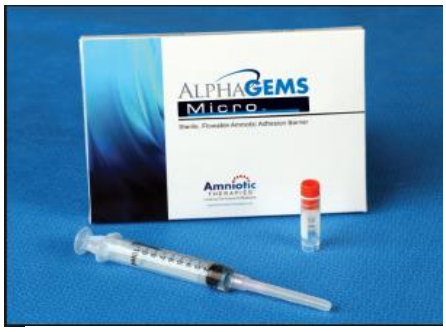
Amnion contains Leukemia Inhibiting Factor (LIF), an interleukin 6 class cytokine that regulates cell growth and inhibits differentiation, preventing MSCs from differentiating prematurely into tenocytes in vitro. Tenocytes, fibroblast-

like differentiated cells that form mature tendon, synthesize the extracellular matrix (ECM) and induce assembly of early collagen fibers which form the basic units of the tendon. The interaction of ECM and MSCs influence the regulation of MSC differentiation and proliferation. Bio-enhanced scaffolding additionally function as native ECM and interact with MSCs for successful tissue regeneration (Bhattacharya and Stubblefield, 2013).

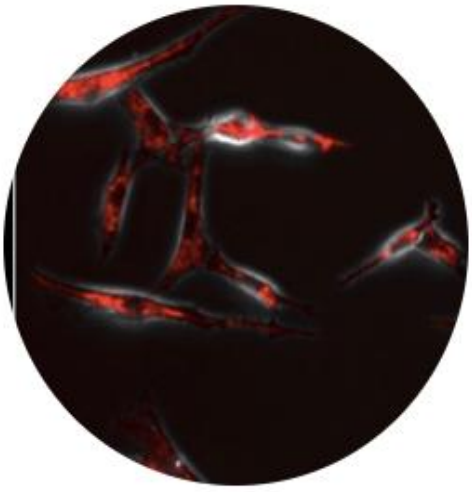
In a normal birth, the placenta, amniotic sack, and the remnant umbilical cord discharge from the mother (called afterbirth) and is typically discarded. Some biotechnology companies, however, like Amniotic Therapies Inc. (ATI, Dallas, TX) led by *Dr. Neil Riordan, procure this tissue from contracted hospitals after normal, healthy births. Once it has been fully tested for infectious diseases, sterility, and immune-privileged compatibility, the tissue is processed. Using proprietary technology, the tissue is separated into very small particles that are cryo-preserved in a patented way that keeps the integrity of the tissue the same as it was when it discharged after the healthy birth of the child. (**Dr. Riordan, the candidate's research mentor and co-founder of Riordan-McKenna Orthopedic Institute (RMI), also serves as founder and chief scientific officer of ATI. ATI specializes in amniotic tissue research and development, with a current product line that includes AlphaGEMS and AlphaPATCH amniotic tissue-based products*). (Riordan et al. 2015; Carlson 1997; Pierpont et al. 2014)



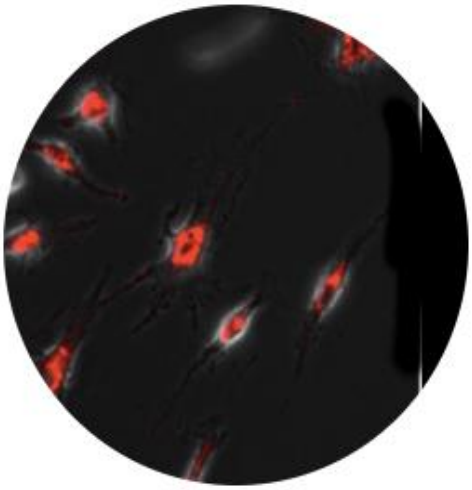
AlphaPATCH *



AlphaGEMS *



Stem Cells with Amnion



Stem Cells without Amnion

On the **left**: cells cultured with amniotic tissue product: have the morphology of young, healthy MSCs; no flattening; red color of mitochondria; and uniform spreading throughout the entire cell body. *

On the **right**: cells not cultured with amniotic tissue product: are flat and fibroblastic; are becoming senescent - the last step before they stop producing growth signals; forming scar tissue. *

Note: The MSCs are identical in both scenarios (i.e. with regard to number of divisions after harvest).

* With permission from/courtesy of Riordan-McKenna Institute and Amniotic Therapies, Inc.

Therefore, how do amniotic tissue products augment a patient's bone marrow stem cells? My study of orthopedic patients' outcomes at the Riordan-McKenna

Institute involved augmentative treatment with their proprietary stem cell therapy called STEMNEXA (RMI 2016).

1.2.4.4 STEMNEXA

Riordan-McKenna Institute's STEMNEXA protocols combine the latest scientific advances in bone marrow harvesting with two complimentary cellular technologies: Bone Marrow Aspirate Concentrate (BMAC) and amniotic tissue product (AlphaGEMS). Possible clinical benefits of STEMNEXA include but are not limited to: shorter healing times; increased vascularization; less pain for the patient; increased safety rate with the harvesting method; reduced inflammation; and decreased risk of infection. By incorporating BMAC and AlphaGEMS amniotic tissue product, STEMNEXA-Rx offers an alternative to nonsurgical candidates via injection directly to the tendinotic site, whereas STEMNEXA-Sx combines advanced orthopedic surgical procedures with cell therapy to yield better patient outcomes (RMI 2016; Thomas et al. 2008).

1.2.4.5 AlphaGEMS

AlphaGEMS, a proprietary, morselized flowable tissue allograft (transplant) derived from human placental amnion, functions as a biologic structural matrix (scaffold) to facilitate and enhance tissue healing and repair. AlphaGEMS contains 108 different growth factors, including WNT-4, prostaglandin, PGE2, and GDF-11. After an injury event, prostaglandin inhibits inflammation, marking

the beginning of the healing process. The faster the inflammatory phase of healing is completed, the sooner the body moves on to the next phase of regenerative healing (Kjaer 2004).

Mesenchymal stem cells (MSCs) secrete PGE2, or Prostaglandin E2, in response to injury. This molecule inhibits fibrosis by way of limiting fibroblast proliferation, migration, collagen secretion, and transforming growth factor (TGF)-induced myofibroblast that can spur fibroblast proliferation (Kjaer 2004). PGE2 also enhances the wound healing process and angiogenesis (Krampera et al. 2006). WNT4, a protein, drives wound healing by way of wound re-epithelialization and cell proliferation (Lian et al. 2007). The creation of new tissue materializes by multiple methods including new blood vessel formation, a critical element for normal wound healing (Maffulli et al. 2005). GDF-11, or growth/differentiation factor 11, has been identified as one of the key molecules propelling the regeneration of skeletal muscle, cardiac muscle, and nervous tissue in aged mice (Nordsletten and Madsen 2006).

Containing more than sixty times the amount of prostaglandin relative to other similar products, AphaGEMS also provides ten times the amount of WNT-4, arguably the single most important molecule required for wound healing (Nordsletten and Madsen 2006). Tissue used for AlphaGEMS is donated after normal, healthy births from contracted healthcare centers. Once fully tested for infectious diseases and sterility, the tissue is processed by using proprietary methods developed by Neil Riordan, PhD.

1.3 Goals and Objectives

This study will examine whether the use of biologics and growth factors enhances tissue healing (measured by post-treatment MRI T1 relative strength intensity (RSI) units), increases homogeneity, decreases echogenicity, and decreases overall time to recovery in connective tissue injuries. Specifically, this dissertation will investigate whether CD34+ stem cells, mesenchymal stem cells, and growth factors found in autologous bone marrow aspirate concentrate (BMAC) in conjunction with amniotic tissue products enhance regeneration of injured connective tissues to normal homogeneity levels.

1.3.1 Goal of Increased Homogeneity:

Homogeneity represents the degree to which the results of studies included in a review are similar. Clinical trial homogeneity, in addition, involves similar or comparable participants, interventions, and outcome measures. Studies are considered statistically homogeneous if their results vary no more than what might be expected by the play of chance (Riordan and McKenna 2014).

1.3.2 Goal of Decreased Echogenicity:

Echogenicity is the ability to bounce an echo as found in returned signals in ultrasound examinations. Echogenicity is *higher* when the surface bouncing the sound echo reflects *increased* sound waves (i.e. injured tissue, lesions). While

scar tissue or injured tissue appears echogenic in MRI's, healthier, more viable tissue display decreased echogenicity (Riordan and McKenna 2014; Stein et al. 2015).

Thus, homogeneity and echogenicity exhibit an inverse relationship: as homogeneity increases, echogenicity decreases (Riordan and McKenna 2014; Chang et al. 2002).

1.3.3 Goal of Achieving Low Relative Signal Intensity (RSI) Units on MRI:

RSI units indicate the relative brilliance of a radiographic image. Signal characteristics can provide considerable diagnostic information. Understanding the signal characteristics of normal anatomic tissue assists in identifying abnormalities (Riordan and McKenna 2014).

For instance, fat, proteinaceous fluid, scar tissue, and lesions appear bright on T1-weighted sequences, yielding higher RSI units. On the contrary, cortical bone, air, and ligaments appear dark on T1 and T2 sequences, yielding lower RSI units. In addition, muscle and cartilage display intermediate brilliance on both T1- and T2-weighted sequences, yielding intermediate RSI units (Riordan and McKenna 2014; Goldspink and Yang 2004).

1.4 Significance of Study

The use of autologous biologics does not alter the pathophysiologic process of healing in the human body. On the contrary, it avoids or shortens the inflammatory phase of healing and hastens the progression to remodeling and restoration of function of the treated tendon or ligament (Riordan and McKenna 2014).

The biologics treatment aims to restore cellular volume and tissue viability to the injured or chronically injured acellular fibrotic (scar) tissue. This compromised tissue is involved in the disease process that further acts to cause pain and limit function (Brittberg et al. 1994).

Therefore, the use of biologics presents a very promising potential of increasing homogeneity while decreasing echogenicity in injured connective tissue. Till date, there exists no previous scientific study that author is aware of that has effectively documented changes of echogenicity of a tendon or ligament toward a healing, normal approaching tendon or ligament, with homogeneity returning at the injured/tendinotic site (Riordan and McKenna 2014; Brittberg et al. 1994).

1.5 Hypotheses and Research Questions

This study in general will attempt to prove that the use of biologics in regenerative medicine treatments will yield enhanced healing and increased therapeutic efficacy in comparison to traditional medical treatments.

1.5.1 Presumptive statement

Use of biologics decreases time to recovery for injured connective tissues treated both surgically and conservatively as seen in Anterior Cruciate Ligament graft and Achilles Tendonitis/ruptures, respectively.

1.5.2 Underlying assumptions:

Decreased time to recovery is directly dependent on the following variables: decreased incorporation time of the graft; increased homogeneity; and decreased echogenicity (measured by post-treatment MRI T1 relative strength intensity (RSI) units in the affected connective tissue). In addition, predisposing factors such as age, gender, previous injuries, recurrent injuries, and comorbidities may influence time to recovery.

Need factors include but are not limited to pre-treatment MRI studies detailing extent of injury and post-MRI studies measuring extent of recovery.

Research Question (RQ1). Is there a relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho1). There is not a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha1). There is a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Research Question (RQ2). Is there a relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho2). There is not a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha2). There is a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

CHAPTER 2 LITERATURE REVIEW

2.1 Literature Search and Search Strings

Literature search consisted of review of substantial peer reviewed articles published on Achilles tendon graft and ACL repair. Studies were identified by searching the MEDLINE, PUBMED, and SPORTDiscus electronic databases. The last search was performed on January 21 2017. The following search terms were used: “Epidemiology of Achilles tendon injury”; “Epidemiology of ACL injury;” “Various grafts used in ACL reconstruction surgery”; “Use of biologics and background of mesenchymal stem cells (MSCs)”; “Healthcare cost and utilization project (HCUP) for ACL and Achilles tendon surgeries”; “ICD-10 codes for Achilles tendon and ACL reconstruction surgeries.”

The author utilized a generalized search algorithm to yield maximum return. Along with the online searches, the author reviewed bibliographies of the included studies to identify additional publications. No date limits were considered for the publications on ACL healing and repair. The author combined citations identified from the searches and excluded duplicates.

2.2 Role of Biologics in Regenerative Medicine

2.2.1 Biologics used in Wound Healing – Literature Review

The author of this dissertation will utilize his own article, "Case report of non-healing surgical wound treated with dehydrated human amniotic membrane," published in the *Journal of Translational Medicine (2015)*, for this section.

As evidenced in the case study, non-healing wounds can pose a medical challenge as in the case of vasculopathic venostasis resulting in a surgical ulcer (Riordan et al. 2015). When traditional approaches to wound care fail, an amniotic patch (a dehydrated tissue allograft derived from human amnion) can function as a biologic scaffold to facilitate and enhance tissue regeneration and rehabilitation (Carlson 1997; Pierpont et al. 2014).

We present a case of a severe non-healing surgical wound in a 78-year-old male, 17 days post right total knee arthroplasty. The full-thickness wound exhibited a mobile flap, measured 4 cm long × 3 cm wide, and showed undermining down to patellar tissue. We treated the wound conservatively for 6 weeks with no evidence of wound healing. Upon failure of the conservative treatment, two amniotic AlphaPatch (Amniotic Therapies Inc., Dallas, TX, USA) were applied to the wound, and the wound healed completely in 10 weeks (Ramirez et al. 2014; Menedez et al. 2014).

In the OR, the wound was irrigated with three liters of double antibiotic solution under pulse lavage. Two dry amniotic AlphaPatch (4 cm × 4 cm) were placed over the wound with Acticoat applied on top.

At the two-week follow-up visit (following the incision and drainage of the wound dehiscence and application of the amniotic AlphaPatch), a central scab had formed centrally in the wound dehiscence area (Maxson et al. 2012; Murphy and Evans 2012; Sun et al. 2014). At the four-week follow-up visit, the wound dehiscence area had completely scabbed over with no open areas left. At the eight-week follow-up visit, the scab had just fallen off, and the wound was healing well with immature skin representing the size of a penny. At the ten-week follow-up visit, the wound was completely healed, the patient demonstrated full knee ROM (120° of flexion and 180° of extension), and patient was released from orthopaedic care.

In this case study, although the 78-year-old patient demonstrated excellent results in ROM and overall decreased pain levels following right total knee arthroplasty, the non-healing status of the surgical wound at even 42 days status post-surgery posed great concern. The vasculopathic venostasis resulting in a surgical ulcer left very little option than to perform dehiscence wound irrigation with pulse lavage and to apply dry amniotic AlphaPatch to the wound.

The amniotic AlphaPatch, a dehydrated amniotic tissue allograft, contains the molecules PGE2, WNT4, and GDF-11. Mesenchymal stem cells (MSCs) secrete PGE2, or Prostaglandin E2, in response to injury. This molecule inhibits fibrosis by way of limiting fibroblast proliferation, migration, collagen secretion, and transforming growth factor (TGF)-induced myofibroblast that can spur fibroblast proliferation (Madrigal et al. 2014; Bozyk and Moore 2014). PGE2 also enhances the wound healing process and angiogenesis (Bozyk and Moore 2014). WNT4, a

protein, drives wound healing by way of wound re-epithelialization and cell proliferation (Syeda et al. 2012). The creation of new tissue materializes by multiple methods including new blood vessel formation, a critical element for normal wound healing (Labus 1998). GDF-11, or growth/differentiation factor 11, has been identified as one of the key molecules propelling the regeneration of skeletal muscle, cardiac muscle, and nervous tissue in aged mice (Mendelsohn and Larrick. 2014).

Thus, sterile, dehydrated amniotic tissue AlphaPatches (containing trophic factors known to enhance wound healing) have proven effective in completely healing an otherwise non-healing wound in a 78-year-old male who failed six weeks of conservative wound care treatment (Syeda et al. 2012; Zhang et al. 2014). The two dry amniotic patches applied on the patient's wound substantially accelerated the wound healing process. The dehisced surgical wound that showed no sign of healing even after 42 days post total knee replacement surgery, demonstrated a central scab formation in the middle of the wound dehiscence area only after 2 weeks of amniotic patch application. After eight more weeks, the wound was completely healed, and the patient was released from orthopaedic care to assume high levels of physical activity and activities of daily living. Although more studies are warranted to further substantiate the therapeutic benefits of this treatment, we suggest unreservedly that dehydrated tissue allograft patches derived from human amnion embody a viable and more effective alternative to current traditional means of wound care management.

