2015

Use of Swine Small Intestine Submucosa as a Dermal Suture

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Master of Biomedical Sciences

USE OF SWINE SMALL INTESTINE SUBMUCOSA AS A DERMAL SUTURE

A Thesis In Biomedical Sciences by Paige E. Black

Submitted in Partial Fulfillment of the Requirements of the Degree of

Master of Biomedical Sciences

August 2015

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ABSTRACT

USE OF SWINE SMALL INTESTINE SUBMUCOSA
AS A DERMAL SUTURE MATERIAL
Master of Biomedical Sciences, July 2016
Philadelphia College of Osteopathic Medicine
Charlotte Greene, Thesis Advisor

The purpose of this preliminary study is to determine if swine small intestine submucosa (SIS) can be used to create a suture that will perform comparably to commercially available chromic gut sutures when placed into a wound in rat dermis. This study is part of an ongoing collaboration that has explored SIS as a biologic scaffold in various tissues and surgical procedures.

In this study, eight Sprague-dawley rats were divided into two groups, each receiving a full thickness skin wound between the scapula, made by a 5mm biopsy punch. The wound was then closed with commercially available chromic gut suture, or SIS suture that was prepared in our laboratory. These sutures were created from swine jejunum with the serosal and mucosal layers removed, leaving behind only the submucosa. The submucosa was sliced into strips comparable to 3.0 sutures, and threaded onto a c-3 reverse cutting needle. The sutures were sterilized in a gentamycin and saline solution and refrigerated until placement. Rats were observed for signs of distress and infection, and euthanized at day 21 post-surgery. Tissue samples were taken, fixed, sectioned, mounted to slides, stained with Toren's reagent, and examined with light microscopy.

The SIS animals were compared to the chromic gut group under the parameters of fibroblast presence, infiltration of native tissue into the suture material, organization of collagen, vascularity, and granulation tissue. At day 21, all animals showed full wound closure, with no signs of scarring. No significant differences were found between the SIS and Chromic Gut groups in any measured parameter.

The results of this study warrant further examination of SIS as a suture material. As the production of low cost, naturally occurring absorbable suture materials declines in favor of the production of more expensive synthetic sutures, third world nations are left with fewer wound closure options. SIS sutures could provide a cost effective option that can be made on site for a variety of surgical applications.

TABLE OF CONTENTS

FIGURES

CHAPTER 1: INTRODUCTION

1.1 Background

This is a report of the final phase of a project to develop an alternative biological graft and suture material for a variety of surgical repairs that is affordable and readily available in resource-poor settings of the developing world. The most notable trends in these locals are the increased number of traumatic injuries combined with treatment constraint that are often related to the availability of medical materials (Spiegal, 2003). Patients are often requested to pay for the materials needed during surgical procedures, thereby limiting their access to necessary medical attention (Freudenberg, Nyonde, Mkony, Bay , & Wilhelm, 2004). Furthermore, 80% of the world's population resides in the developing world and studies have shown that it may be counterproductive to train surgeons from the developing world in the developed world, and then return them to an environment where modern conventional resources are unavailable (Eyer-Brook, 1986). An alternative effort would be to train local providers in their home country, based upon the pathology and resources available. Dedicated surgeons, for example, face a large volume of complex problems within a setting where the need for graft and suture material is pressing but unattainable or often prohibitively expensive. This is the primary rationale for this tactical study; to provide information concerning the preparation of a plentiful and functional material that can be harvested from locally grown animals, safely prepared in the fresh state, with the flexibility to be adapted for placement in a variety of procedures. Hopefully, the significance of this work lies beyond these preliminary studies.

1.2. Present Needs in the Developing World Related to the Preceding Phases of this Ongoing Small Intestine Submucosa (SIS) Study

1.2.1. Corneal Transplants

75% of world blindness currently occurs in Asia and Africa. Corneal diseases are second only to cataracts in causing blindness worldwide. Visual rehabilitation in many of these cases is possible with corneal transplantation. However, a review of published reports clearly shows that 80% of blind people live in countries where chronic economic deprivation is exacerbated by the added challenge of failing vision. Many of those currently visually impaired due to corneal diseases could be visually rehabilitated by corneal transplantation.

However, in India, for example, with a population close to one billion, only 20,514 donor corneas were procured in the year 2003, representing only 5% of the current need. Of those donated, only 8,426 could be utilized for transplantation. This clearly shows that there is a huge gap between demand and supply of donor corneal tissues in countries where corneal blindness is most prevalent. This may not only affect the number of transplants that can be performed but also surgical outcomes

(Garg, Krishna, Stratis , & Gopinathan, 2005) (Cornea Donor Study Group, 2005) (Ilhan-Sarac & Akpek, 2005).

