Basic and Translational Science

A Comparative Study Evaluating the In Vivo Incorporation of Biological Sling Materials

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OBJECTIVES
To comparatively investigate biological tissues that are clinical products currently used for implantation in urological reconstruction. Specifically, we examined biological materials in vivo and evidence regarding the tissue response observed. Biological tissues are widely used in urological surgeries to treat conditions such as pelvic organ prolapse and stress urinary incontinence.

METHODS
Histologic data from 4 biological sling materials, that is, small intestinal submucosa (SIS), cadaveric fascia lata, cadaveric dermis, and porcine dermis, implanted within mice (n = 64) were evaluated at 2, 4, 8, and 12 weeks. Recovered tissue was assessed by several biocompatibility parameters such as capsule formation (collagen deposition), cellular number, cell morphology, and angiogenesis.

RESULTS
Data provide a scientific depiction of the cellular response to these biomaterials through a 12-week evaluation. SIS had a significantly higher level of angiogenesis and cell infiltrate as compared with all other material tested. Collectively, the data suggest that SIS has improved biocompatibility over other tested materials.

CONCLUSIONS
This study compared SIS with other biological tissues in an animal model and was found to have superior biocompatibility as seen in humans. This may be helpful for clinicians while selecting a particular biological material. The study provides evidence of the varying stages of remodeling each implant, with hopes to better understand the material response in vivo.

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Biological tissues are widely used in urological surgeries to treat conditions such as pelvic organ prolapse and stress urinary incontinence (SUI). SUI is a major urological health problem that affects millions of women in the United States. Treatment options for SUI are designed to prevent the involuntary loss of urine from the urethra during increases in intra-abdominal pressure that occur during physical activity, coughing, or sneezing. However, there are few effective nonsurgical and pharmacologic treatment options. Although these approaches are less invasive, they produce relatively low cure rates and yield limited improved function. None of them currently provide a simple or long-term remedy for incontinence. Surgical techniques, in contrast, produce higher cure rates (85%-90%), but they are invasive and accompanied by all the morbidity associated with major surgery. In addition, it is also associated with significant potential complications, such as impaired bladder emptying and an overactive bladder.

Surgical treatment for SUI involving suburethral sling placement is one of the most common forms of treatment. With the introduction of synthetic mid-urethral tension-free tapes in the last decade, major changes in clinical practice were observed. Although these tension-free tapes have become a common treatment of choice for female SUI, long-term follow-up is still needed as complications are still being reported. Even though the synthetic materials, such as polypropylene mesh, are most commonly used, the biological materials still remain in the surgeons’ armamentarium. A number of biological materials, such as xenograft dermis, allograft dermis, and allograft fascia, are approved by the US Food and Drug Administration and are available “off the shelf” for human use. The suburethral sling treats SUI by supporting the bladder neck and urethra in a normal position and assisting the urethra to close more tightly. The sling acts as a hammock that cradles the bladder neck and urethra; thus, the bladder is well supported and urethra has a solid point to rest on and press against, allowing the sphincter to close more effectively.

Research is ongoing to enhance sling biomaterials to meet both the biological and mechanical demands placed on them. In an attempt to guide new tissue ingrowth and structural organization, biological materials are frequently used as scaffolds for urological tissue engineering.
torgrafts are a good choice as there is a smaller chance of graft rejection and present few health risks. They are advantageous with regard to the lack of vaginal erosion, still their mechanical properties are weak and can be accompanied with voiding dysfunction, postoperative pain, and morbidity.9 Allograft materials, those from another species, present a low risk of erosion and infection; however, reports of early failure with these materials and recurrences of incontinence are noted.2,7

The evidence base for determining practice decisions for patients with SUI is growing; however, there is a lack of studies that comparatively investigate the biological aspects of commercially available biological tissues.2 Despite the fact that there have been several reports on the mechanical and cellular response to each biomaterial individually,2,10,11 and a wide published data on synthetic sling materials,12-14 none have focused specifically or comparatively evaluated the biocompatibility of the biological sling tissues in vivo. This study was conducted to provide knowledge, which is lacking in the scientific community in regard to urological bio-tissue currently used clinically as treatment for urological reconstruction.

MATERIAL AND METHODS

Tissue Preparation and Implantation
Four groups of biological tissue samples were used for this study; 4-ply small intestinal submucosa (SIS) (Cook Biotech, Inc, West Lafayette, IN), human cadaveric tissues fascia lata (FL) and fascia dermis (FD) (Mentor Corporation, Santa Barbara, CA), and Pelvicol (P) (C. Bard, Murray Hill, NJ), which is a porcine dermis tissue. Prior to implantation the materials were cut into 1.5-cm sections and incubated in sterile phosphate-buffered saline for 1 hour. A total of 64 female BALB/c mice (Jackson Laboratories, Bar Harbor, ME), weighing at least 22 g at surgery, were anesthetized by intraperitoneal injection of a xylazine and ketamine mixture. The abdomen of the mice was shaved, disinfected, and a 1.5-cm midline incision was made. After the skin incision, the linea alba was incised and the abdominal muscles were retracted to expose the pelvic area. The biological tissue was placed at the bladder neck, anchored to the surrounding tissues with 4-0 nylon suture and the skin incisions were closed. From each group, 4 mice were killed at 2, 4, 8, and 12 weeks. Both the care and use of laboratory animals followed the National Institute of Health guidelines and were approved by the Wayne State University Animal Investigation Committee.