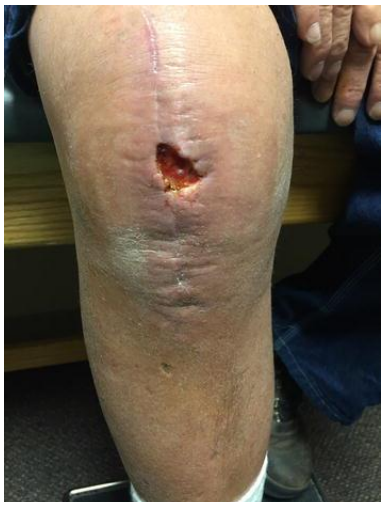


Fig. 1 (Pre-lavage)

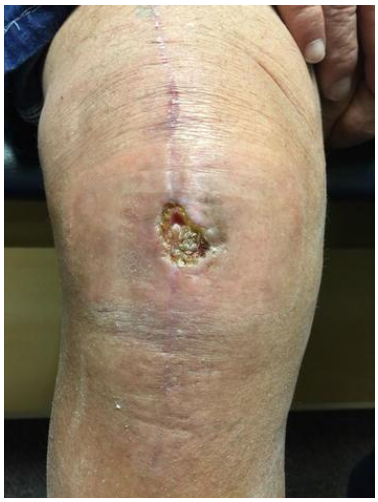


Fig. 2 (2 weeks post-lavage and amniotic Alpha Patch application)

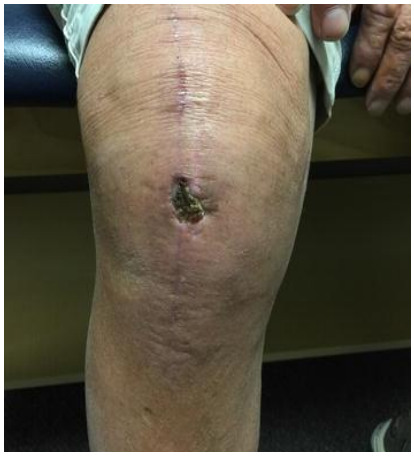


Fig. 3 (4 weeks post-lavage and amniotic Alpha Patch application)

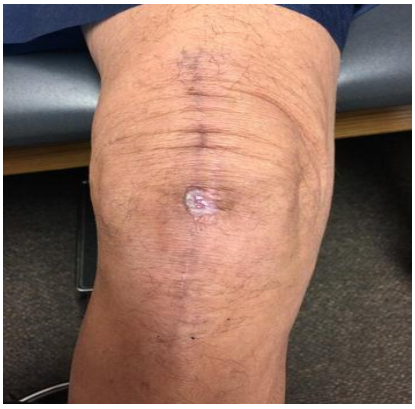


Fig. 4 (8 weeks post-lavage and amniotic Alpha Patch application)

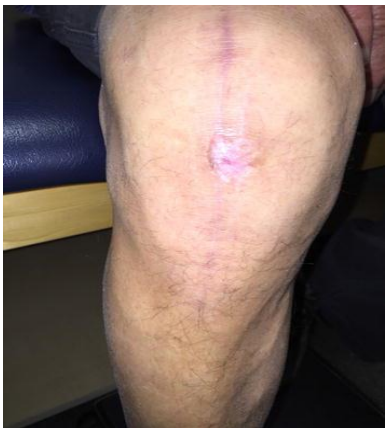


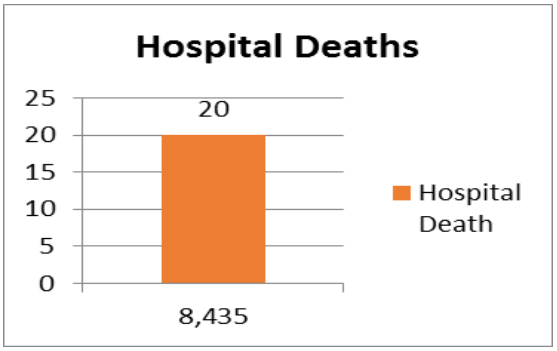
Fig. 5 (10 weeks post-lavage and amniotic Alpha Patch application)

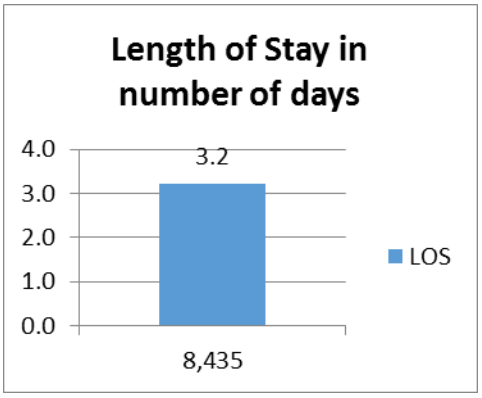
2.2.2 Biologics used in Achilles Tendon – Literature Review

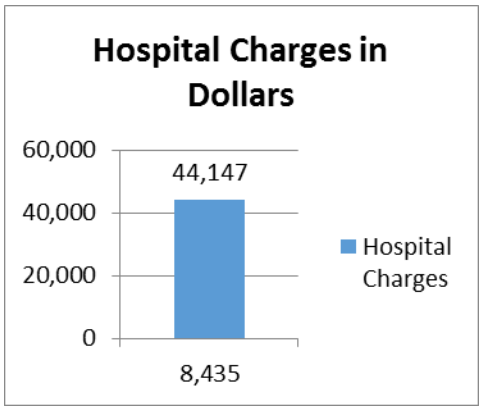
Injuries of the Achilles tendon are relatively common with potentially devastating outcomes. Unlike bone, tendon healing does not result in tissue homologous to its prior uninjured state. Instead, a fibrovascular scar is formed which results in a tendon mechanically weaker than the native tendon which can result in further injuries (Williams 1986).

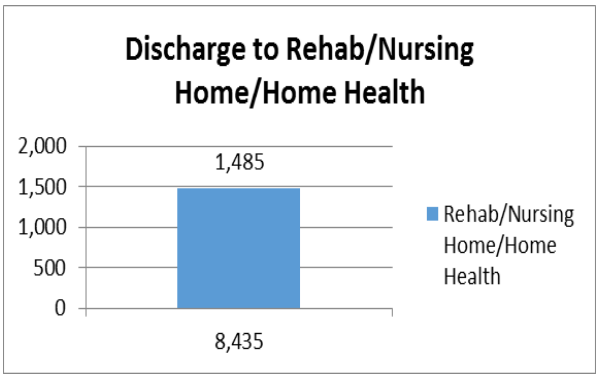
Potential complications specific to Achilles tendon surgery include wound infection, delayed wound healing, and re-rupture. Scarring of the tendon or thickening of the surgical scar may occur, further weakening the tendon (Kvist 1991; Rowe et al. 2012).

The American Orthopaedic Foot and Ankle Society (AOFAS) has documented the following after Achilles tendon surgery: pain decrease in six months with endoscopic incisions; improvement in tendon quality after one year; and return to sports after two years (Sangeorzan 2015).









Y-axis (mean values) vs. x-axis (total number of Achilles T. surgery patients)

Achillotenotomy (**2013 National Statistics**) according to HCUP *National (Nationwide) Inpatient Sample (NIS)*

http://www.hcup-us.ahrq.gov/nisoverview.jsp

2.2.2.1 Nonsurgical Success of Achilles Tendon Treatment with BMAC/STEMNEXA: Riordan McKenna Institute Case Study – Literature Review (Riordan and McKenna 2014):

The author followed the case of a 56-year old female tennis player who presented to RMI clinic, suffering chronic Achilles tendinopathy for ten years. The patient's

pain limited her ADL's like shopping, limited her standing to thirty minutes, restricted her footwear to sandals, and restrained her from participating in court sports. She had followed ten years of failed, standard conservative treatments like therapeutic ultrasound, anti-inflammatory medications, and stretching exercises (Riordan and McKenna 2014; Stein et al. 2015).

In short, the practitioner injected the combination injection therapy of BMAC and STEMNEXA Rx directly to the area of Achilles tendinosis via ultrasound guidance in the clinic. The patient amazingly returned to high intensity tennis at eight weeks post-non-surgical intervention. This result stands in stark contrast to the data reported by the American Orthopaedic Foot and Ankle Society (AOFAS): Achilles tendon surgical intervention yields decrease in pain at twenty-four weeks and complete recovery at two years (104 weeks) (Riordan and McKenna 2014; Sangeorzan 2015).

Post Intervention results:

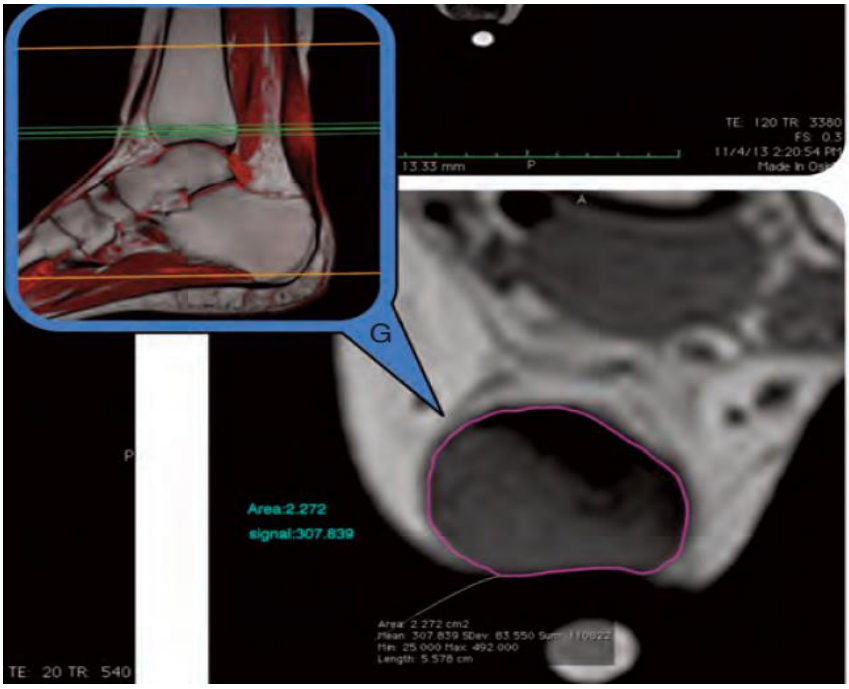
Using the Visual Analog Scale (VAS), the case study patient demonstrated far superior recovery results as depicted in Table 2.

Table 2 [Scores of the Visual Analog Scale (VAS) for pain at rest, on walking and at toe-raising and dorsiflexion before and after the intervention. Courtesy of RMI clinic.].

	Pre-intervention	Post-intervention	
	t=0	t=8 weeks	t=32 weeks
At rest	2-3	0	0
Walking	9	2	0
Toe raising	8	0	0

MRI results

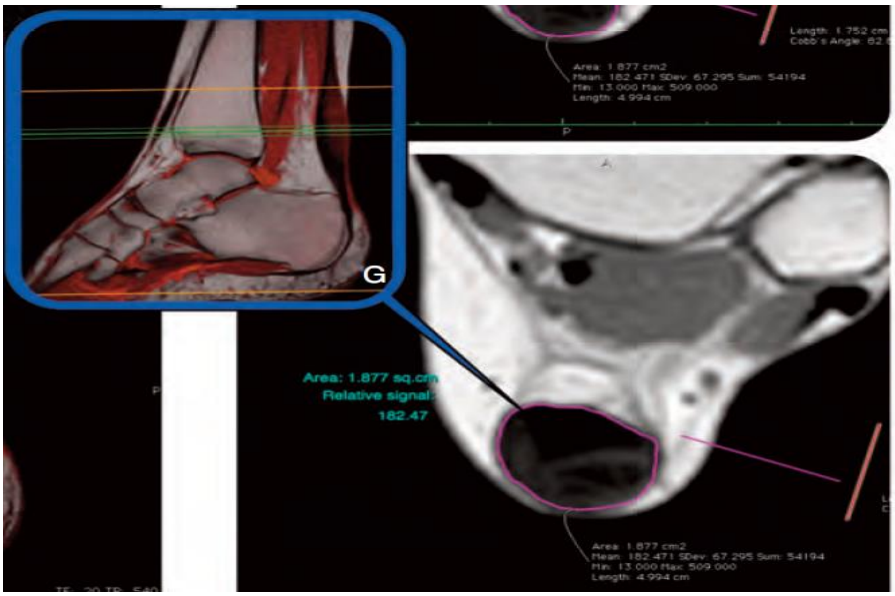
Pre-intervention



G, Scout view – center green line represents the image at the most involved portion of the tendon.

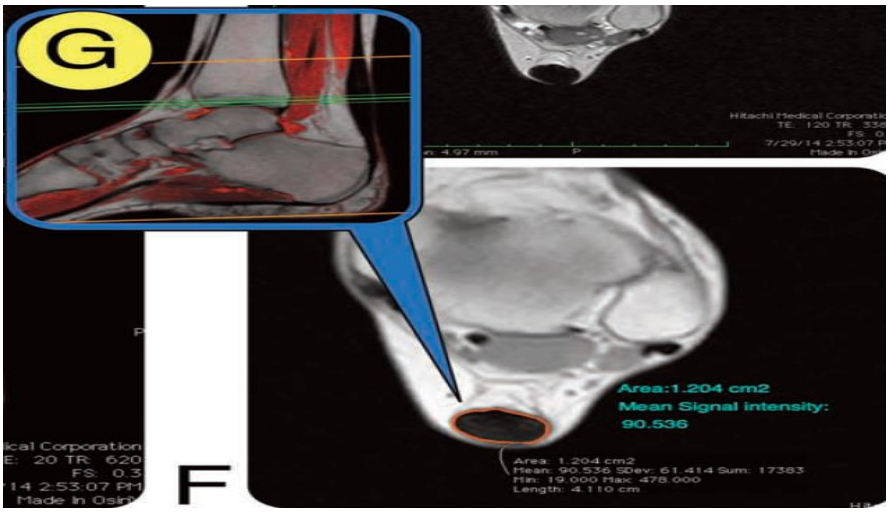
Courtesy of RMI clinic with special thanks to Dr. Paul Marsh, D.O. of Monticello Diagnostic Imaging, for reading these images.

10 weeks Post-intervention



G, Scout view – center green line represents the image at the most involved portion of the tendon.

32 weeks Post-intervention



G, Scout view – center green line represents the image at the most involved portion of the tendon.

Courtesy of RMI clinic with special thanks to Dr. Paul Marsh, D.O. of Monticello Diagnostic Imaging, for reading these images.

	Pre-intervention	10 weeks	32 weeks	Unaffected tendon
Area of Lesion (cm sq)	2.272	1.877	1.204	N/A
RSI units	307.83	182.47	90.536	72.536

* Figure below shows Relative Signal Intensity (RSI) values before and after treatment.

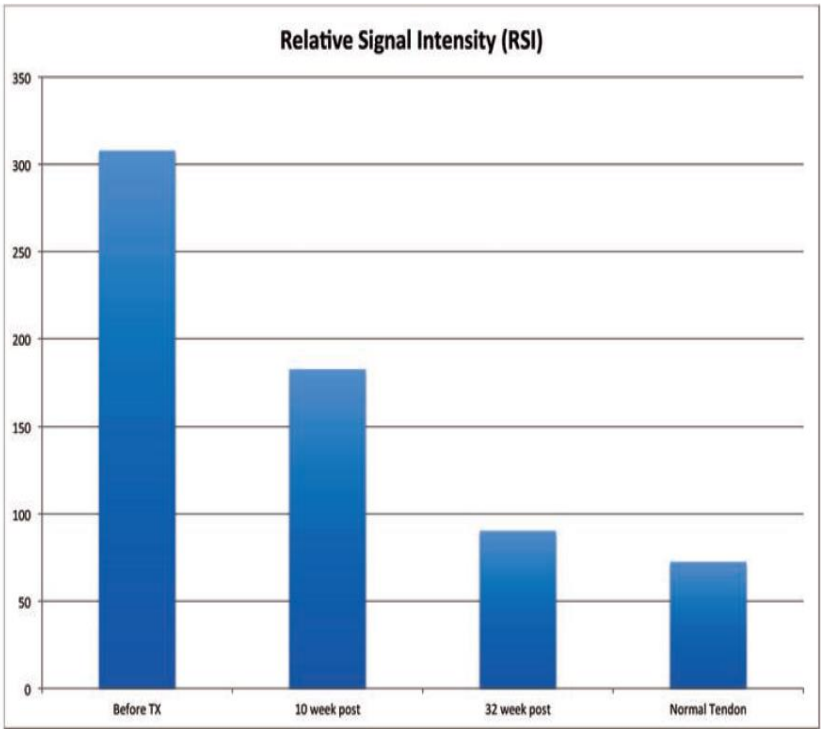


Figure 4. Relative Signal Intensity (RSI) values pre-treatment (Before TX), at 10 weeks post-treatment (10 week post) and 32 weeks post treatment (32 weeks post). The value of a normal tendon area at 32 weeks is also provided for comparison.