The related study, *Small Intestine Submucosa for Repair of Rabbit Cornea,* hypothesized that an SIS xenograft could be used globally to treat ocular surface disorders if it facilitated corneal reepithelization without eliciting an immune response. This pilot study explored the SIS xenograft in a New Zealand White Rabbit, which has a comparable eye structure and physiology to humans. Synopsis of results: Successful repair was clinically observed in all five injured corneas. Corneal surface damage caused by chemical injury is devastating and difficult to manage in human patients. The noted smooth and avascular surface, clear stroma and good vision, as judged by clear iris detail and intact pupillary reflex, was preliminary evidence that a SIS xenograft is an efficacious treatment for corneal injuries (Cornea Donor Study Group, 2005) (Perenich, McCormak, & Greene, 2010). SIS is available in areas where pigs are raised and can be prepared by the ophthalmologist.

1.2.2. Arterio-Venous (A-V) Fistula Access for Dialysis

Fistula failure is common, not only due to poor surgical techniques, but because the fistulae are often put into routine use before full maturation. Arteriovenous grafts are even less frequently used, due to the cost of Gortex grafts, which are vascular grafts frequently used in fistula formation. A 2003 Study evaluating dialysis in St. Nicholas Hospital in Lagos, Nigeria found that the only patient with a Gortex graft in the hospital had it placed in the USA prior to commencing dialysis in their dialysis unit (Bamgboye, 2003).

A previous phase of the present study explored the use of SIS as an aortic graft. The swine intestinal submucosal and serosal cellular layers were essentially removed, the remaining SIS sterilized in gentamycin, and the grafts surgically inserted using a technique similar to that used for insertion of a synthetic vascular graft. The most striking feature observed in the SIS allografts was that the native vascular endothelium "seeded" over the SIS graft, vascularizing and reendothelializing it, so that the graft site eventually presented microscopically similar to vascular tissue. The SIS supported host tissue ingrowth and fostered cellular differentiation. In addition to the histologic changes, this material appeared less subject to rejection, infection, and incomplete endothelialization than other studied graft materials.

It was found that tensile strength can be maintained with proper graft preparation, and thrombosis can be minimized with appropriate prophylaxis. We were able to demonstrate The feasibility of carrying out the procedure under more strenuous surgical conditions than would ordinarily be encountered clinically was demonstrated by placing the SIS grafts in 300 lb preadolescent swine whose vessels are extremely delicate (Suzan E. Marshall, et al., 2000) (Selgrath, Lynch, & Greene, 1996) (Costa, Greene, Higgins, Lynch, & Prier, 1998) (White).

1.2.3. Urethral Grafts

Long anterior urethral strictures are fairly common in the developing world. Although dorsal on-lay buccal mucosa graft (BMG) urethroplasty has shown the highest success rate across studies and is the gold standard for anterior urethral strictures, it might not always be feasible to perform BMG urethroplasty due to lack of healthy buccal mucosa owing to tobacco chewing or the need for very long graft material (Sharma, Ratkal, Shivlingaiah, Girish, Sanjay, & Venkatesh, 2013).

A previous phase of the present study explored the use of SIS as a replacement for an 11-mm segment of native ureter excised from eight New Zealand White rabbits. The SIS graft was circumferentially wrapped around a ureteral stent. The grafts were harvested at either 11 days or 35 days post-implantation. This preliminary study demonstrated that SIS xenografts will epithelialize when used as a ureteral replacement material. The repair mechanism occurred through a regenerative healing process rather than by scar formation. With further studies, this material may prove to be a useful treatment option in patients with ureteral injuries (Jaffee, et al., 2001).

1.2.4. Nerve Regeneration/Lengthening

Injection nerve palsy, which is injury to the peripheral nerves from accidental injection of drugs, is an adverse outcome of intramuscular injections.

Intramuscular injection is an often-abused form of medical intervention in developing countries, making it the most common cause of iatrogenic nerve injuries. An African study, found that chloroquine, novalgin, penicillin, and sulfadoxine-pyrimethamine, drugs commonly given intramuscularly in children resulted in nerve injury. Certain drugs are much more damaging than others when injected into a peripheral nerve (Katki, Bhat, Bhagavathula, & Shukla, 2013).

Traumatic peripheral nerve injury in Turkey, a developing country, commonly occurs at the brachial plexus, and constitutes a major medical and public health problem. Despite dramatic advances in surgical techniques and an increased awareness of the importance of nerve grafting in peripheral nerve repair, the long-term prognosis for patients with severe nerve injuries remains gloomy (Uzun, et al., 2006).