Material Recovery and Histology
At the time of killing, implants were recovered and the area of implantation was visually inspected for evidence of any tissue reaction or inflammation. Recovered implants were placed in 10% buffered formalin, dehydrated, and embedded in paraffin blocks. Sections of size 10 μm were cut along the longitudinal axis and mounted. Sections were stained with Carazzi haematoxylin and eosin (H&E) to evaluate cell number and morphology, Masson trichrome to determine collagen fiber deposition, and CD31 to determine angiogenesis. After staining, the slides were permanently bonded with coverslips. A minimum of 3 separate stained sections per specimen were examined microscopically and histologic parameters were measured using the Image-Pro Plus software package (Media Cybernetics, Silver Spring, MA). Cell counts, cell morphology, capsule thickness, and angiogenesis were determined from five 20× objective fields for each sample. Total numbers of cells, based on nucleus count, were determined as cells per square millimeter. The cell morphology was determined by the mean cell aspect ratio (cell width divided by its height) with fibroblastic cells having a greater aspect ratio compared with inflammatory cells. Capsule thickness was analyzed by measuring the distance from the implant surface to the adjacent tissue. Angiogenesis was determined during CD31 immunostaining, which identifies endothelial cells.

Statistical Analyses
Statistical analyses were conducted using SPSS (SPSS Inc, Chicago, IL). To evaluate differences between or among groups, analysis of variance was performed with post hoc pairwise testing, when necessary, using the Scheffé test. An alpha level of .05 was selected for significance for all statistical tests.

RESULTS
At all time points, tissue extracts were recovered with no noticeable macroscopic inflammatory signs. Animal bladders were all histologically determined to be normal. At no time point were the bladders found to have significant inflammatory cells or erosion. However, the histologic responses to the individual implants were quite different. Figure 1 depicts the capsule thickness over time for each biological tissue tested. SIS implants were the only group to show a significant decrease in capsule thickness over the 12-week time point (*P <.01). SIS indicates small intestinal submucosa; FL = cadaveric fascia lata; FD = cadaveric fascia dermis; P = Pelvicol porcine dermis.
with a high cell count at the 2-week time point as compared with Pelvicol, which demonstrated a low level of cell infiltration (Fig. 2). Interestingly, the aspect ratio of SIS and Pelvicol increased, whereas FL and FD demonstrated a decreasing aspect ratio with respect to time. The results demonstrated that angiogenesis occurred during the implantation period. At 2 weeks, SIS shows a significant number of capillaries per square micron (12.8 (4.1)) as compared to the other groups (average of groups was 3.9 (1.6)) at 2 weeks (P < .05). However, no significant difference was observed between the groups at the other time points evaluated. Overall, Pelvicol demonstrated the lowest measure of angiogenesis.

The tissue slides were stained with Masson trichrome stain and pictures were taken at 20× magnification. Masson trichrome will stain collagen a blue color and stain and pictures were taken at 20× magnification.

Figure 2. The number of infiltrating cells was quantified with H&E staining. Individually, none of the implants displayed a significant change in cell number during the 12 weeks. However, Pelvicol (P) showed significant decrease in cell number as compared with all other groups at both 2 and 12 weeks (* = P < .05).

**COMMENT**

The purpose of comparing biological tissues for urological reconstruction was to assess the biocompatibility of biologically derived implants within its urological environment. In this study, biocompatibility was assessed by analysis of capsule formation, cell number and morphology, and angiogenesis.

Allograft and xenograft tissues are processed and sterilized using different patented techniques that work to eliminate the cellular content and inactivate infection/disease-causing agents. However, several cellular reactions occur after implantation. Historically, autologous FL was among the most commonly used sling materials; however, human cadaveric tissues (dermis and fascia), and xenograft tissues have become prevalent because of their “off the shelf nature.” Although there are many biological materials available to the clinicians, acellular biological tissue matrices such as porcine small intestine submucosa have been proven to support cellular ingrowth and regeneration of genitourinary tissues, including the urethra and bladder tissues.4,15 Emphasis has also been placed on using cadaveric fascia due to the reduction in operative and recovery times. However, there is concern regarding the lack of long-term efficacy data on sling materials other than autologous fascia and synthetic materials.2,7,16 Clinical studies suggest that there may be issues with durability.10,16 However, little information exists on in vivo histologic characteristics of the various biomaterials.