*Courtesy of and credit given to RMI clinic

2.2.2.2 Surgical Success of Achilles Tendon (AT) using BMAC – Literature Review

Literature also supports evidence of surgical success of AT repair with BMAC augmentation as seen in Stein's article, "Outcomes of acute Achilles tendon rupture repair with bone marrow aspirate concentrate (BMAC) augmentation (Stein et al. 2015)."

In this article, Stein discusses how optimal treatment of acute Achilles tendon ruptures remains controversial. He reports that although using stem-cell-bearing concentrates have been successful with other soft-tissue repairs, this study is novel because no other studies exist on outcomes of bone marrow aspirate concentrate (BMAC) augmentation in primary Achilles tendon repair (Stein et al. 2015).

The researchers reviewed twenty-seven patients with sport-related Achilles tendon ruptures treated via open repair augmented with BMAC injection from 2009 to 2011. The authors examined the Achilles tendon Total Rupture Score (ATRS) along with data from the following variable measures: operative complications; strength; range of motion; rerupture; calf circumference; and functional improvement through progressive return to sport (Stein et al. 2015).

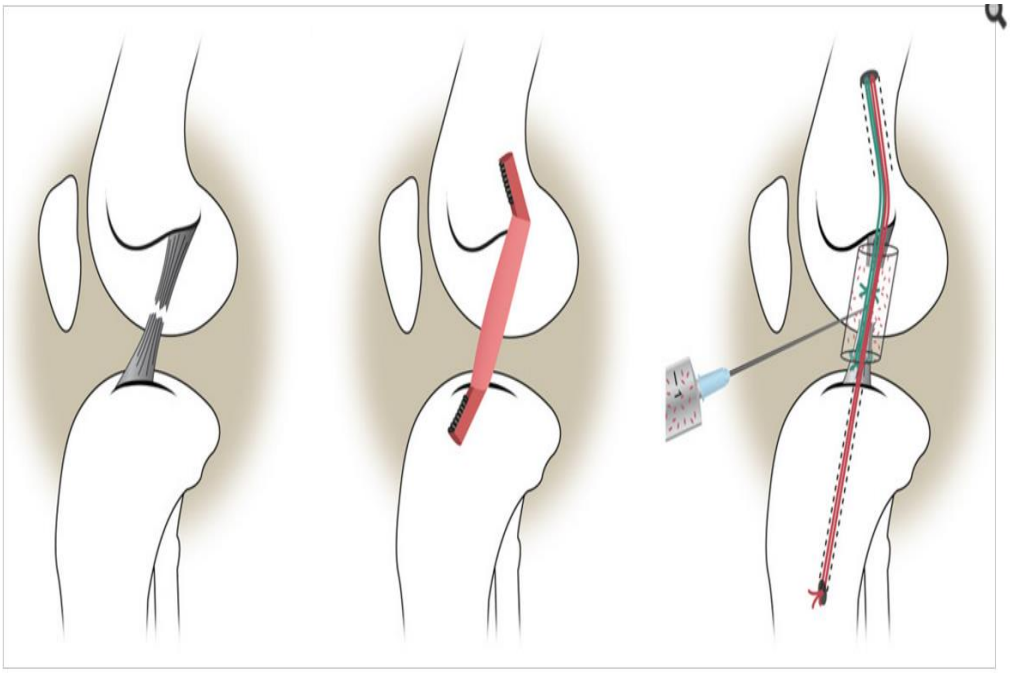
The following results were noted:
"A total of 27 patients (28 tendons) treated with open repair and BMAC injection were identified (mean age 38.3 ± 9.6 years). At mean follow-up of 29.7 ± 6.1 months, there were no reruptures. Walking without a boot was at 1.8 ± 0.7 months, participation in light activity was at 3.4 ± 1.8 months and 92% (25 of 27) of patients returned to their sport at 5.9 ± 1.8 months. Mean ATRS at final follow-up was 91 (range 72-100) points. One case of superficial wound dehiscence

healed with local wound care. No soft-tissue masses, bone formation or tumors were observed in the operative extremity (Stein et al. 2015)."

The researchers embraced the superior results of the open Achilles tendon repair augmented by BMAC, especially noting that no re-ruptures occurred in the 27 cohort athletes with early mobilization. In addition, the authors concluded that the clinical significance of BMAC administration in the surgical repair of acute AT ruptures warrants further study, particularly in the absence of observed adverse outcomes of this biologic treatment protocol (Stein et al. 2015).

2.2.3 ACL Literature Review

ACL injuries not only can present immediate and long-term effects on quality of life, but can also make the individual extremely susceptible to post-traumatic osteoarthritis (Griffin et al. 2000). Historically, operative repair of the ACL, as a result of failing in over 90% of patients, paved the way for the current gold standard of treatment, ACL reconstruction, which involves removal and replacement of the ligament with a tendon graft (Hewett et al. 2005). ACL reconstruction patients, however, continue to exhibit progressive articular cartilage and joint damage in the injured knee. In fact, a recent prospective cohort study reveals that 62% of ACL reconstructed patients with an isolated ACL injury presented with radiographic evidence of post-traumatic osteoarthritis ten to fifteen years post-surgery (Arendt and Dick 1995). As many individuals sustain ACL injuries before the age of sixteen, these injuries may place young patients at risk for premature post-traumatic osteoarthritis, even with best treatment protocols.



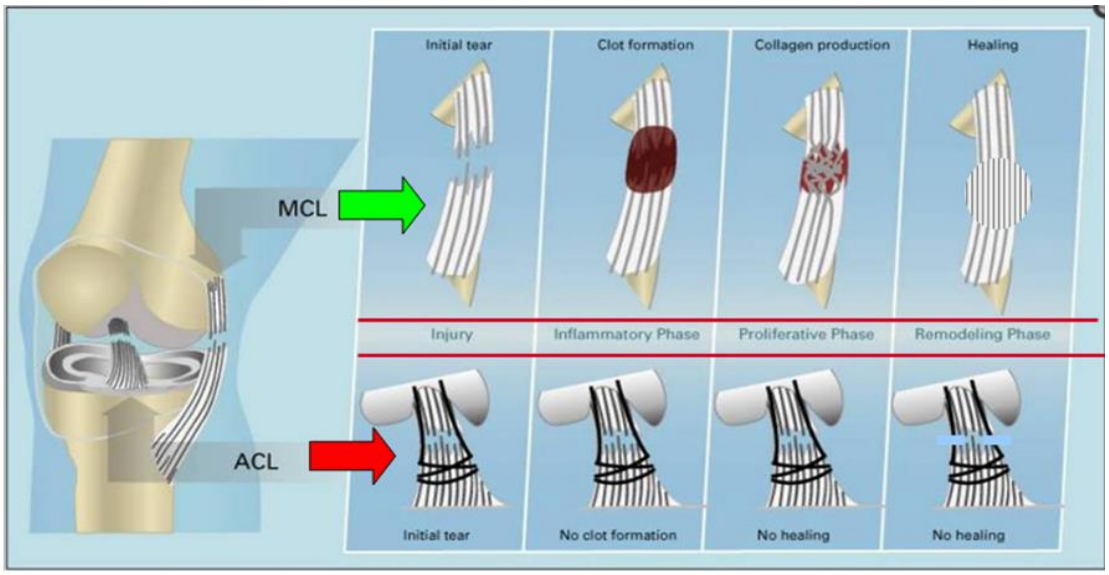
Schematic demonstrating an ACL tear (left panel), our current method of treatment with removal of the ACL and replacement with a tendon graft (ACL reconstruction, middle panel) and the novel treatment of repair and regeneration we have developed for this injury ("Bio-enhanced ACL repair", right panel) (Adapted with permission from Murray & Fleming[36]).

Figure 6 (Courtesy of and credit given to Murray and Fleming)

Having observed the long-term complications of ACL reconstruction surgery, researchers set out to design biologically-enhanced ways of repairing the ACL to overcome the underlying mechanisms that prevent the injured ACL to heal back to full homogeneity (Yu et al. 2002).

A series of experiments in the literature review shed light on why a torn ACL does not heal with full integrity and homogeneity. These experiments differentiated the following factors: intrinsic cellular deficiency of ACL fibroblasts; vascular deficiency; local environmental factors such as the bathing of the damaged ends of the ligament by synovial fluid; or an inability to bring opposing ends of the torn ligament sufficiently close together using suturing or other stabilizing techniques (Yu et al. 2002; Kirkendall and Garrett, Jr 2000).

Researchers studied ACL fibroblasts relative to the fibroblasts obtained from the medial collateral ligament (MCL), an extra-articular ligament that has demonstrated high success rates of healing with conservative treatment. Cell culture assays (*in vitro)* along with histological and immunohistological (*in vivo)* techniques revealed that MCL and ACL cells within injured ligaments have comparable rates of proliferation and the ability to revascularize after rupture (Yu et al. 2002; Kirkendall and Garrett, Jr 2000). In addition, both the ACL and MCL ligaments yielded very similar collagen production up to one year after injury (Boden et al. 2000). The chief discovery, however, highlighted the presence of a provisional scaffold in the wound site of the MCL and other extra-articular ligaments, whereas the ACL injury site contained no such provisional scaffold (Boden et al. 2000).



Differences in the intrinsic healing response of the ACL (top row) and medial collateral ligament of the knee (MCL, bottom row). The ACL is injured, but no blood clot forms in the injury site, likely due to the synovial fluid which bathes the ligament washing the clot out. In contrast, in the MCL, blood clot forms at the site of the tear and stabilizes the two ligament ends. The MCL tissue can then grow into this provisional scaffold and the defect can be healed. The loss of the provisional scaffold in the ACL is likely the key mechanism behind its failure to heal.

Figure 7 (Courtesy of and credit given to Murray and Fleming)

Difference in mechanical stabilization between the ACL and altered environments surrounding the torn ligaments, therefore, serves as explanation for the poor healing response of the ACL (Fig. 7). For instance, synovial fluid surrounds the ACL, not the MCL and all other extra-articular ligaments. Thus, while the proliferative and revascularative healing response of the injured ACL matches that of the MCL, the bathing away of the regenerative clot by the synovial fluid in the ACL negates the opportunity for a matrix scaffold to be formed in order to assist in rejoining the proximal and distal ends of the ligament to be remodeled by invading cells (Fig. 8). These findings have led scientists to hypothesize that the lack of a provisional scaffold between the opposite ends of the torn ACL plays the critical role behind the failure of this tissue to heal,

relative to the MCL and other extra-articular ligaments (Ferretti et al. 1992; Ekegren et al. 2009).

After establishing that no provisional scaffold formation exists between the ends of the ruptured human ACL, researchers hypothesized concerning a quantitative difference in the amount of wound site filling in the ACL and MCL, particularly in molecular deposition of the following: fibrinogen; fibronectin; PDGF-A; TGF-β1; FGF-2; and vWF (Kirkendall and Garrett, 2000; Ekegren et al. 2009; Boden et al. 2010). To test this hypothesis, scientists inflicted central defects (Fig. 8) in the MCL, patellar ligament, and ACL in canine knees. They examined histologic response to the injury at 3 days, 7 days, 21 days, and 42 days. The scientists concluded that the extra-articular MCL and patellar ligament defects exhibited far greater filling of the wound site (with increased presence of fibrinogen, fibronectin, PDGF-A, TGF-β1, FGF-2 and vWF) when compared to intra-articular ACL defects at all interval points (Fig. 8) (Kirkendall and Garrett, Jr 2000). The data, therefore, not only supports the hypothesis of a lack of a provisional scaffold within the intra-articular injury site of the ACL, but also implicates this loss to a decreased presence of important extracellular matrix proteins and cytokines within the ACL injury site.

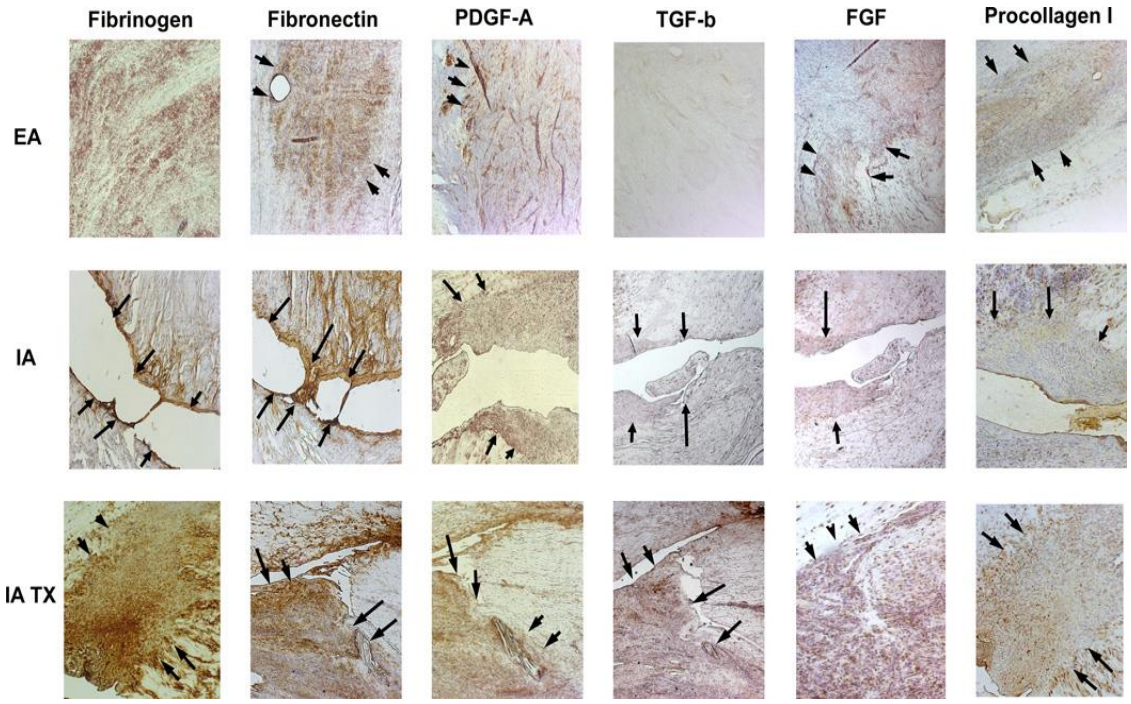
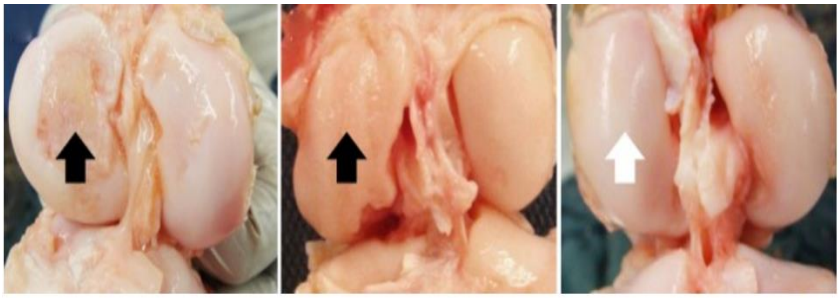


Figure 8: Representative photomicrographs of the patellar ligament wounds (extra-articular (EA; first row)) untreated ACL wounds (intra-articular (IA; second row)) and ACL wounds treated with collagen-platelet scaffold (intra-articular (IA TX; third row)) 21 days after wounding (10x). Similar distributions of protein presence were noted in the treated ACL wounds and the healing patellar ligament wounds. The untreated ACL wounds remain relatively empty of any substratum (Used with permission from Murray et al7). +

Figure 8 (Courtesy of and credit given to Murray and Fleming)

After linking the failure of ACL healing to the premature loss of the provisional scaffold, there arose a critical need to develop an easily implantable substitute scaffold capable of providing the growth factors and enzymes required to optimize fibroblast activity and capable of withstanding the physical conditions of the intra-articular environment. This led to a search for a more physiologically relevant cytokine and growth factor delivery system, wherein delivering platelets to an ACL injury site using a stable carrier (scaffold) would be evaluated for clinical results consistent with healing found in the MCL. In a series of in vitro experiments, the researchers discovered that the concentration of growth factors

released from platelet rich plasma (PRP) depended on the platelet concentration and that the timing of that release depended on the activation method for the platelets (Kiapour and Murray 2014; Gulati et al. 2015). While the application of thrombin resulted in an immediate release of the platelet-derived growth factors, the addition of collagen as an activator resulted in a more sustained release of multiple platelet-derived growth factors (Riordan and McKenna 2014). In addition, maintaining the platelets in their physiologic plasma yielded an environment in which the platelets stimulated collagen synthesis by ACL fibroblasts (Stein et al. 2015).