A previous phase of the present study explored the use of SIS with added laminin and/or fibronectin to determine whether sciatic nerve regeneration improved. The experimental group had a 10 mm segment of the right sciatic nerve resected, followed by placement and suturing of an SIS graft plus laminin and/or fibronectin surgically inserted into the gap as a conduit for nerve regeneration. The right sciatic nerve was resected and reattached directly in the control group. Schwann cell growth and migration, as well as nerve regeneration were assessed using anti-s100 antibody, and fast cresyl violet stain respectively (Oxenberg, Smith, Troutman, Kriebel, & Greene, 2006).

Preliminary results indicated that: Schwann cell migration and accompanying neuron infiltration occurred up to approximately 2.5 cm over the 6-month healing time in experimental animals. The healing in control animals was observed to be inhibited by the formation of collagen scar tissue. Gait analyses showed increased sciatic function in experimental groups of laminin and/or fibronectin compared to control group (Oxenberg, Smith, Troutman, Kriebel, & Greene, 2006).

1.3. Current Study

This study represents the next phase in the exploration of the utilization of native porcine SIS by evaluating its suitability as a cutaneous repair suture material. Determining the efficacy of SIS as a dermal bioscaffold will fill a missing piece of this ongoing collaborative study. Based on previous success with this material, it was hypothesized that swine intestine submucosa will produce outcomes that are consistent with chromic gut sutures when used as a dermal suture.

SIS is a biomaterial derived from swine jejunum (Chen & Badylak, 2001). Presently there is little published information regarding the use of fresh, native SIS as a suture material. Since SIS has been shown to behave as a template that encourages ingrowth of adjoining approximated tissue at wound/incision sites, sutures made from fresh SIS may allow faster healing of the skin with fewer complications than observed with current suture materials (Chen & Badylak, 2001).

Presently available suture materials pose the problems of hypersensitivity, infection, and cost, which make them especially prohibitive to obtain for use by physicians in the developing world (Engler, Weber, & Turnicky , 1986). The development of a technique to create effective sutures from fresh, native SIS could be an effective and cost efficient alternative.

1.4. Healing Process

Wound healing in general has been recognized as important to human health for over 5,000 years. The ancient Egyptians recorded wound care procedures, including the use of compression for hemostasis (Reinke & Sorg, 2012). Galen and Celsius described the principles of wound healing during the Roman Empire, focusing on preventing infection (Reinke & Sorg, 2012). In the modern era scientists and physicians rediscovered some of this lost information, and built upon it.

The advancement of antisepsis, the discovery of microorganisms, and the development of antibiotics had a massive impact on the progress of wound healing (Reinke & Sorg, 2012). After centuries of exploring wound therapy the goals remain the same: fast and straightforward wound closure, while reducing scar formation.

1.4.1. Healing Process with Chromic Gut Sutures

A study using Wistar rats examined the histology of epidermal wound healing using chromic gut sutures. Tissue necrosis was observed within the first 24 hours after the suture was placed. Neutrophils infiltrated the tissue after 48 hours. Fibroblasts, representing the formation of granulation tissue, were noted after 73 hours. Lymphocytes and some fibroblasts were present in the tissue on the 7th post-operative day. The granulation tissue began to give way to organized collagen fibers, and by the 14th day, the majority of the cells in the wound area were mature fibroblasts. By this time, the fibers were more numerous, but loosely arranged, and granulation tissue was still observed (Andrade, Weissman, & Reis, 2006).

1.4.2. Healing Process with SIS Material

SIS is a naturally occurring extracellular matrix that has been demonstrated in previous research to induce site-specific remodeling of the organ or tissue in which it is placed by serving as a bioscaffold for tissue regeneration (McPherson & Badylak, 1998). Successful studies have been performed to test the use of fresh SIS to create de novo abdominal wall skeletal muscle, and other tissues (Chen & Badylak, 2001) (Wang & Liao, 2014) (Valentin, Turner, Gilbert, & Badylak, 2010). SIS has been proven to be effective in abdominal wall repair (Chen & Badylak, 2001), as well as repairing muscle injuries (Dejardin, Arnoczky, Ewers, Haut , & Clarke, 2001). Studies using SIS as a wound dressing in combination with standard suture procedures have shown that the material has the same bioscaffolding effect in skin cells (Prevel, et al., 1995), although a study examining the use of freshly prepared SIS as a suture material had not been previously published.

When placed in contact with a wound, SIS stimulates angiogenesis, connective and epithelial tissue growth and differentiation. It also encourages the deposition, organization, and maturation of extracellular matrix (ECM) (Hodde & Allam, 2007). The healing process is enabled to proceed normally, and the SIS will take on the histology of the tissue in which it is placed as the SIS is scaffolding supports the integration of regenerating tissue into the host tissue (Hodde & Allam, 2007).

