Evaluation of a Novel Hybrid Viable Bioprosthetic Mesh in a Model of Mesh Infection

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Background: The reported incidence of mesh infection in contaminated operative fields is as high as 30% regardless of material used. Our laboratory previously showed that augmenting acellular bioprosthetic mesh with allogeneic mesenchymal stem cells (MSC) enhances resistance to bacterial colonization in vivo and preserves mesh integrity. This study's aim was to determine whether augmentation of non-crosslinked porcine dermis (Strattice) with commercially available, cryopreserved, viable MSC-containing human placental tissue (Stravix) similarly improves infection resistance after inoculation with *Escherichia coli* (*E. coli*) using an established mesh infection model.

Methods: Stravix was thawed per manufacturer's instructions and 2 samples were tested for cell viability using a Live/Dead Cell assay at the time of surgery. Rats (N = 20) were implanted subcutaneously with 1 piece of Strattice and 1 piece of hybrid mesh (Strattice + Stravix sutured at the corners). Rats were inoculated with either sterile saline or 10^6 colony-forming units of *E. coli* before wound closure (n = 10 per group). At 4 weeks, explants underwent microbiologic and histologic analyses.

Results: In *E. coli*–inoculated animals, severe or complete mesh degradation concurrent with abscess formation was observed in 100% (10/10) hybrid meshes and 90% (9/10) Strattice meshes. Histologic evaluation determined that meshes inoculated with *E. coli* exhibited severe acute inflammation, which correlated with bacterial recovery ($P < 0.001$). Viability assays performed at the time of surgery failed to verify the presence of numerous live cells in Stravix.

Conclusions: Stravix cryopreserved MSC-containing human umbilical tissue does not improve infection resistance of a bioprosthetic mesh in vivo in rats after inoculation with *E. coli*. (*Plast Reconstr Surg Glob Open 2017;5:e1418; doi: 10.1097/GOX.0000000000001418; Published online 10 August 2017.*)

INTRODUCTION

Mesh has been used for decades in the diverse field of reconstructive surgery. Despite advances in the development of synthetic and bioprosthetic materials and improvements in surgical techniques, mesh infection remains a challenging and costly complication, particularly in abdominal wall reconstruction.[1] Although mesh placement has reduced the incidence of ventral hernia recurrence by 50% over suture repair alone,[2] published rates of infection following this procedure range from 4% to 16%.[3] Infection has been reported in as many as 30% of cases following mesh use in contaminated operative fields regardless of the material used.[4–12] In addition, infection is known to be an independent risk factor for hernia recurrence and with each procedure, both cost and recurrence risk increase.[13] An estimated 365,400 ventral hernia repairs are performed annually in the United States at a cost of $3.2 billion, with a 3% increase in procedures projected each year.[14] Mesh usage is also expected to rise with emerging evidence supporting prophylactic insertion during the index operation.[15,16]

Mesenchymal stem cells (MSC) seeded onto the surface of bioprosthetics have been found to enhance mesh incorporation into surrounding host tissue, increase neo-

vascularization, and improve mechanical properties of meshes.[17–22] Recently, our laboratory demonstrated that MSC-seeded bioprosthetic mesh is more resistant to infection in vivo when compared with unseeded materials in the setting of *Escherichia coli* (*E. coli*) contamination.[23] MSCs have been shown to benefit wound healing not only by promoting tissue regeneration and increasing the recruitment of macrophages and endothelial cells into the wound but also by possessing immunomodulatory and antimicrobial activity.[24–26] Animal studies have determined that the administration of MSCs in a variety of in vivo conditions augments antibacterial responses against both Gram-negative and Gram-positive pathogens, decreases inflammation, and leads to faster bacterial clearance.[25–29]

Although MSC-seeded bioprosthetic materials have shown a great deal of promise in the laboratory for their enhanced antimicrobial and wound healing properties, that promise has yet to be realized in clinical practice due to logistical and economic hurdles of traditional stem cell therapy using cells typically derived from bone marrow or adipose tissue to generate MSC-seeded bioprosthetics. Two commercially available products by Osiris Therapeutics, Inc. (Columbia, MD) containing viable, nonimmunogenic human MSC (Grafix Prime Cryopreserved Placental Membrane and Stravix Cryopreserved Placental Tissue) have the potential to overcome these obstacles. Grafix has demonstrated safety and efficacy in a clinical trial for healing chronic wounds and reducing rates of wound infection.[30] These placental products, however, lack the tensile strength necessary for successful hernia repair. The objective of this study was to evaluate whether augmentation of an acellular bioprosthetic mesh frequently used in contaminated hernia repair (Strattice; LifeCell Corp., Branchburg, N.J.) with commercially available, viable human MSC-containing wound matrices improves infection resistance in vivo after inoculation with a common Gram-negative pathogen.