Photographs showing the distal femoral cartilage at one year after a) an untreated anterior cruciate ligament (ACL) rupture, b) after conventional ACL reconstruction, and c) bio-enhanced ACL repair. Note the damage to the medial femoral condyle in the untreated and ACL-reconstructed knee (black arrows). No damage to the medial femoral condyle in the bio-enhanced ACL-repair knee (white arrow) was observed (adapted and modified with permission from Murray and Fleming [182]).

Figure 9 (Courtesy of and credit given to Murray and Fleming)

2.2.4 MSC Labeling

The 2006 Carstangen study of an adult horse with a soft palate defect elucidates the viability and efficacy of MSCs *in vivo*. The scientists isolated, cultured, and labeled equine MSCs and then implanted the mesenchymal cells in the surgically repaired soft palate of an adult horse. The MSCs were isolated, cultured in monolayers, and labeled with 5-bromo-2-desoxymidine (BrdU) and chloromethylbenzamido-DiI-derived (cm-DiI) before transplantation. After repairing the soft palate defect by mandibular symphysiotomy, the practitioners injected the labeled MSCs in to the repaired soft palate. Two weeks after transplantation, the examiners identified the stained autologous MSCs in the soft palate tissue (Carstangen B et al. 2006).

Furthermore, postmortem examination revealed not only that 90% of the soft palate defect had been sutured, but clearly showed intense staining by BrdU and cm-DiI in the soft palate tissue. In fact, the scientists detected the labeled MSCs in tissue slices from the injection sites with cell organization commensurate with that of the native soft palate tissue, signaling successful engraftment (Carstangen B et al. 2006).

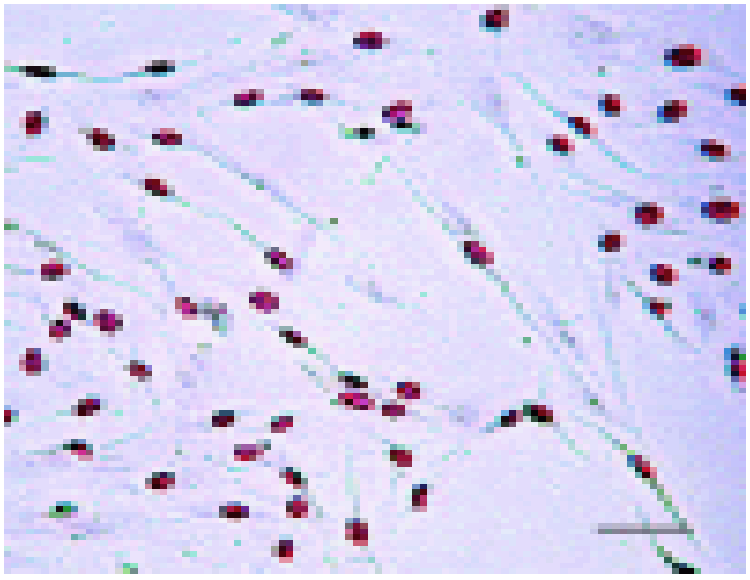


Figure 10 - Phase-contrast photomicrograph of labeled mesenchymal stem cells (MSCs) that have integrated 5-bromo-2-desoxymidine (BrdU), expressed in the nucleus (brown staining). (Carstangen B et al. 2006)

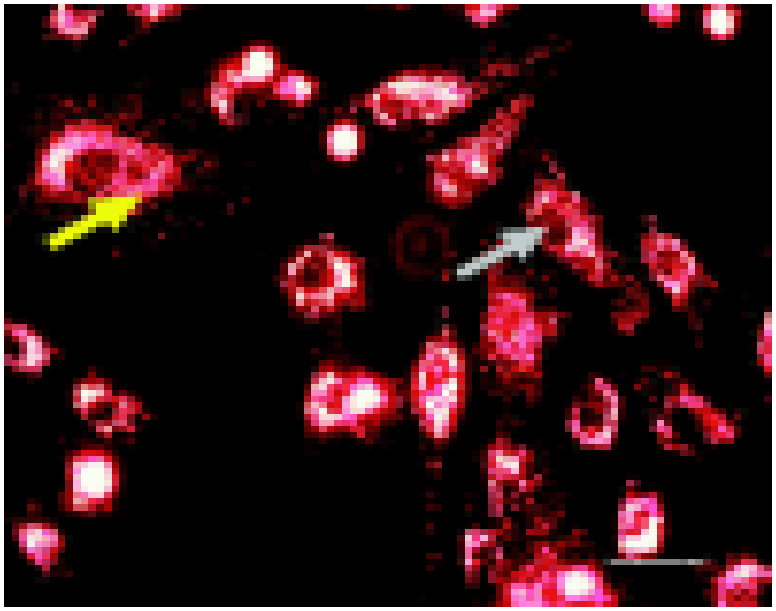


Figure 11 - Fluorescent photomicrograph of labeled MSCs that have integrated chloromethylbenzamido-DiI-derived (cm-DiI), expressed in the cell membrane (yellow arrow) but not the nucleus (grey arrow). (Carstangen B et al. 2006)

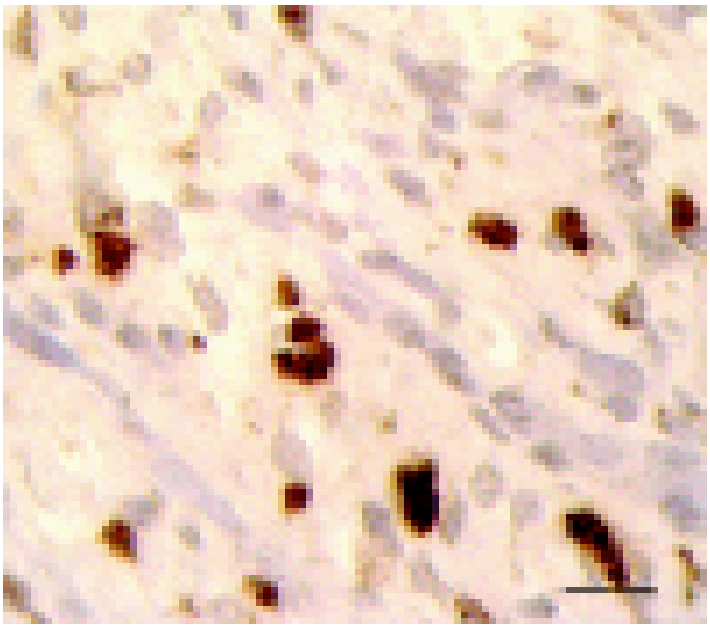


Figure 12- Photomicrograph of a 5-µm-thick frozen-tissue slice of soft palate, showing the grafted MSCs that have integrated into soft palate layers (brown nuclei). (Carstangen B et al. 2006)

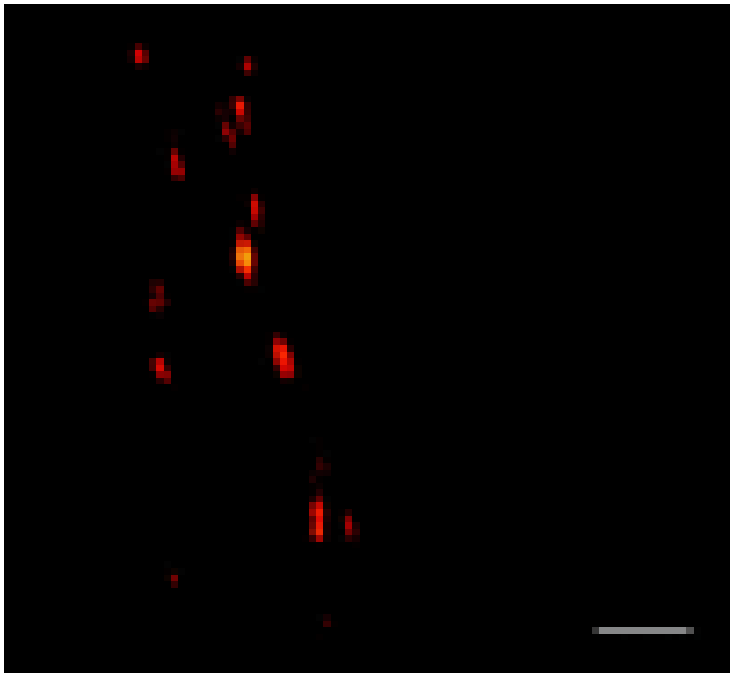


Figure 13 - Photomicrograph of a 5-µm-thick frozen-tissue slice of soft palate, showing the red cm-DiI autofluorescence of grafted MSCs. (Carstangen B et al. 2006)

CHAPTER 3 METHODOLOGY

3.1 Research Overview

Injuries to the Achilles Tendon and Anterior Cruciate Ligament pose a great challenge for treatment and time to recovery in sports medicine.

3.1.1 Etiology

ACL injuries occur typically in the third decade of life in active individuals. Seventy percent of these injuries result from sports participation, thirty percent from direct contact and seventy percent from non-contact sports. Interestingly, female athletes are more likely to sustain ACL tears (Kiapour and Murray 2014). Achilles tendinopathy/tears, on the other hand, generally occur in males in the third to fifth decade of life during participation in recreational sports (Paavola et al. 2002).

3.1.2 Prevalence

With an annual incidence of more than 200,000 ACL injuries in the USA, approximately half of these cases require reconstruction surgery. The majority of ACL injuries occur while playing agility sports, with high prevalence (1 in 1,750 persons) in active 15-45 year-olds and in females who are two to eight times more likely to get injured than males (Gordon and Steiner 2004; Kiapour and Murray 2014).

The incidence of Achilles tendon rupture in the general population is 7 per 100,000. Over 80 percent of ruptures occur during recreational sports. Approximately 10 percent of patients who sustain an Achilles tendon rupture had preexisting Achilles tendon pathology (Jarvinen et al. 2005). Observational data suggest that competitive athletes have a lifetime incidence of Achilles tendinopathy of 24 percent, with 18 percent sustained by athletes younger than 45 years. Tendon rupture occurs in 8.3 percent. Among competitive runners, the lifetime incidence of Achilles tendinopathy may be as high as 40 to 50 percent (Maffulli et al. 2003; Maffulli et al. 2004).

Therefore, the purpose of this correlational, quantitative study is to examine the role of biologics in decreasing time to recovery and increasing homogeneity for injured connective tissues treated both surgically and conservatively as seen in Anterior Cruciate Ligament reconstruction surgery and Achilles tendonitis/ruptures, respectively.

Chapter 3 will present an overview of the methodology used for this study. This overview will include the following: study design, population, sampling method, sample size, instrumentation, and data analysis methods.

3.2 Research Design

This study will follow a quantitative, correlational design. A quantitative correlational design seeks to examine potential relationships between

independent and dependent variables. Further insight into why this design selection is appropriate for this study can be seen by examining the two parts of the design separately (quantitative and correlational).

Quantitative research attempts to identify relationships between variables using trends, meanings, and suggested characteristics of the individual variables. Using a quantitative design for this study will allow the researcher to explore the relationship between decreased time to recovery and the following variables: decreased incorporation time of the tendon/graft; increased homogeneity; and decreased echogenicity (measured by post-treatment MRI T1 relative strength intensity (RSI) units in the affected connective tissue).

Correlational studies should be used when independent variable variation has occurred without researcher control. In this study, the researcher is not able to control any of the independent variables; the variation within the independent variables occurred prior to data collection. All data is retrospective and; therefore, the researcher is unable to introduce any type of intervention, only examine the relationships between variables. The basic purpose of a correlational study is to determine the relationship between variables, but not the cause of this relationship. According to Triola (1998), researchers must avoid concluding that the results of a correlational study imply causality (Triola 1998).

3.3 Research Questions and Associated Hypothesis

This study will be driven by the following research questions and associated statistical hypotheses:

Research Question (RQ1). Is there a relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho1). There is not a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha1). There is a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Research Question (RQ2). Is there a relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho2). There is not a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha2). There is a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

3.4 Population and Sample Criteria

The focus of this study will be N (non-surgical) = 1 Achilles tendinopathy and N (surgical) =20 ACL reconstruction patients between the ages of 17 and 57 in the United States from 2013 to 2016. For this study, Healthcare Cost and Utilization Project (HCUP) data will be used as supporting evidence. The HCUP data contains the Nationwide Inpatient Sample (NIS). The NIS contains data on more than seven million hospital stays each year. The NIS is sampled from the State Inpatient Database (SID) which contains all inpatient data that are currently contributed to HCUP.

3.4.1 Sample Size Calculation

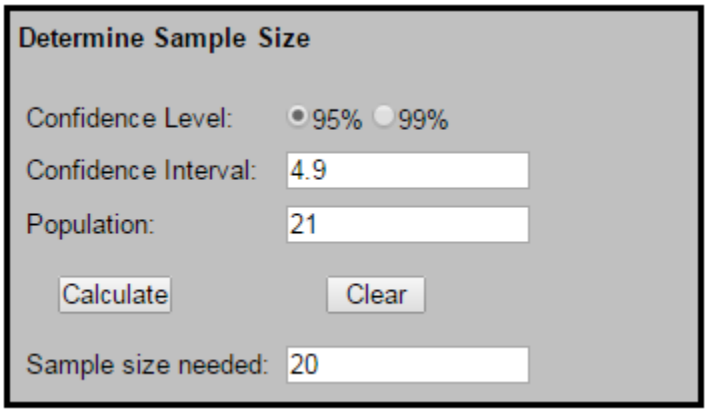
Given the composite outcome measures derived in the meta-analysis, the researcher determined the number of patients from this cohort study to detect statistically significant differences between traditional methods versus use of biologics in Achilles tendinopathy and ACL reconstruction for various outcome measures. For these calculations, the researcher set power at 0.80 and alpha at 0.05. The researcher calculated the sample sizes using a confidence level of 95% and confidence interval of 4.9.

3.4.2 Sample Size Calculator Terms: Confidence Interval & Confidence Level

The confidence interval (also called margin of error) is the plus-or-minus figure usually reported in polling results. For example, in the above scenario, if 47% percent of the sample picks an answer, one can be certain that the population between 43% (47-4) and 51% (47+4) would have picked that answer.

The confidence level tells a researcher how sure he/she can be. It is expressed as a percentage and represents the percentage of the population who would likely pick an answer that lies within the confidence interval. The 95% confidence level means one can be 95% certain of the result; the 99% confidence level means one can be 99% certain of the result. Most researchers traditionally use the 95% confidence level.

When the researcher combines the confidence level and the confidence interval together, he can say that he is 95% sure that the true percentage of the population is between 43% and 51%. The wider the confidence interval that he is willing to accept, the more certain he can be that the total population answers would be within that range.

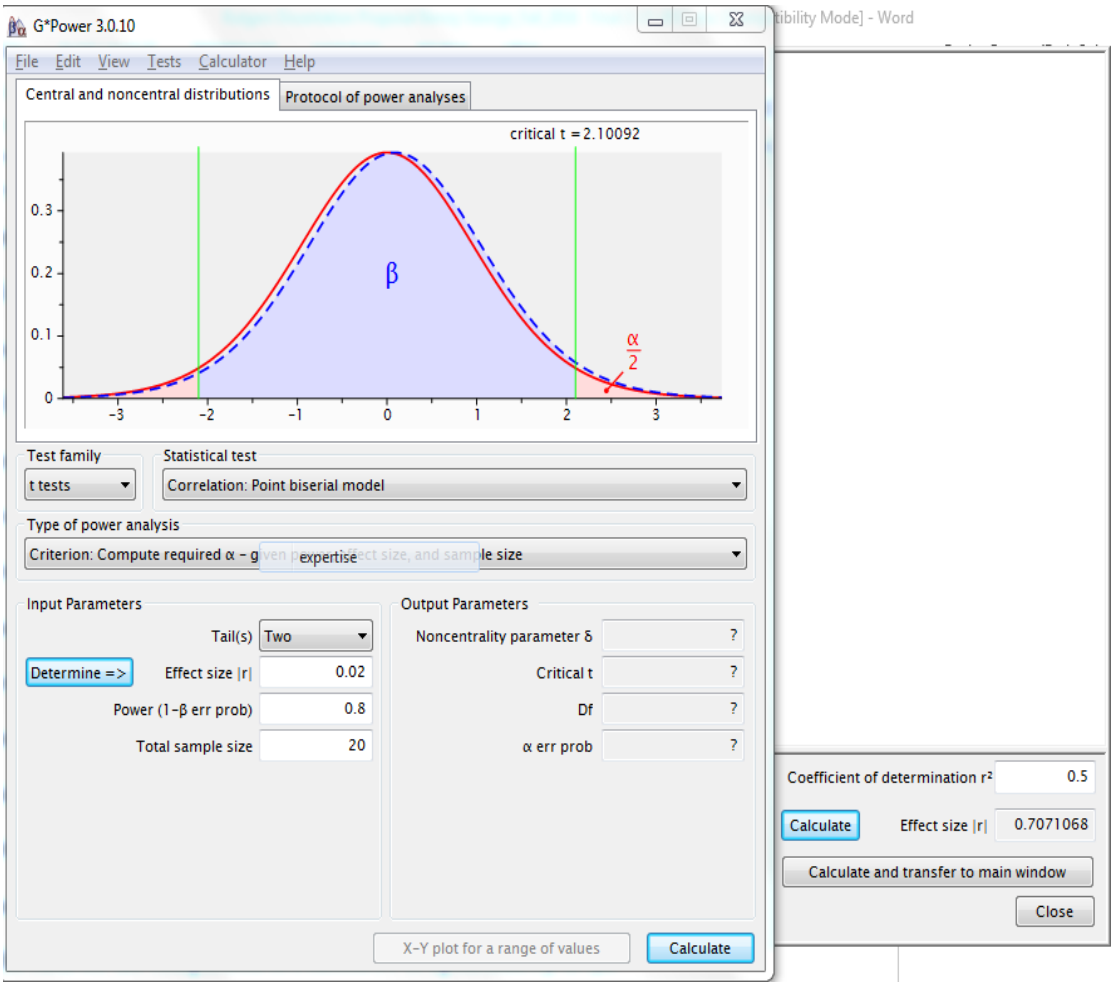


A good sample size calculation required to attain a confidence level of 95% is 20.