This study used freshly prepared native SIS, as opposed to commercially available SIS, which has undergone crosslinking to inhibit collagen degeneration, and other procedures to prolong shelf life (Wounds International , 2010). In the processing of one widely used commercially available SIS wound matrix, produced by Cook Biotech (West Lafayette, IN), the tissue undergoes disinfection with peracetic acid, lyophilized, and then sterilized with ethylene oxide (McDevitt, Wildey, & Cutrone, 2003).

While commercially available SIS products have been widely researched, their cost can be prohibitive for use in developing countries. Currently, 5 sheets of

7cm x 10 cm Healthpoint Oasis Wound Matrix, a commercial SIS product, retails for $4,100 (Amazon).

This study examined a cost effective option, by utilizing sutures made from fresh, native SIS, prepared prior to surgery.

1.5. Sutures

Currently in the United States the most commonly used suture materials are synthetic, most commonly made from aliphatic polyesters, mainly being polyglycolic acid, co-polymers of glycolic and lactic acid and polydioxanone. Although major advancements have been made in synthetic suture materials, they are not without complications (Scientific Committee on Medicinal Products and Medicinal Devices, 1998). Synthetic materials are often coarse and braided, leaving them little room to stretch and remain permanently embedded in the tissue. This can lead to impaired healing and scaring in delicate tissues (International Newsletters, 2001). Synthetic sutures are more expensive than natural absorbable suture materials. Less effective absorbable chromic gut sutures are still used in developing countries, because synthetic sutures are up to 40% more expensive. However, chromic gut is being produced less frequently, as developed countries that manufacture it have begun to phase it out (Semchyshyn & Elston, 2009). Chromic gut also poses its own unique problems, causing higher rates of infection and hypersensitivity (Engler, Weber, & Turnicky , 1986).

Rather than use commercially available SIS for this study, fresh porcine jejunum was used in our laboratory to prepare SIS sutures. Native SIS following gentamycin sterilization is essentially cell-free thus decreasing the opportunity for an immunogenic response. It also contains naturally occurring growth factors. Among the growth factors found in the SIS membrane are basic fibroblast growth factor (b-FGF), transforming growth factor β (TGFb) and vascular endothelial growth factor (VEGF) (Cartlidge & Elder, 1989) (Marikovsky, et al., 1993) (Greca, Noronha, Bendhack, Feres, Soccol, & Joao R. Duda, 2004) (Irela-Arispe & Sage, 1993). The SIS tissue was formed into 3-0 sutures by sectioning and debriding the mucosal and serosal layers of fresh swine jejunum.

Since this procedure is relatively straightforward and inexpensive, it could easily be introduced to developing countries as an alternative suture material. When used in combination with other applications of SIS as a graft to repair various organs and tissues as previously stated its use as an epidermal suture material could allow for entire procedures such as limb replacement procedures to be completed using SIS material.

Chromic gut absorbable sutures are available commercially and are composed of collagen from the serosal layer of beef or the submucosal layer of sheep, coated with a chromic salt solution. Chromic gut is processed to provide greater resistance to absorption and is indicated for use in general soft tissue approximation and ligation.

(Ethicon, 2007). The sutures are composed of monofilaments, created by twisting together purified collagen strands from bovine or sheep small intestinal submucosa. Chromic salts are used to increase the holding time of these sutures, which typically last about 14 days. Absorption of the chromic gut sutures, due to proteolysis and macrophages, occurs in approximately 21 days (Hochberg, Meyer, & Marion, 2009). Specific information about the manufacturing of chromic gut sutures used in the present study was not available due to proprietary reasons.

There can be many complications with chromic gut sutures. Variations in the monofilaments make it hard to standardize the diameter and tensile strength. This tensile strength is then lost at a rate that is difficult to determine. The by-products of the collagen's degradation may cause toxic and allergic reactions, and the material helps to maintain the infections it causes. Once infected, the pH of the wound drops, causing the suture to lose tensile strength at a faster rate. Due to these factors, a strong correlation has been seen between the use of chromic gut and postoperative complications such as infections, wound dehiscence, and hernias (Scheidle & Hohl, 1987).

1.6. Current Studies with SIS

Many investigations have examined the use of commercially prepared SIS for tissue repair. One such study explored the use of commercial SIS as a graft for orbital reconstruction following tumor resections. Low levels of surgical complications were seen, and SIS was found to be effective in repairing the orbital wall defects. Only one participant in the 17-person trial experienced any significant complications (Phillips, Riley, & Woodworth, 2014 June).

Conversely, another study found that placement of a commercially processed SIS mesh after an esophageal mucosectomy negatively affected outcomes and resulted in esophageal stricture formation. All groups that received mesh with a stent, including the SIS group, experienced more stricture formation than the stent only group (Schomisch, et al., 2014). These studies demonstrate that while commercial SIS has widespread application in wound healing, it is not appropriate in some settings.