We examined biological implant histology at several time points through various assessment parameters to correlate with biocompatibility. When measuring implant encapsulation, the thickness of the fibrous tissue capsule generally correlates with the severity of the inflammatory response. At 2 weeks, SIS had the thickest capsule formation, followed by FL, FD, and Pelvicol having the thinnest capsule. At 12 weeks of implantation, SIS demonstrated minimal encapsulation with near complete cell infiltration throughout the implant. FL and FD maintained a thicker capsule with minimal cell infiltration throughout the implant. Pelvicol continues to display a very thin capsule with little to no cell infiltration into the implant. A decrease in capsule thickness with time indicates improved biocompatibility as a consistently moderate capsule indicates a continual immune response potentially leading to a granuloma formation. The lack of fibrous capsule formation denotes minimal, if any, inflammatory reaction to the material. When examining cell number, we assess the 2- and 12-week time points for comparison. SIS, FL, and FD all displayed relatively high cell infiltration at 2 weeks, whereas Pelvicol did not seem to attract the high level of inflammatory cells. Early recruitment of inflammatory cells into the implant area has been shown to be important for the development of angiogenesis.11 This would appear to correlate with current data as the 3 implants with high cell numbers did have a higher level of angiogenesis as compared with Pelvicol.

In wound healing, cells and their products interact to repair damaged tissue. If an implant is present in the tissue, this sequence of events is disrupted to varying degrees, resulting in a visible change in tissue morphology. Many types of cells are involved in normal wound healing, including inflammatory cells, which can be identified when determining the aspect ratio of the cells. Aspect ratio correlates with cell morphology because smaller round cells indicate inflammatory cells and longer cells indicate a fibroblastic type of cell. We found that
the infiltrating cells of SIS and Pelvicol consistently had a higher aspect ratio than FL and FD. These data portray SIS and Pelvicol to contain a more fibroblastic cell type, whereas FL and FD were found to contain more inflammatory cells. Because the process of inflammation normally leads to angiogenesis and fibrosis, it is important for inflammatory type cells to be replaced by fibroblasts as they lay down the collagen matrix, which acts as a scaffold for new tissue formation. At 2 weeks, SIS had a significantly higher capillary count than all the other tested materials. This high count could be due to the high initial inflammatory response seen at 2 weeks with a thick capsule and high cell number, which is important for angiogenesis. Capillary ingrowth is necessary to deliver nutrients to the matrix and is essential for the survival of the implant. FL and FD displayed a moderate number of capillaries formed at 2 and 12 weeks of implantation, whereas Pelvicol showed minimal capillary formation throughout implantation. Although SIS displayed an acute inflammatory response, it diminished such that the implant ultimately became indistinguishable from native tissue. The increase in angiogenesis with SIS may be linked to tissue remodeling and new tissue formation. These results are thought to be useful clinically, especially when considering the durability of slings and their clinical outcome. Collectively, these data suggest that SIS has improved biocompatibility over the other tested materials.

In comparing biological tissues for urological reconstruction, we assessed the biocompatibility within the urological environment. Through commercial processing, tissues are claimed to be devoid of cells. However, other antigens may be present, which elicit inflammatory reactions, thus limiting the implant incorporation and use for long-term urological therapies. Zheng et al17 demonstrated that although SIS is an acellular material, levels of porcine DNA were found to be present, suggesting this may contribute to the inflammatory response that they

Figure 3. Masson trichrome staining demonstrating capsule thickness at 2 and 12 weeks after implantation (20× magnification).
noted with their in vivo study. In our study, SIS was shown to have the highest acute inflammatory response; yet, the final assessment of SIS depicted good tissue remodeling processes as compared with the other materials. Because the final assessment was conducted at 12 weeks, more studies should be carried out to evaluate the long-term histological outcome.

Although we acknowledge that there are certain limitations with this study, such as the difficult in translating the knowledge gained from the rodent model directly to humans, it is necessary for these comparative types of biocompatibility studies to be conducted in a controlled environment to accurately evaluate the response to the biological materials. In addition, animal studies are necessary to establish appropriate biocompatibility standards adapted to new material assessments. This study provides evidence of the varying stages of remodeling each implant, in hopes to better understand the material response in vivo.

CONCLUSIONS

The ideal biological material would provide good urethral support by promoting organized fibrosis and new tissue formation. A number of reports have described varying failure rates using both porcine and cadaveric sling material. Improved success in urological reconstruction has been reported since the introduction of SIS as a sling material. Histological studies in humans have shown SIS to exhibit good biocompatibility without significant foreign body reaction. This study compared SIS with other biological tissues in an animal model and was found to have superior biocompatibility as seen in humans. This may be helpful for clinicians while selecting a particular biological material.

References


Figure 4. CD31 immunohistochemistry demonstrates capillary formation at 12 weeks after implantation. Examples of capillaries (positive stained brown) are noted by arrow heads (20x magnification).