3.4.3 Power Analysis

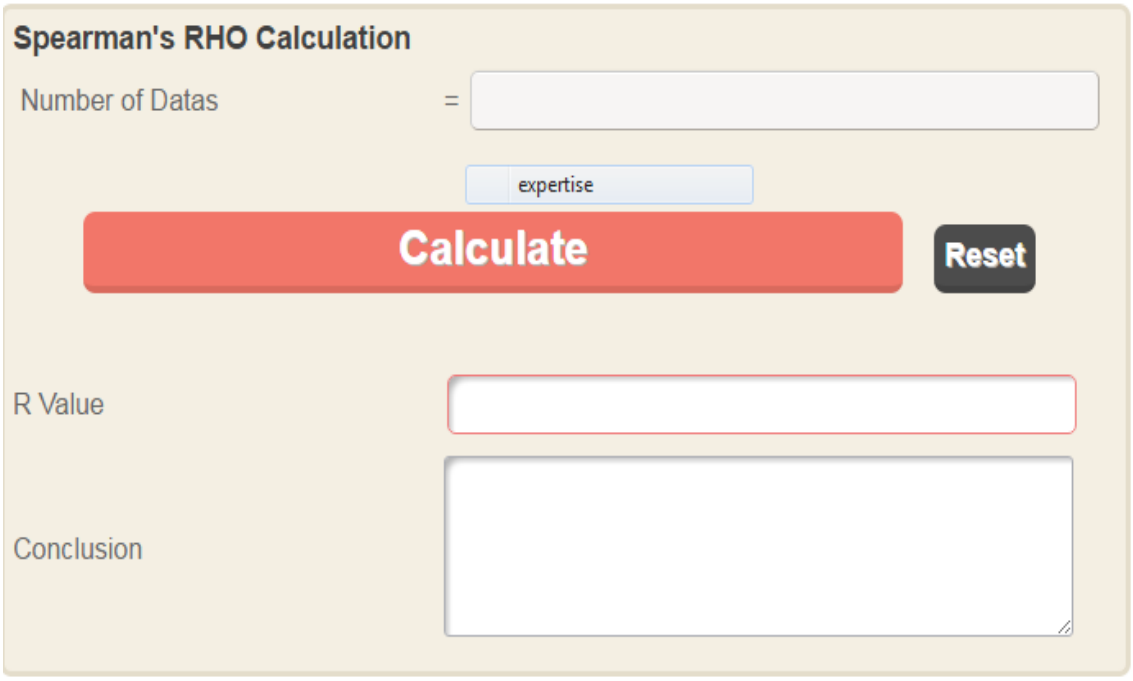
Power analysis for multiple regression was performed using G*Power 3.1.9.2 using a two-tailed test, a small effect size of .02, an alpha of .05, and a power of .80.

Power analysis was also performed for correlational analysis using G*Power 3.1.9.2 using a two-tailed test, a small effect size of .10, an alpha of .05, a power of .80, and a correlation for the null hypothesis of 0.



For some of the variables in this study, Spearman's rank order correlations (RHO calculator) will be used due to the use of ordinal variables. The sample size required for Pearson's r correlations will be used in tandem with the asymptotic relative efficiency (ARE) in order to calculate the required sample size for Spearman's rank order correlation. According to Prajapati, Dunne, and Armstrong (2010), the ARE for the comparison of Pearson's r to Spearman's ρ is .91 (Prajapati et. al. 2010).

RHO calculator helps to calculate the Spearman's Correlation rank value of coefficient using given number of the data. Rank value (R) concludes positive and negative values (Prajapati et. al. 2010).



The two-way analysis of variance, an extension to the one-way analysis of variance, involves two independent variables (hence the name two-way).

Assumptions:

1. The populations from which the samples were obtained must be normally or approximately normally distributed.
2. The samples must be independent.
3. The variances of the populations must be equal.

Hypotheses

Two-way ANOVA test contains three sets of hypothesis. The null hypotheses for each of the sets are given below:

1. The population means of the first factor are equal, like the one-way ANOVA for the row factor.
2. The population means of the second factor are equal, like the one-way ANOVA for the column factor.
3. No interaction exists between the two factors, similar to performing a test for independence with contingency tables.

Factors

The two independent variables in a two-way ANOVA, called factors, affect the dependent variable. Each factor will have two or more levels within it, and the degrees of freedom for each factor is one less than the number of levels.

3.4.4 Instrumentation

- *MRI, MRI readings*
- *RSI for graft, RSI for unaffected tendon (i.e. Posterior Cruciate Ligament)*
- Dr. Paul Marsh, Monticello Imaging

For comparison study, the researcher also employed the NIS dataset from HCUP database and patient information collected from Riordan-McKenna Institute. HCUP data is collected using discharge records from participating states. The discharge documentation was originally submitted to the state from hospitals as required by law or for hospital reimbursement reasons. According to Al-Halal, Kezouh, and Abenhaim (2013), no studies exist regarding validity of the HCUP-NIS database. Nevertheless, HCUP-NIS is a trusted database which has been used in a large number of studies (i.e. Studies by Bao and Sturm (2011), Ritchie, Maynard, and Chapko (1999), and Rutledge (1997)) (Al-Halal et al. 2013).

According to Al-Halal, Kezouh, and Abenheim (2013), the HCUP-NIS is considered to be the largest and only publicly available all-payer inpatient database. The NIS, sampled from the State Inpatient Databases (SID), represents another HCUP database that contains all inpatient data (Al-Halal et al. 2013). According to Carlton (2009), quality control of this database is conducted by an independent contractor and through automated quality control checks. Accomplished by verifying the data against standardized norms and against data conflicts, some data are set to special missing values so that it can be investigated for data anomalies. By comparing values of similar data elements, the internal consistency of data is finalized. Carlton (2009, 52) then goes on to cite the Agency for Healthcare Research and Quality to explain that “when conflicts occur, data are managed by established protocols.”

NIS depends on the hospitals to ensure accuracy, completeness, and consistency of the data. Validity and reliability of the data from the hospitals to HCUP is out of the researcher's realm of control. Despite this lack of control, due to the federal protocols and its use in a large number of studies, the NIS proves to be well documented and reviewed prior to use by the researcher for this study.

3.5 Measures

The variables utilized in this study, both from the HCUP database as well as from the RMI clinic can be found in the appendix section.

3.6 Collection Method/Procedures

Patient data on ACL reconstruction and AT tendonitis is collected from Riordan-McKenna Institute (http://www.rmiclinic.com).

MRI images and impressions are obtained from Dr. Paul Marsh (Monticello Diagnostic Imaging).

3.6.1 Non-surgical method (AT BMAC injection (STEMNEXA-Rx) at the RMI clinic)

In an office setting, 60 cc of bone marrow was aspirated from the metaphyseal area of the medial tibia and concentrated with a closed system. The resulting BMAC plus AlphaGem concoction was injected intralesionally into the affected tendinotic mid-substance area under ultrasound guidance.

3.6.2 Surgical method (ACL reconstruction (STEMNEXA-Sx))

In an Operation room setting, 60 cc of bone marrow was aspirated from the iliac

crest of the patient and was then placed in the Magellan centrifuge to spin down the cells to help harvest the stem cell and plasma platelet rich areas of the blood. The resulting BMAC is then injected at the time of surgery into the base of the graft (proximally and distally) directly following injection of 1 cc of AlphaGems into the same areas. This STEMNEXA-Sx administration helps aid faster healing and incorporation of the graft.

Searches via PubMed and Google Scholar were performed to identify both scientific investigations and review articles to ensure inclusion of pertinent data.

3.7 Data Analysis

All statistical analysis will be performed using Stata v12. All inferential tests will be two-sided and will utilize a 95% significance level.

Hypothesis testing will be performed using Spearman's rank order correlational analysis. A correlation table will be created containing all variables. This correlation table will also be used to choose control variables to be used in hypothesis testing for RQ. Any potential control variable with an absolute correlation at or above .30 with time to recovery will be included in hypothesis testing for RQ. Prior to correlational analysis, the assumption of a monotonic relationship between compared variables will be tested using scatterplots of the variable combinations.

Two separate multiple regressions will be used to address RQ. Prior to hypothesis testing, the assumptions (absence of outliers, normality, and linearity) will be

checked for both models. Visual inspection of a histogram and Normal Q-Q plots will be performed on the dependent variable of the study to check for deviations from normality. If the normality assumption is severely violated (mean and 5% trimmed mean vary greatly, and the mean and median vary greatly), then logarithmic or other transformation of the dependent variable will be considered to meet the normality assumption. Multicollinearity will be checked using the correlation table created for hypothesis testing of RQ as well as the selection of control variables. If multicollinearity is detected (defined as a correlation of .90 or greater according to Tabachnick and Fidell) between any pair of independent variables, then it will be determined if omission of one of the variables is necessary, or if retention of both variables is more appropriate (Tabachnick and Fidell 2007).

One of the two multiple regressions will be used to address RQ. This multiple regression will use echogenicity and homogeneity as the dependent variable in analysis and employ the following as independent variables: (a) age; (b) gender; and (c) time period between surgery to post-surgical MRI study (with a correlation of .30 or greater with time to recovery to be used as control variables).

3.8 Ethical Considerations

Ethical considerations for the use of this data are assured by IRB approval and the required HCUP Data Use Agreement (DUA) training. The DUA has specific requirements which must be followed by the researcher in order to preserve

patient rights. The DUA emphasizes the importance of data protection and makes this an individual responsibility of the researcher. The DUA focuses on protection of individual identities. All data elements which can be used to directly identify an individual have been previously removed. Hospital/patient names will not be reported.

The HCUP-DUA training is done online, and can be found at http://www.hcup-us.ahrq.gov/tech_assist/dua.jsp. The course takes approximately 15 minutes to complete.

3.9 Summary

Chapter 3 defines the methodology process to be used for this quantitative, correlational study. Patients between the ages of 17 and 57 make up the population of this study. Data will be taken from Riordan-McKenna Institute and Monticello Diagnostic Imaging between the years 2013 and 2016.

Hypothesis testing will be done using two multiple regressions on survey data to analyze the relationship between the variables, the details of which can be found in Appendix A. Chapter 4 will explore and report the preliminary findings of this study.

CHAPTER 4 RESULTS

In this chapter, preliminary results of measures of central tendency and frequencies and percentages of descriptive statistical analyses performed by the methods described in chapter 3 are reviewed and discussed. Descriptive statistics for each variable were performed separately; frequencies percentages for all categorical variables, and measures of central tendency for all continuous variables and counts were performed and are noted below.

4.1 Measures of Central Tendency for Continuous Variables and Counts

Measures of central tendency for continuous variables include: LOS; cost; and age. The researcher calculated mean, median, standard deviation, minimum, and maximum with respective variances on the three variables. Highly-skewed, dependent variables would require a transformation of data to undergo a regression analysis. LOS and cost variables are observed to be highly skewed where 89% of data is not within three standard deviations. For LOS, the min to max range is 0 to 10, with a high skewness of 10.886. Cost variable yields a min value of 500K to a max of 7 M, averaging a cost of 2M and skewness of 9.842, thus indicating outliers. LOS and cost variables will, therefore, need to be cleaned to remove outliers.

4.2 Frequencies Percentages for Categorical and Ordinal Variables

Measures of frequencies percentages for categorical and ordinal variables include: gender; census division; region; and payer-owner. The researcher calculated frequency, percent, valid percent, and cumulative percent against the above-stated variables.

The percent of primary payers projects that income and age may be correlated with the type of payers. Medicare comprises 25% while Medicaid reflects 20% of the primary pay. Private insurance contributes up to 37%, while self-pay generates 18%.

4.3 Further Work

The preliminary analysis revealed that more research will need to be developed and improved for the dissertation. At a minimum, the research must perform an aggregation of groups to remove records, outliers, and clean up the data. After performing a correlational analysis, the results will determine which control variables will be included in the multiple regression testing. If correlations are found to be strong to the research questions, the independent variable will be a control variable for the multiple regression.

In addition, preliminary research revealed a need to divide hospitals by region. Therefore, a census region of the hospital wherein each individual received

treatment will be used as a control for the location within the United States. The four categories for census region will be (a) West, (b) Midwest, (c) South, and (d) Northeast.

4.4 Sample and model specifications

The candidate performed initial statistical analysis on the data obtained from the HCUP database. This data retrieved from NIS database includes Achilles tendinopathy as well as ACL reconstructions. The researcher used this data to analyze the burden applied to healthcare systems and individuals by way of medical costs and length of stay (LOS).

A total of N = 10K records were retained for analysis. SPSS v22 helped to compute the descriptive measures of the variables. Stata 12 software performed all the inferential tests on the above variables. The candidate applied two-sided inferential tests with 95% significance level to determine statistical significance. Furthermore, the researcher utilized multiple regression analyses and correlational analyses to address the research questions based on the data received from RMI clinic.

4.5 Descriptive Statistics

Table 3*: Measures of Central Tendency for Continuous Variables of Study, for the Sample and the Population*

Variable	***M***	***SD***	***Mdn***	**Range**
LOS				
Sample	4.15	5.64	3.00	0 - 333
Population	4.15	5.66	3.00	0 - 333
Cost				
Sample	25,502.19	41,426.80	15,010.00	106 - 1,469,196
Population	25,665.26	41,772.48	15,075.00	106 - 1,469,196
Age				
Sample	37.21	10.54	37.00	21 - 55
Population	37.23	10.54	37.00	21 - 55

Note. Sample N = 758,874; Population N = 3,794,690; M = Mean; SD = Standard Deviation; Mdn = Median.

Table 3 presents the measures of central tendency (mean, median, standard deviation, and range) for sample and the weighted population data, for the continuous variables in the study. As expected, the measures are similar between the sample of data and the weighted population findings. The patients in the

weighted population ranged in age from 1 to 84 years (*M* = 37.23 years, *SD* = 10.54 years). The patient length of stay (LOS) ranged in value from 0 to 333 days (*M* = 4.15 days, *SD* = 5.66 days). The patients had approximately 2 procedures on average, and presented with approximately 3 chronic conditions on average. The total charges (Cost) ranged from $106 to over 1 million dollars (*M* = 25,665.26, *SD* = $41,772.48).

Table 4: *Measures of Central Tendency (mean, median, standard deviation, and range) for sample and the weighted population data, for the continuous variables*

Variable	***M***	***SD***	***Mdn***	**Range**
LOS				
Sample	4.52	6.99	3.00	0 - 248
Population	4.53	7.01	3.00	0 - 311
Cost				
Sample	30,708.52	49,500.68	17,932.00	142 - 1,469,196
Population	31,091.34	50,285.21	18,082.00	142 - 1,469,196
Age				
Sample	37.89	10.32	38.00	21 - 55
Population	37.89	10.32	38.00	21 - 55

Note. Sample *N* = 155,026; Population *N* = 800,980; *M* = Mean; *SD* = Standard Deviation; *Mdn* = Median.