2. MATERIALS AND METHODS

2.1 Experimental Animal

This study was approved by the Philadelphia College of Osteopathic Medicine Institutional Animal Care and Utilization Committee (IACUC).

Eight adult, male, 300g Sprague-Dawley Rats (Charles River Laboratories, Wilmington, MA) were utilized in this study. The animals were stabilized for 24 hours upon arrival and confirmed to be in good health and maintained on a diet of normal rat chow over the length of study.

The animals were randomly subdivided into two groups of four animals each.

Group I. Full thickness skin wound repaired with SIS suture

Group II. Full thickness skin wound repaired with chromic gut suture (Ethicon Corporation, Somerville, NJ).

A punch biopsy was used to create full-thickness skin wounds in the rats between the scapulae that were sutured with either SIS, or chromic gut.

Infection and inflammation, and histology were monitored for both groups

2.2. Production of SIS Sutures

1. Fresh porcine small intestine was obtained from a USDA approved vendor.
2. The jejunum was sectioned and sliced longitudinally to produce a sheet.
3. The sheet was positioned serosa side up, and the serosa and muscularis layers were peeled from the submucosa and discarded.
4. The sheet was inverted and the mucosal surface was denuded.
5. The sheet was thinly sliced, with thickness comparable to commercial 3.0 sutures, to be used as suture material.
6. The sutures were threaded onto a reusable C-3 reverse cutting needle one day prior to surgery, and then placed into a sterilizing, chilled 10% gentamycin: 0.9% saline solution until placement.

2.3. Surgical procedure

1.Rats were anesthetized to a surgical plane of anesthesia (Ketamine 40 mg/kg, IM/Xylazine 2 mg/kg, IM).

2. Standard techniques were be used to prepare the animals for aseptic surgery, and appropriate draping was applied around the surgical site.

3. A sterile, disposible 5mm diameter biopsy punch was used to create a single midline, full skin thickness excision between the scapulae of each rat.

4. Butorphanol, 2.0 mg/kg was administered subcutaneuously intraoperatively at least 30 minutes before recovery from anesthesia and again 4 hours post surgery.

5. Group I incisions were closed with the SIS material, using standard suturing techniques.

6. Group II incisions were closed with 3-0 chromic gut sutures and standard suturing techniques.

7. Both groups were visually monitored until they resumed dorsal recumbent position and stabilization of vital signs.

8. Animals were then monitored on twice daily basis for cleanliness, dryness, and closure. Their level of distress was assessed by observing for normal feeding behavior, amount and quality of feces, and activity level.

2.4. Tissue Collection and Preparation

1. On post surgical day 21, all rats were euthanized via CO2 inhalation in a closed chamber.
2. An area of epithelium containing the surgical site was excised and placed in formalin, paraffin embedded, and sectioned according to standard histological methods.
3. The tissues were stained using Toren's Method (Electron Microscopy Sciences) for the presence of mast cell granules, collagen and elastic fibers, fibrin, colloid, keratin and erythrocytes.

4. A Nikon Eclipse 50i Microscope (Philadelphia, PA) was used to image the tissue.

2.5. Methods of Analysis

Analysis of fibroblasts was completed by placing a 2.01 by 2.01 inch sampling box in the bottom right corner of each 20X image that contained suture material. Fibroblasts were counted within the box, and a Student t-test was applied to determine statistical difference between the two groups.

The size of the sampling box was determined by choosing the largest box possible that would allow each fibroblast to be accounted for. The density of fibroblasts within the full sample made attempts to count each cell inaccurate.

Evaluation of infiltration was determined based on a system of 0 representing no infiltration of the animal's native tissue into the suture material. A rating of 1 represented that the animal's tissue had some degree of infiltration into the suture material under microscopic visualization at 10X.

Collagen organization was also imaged and compared at 10X amongst sections from all animals.

3. RESULTS

3.1. Physical Appearance

On day 21 post-surgery there were no significant differences on gross inspection between the SIS and the chromic gut sutured groups. All animals demonstrated full hair regrowth at the operative site with no signs of cutaneous scarring or infection.

3.2. Histological Evaluation

A sample of the histological evaluation of the sectioned tissue is exhibited in figures 3.1 and 3.2.

