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# Histologic and Biomechanical Evaluation of Biologic Meshes following Colonization with *Pseudomonas aeruginosa*

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***Background.*** **Biologic meshes have become increasingly popular for the repair of abdominal wall defects, especially in contaminated sites. The purpose of this study was to evaluate the histologic and biomechanical properties of biologic mesh in response to a bacterial encounter.**

***Material and Methods.*** **A rat model of *Pseudomonas aeruginosa* colonization and infection of subcutaneously implanted biologic mesh was used. Samples of biologic meshes [acellular human dermis (ADM) and porcine small intestine submucosa (SIS)] were inoculated with *P. aeruginosa* ($10^5$ or $10^9$ cfu) or saline as a control prior to wound closure ($n = 6$ per group). After 10 or 20 d, the meshes were harvested. The recovered meshes were analyzed for histologic changes and bacterial recovery as well as the material strength properties. Statistical significance ($P < 0.05$) was determined using 1-way analysis of variance or Mann-Whitney test.**

***Results.*** **ADM and SIS colonized with $10^9$ cfu *P. aeruginosa* showed an increased inflammatory response with an associated decrease in neo-vascularization ($P < 0.05$) at 20 d post-implantation compared with controls. *P. aeruginosa* had no effect on the tensile strength of ADM, but the tensile strength and modulus of elasticity were reduced for SIS compared with controls at 20 d.**

***Conclusion.*** **Bacterial colonization of ADM and SIS with $10^9$cfu *P. aeruginosa* negatively effected neovascularization and cellular re-population of the material over time but only SIS showed alterations in their biomechanical properties in response to this gram-negative bacterial challenge.** 

***Key Words:*** **biologic mesh; *Pseudomonas aeruginosa*; mesh infection; biomechanical properties; neovascularization.**

## INTRODUCTION

Abdominal wall herniorrhaphy is one of the most common operations worldwide that has been revolutionized by the use of meshes. Biologic prostheses are becoming increasingly more popular in the repair of these hernias, in part due to the widely held belief that they are superior to synthetic materials when used in an infected or contaminated field. However, there is limited research on the performance profile of these materials in terms of their response to a bacterial encounter.

Acellular biologic materials are derived from the collagen-rich tissues of human or animal sources and provide an extracellular bioscaffold that becomes repopulated with new collagen deposition and blood vessels while still maintaining enough mechanical integrity to prevent hernia recurrence [1]. Outcomes related to these materials appear to be associated with the preservation, processing methods (including decellularization and sterilization), and structural designs (whether or not they are intentionally crosslinked) [2, 3]. Two of the more commonly used biologic meshes are human acellular dermal matrix (ADM) and porcine small intestine submucosa (SIS).

Once any mesh becomes infected or colonized with bacteria, it is difficult if not almost impossible to overcome. Although there have been numerous claims that these biologic materials are "resistant" to

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infections, there is little data to support this claim. Experimental studies have shown that microorganisms are able to thrive and adhere to the surfaces of these collagen-based prostheses [4, 5], and clinical studies have reported that biologic meshes continue to be plagued with bacterial infection and colonization, often leading to implant removal and structural failure [6, 7]. When this happens, the patients are left with their own previous hernia, requiring additional operations and substantial healthcare expenditures.

Infections associated with biologic meshes are most often attributed to gram-positive cocci (*Staphylococcus*) and/or gram-negative bacilli (*P. aeruginosa*) [8–10]. In a recent animal study, it has been shown that colonization of biologic mesh with a gram-positive bacterium (*Staphylococcus aureus*) caused a significant degradation of the materials [5]. However, no data exist for the effects of a gram-negative bacterial colonization, such as with *Pseudomonas aeruginosa*, on biologic mesh. Since infection with this organism continues to be observed clinically following the implantation of these materials, it is important to have a thorough understanding of mesh response to this type of bacterial encounter. The purpose of this study is to further characterize the effect of bacterial colonization, with *P. aeruginosa*, on the histologic and biomechanical properties of two commonly used biologic meshes.

## MATERIALS AND METHODS

### Bacterial Inoculum Preparation

The bacterial strain, *Pseudomonas aeruginosa* strain PAO1, was obtained from American Type Culture Collection (ATCC #47085). One day prior to implant, an aliquot of this strain was thawed from frozen stock and grown overnight in Luria-Bertani broth (LB) for 16–19 h. The overnight cultures was diluted 1:100 into fresh LB and grown for 3 h. The cultures were washed in saline, and the culture concentration was determined by spectrophotometry (OD600) and compared with a predetermined growth curve. Each culture was brought to the desired concentration and bacterial colony-forming units (cfu) were verified by plating serial dilutions of the final solution on LB agar.