Table 4 presents the measures of central tendency (mean, median, standard deviation, and range) for sample and the weighted population data for the continuous variables in the study for the Northeast region. The weighted population of patients in the Northeast ranged in age from 1 to 84 years (*M* = 37.89 years, *SD* = 10.32 years). The patient length of stay (LOS) ranged in value from 0 to 311 days (*M* = 4.53 days, *SD* = 7.01 days). The patients had approximately 2 procedures on average. The total charges (Cost) ranged from $142 to over 1 million dollars (*M* = $31,091.34, *SD* = $50.285.21).

Table 5: Measures of Central Tendency for Continuous Variables of Study, for the Sample and the Population, for the South Region

Variable	***M***	***SD***	***Mdn***	**Range**
LOS				
Sample	4.04	5.16	3.00	0 - 333
Population	4.03	5.17	3.00	0 - 333
Cost				
Sample	22,437.26	35,558.83	13,431.50	106 - 1,454,410
Population	22,584.35	35,814.81	13,498.00	106 - 1,454,410
Age				
Sample	36.77	10.55	36.00	21 - 55
Population	36.78	10.55	36.00	21 - 55

Note. Sample *N* = 428,936; Population *N* = 2,121,483; *M* = Mean; *SD* = Standard Deviation; *Mdn* = Median.

Table 5 presents the measures of central tendency (mean, median, standard deviation, and range) for sample and the weighted population data for the continuous variables in the study for the South region. The weighted population of patients in the South ranged in age from 1 to 84 years (*M* = 36.78 years, *SD* = 10.55 years). The patient length of stay (LOS) ranged in value from 0 to 333 days (*M* = 4.03 days, *SD* = 5.17 days). The patients had approximately 2 procedures on average. The total charges (Cost) ranged from $106 to over 1 million dollars (*M* = $22,584.35, *SD* = $35,814.81).

***Table 6**: Measures of Central Tendency for Continuous Variables of Study, for the Sample and the Population, for the Midwest Region*

Variable	***M***	***SD***	***Mdn***	**Range**
LOS				
Sample	4.04	4.76	3.00	0 - 275
Population	4.03	4.73	3.00	0 - 275
Cost				
Sample	23,332.63	37,220.83	14,414.50	116 - 1,438,090
Population	23,212.67	36,959.22	14,362.00	116 - 1,438,090

Age				
Sample	37.75	10.65	38.00	21 - 55
Population	37.77	10.65	38.00	21 - 55

Note. Sample *N* = 113,734; Population *N* = 577,301; *M* = Mean; *SD* = Standard Deviation; *Mdn* = Median.

Table 6 presents the measures of central tendency (mean, median, standard deviation, and range) for sample and the weighted population data for the continuous variables in the study for the Midwest region. The weighted population of patients in the Midwest ranged in age from 1 to 84 years (*M* = 37.77 years, *SD* = 10.65 years). The patient length of stay (LOS) ranged in value from 0 to 275 days (*M* = 4.03 days, *SD* = 4.73 days). The patients had approximately 2 procedures on average. The total charges (Cost) ranged from $116 to over 1 million dollars (*M* = $23,212.67, *SD* = $36,959.22).

***Table 7**: Measures of Central Tendency for Continuous Variables of Study, for the Sample and the Population, for the West Region*

Variable	***M***	***SD***	***Mdn***	**Range**
LOS				
Sample	4.21	6.46	3.00	0 – 260
Population	4.23	6.50	3.00	0 – 260
Cost				
Sample	37,831.69	57,818.33	22,941.50	135 -

				1,457,016
Population	37,891.38	58,086.51	22,941.00	135 - 1,457,016
Age				
Sample	37.54	10.67	37.00	21 – 55
Population	37.56	10.67	37.00	21 – 55

Note. Sample N = 61,178; Population N = 294,925; M = Mean; SD = Standard Deviation; *Mdn* = Median.

Table 7 presents the measures of central tendency (mean, median, standard deviation, and range) for sample and the weighted population data for the continuous variables in the study for the West region. The weighted population of patients in the West ranged in age from 1 to 84 years (M = 37.56 years, SD = 10.67 years). The patient length of stay (LOS) ranged in value from 0 to 260 days (M = 4.23 days, SD = 6.50 days). The patients had approximately 2 procedures on average. The total charges (Cost) ranged from $135 to over 1 million dollars (M = $37,891.38, SD = $58,086.51).

***Table 8**: Frequencies and Percentages of Sample and Population Findings for Life Factor Variables*

	Sample		**Population**	
Variable	**Frequency**	**Percent**	**Variable**	**Frequency**
SES				
0 to 25,000	366,753	48.3	367,399	48.4
25,001 to 30,000	169,318	22.3	168,875	22.3
30,001 to 35,000	132,580	17.5	132,244	17.4
35,001 and above	90,223	11.9	90,355	11.9
Medicare				
Yes	96,650	12.7	96,943	12.8
No	662,224	87.3	661,931	87.2
Medicaid				
Yes	309,301	40.8	309,177	40.7
No	449,573	59.2	449,697	59.3
PrivInsurance				
Yes	257,240	33.9	257,402	33.9
No	501,634	66.1	501,472	66.1
SelfPay				
Yes	63,091	8.3	63,141	8.3
No	695,783	91.7	695,733	91.7
NoCharge				
Yes	6,833	0.9	6,813	0.9

No	752,041	99.1	752,061	99.1
Other				
Yes	25,759	3.4	25,398	3.4
No	733,115	96.6	733,476	96.7

Table 8 presents the frequencies and percentages of the life factor variables for the sample data and the weighted population. Almost half of the patients in the weighted population had an income of $25,000 or less (48%). Approximately 40% of the patients in the weighted population had Medicaid.

Table 9: *Frequencies and Percentages of Sample and Population Findings for Life Factor Variables, According to Region*

	Sample		**Population**	
Variable/Strata	**Frequency**	**Percent**	**Variable**	**Frequency**
SES – Northeast				
0 to 25,000	79,574	51.3	79,885	51.5
25,001 to 30,000	26,770	17.3	26,434	17.1
30,001 to 35,000	25,078	16.2	25,094	16.2
35,001 and above	23,604	15.2	23,613	15.2
SES – South				
0 to 25,000	205,864	48.0	205,411	47.9
25,001 to 30,000	99,973	23.3	100,073	23.3
30,001 to 35,000	73,679	17.2	73,805	17.2
35,001 and above	49,420	11.5	49,647	11.6
SES – Midwest				
0 to 25,000	63,364	55.7	63,396	55.7
25,001 to 30,000	26,408	23.2	26,490	23.3
30,001 to 35,000	17,517	15.4	17,446	15.3
35,001 and above	6,445	5.7	6,403	5.6
SES – West				
0 to 25,000	17,951	29.3	17,978	29.4
25,001 to 30,000	16,167	26.4	16,275	26.6

30,001 to 35,000	16,306	26.7	16,188	26.5
35,001 and above	10,754	17.6	10,737	17.6
Medicare				
Northeast	17,737	11.4	17,739	11.4
South	53,653	12.5	53,919	12.6
Midwest	17,660	15.5	17,674	15.5
West	7,600	12.4	7,615	12.5
Medicaid				
Northeast	67,494	43.5	67,785	43.7
South	168,368	39.3	167,865	39.1
Midwest	45,367	39.9	45,359	39.9
West	28,072	45.9	28,067	45.9
PrivInsurance				
Northeast	54,697	35.3	54,416	35.1
South	146,713	34.2	147,103	34.3
Midwest	38,088	33.5	28,028	33.4
West	17,742	29.0	17,711	29.0

	Sample		Population	
Variable/Strata	**Frequency**	**Percent**	**Variable**	**Frequency**
SelfPay				
Northeast	11,537	7.4	11,493	7.4
South	39,272	9.2	39,307	9.2
Midwest	8,811	7.8	8,854	7.8
West	3,471	5.7	3,527	5.8
NoCharge				
Northeast	268	0.2	274	0.2
South	5,545	1.3	5,545	1.3
Midwest	825	0.7	837	0.7
West	195	0.3	204	0.3
Other				
Northeast	3,293	2.1	3,319	2.1
South	15,385	3.6	15,197	3.5
Midwest	2,983	2.6	2,983	2.6
West	4,098	6.7	4,055	6.6

Table 9 presents the frequencies and percentages of the life factor variables for the sample data and the weighted population. Almost half of the patients in the weighted population had an income of $25,000 or less for the Northeast, South, and Midwest regions. The patients in the West region were more evenly

distributed across the four SES income levels. The majority of patients in all four regions, from 73% to 79% of patients, had Medicaid or private insurance.

4.6 Assumptions

The researcher investigated the data set to ensure that it satisfied the assumptions of the multiple regression and correlational analyses of this study: absence of missing data; absence of outliers; normality; homoscedasticity; and linearity as they relate to the two dependent variables of LOS and Cost.

Using only complete record sets, the absence of missing data assumption was met. Outliers in a dataset have the potential to distort results of an inferential analysis. A check of standardized scores and box plots for the LOS and Cost variables was performed to inspect the data for outliers. LOS and Cost values with a standardized score of a magnitude of 3 or greater were classified as outliers. 11,446 records (1.5% of all records) had outliers on the LOS variable. 11,032 records (1.5% of all records) had outliers on the Cost variable. Multiple regression analyses are robust to the presence of outliers if the homoscedasticity assumption is met. The researcher investigated homoscedasticity of the residuals by computing regression models using the raw data and by visually inspecting scatterplots and histograms of the residual distributions. The assumption of homoscedasticity was met for both the LOS and Cost models.

Additionally, all outlying values remained within the acceptable ranges of their associated variables. By meeting the homoscedasticity assumption and by maintaining acceptable ranges of the LOS and Cost variables for all outliers, all records were retained for analysis, and the outlier assumption was considered not violated.

Normality for the LOS and Cost variables was investigated with SPSS Explore. The Kolmogorov-Smirnov test (K-S) for normality indicated that both the LOS and Cost variables were not normally distributed ($p < .01$). However, statistical tests performed on a very large sample, like the one in this study, will return significance even on very small effects. A visual check of histograms and Normal Q-Q plots indicated that both LOS and cost variables skewed towards the right. A comparison of the median and mean values for the LOS variable indicated that both of the measures of central tendency were similar in value, suggesting that the skew, outliers, and non-normality were not adversely affecting the distribution of the LOS variable ($Mdn = 3.0$, $M = 4.15$).

However, the median and mean for the Cost variable were not close in value ($Mdn = 15{,}010.00$, $M = 25{,}502.19$), indicating that the right skew was pulling the mean higher than the true center of the distribution for the Cost variable. Logarithmic and square root transformations performed on the Cost variable did not improve the distribution. Regression models computed for the raw and transformed Cost variables along with the model findings proved to be similar across all models. Therefore, since the assumption of homoscedasticity was met

for both the LOS and Cost variables and the transformation of the Cost variable did not improve the data distribution, the assumption of normality was considered acceptable for the study. The researcher conducted all inferential tests using raw and untransformed data. The researcher used STATA and the "svy" command to ensure that no violations occurred, thereby assuming the absence of multicollinearity.

Due to the large sample size of this study, the significance was found on a very small effect size. Therefore, in addition to reporting the p-values of the inferential tests, the investigator also reported the effect size of each test.

4.7 Multiple regression analysis for Research Questions

Research Question (RQ1). Is there a relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho1). There is not a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha1). There is a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Research Question (RQ2). Is there a relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho2). There is not a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha2). There is a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

ANOVA testing for RQ1:

Correlation Matrix

	X1	Y
X1	1	0.783
Y	0.783	1

Regression Coefficients:

The multiple regression equation is of the general form

$Y = a + b_1X_1 + b_2X_2 + \cdots + b_kX_k$

where **a** is a starting-point constant analogous to the intercept in a simple two-variable regression, and $\mathbf{b_1}$, $\mathbf{b_2}$, etc., are the unstandardized regression weights for X_1, X_2, etc., each analogous to the slope in a simple two-variable regression. In the present analysis, **a** = 1480.0257 and the values of **b** are as indicated below. The values listed as **B** are the standardized regression weights.

	b	B	B x r_{xy}
X1	52.6626	0.7833	0.6135
Multiple $\mathbf{R^2}$ = 0.6135			
Adjusted Multiple $\mathbf{R^2}$ = 0.5362			
Standard Error of Multiple Estimate	455.0614		

ANOVA Table

Source	SS	df	MS	F	P
Regression	1972176.2287	1	1972176.2287	7.94	0.0372
Residual	1242485.4856	5	248497.0971		
Total	3214661.7143	6			

Names of Variables:

X1. Weeks Elapsed between surgery and post MRI

Y. ACL RSI

ANOVA testing for RQ2:

Correlation Matrix

	X1	Y
X1	1	0.961
Y	0.961	1

Regression Coefficients:

The multiple regression equation is of the general form

$Y = a + b_1X_1 + b_2X_2 + \cdots + b_kX_k$

where **a** is a starting-point constant analogous to the intercept in a simple two-variable regression, and $\mathbf{b_1}$, $\mathbf{b_2}$, etc., are the unstandardized regression weights for X_1, X_2, etc., each analogous to the slope in a simple two-variable regression. In the present analysis, **a** = 615.4854 and the values of **b** are as indicated below. The values listed as **B** are the standardized regression weights.

	b	B	B x r_{xy}
X1	0.5489	0.9611	0.9236
Multiple $\mathbf{R^2}$ = 0.9236			
Adjusted Multiple $\mathbf{R^2}$ = 0.9141			

Standard Error of Multiple Estimate	162.7731

ANOVA Table

Source	SS	df	MS	F	P
Regression	2883472.3155	1	2883472.3155	96.74	<.0001
Residual	238455.7845	8	29806.9731		
Total	3121928.1	9			

Names of Variables:

X1. ACL RSI

Y. PCL RSI

ANOVA testing (age/RSI):

Correlation Matrix

	X1	Y
X1	1	0.02
Y	0.02	1

Regression Coefficients:

The multiple regression equation is of the general form

$Y = a + b_1X_1 + b_2X_2 + \cdots + b_kX_k$

where **a** is a starting-point constant analogous to the intercept in a simple two-variable regression, and $\mathbf{b_1}$, $\mathbf{b_2}$, etc., are the unstandardized regression weights for X_1, X_2, etc., each analogous to the slope in a simple two-variable regression. In the present analysis, **a** = 1846.5798 and the values of **b** are as indicated below. The values listed as **B** are the standardized regression weights.

	b	B	B x r_{xy}
X1	1.2505	0.0198	0.0004
Multiple $\mathbf{R^2}$ = 0.0004			
Adjusted Multiple $\mathbf{R^2}$ = 0			
Standard Error of Multiple Estimate	1030.908		

ANOVA Table

Source	SS	df	MS	F	P
Regression	3746.3757	1	3746.3757	0	1
Residual	9564942.5243	8	1195617.8155		
Total	9568688.9	9			

Names of Variables:

X1. AGE

Y. ACL RSI

4.8 Correlational analysis findings for Research Questions

Research Question (RQ1). Is there a relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho1). There is not a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha1). There is a statistically significant relationship between the use of biologics and decreased time to recovery/healing of injured connective tissue as compared to traditional treatment methods.

Research Question (RQ2). Is there a relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods?

Null Hypothesis (Ho2). There is not a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

Alternate Hypothesis (Ha2). There is a statistically significant relationship between the use of biologics and increased homogeneity of injured connective tissue as compared to traditional treatment methods.