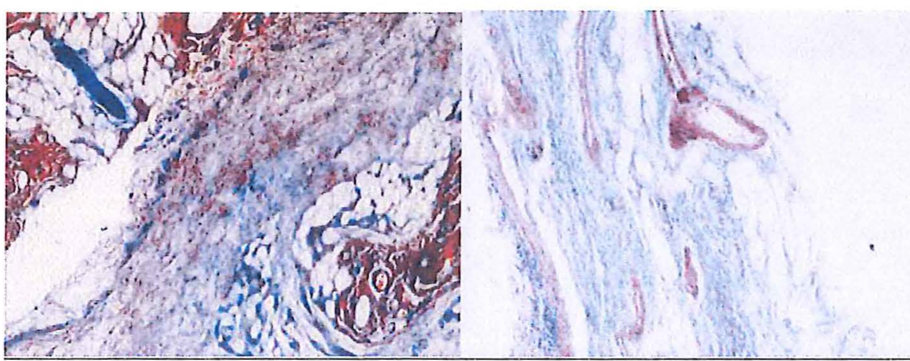

Figure 3.1. Left: SIS repaired site at day 21 post-surgery stained with Toren's reagent at 10x magnification. Right: Toren's reagent stained SIS tissue at 10x magnification.

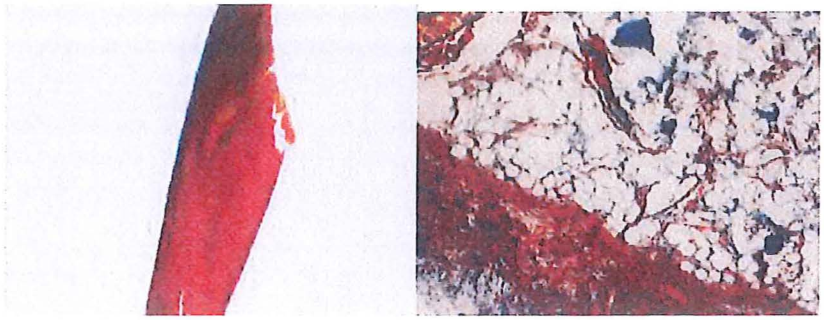

Figure 3.2. Left: Chromic Gut suture stained with Toren's reagent viewed at 10x magnification. Right: Chromic Gut repaired site stained with Toren's reagent at day 21 post surgery at 10x magnification.

3.3. Vascularity

No observable differences in vascularity were found between the SIS and chromic gut sutured groups. None of the tissue sections in the area of the wound that contained the suture materials had any evidence of vascular infiltration.

3.4. Collagen

The degree of organized collagen found in tissue from both types of repairs was similar with comparable arrangements of parallel collagen fibrils.

3.5. Infiltration of Native Tissue into the Suture Material

Samples were rated 0 or 1 based on no infiltration or presence of infiltration respectively. Multiple samples from the same animal were rated and then averaged.

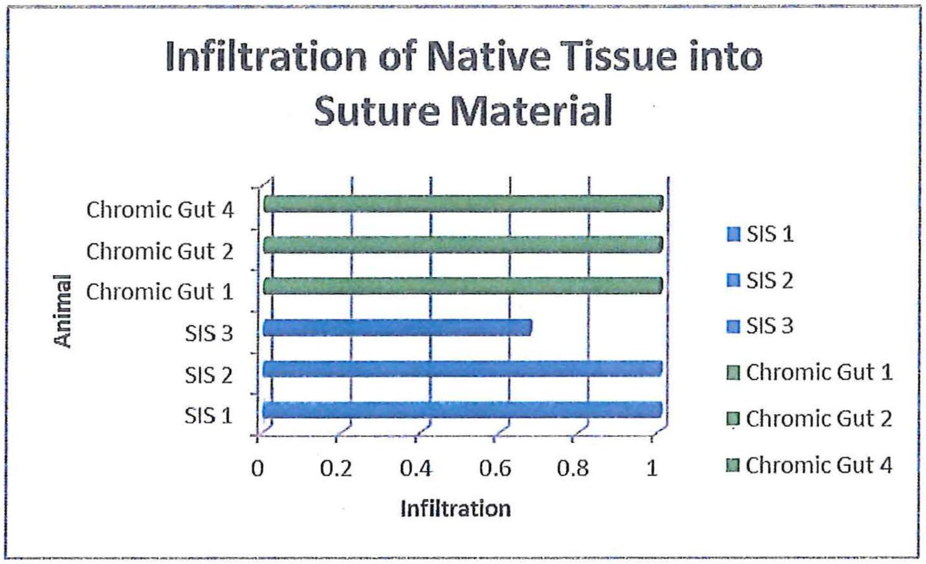

Figure 3.3. This table demonstrates the presence or absence of native tissue infiltration into the suture material.

A Fisher Exact test was performed to determine if there was a statistical difference between the SIS and Chromic gut in the proportion of suture material-containing samples that were observed to be infiltrated by native tissue.

The Fisher exact test statistic value was calculated to be 0.18, which is not significant at $p<0.05$. There was no significant difference found between SIS and chromic gut sutures when evaluating native tissue infiltration.

3.6. Fibroblasts

Fibroblasts were counted in a 2.01 by 2.01 sampling square superimposed upon each 10X micrograph. The average number of fibroblasts per animal was calculated, and compared using a Student's t test. The t statistic was calculated as 0.07 with a P value of 0.95, which is not significant at the P> 0.05 level.