### Experimental Animals and Design

Male Sprague Dawley rats (Charles River Laboratories International, Inc., Wilmington, MA), each ranging from 300 to 500 g were randomly assigned to receive a rectangular implant (2.5 × 1.5 cm) of either acellular dermal matrix (1.04–2.28 mm in thickness) (ADM; AlloDerm; Life Cell, Branchburg, NJ) or multi-laminate (eight-layer) porcine small intestinal submucosa (SIS; Surgsis Biodesign; Cook Biotech; Bloomington, IN). The control (non-colonized) animals ($n = 6$) received a piece of rehydrated ADM or SIS and 200 mL of sterile normal (0.9%) saline into the surgical wound. The experimental (colonized) animals received a piece of rehydrated ADM or SIS inoculated with a 200 $\mu$L bacterial suspension of $10^5$ or $10^9$ cfu *P. aeruginosa* into the surgical wound before skin closure. All animals were then monitored for 10 or 20 d and then sacrificed. This study was approved and monitored by Tulane University's Institutional Animal Care and Use Committee, and all animals were cared for in accordance with guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care International.

### Mesh Infection Rat Model: Implantation Procedure

The biologic meshes were implanted under dorsal skin flaps, according to a previously described model [5]. Briefly, under sterile conditions, two dorsal subcutaneous pockets were developed 3 cm in length and 1cm to either side of the spine. One piece of rehydrated mesh was then placed in either pocket and saturated with the challenge inoculum or saline. After skin closure, buprenorphine (0.02–0.05 mg/kg) was administered intramuscularly every 12 h for 3 d. After achieving sternal recumbence, the rats were housed individually and left for either 10 or 20 d with available food and water. The animals were monitored daily for signs of pain, distress, erythema, local infection, and sepsis. Incisions were observed to detect macroscopic findings of infection such as seroma formation, wound dehiscence, and purulent drainage.

### Harvest: Collection of Samples

At 10 and 20 d postoperatively, the animals were anesthetized, clinical observations were made and a careful dissection was performed to open the dorsal flap and excise the mesh under sterile conditions. One implant per animal was placed in a Petri dish containing 2 mL of 0.9% saline to remain hydrated prior to biomechanical analysis. The other implant was cut into two equal pieces. One piece was fixed for 24 h in 10% buffered formalin (Fisher Scientific, Fair Lawn, NJ) and processed according to conventional procedures for histologic assessment and the other piece was placed in a tube containing 1 mL sterile saline and immediately analyzed by serial dilution plating for any bacteria present.

A cardiac puncture was then taken, after which the animal was humanely euthanized. This blood drawn at study termination was used to determine if there was any hematogenous dissemination of the bacteria, which could lead to multi-device colony counts in the same animal. A 100-$\mu$L aliquot of whole blood was inoculated on a blood agar plate. Bacterial growth was assessed after the plates were incubated at 37°C for 48 h.

### Bacterial Recovery at Explant

Several initial *in vitro* bacterial sampling methods, on both biomaterials, were performed prior to our *in vivo* experiments to determine the most consistently effective method of bacterial recovery. In the end, bacterial recovery from both materials was found to be greatest after vortexing the material in 0.9% saline. For these studies, two independent experiments demonstrated that the mean percent recovery of *P. aeruginosa* was 98.1% for ADM and 98.9% for SIS.

For the *in vivo* experiments, the explanted biologic mesh was submerged in 1 mL of sterile saline. The sample was then vortexed to dissociate adherent bacteria, and serial dilutions were plated on LB agar. After 24 h, bacterial counts were performed in triplicate. If bacteria grew from the cultured sample, they were scored as positive.

### Histologic Analysis

Six sections, 5 $\mu$m thick, were cut from each sample, stained with hematoxylin and eosin (H&E), and examined using light microscopy. Each slide was assessed and subjectively graded by a pathologist blinded to the treatment group for the following characteristics: inflammation, depth of inflammatory response, neovascularization, and cellular re-population response. Histologic grading was performed as shown in Table 1. The inflammatory response is represented by polymorphonuclear leukocytes per high power field for acute inflammation. Other inflammatory cells (macrophages, lymphocytes, eosinophils) were not counted. For the depth of the