Regression testing between Weeks Elapsed and ACL RSI

SISA

Correlation/Regression procedure

For help go to SISA

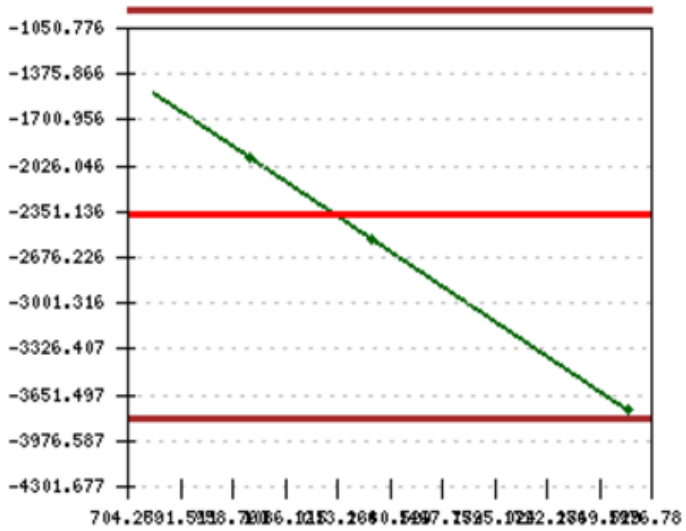
Overview

z for 95% CI= 1.96
Invalid: 5
Cases-N: 9

r (Days.Elapsed)= -0.6301
p= 0.9655 more
N.S. Calculate Sample Size

Anova table				
Source	Σ of Sq.	%	Mean Σ-sq	df
Explained	249.08	39.7	249.08	1
Unexplained	378.29	60.3	54.042	7
Total	627.37	100%		8
F-value: 4.609; p-value: 0.06893				
Residual standard error: 19.45				

X-axis (Weeks Elapsed); Y-axis (ACL RSI)



Bland-Altman table				
	value	sd	95% C.I.	
Mean	1662.8	837.22	1115.8	2209.7
Mean - 2SD	-11.678	1450.1	-959.066	935.71
Mean + 2SD	3337.2	1450.1	2389.8	4284.6
BIAS: 1662.8; t=5.9582, df=8; p=0.0002				
E(Days-Elapsed)=-59.871(se=11.85)+2.027(0.013)*(Days+Elapsed)/2				

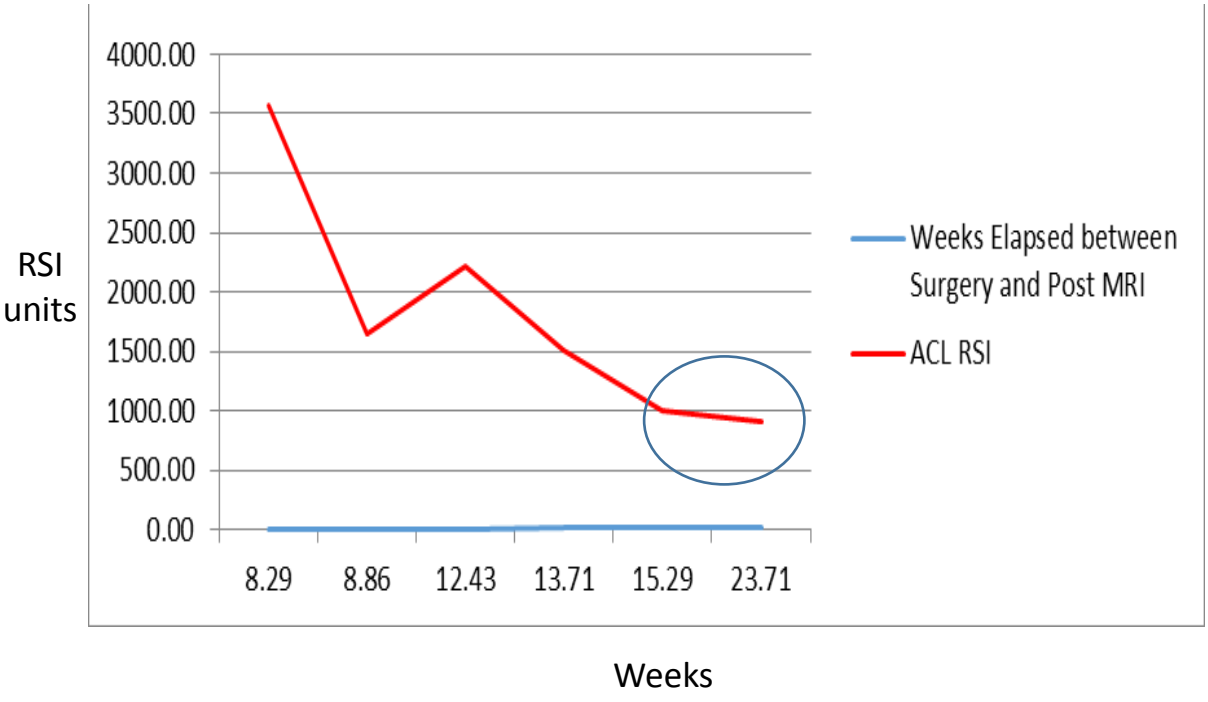
Descriptives for Days

Mean: 1681.33
Sum: 15132
Variance: 691579
sd: 831.612
se: 277.204
95% CI: 1138 >1681> 2225

Descriptives for Elapsed

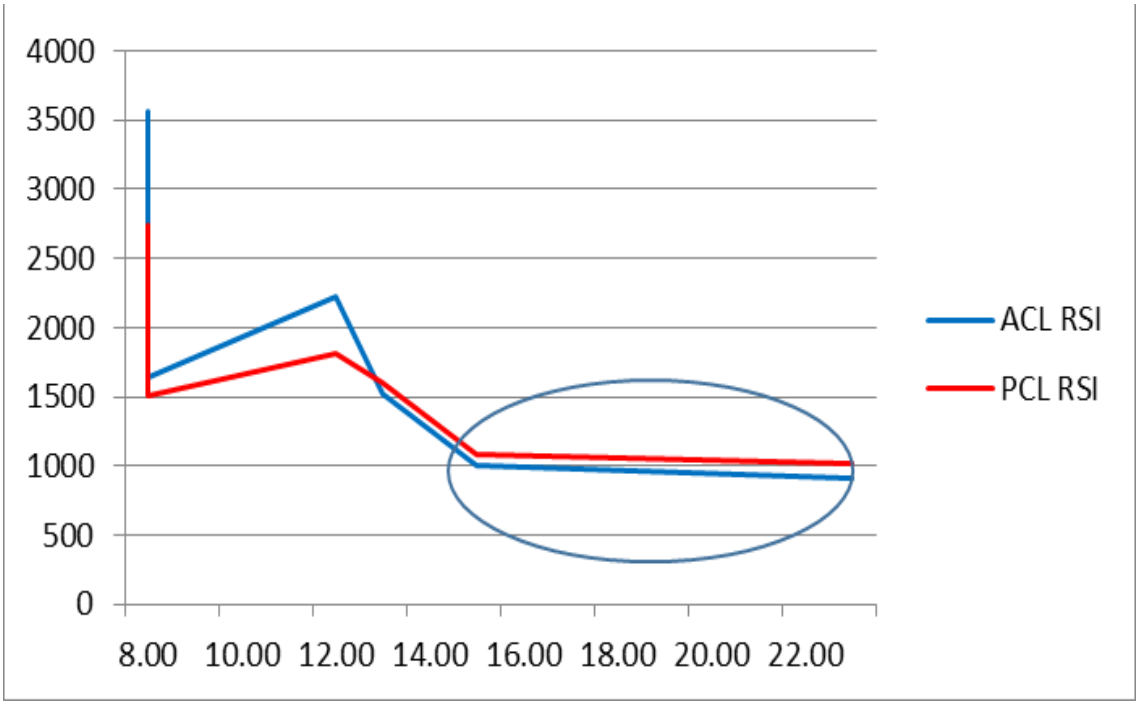
Mean: 18.5711
Sum: 167.14
Variance: 78.4211
sd: 8.85557
se: 2.95186
95% CI: 12.79 >18.6> 24.36

Figure 14: Reconstructed ACL RSI vs. Weeks Elapsed between bio-enhanced ACL Reconstruction Surgery and Post MRI



- **Figure 14 -** Between 16 and 24 weeks, notice not only a decrease in the RSI, but also a level of saturation (plateau) indicating **incorporation of the graft (compared to graft incorporation at 1-2 years for traditional ACL reconstruction surgery).**

Figure 15: Reconstructed ACL RSI vs. Intact PCL RSI post bio-enhanced ACL Reconstruction Surgery



- **Figure 15** - The RSI for ACL and PCL closely converge between 16 and 24 weeks indicating **homogeneity** between the healing ACL graft and intact PCL.

CHAPTER 5 DISCUSSION, CONCLUSION, NEXT STEPS

Interest in the use of biologics in regenerative medicine has been increasing exponentially over the past recent years. Although bone autograft and allograft techniques have been established for decades in orthopaedic care, bio-enhanced therapeutics, have, however, only reached the level of being clinical procedures in the last few years. Furthermore, local application of stem cells is currently only being performed at highly-specialized centers like the Riordan-McKenna Institute. The past forty years have shown no ground-breaking developments or advanced techniques in orthopaedic care. The engineering of biologics in orthopaedic regenerative medicine, on the contrary, may very well revolutionalize the landscape of medicine as we know it and propel us into a world of endless possibilities in personalized and translational medicine. In fact, the researcher has keen interest to pursue post-doctoral research in stem cell, bio-enhanced applications for the following debilitating conditions: autism; cerebral palsy; multiple sclerosis (MS), spinal cord injury; osteoarthritis (OA); rheumatoid arthritis (RA); heart failure; and autoimmune diseases.

The researcher has found promising potential of bio-enhanced therapeutics, particularly in the area of healing and regeneration of soft tissue, tendons, and ligaments. Specifically, the use of biologics (BMAC plus STEMNEXA) in both the injection for AT tendinopathy and surgical ACL graft reconstruction cases has shown to decrease time to recovery, decrease echogenicity, and increase homogeneity of injured connective tissues toward complete healing when

compared to traditional nonsurgical and surgical interventions. At a minimum, these phenomenal clinical results should substantiate the critical need for continued study and spur further research on bio-enhanced treatment (to be applied conservatively or surgically) to an array of acute and chronic maladies.

The explicit results following the employment of biologics and amniotic growth factors in the ACL reconstruction study has led the researcher to reasonably surmise:

1) early loss of a provisional scaffold inhibits ACL healing, 2) that placement of a substitute provisional scaffold can restore functional healing, and 3) that growth factor delivery systems can be specifically designed for use in the joint. In addition, the research has demonstrated that the healed ligament following bio-enhanced ACL repair matches or exceeds the strength of the graft after traditional ACL reconstruction (measured by incorporation time, decreased recovery time, and increased homogeneity), and that it also minimizes post-traumatic osteoarthritis following ACL injury and reconstruction.

The researcher analyzed data using correlation and regression analyses to assess the correlation between several variables. The researcher conducted the study, seeking to examine 1) whether a correlation exists between augmenting biologics with standard treatment and decreased recovery time 2) whether a correlation exists between the use of biologics and restoring tissue and ligament to their homogenous state 3) whether a correlation exists between life factors such as age, gender, and recovery time.

In the present study, recovery time decreased for patients who were treated with biologics (p <.05). The resulting RSI was compatible with the RSI of the uninjured, intact tendon/ligament (p<.05), thereby proving that homogeneity increased with the use of biologics. No correlation was observed between independent factors such as age and gender vs recovery time (p=1).

In light of the data presented herein, this candidate believes, unequivocally, that sufficient, and, in fact compelling, evidence exists to support further research into the administration of mesenchymal stem cell-based biologics and amniotic growth factors in the management and treatment of connective tissue injuries in regenerative medicine.

5.1. Limitations

Study limitations included but were not limited to: funding challenges for repeat MRI studies for Achilles Tendonitis and ACL reconstruction patients (ten patients on waiting list); exclusion of five post-ACL MRIs due to re-tears and focal signal abnormalities (FSA) that altered the RSI readings; and magic angle phenomenon in MRI reading.

REFERENCES

A Bernhardt et al.2008. “Mineralised collagen: an artificial, extracellular bone matrix–improves osteogenic differentiation of bone marrow stromal cells,” *J Mater Sci Mater Med*. 19(2008):269–1275.

A Ferretti et al. 1992. “Knee ligament injuries in volleyball players.” Am J Sports Med 20: 203-207.

A Giordano et al. 2007. “From the laboratory bench to the patient’s bedside: an update on clinical trials with mesenchymal stem cells,” *J Cell Physiol*. 211(1):27–35.

A. Mahnik et al. 2013. “Current practice variations in the management of anterior cruciate ligament injuries in Croatia,” *World J Orthop*. 4(2013):309–315.

A Selvanetti et al. 1997. “Overuse tendon injuries: basic science and classification,” *Operative Tech Sports Med*. 5(1997): 110-117.

AD Mazzocca et al. 2012. “Platelet-rich plasma differs according to preparation method and human variability,” *J Bone Joint Surg Am*. 94(2012):308–16.

Akanematsu et al. 2004. “Collagenous matrices as release carriers of exogenous growth factors,” *Biomaterials*.25(2004):4513–4520.

Alfredson H. “Chronic midportion Achilles tendinopathy: an update on research and treatment,” *Clin Sports Med*. 22(2003): 727-741.

Arendt E and R Dick.1995. Knee injury patterns among men and women in collegiate basketball and soccer. NCAA data and review of literature. Am J Sports Med 23 (1995): 694-701.

B Demirağ et al. 2011. “Incidence of meniscal and osteochondral lesions in patients undergoing delayed anterior cruciate ligament reconstruction,” *Acta Orthop Traumatol Turc*. 45(2011):348–352.

B Han et al. 2009. “The effect of thrombin activation of platelet-rich plasma on demineralized bone matrix osteoinductivity,” *J Bone Joint Surg* Am. 91(2009):1459–14570.

B Prajapati et al. 2010. “Sample size estimation and statistical power analyses.” Optometry Today, July 17 2010.

B Yu et al. 2002. “Lower extremity motor control-related and other risk factors for noncontact anterior cruciate ligament injuries.” Instr Course Lect 51: 315-324.

B Zhang et al. 2014. “HucMSCexosome mediated-Wnt4 signaling is required for cutaneous wound healing." Stem Cells. doi:10.1002/stem.1771

Bishop GB and TA Einhorn. “Current and future clinical applications of bone morphogenetic proteins in orthopaedic trauma surgery,” *Int Orthop*. 31(2007):721–727.

BP Boden et al. 2000. “Mechanisms of anterior cruciate ligament injury.” Orthopedics 23 (2000): 573-578.

BP Boden et al. 2010. “Noncontact anterior cruciate ligament injuries: mechanisms and risk factors.” J Am Acad Orthop Surg 18 (2010): 520-527.

Bruce Sangeorzan, “Excellence in orthopaedic foot and ankle education” (paper presented at the AOFAS annual meeting 2015, Long Beach Convention Center, Long Beach, California July 15-18, 2015).

C Vater et al. 2011. “Culture media for the differentiation of mesenchymal stromal cells,” *Acta Biomater*. 7(2011):463–477.

Caplan AI 2000. “Mesenchymal stem cells and gene therapy,” C*lin Orthop Relat Res*.379 (2000) :67–70.

Carlson MA .1997. Acute wound failure. Surg Clin N Am 77(3):607–636

CL Ekegren et al. 2009. “Reliability and validity of observational risk screening in evaluating dynamic knee valgus.” J Orthop Sports Phys Ther 39:665-674.

Carstangen B et al. 2006. “Successful engraftment of cultured autologous mesenchymal stem cells in a surgically repaired soft palate defect in an adult horse”. *Canadian Journal of Veterinary* Research 70(2):143-7.

CE Gross et al. 2013. “Injectable treatments for noninsertional achilles tendinosis: a systematic review,” *Foot Ankle Int*. 34(2013): 619-628.

DA Shaerf et al. 2014. “Anterior cruciate ligament reconstruction best practice: A review of graft choice,” *World J Orthop*. 5(2014):23–29.

DP Go et al. 2011. “Multilayered microspheres for the controlled release of growth factors in tissue engineering,” *Biomacromolecules.* 12(2011):1494–1503.

DT Kirkendall and WE Garrett, Jr. 2000. “The anterior cruciate ligament enigma. Injury mechanisms and prevention.” Clin Orthop Relat Res : 64-68.

E Romanini et al. 2010. “Graft selection in arthroscopic anterior cruciate ligament reconstruction,” A.*J Orthop Traumatol*. 11(2010):211–219.

EM Horwitz et al. 1999. “Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta,” *Nat Med*. 5(1999):309–13.

EM Horwitz et al. 2002. “Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone,” *Proc Natl Acad Sci* 99(2002):8932–8937.

F Granero-Moltó et al.2011. “Mesenchymal stem cells expressing insulin-like growth factor-I (MSCIGF) promote fracture healing and restore new bone formation in Irs1 knockout mice: analyses of MSCIGF autocrine and paracrine regenerative effects,” *Stem Cells*.29(2011):1537–1548.

G Filardo et al. 2011. “Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis,” *Knee Surg Sports Traumatol Arthrosc*.19 (2011):528–535.

G Hughes et al. 2006. “A risk-factor model for anterior cruciate ligament injury.” Sports Med. 2006; 36:411–428.

GB Maletis et al. 2013. “Reconstruction of the anterior cruciate ligament: association of graft choice with increased risk of early revision,” *Bone Joint J*. 95-B (2013):623–628.

Goldspink G and S.Y Yang. 2004. The splicing of the IGF-1 gene to yield different growth factors. Advances in Genetics 52: 23-47.

Gordon MD and Steiner ME. 2004. Anterior cruciate ligament injuries. In: Orthopaedic Knowledge Update Sports Medicine III, Garrick JG. American Academy of Orthopaedic Surgeons, Rosemont.