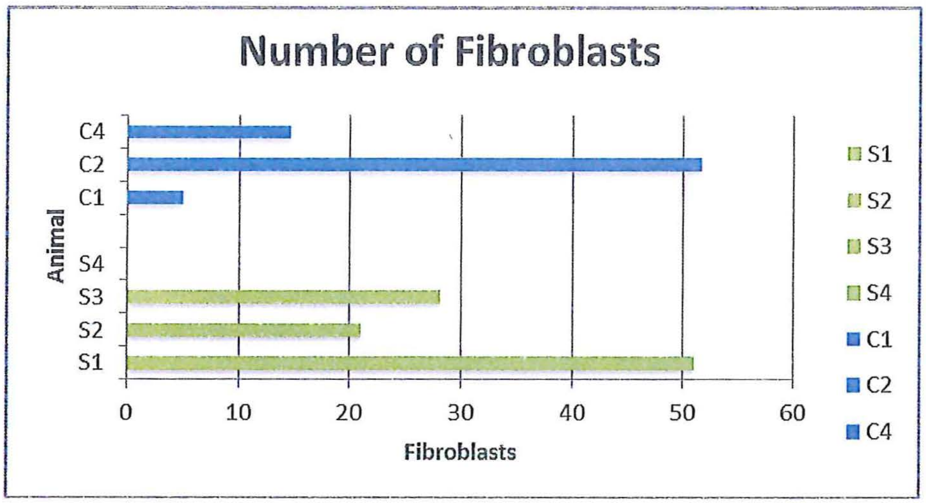

Figure 3.4. This graph represents the average number of fibroblasts per animal within a 2.01 x 2.01 inch sampling square in the lower right corner of each slide containing suture material.

4. DISCUSSION

After evaluating samples grossly and by light microscopy, no statistically significant differences were found between SIS and chromic gut sutures. In all evaluated parameters of healing, it appears that SIS sutures performed as well as commercially available chromic gut suture material.

4.1. Scar Formation and Hair Growth

Lack of hair and a pale and somewhat shiny epidermal tissue is indicative of scarring in rats (Stewart, Paderi, Snyder, Freeman, & Panitch, 2011); neither of which were noted in any of the animals in either group. By day 21, full hair regrowth over the incision site was observed, and there were no changes in dermal texture or pigmentation in either group. The presence of hair is indicative of the successful wound healing, since various growth factors involved in the healing process also stimulate hair growth. The epithelium adjacent to the wound is an example of growth promotion influencing organ specific growth (Argyris & Argyris , 2005). Lack of discernable scar tissue indicates an increasing wound tensile strength, and decreasing granulation tissue formation over the repaired wound site (Stewart, Paderi, Snyder, Freeman, & Panitch, 2011).

4.2. Granulation Tissue

The typical course of wound healing following cutaneous surgery initially involves the appearance of a hematoma that is replaced by granulation tissue during the first few days. This tissue is composed of new capillaries, fibroblasts, and a primitive extracellular matrix, which persist until collagen deposition begins to predominate. Granulation tissue is more prominent in burns, infected wounds, and incisions that are left to heal by secondary intention (Doherty, 2010). Since this study utilized primary closure, little granulation tissue formation was expected, unless complications such as wound dehiscence or infection occurred.

When evaluating tissue histology for the presence of granulation tissue, no significant difference was noted between the SIS and Chromic gut suture groups. None of the tissue samples from either group were observed to contain any measurable amounts of granulation tissue on histological examination. This implies that both groups experienced similar, minimal exposure to infection and tensile stress over the incision sites.

4.3. Collagen

At the time the wound samples were obtained, the remodeling phase of wound healing had begun. During this phase, fibroblasts organize the collagen which results in an increase in wound strength. Type III collagen also is being replaced by type I collagen, which is consistent with the type of collagen found in normal dermal tissue (Andrew Hsu & Mustoe, MD, 2010).

The remodeling phase can last from 2 weeks to two years post injury. During this time collagen production will peak, then synthesis and degradation eventually reach a state of equilibrium (Andrew Hsu & Mustoe, MD, 2010). Collagenase enzymes from granulocytes, macrophages, epidermal cells, and fibroblasts contribute to the degradation of collagen and can amplify the normal remodeling process (Clark, 1985).

There was no significant difference found in the organization of collagen between the SIS and chromic gut experimental groups, which is consistent with expectations at this stage of wound healing. Since the rate of wound healing can be impacted by factors such as infection and other exogenous factors (Andrew Hsu & Mustoe, MD, 2010), this finding is a good indicator that neither group experienced any measurable delay in wound healing.