Gwinn DE et al. 2000. “The relative incidence of anterior cruciate ligament injury in men and women at the United States Naval Academy.” Am J Sports Med (2000) 28:98–102.

H Al-Halal et al. 2013. “Incidence and obstetrical outcomes of cervical intraepithelial neoplasia and cervical cancer in pregnancy.” Archives of Gynecology and Obstetrics 287 (2013): p. 245-250.

H Alfredson et al. 1998. “Heavy-load eccentric calf muscle training for the treatment of chronic Achilles tendinosis,” *Am J Sports Med*. 26(1998): 360-366.

H Thermann et al. 2009. “Endoscopic treatment of chronic mid-portion Achilles tendinopathy: novel technique with short-term results,” *Knee Surg Sports Traumatol Arthrosc.* 17(2009): 1264-1269.

Hart L. “Corticosteroid and other injections in the management of tendinopathies: a review,” *Clin J Sport Med*. 21(2011): 540-541.

HHK Xu et al.2004. “Synergistic reinforcement of in situ hardening calcium phosphate composite scaffold for bone tissue engineering,” *Biomaterials*. 25(2004):1029–1037.

HO Mayr et al. 2004. “Arthrofibrosis following ACL reconstruction--reasons and outcomes.” Orthop Trauma Surg. 2004; 124:518–522.

Hootman JM et al. 2007. “Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives”. 42 (2007): p. 311–319.

Hutchings Pierce, 2014. Injury Prevention and Athletic Performance, Chicago, Illinois (Greater Chicago Area), USA.

ID Xynos et al.2001. “Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution,” *J Biomed Mater Res*. 55(2001):151–157.

International Olympic Committee Consensus statement, Available at https://stillmed.olympic.org/media/Document%20Library/OlympicOrg/IOC/Who-We-Are/Commissions/Medical-and-Scientific-Commission/EN-IOC-Consensus-Statement-Molecular-Basis-of-Connective-Tissue-and-Muscle-Injuries-in-Sport.pdf

J Kennedy et al. 2010. "Timing of reconstruction of the anterior cruciate ligament in athletes and the incidence of secondary pathology within the knee". Bone Joint Surg. 2010; 92:362–366.

J Shaginaw et al. 2014. "ACL reconstruction - it's all about timing". Int J Sports Phys Ther 9:pp. 268–273.

JC Albright et al. 1999. Knee and leg: soft tissue trauma. In: Orthopaedic Knowledge Update. American Academy of Orthopaedic Surgeons, Rosemont.

JD Rompe et al. 2008. "Mid-portion Achilles tendinopathy–current options for treatment," *Disabil Rehabil*. 30(2008): 1666-1676.

JRV Ninet al. 2009. "Has platelet-rich plasma any role in anterior cruciate ligament allograft healing?" *Arthroscopy*. 25(2009):1206–1213.

K Gaweda et al. 2010. “Treatment of Achilles tendinopathy with platelet-rich plasma,” *Int J Sports Med*.31(2010):577–583.

KD Shelbourne et al. 1991. “Arthrofibrosis in acute anterior cruciate ligament reconstruction. The effect of timing of reconstruction and rehabilitation.” Am J Sports Med. 1991; 19:332–336.

KG Silbernagel et al. 2007. “Continued sports activity, using a pain-monitoring model, during rehabilitation in patients with Achilles tendinopathy: a randomized controlled study,” *Am J Sports Med*. 35(2007): 897-906.

Kiapour AM and Murray MM. 2014. “Basic science of anterior cruciate ligament injury and repair.” Bone Joint Res (2014) 3(2):20-31.

Kim HJ et al. 2004. “Vascular endothelial growth factor-induced angiogenic gene therapy in patients with peripheral artery disease.” Experimental and Molecular Medicine (2004) 36(4): 336-44

Kjaer M. 2004. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiology Review. 84: 649-98.

KM Khan et al. 1998. “Tendinopathy: an Achilles’ heel for athletes and clinicians. *Clin J Sport Med*. 8(1998): 151- 154.

Kvist, M. 1991. “Achilles tendon injuries in athletes.” Ann Chir Gynaecol 80(2): 188-201.

LC Almekinders et al. 1995. “Post-operative problems following anterior cruciate ligament reconstruction.” Knee Surg Sports Traumatol Arthrosc. 3(1995):78–82.

Lian O and Scott A Engebretsen L, et al. 2007. Excessive apoptosis in patella tendinopathy in athletes. American Journal of Sports Medicine. Tendon and skeletal muscle repair. Bone 39 (2007): 78-83.

LP Granan et al. 2009. “Timing of anterior cruciate ligament reconstructive surgery and risk of cartilage lesions and meniscal tears: a cohort study based on the Norwegian National Knee Ligament Registry”. *Am J Sports Med*. 37 (2009):955–961.

LY Griffin et al. 2000. “Noncontact anterior cruciate ligament injuries: risk factors and prevention strategies.” J Am Acad Orthop Surg 8: 141-150.

M Brittberg et al. 1994. “Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation,” *New England Journal of Medicine* 331 (1994).

M Goddard et al. 2013. “Incidence of graft rupture 15 years after bilateral anterior cruciate ligament reconstructions,” Bone *Joint J*.95-B (2013):798–802.

M Hakimi et al. “Current UK Practice; ACL Reconstruction-Current UK Practice,” *J Bone Joint Surg Br. 94B* (2012):42.

M Ito et al. 1999. “Effect of hydroxyapatite content on physical properties and connective tissue reactions to a chitosan-hydroxyapatite composite membrane,” *J Biomed Mater Res*. 45(1999):204–208.

M Jäger et al. 2009. “Bone marrow concentrate: a novel strategy for bone defect treatment,” *Curr Stem Cell Res Ther*. 4(2009):34–43.

M Korda et al. 2008. “Use of mesenchymal stem cells to enhance bone formation around revision hip replacements,” *J Orthop Res*. 26(2008):880–885.

M Krampera et al. 2006. “Mesenchymal stem cells for bone, cartilage, tendon and skeletal muscle repair” Bone 39 (2006):678-683.

M Madrigal M et al. 2014. “A review of therapeutic effects of mesenchymal stem cell secretions and induction of secretory modification by different culture methods,” *J Transl Med*. 12(2014):260.

M Paavola et al. 2002. “Surgical treatment for chronic Achilles tendinopathy: a prospective seven month follow up study.” Br J Sports Med (2002) 36(3): 178-182.

M Paavola et al. 2002. “Achilles tendinopathy,” *J Bone Joint Surg Am*. 84-A(2002): 2062-2076.

M Razi et al. 2013. “Timing of anterior cruciate ligament reconstruction and incidence of meniscal and chondral Injury within the knee”. Int J Prev Med. 2013; 4:0.

M Ryan et al. 2010. “Favorable outcomes after sonographically guided intratendinous injection of hyperosmolar dextrose for chronic insertional and midportion achilles tendinosis,” *AJR Am J*. 194(2010): 1047-1053.

M Sanchez et al.2009.“Nonunions treated with autologous preparation rich in growth factors,” *J Orthop Trauma*.23(2009):52–59.

MB Labus et al. 1998. “Expression of Wnt genes in early wound healing.” Wound Repair Regen (1998) 6(1):58–64

McKenna R. W. and N. H. Riordan. 2014. “Minimally invasive autologous bone marrow concentrate stem cells in the treatment of the chronically injured Achilles tendon: a case report,” CellR4 2(2014): 1100

Mendelsohn AR, and JW Larrick. 2014. “Systemic factors mediate reversible age-associated brain dysfunction.” *Rejuvenation Res*. 17(2014):525-528

Mishra A and T Pavelko. “Treatment of chronic elbow tendinosis with buffered platelet-rich plasma,” *Am J Sports Med*. 34(2006):1774–1778.

MJ Yelland et al. 2011. “Prolotherapy injections and eccentric loading exercises for painful Achilles tendinosis: a randomised trial,” *Br J Sports Med*. 45(2011): 421-428.

MM Syeda et al. 2012. “Prostaglandin transporter modulates wound healing in diabetes by regulating prostaglandin-induced angiogenesis.” Am J Pathol 181(1):334–346.

MV Paterno et al. 2012. “Hamstrings Autograft; A between sex comparison of anterior-posterior knee laxity after anterior cruciate ligament reconstruction with patellar tendon or hamstrings autograft: a systematic review,” *Sports Med*. 42 (2012)135–152.

N Maffulli et al. 2004. “Achilles tendinopathy: aetiology and management.” J R Soc Med (2004) 97(10): 472-476.

N Maffulli et al. 2003. “Types and epidemiology of tendinopathy.” Clin Sports Med (2003) 22(4): 675-692.

N Maffulli et al. 2005. “Tendon injuries: Basic and clinical aspect.” Springer Verlag, London. pp. 1-252

N Saito et al. 2005. “Synthetic biodegradable polymers as drug delivery systems for bone morphogenetic proteins,” *Adv Drug Deliv Rev*. 57(2005):1037–1048

Niranjan Bhattacharya and Phillip Stubblefield, *Human Fetal Tissue Transplantation*. London: Springer-Verlag, 2013.

NJ Maxwell et al. 2007. “Sonographically guided intratendinous injection of hyperosmolar dextrose to treat chronic tendinosis of the Achilles tendon: a pilot study,” *AJR Am J*. 189(2007): 215-220.

Nordsletten L, Madsen J.E. 2006. The effect of bone morphogenetic proteins in fracture healing. Scandinavian Journal of Surgery 95: 1-4.

Online Pearson's calculator

http://www.socscistatistics.com/tests/pearson/Default2.aspx

P Kalia et al. 2006. "Do autologous mesenchymal stem cells augment bone growth and contact to massive bone tumor implants?" *Tissue Eng*. 12(2006):1617–1626.

Partridge KA and Oreffo ROC 2004. "Gene delivery in bone tissue engineering: progress and prospects using viral and nonviral strategies," *Tissue Eng*. *10 (*2004):295–307.

PN Chalmers et al. 2014. "Does ACL reconstruction alter natural history? A systematic literature review of long-term outcomes," *Bone Joint Surg Am*. 96(2014):292–300.

Pochampally RR et al. 2005. "Correction of a mineralization defect by overexpression of a wild-type cDNA for COL1A1 in marrow stromal cells (MSCs) from a patient with osteogenesis imperfecta: a strategy for rescuing mutations that produce dominant-negative protein defects," *Gene Ther*. 12(2005):1119–1125.

R Dimitriou et al.2011. "Bone regeneration: current concepts and future directions," *BMC Med*. 9(2011):66.

RA Magnussen et al. 2009. “Nonoperative treatment of midportion Achilles tendinopathy: a systematic review,” *Clin J Sport Med*. 19(2009): 54-64.

Raju Vaishya et al. 2015. “Current Trends in Anterior Cruciate Ligament Reconstruction: A Review,” *Cureus* 2015; 7(2015): 378.

RH Wittenberg et al. 1998. “A comparison of conservative and delayed surgical treatment of anterior cruciate ligament ruptures.” A matched pair analysis:” *Int Orthop*. 22(1998):145–148.

Riley G. 2003. “The pathogenesis of tendinopathy. A molecular perspective:” *Rheumatology*. 42(2003):1-14.

Riordan Neil H et al. 2015. “Case report of non-healing surgical wound treated with dehydrated human amniotic membrane,” Journal of Translational Medicine 13(2015):242.

RJ de Vos et al. 2012. “Tendon structure’s lack of relation to clinical outcome after eccentric exercises in chronic midportion Achilles tendinopathy,” *J Sport Rehabil*. 21(2012): 34-43.

RMI website. Riordan McKenna Institute “Stemnexa”. Accessed July 2016. http://www.rmiclinic.com/non-surgical-stem-cell-injections-joint-pain/stemnexa-protocol/

S Church et al. 2005. “Reconstruction of the anterior cruciate ligament: timing of surgery and the incidence of meniscal tears and degenerative change,” *Bone Joint Surg*. 87(2005):1639–1642.

S de Jonge et al. 2011. “One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial,” *Am J Sports Med*. 39(2011):1623–1629.

S Wakitani et al.2007. “Repair of articular cartilage defects in the patello-femoral joint with autologous bone marrow mesenchymal cell transplantation: three case reports involving nine defects in five knees,” *J Tissue Eng Regen Med*. 1(2007):74–79.

SE Haynesworth et al. 1992. “Characterization of cells with osteogenic potential from human marrow,” *Bone*. 13(1992):81–98.

September et al. 2007. “Tendon and ligament injuries: The Genetic component,” *British Journal of Sports Medicine*. *BMJ* (2007) 41:4

Shimokochi Y and Shultz SJ. 2008. Mechanisms of noncontact anterior cruciate ligament injury. *J Athl Train* 43(2008): 396-408.

SJ Shultz et al. 2012. “ACL Research Retreat VI: an update on ACL injury risk and prevention.”*Athl Train*. 47 (2012):591–603.

SP Bruder et al. 1998. “Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells,” *J Orthop Res*. *1*6(1998):155–162.

Stein BE et al. 2015. “Outcomes of acute Achilles tendon rupture repair with bone marrow aspirate concentrate (BMAC) augmentation”. *Int Orthop* 39(2015):901-905.

“Stem cell therapy for autism,” Stem Cell Institute, accessed March 1, 2017, https://www.cellmedicine.com/stem-cell-therapy-for-autism/

T Gosens et al. 2011. “Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up,” *Am J Sports Med*. 39(2011):1200–1208.

T Ichim et al. 2008. “Placental mesenchymal and cord blood stem cell therapy for dilated cardiomyopathy,” *Reproductive BioMedicine Online* 16 (2008): 898-905.

TA Jarvinen et al. 2005. “Achilles tendon disorders: etiology and epidemiology.” *Foot Ankle Clin*. 10(2005): 255-266.

TE Hewett et al. 2005. “Biomechanical measures of neuromuscular control and valgus loading of the knee predict anterior cruciate ligament injury risk in female athletes: a prospective study.” *Am J Sports Med* 33 (2005): 492-501.

TN Castillo et al. 2011. “Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems,” *Am J Sports Med*. 39(2011):266–271.

TO Smith et al. 2010. “Early versus delayed surgery for anterior cruciate ligament reconstruction: a systematic review and meta-analysis”. Knee Surg Sports Traumatol Arthrosc. 2010; 18:304–311.

Triola, M., Elementary Statistics. 7th ed. 1998, Reading, MA: Addison Wesley Longman, Inc.

T.W. Carlton, “Comparing Hospital-Acquired Patient Outcomes/Complications in Magnet, Non-Magnet and Magnet In-Process Hospitals.” (PhD diss, University of Colorado health sciences, 2009.)

UG Longo et al. 2008. “Minimally invasive stripping for chronic Achilles tendinopathy,” *Disabil Rehabil*. 30(2008): 1709-1713.

Ullmark G and KJ Obrant. “Histology of impacted bone-graft incorporation,” *J Arthroplasty*.17 (2002):150–157.

V Rowe et al. 2012. “Conservative management of midportion Achilles tendinopathy: a mixed methods study, integrating systematic review and clinical reasoning.” Sports Med 42(2012): 941-967.

Vaishya R et al. 2013. “Joint hypermobility and anterior cruciate ligament injury.” *Orthop Surg (Hong Kong)* 21(2013):182–184.

VB Duthon et al. 2011. Noninsertional Achilles tendinopathy treated with gastrocnemius lengthening,” *Foot Ankle Int*. 32(2011): 375-379.

Vivek Gulati et al. 2015. “Management of achilles tendon injury: A current concepts systematic review,” *World J Orthop* 6(2015):380-386.

Williams, JG. 1986. “Achilles tendon lesions in sport.” Sports Med 3(1986): 114-135.

Woo S et al. 2007. “Tendinopathy in athletes.” IOC Medical Commission Publication. Blackwell Publishing.

APPENDIX A

Table 1

Operationalization of Variables of Study

Variable Name	Description	Type	Coding	HCUP Variable Derived From
LOS	Length of stay in hospital, measured in days. This Variable will be used as the dependent variable in one of the two multiple regression models and the correlational analysis.	Continuous	Range from 0-365	LOS
Cost	Monetary cost for hospital care, measured in US dollars. This variable will be used as the	Continuous	Range from 0-1,500,000	TOTCHG

	dependent variable in the other multiple regression model and the correlational analysis.			
Age	Measures the age in years of the patient. This variable will be used in the correlational analysis. If a strong correlation with LOS and/or Cost is indicated, then it will be included as an independent (control) variable in the multiple regression which includes the variable with the strong correlation as the dependent variable.	Continuous	Range from 21-55	AGE