4.4. Fibroblasts

Fibroblasts produce collagen, which provides structure to healing wounds. They become the predominant cell type during the proliferative phase. During the remodeling phase, fibroblasts organize and cross link the collagen, increasing wound strength. As the tensile strength of the wound increases, fibroblast presence decreases. The duration of fibroblast persistence in the wound can be affected by infection and other exogenous factors (Andrew Hsu & Mustoe, MD, 2010).

Since there was no statistically significant difference in the presence of fibroblasts between SIS and chromic gut samples, it can be inferred that both groups were at similar stages of healing. Combined with the similar degree of collagen organization, it can be deduced that all samples would have similar tensile strength.

4.5. Neutrophils

Contamination to the wound results in a prolongation of the inflammatory stage of wound healing, and can lead to increased inflammatory response and tissue destruction as the immune system clears the infection (Clark, 1985). If no contamination has occurred, neutrophil infiltration will resolve within the first few days after injury. The lack identifiable neutrophil invasion in any of the tissue seems to indicate normal tissue healing, without a prolonged immune response in either groups.

4.6. Angiogenesis

Blood vessels are necessary to deliver oxygen and nutrients to the healing tissue. Since hypoxic wounds do not heal, angiogenesis is a vital part of wound healing (Reed & Clark, 1985).

Although tissue samples harvested in proximity to the suture lines did not contain any blood vessels, it is pertinent to note that wound healing is not linear and after the surgery the blood vessels in the wound bed contract, then during proliferation, the wound is 'rebuilt' with new granulation tissue which is comprised of collagen and extracellular matrix into which a new network of blood vessels develop (Leaper & Harding). The inability to identify angiogenesis in this otherwise healthy appearing tissue may simply reflect the premature stage of tissue healing at the time of sampling. Likewise it may be that the invasion of vessels had not yet reached the immediate area that was sampled.

4.7. Cost Effectiveness

While keeping costs low is important, it is also vital to create a suture that is sterile. The use of gentamycin as a sterilizing solution allows SIS sutures to be a cost effective option. Gentamicin sulfate is commercially available for $8 per milliliter. Several milliliters of solution can be used to sterilize a batch of several sutures.

Utilizing a reusable suture needle also keeps costs low when creating SIS sutures. A pack of 12 reusable needles can be purchased for $31.99. These needles can be reused as long as they remain sharp. Sterilization can be achieved by steam, dry heat, ethylene oxide or by irradiation according to internationally accepted standards (Center for Disease Control and Prevention, 2008).

While they are cheaper to use, reusable needles must contain an eye to allow for threading the suture material. This means that the eye will be greater in diameter than the suture material, increasing trauma to the tissue. However, it is important to note that surgical technique plays a bigger part in wound closure than the sutures (Balaki, 2007). With proper training, physicians could learn to compensate for the wider needle, if it would mean that resources could allow them to treat a greater number of patients and perform a wider variety of procedures. Because of the large number of traumatic wounds that must be addressed in resource poor situations, even small differences in treatment options post-operative morbidity that can be reduced by the availability of locally produced suture materials could translate into improved health for a substantial number of individuals and significant savings of cost and health services resources.

4.8. Future Studies

This preliminary study is part of a larger collaborative effort, including dozens of studies over the past few decades. SIS has been successfully used in many other aspects of wound healing, and finding proof of its potential use in dermal tissue suturing is one more piece of the component availability of materials necessary before attempting multi-tissue repair using SIS, such as limb replacement.

This study shows promising results for SIS use as a dermal suture, however additional studies will be needed to confirm its significance. Future studies should incorporate a larger number of animals, and evaluate the healing process at various points, rather than a single point at 21 days. Future studies would also be necessary to assess the effect of the SIS suture on the tensile strength of the wound at various stages of healing to determine how successful it would be in high stress areas of the dermis.

Although no significant difference in outcomes in any measured parameter was found between SIS and chromic gut sutures, further study is still warranted, due to the local availability, cost effectiveness, and ease of creating SIS sutures. If other investigations are able to determine that SIS is as effective as chromic gut sutures in the dermis, the potential benefit of SIS sutures in developing countries as the production of chromic gut and other more affordable suture materials decreases, it justifies the continuing pursuit of SIS applications for which such a vulnerable population could benefit.

5. CONCLUSION

This preliminary study found no significant differences in outcome between wounds repaired with SIS and those repaired with commercially available chromic gut sutures. Although this study did not determine SIS to be superior to chromic gut, there is a population that could benefit from this research. Since SIS sutures can easily be made onsite in nearly any location where pigs are available, it is more accessible than presently used materials. If future studies continue to demonstrate no differences in outcomes, SIS could make an impact on healthcare in third world countries. The possibility of a suture material that is safe, cost effective, easy to produce, and potentially not inferior to currently acceptable materials, deserves a closer evaluation.

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