MATERIALS AND METHODS

Animals
A total of 20 male Sprague-Dawley rats weighing approximately 350 g were used. Animals were obtained from Charles River Laboratories (Wilmington, Mass.) and housed in the Tripler Army Medical Center animal facility. The study protocol was approved by the Institutional Animal Care and Use Committee at Tripler Army Medical Center. Investigators complied with the policies as prescribed in the U.S. Department of Agriculture Animal Welfare Act and the National Research Council's Guide for the Care and Use of Laboratory Animals. Facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Study Design
An established rat infection model[23,31–33] was utilized to evaluate whether co-implantation of cryopreserved human amniotic membrane (Grafix Prime, Osiris Therapeutics, Inc.) or cryopreserved human umbilical amnion and Wharton's Jelly (Stravix) with acellular, non-crosslinked porcine dermis (Strattice, LifeCell Corp.) improves resistance to bacterial contamination after inoculation with *E. coli*. To verify viability of endogenous cells in Grafix Prime and Stravix, cryopreserved human placental matrices were thawed per the manufacturer's instructions on the day of surgery, and 2 different samples each of Grafix Prime and Stravix were tested using a LIVE/DEAD cell viability assay (Life Technologies Corp., Grand Island, N.Y.) and visualized with an Olympus IX71 fluorescent microscope (Olympus America Inc., Center Valley, Pa.). Due to its difficult handling properties and tendency to shear, the utility of Grafix Prime for surgical applications appeared questionable and we decided not to evaluate Grafix Prime in vivo. Stravix and Strattice were cut into 2.5 × 1.5 cm strips using a precut plastic sterile template. Twenty rats underwent subcutaneous implantation in the dorsum with 2 pieces of mesh, with each rat receiving 1 piece of Strattice and 1 piece of "hybrid" mesh containing Strattice plus Stravix sutured together at the corners using 5-0 polydioxanone (Wharton's Jelly adjacent to Strattice) before implantation. Mesh type was varied on the left and right sides of the rats (e.g., hybrid mesh was placed on the left side in odd numbered animals). Experimental (colonized) animals (n = 10) received 200 μl bacterial suspension containing 10^6 colony-forming units (cfu) of *E. coli* into each surgical wound after mesh implantation but before skin closure to simulate a contaminated surgical field. Control (noncolonized) animals (n = 10) received 200 μl sterile saline instead of the bacterial suspension. Four weeks after surgery, explants underwent microbiologic and histologic analyses. This time point was chosen because we wished to examine a period during which meshes should be actively remodeled and acute surgical wounds should have healed.

Bacterial Inoculum Preparation
E. coli was chosen as the contaminant for this study, given its clinical relevance as a common enteric organism and based on prior work in our laboratory demonstrating profound mesh degradation and clinically apparent abscess formation after *E. coli* colonization on non-crosslinked porcine dermis and bovine pericardium.[23,31] *E. coli* (ATCC #25922) was obtained from American Type Culture Collection (Manassas, Va.). Two days before surgery, an aliquot was thawed from frozen stock and cultured on blood agar plates for 48 hours with a minimum of 1 passage between plates. Culture concentration was determined by spectrophotometry (optical density at 600 nm) and compared with a predetermined growth curve. Cultures were brought to the desired concentration in 0.9% sterile saline and verified by plating serial 10-fold dilutions (in triplicate) of the final solution used during surgery.

Surgery and Tissue Collection
Surgery, anesthesia, and analgesia was performed as described previously.[23,31] Briefly, bilateral 3 cm dorsal incisions were made 1 cm lateral to the spine. A subcutaneous pocket was created at each incision site and 1 piece of mesh was placed into each pocket such that each rat received a piece of Strattice and a piece of hybrid mesh

(Stravix side adjacent to subcutaneous tissue). The bacterial inoculum (200 µl suspension of 10^6 cfu *E. coli*) or sterile saline (200 µl) was pipetted onto each implanted mesh before skin closure with sterile stainless steel clips (Braintree Scientific, Braintree, Mass.). Animals were evaluated daily for signs of local infection, sepsis, pain or distress, or wound complications.

On postoperative day 28, rats were deeply anesthetized using a combination of ketamine (75 mg/kg) and dexmedetomidine (250 µg/kg) administered intraperitoneally, and cardiac puncture was performed to determine bloodstream infection rates as described.[23,31] Rats were killed by intracardiac injection of a pentobarbital-based euthanasia solution, and meshes were carefully excised under sterile conditions. In the event that the mesh could not be readily identified, the subcutaneous space was explored from the dorsal midline to the anterior axillary line to rule out migration and to confirm complete degradation. Length and width of each explanted mesh were measured to evaluate contraction (decrease in surface area) of the implant, and each mesh (if present) was divided into 2 equal pieces for bacterial recovery and histologic analyses.

Bacterial Recovery at Explant

Explanted meshes were submerged in 1 ml 0.9% sterile saline and vortexed for 1 minute to dissociate adherent bacteria as described.[23,31] Serial 10-fold dilutions were plated in triplicate on blood agar and incubated at 37°C for 24 hours before counting colonies. Gram stains were performed and meshes were scored as positive if the clinical isolates were Gram-negative. Bacterial clearance was defined as the number of animals with sterile cultures divided by the total number of inoculated animals and expressed as a percentage.

Histology

Samples were fixed in formalin, embedded in paraffin, and 5 µm sections were stained with hematoxylin-eosin. Specimens were evaluated by 2 blinded investigators at 40× to 200× magnification. They were graded for cellular infiltration, cell types present, extracellular matrix deposition, and neovascularization according to a modified scale used by Jenkins et al.[34] and used previously by our laboratory.[23,35] Higher scores on this scale represent more favorable implant remodeling characteristics. A composite histologic score was also calculated for each sample by taking the average of each of the scores in each of the subcategories.

Statistical Analysis

All results were reported as mean ± SEM. A McNemar's test was used to compare abscess formation for Strattice versus hybrid mesh either in a contaminated surgical field (*E. coli* inoculum) or sterile environment (saline inoculum). Total wound complications, reduction in surface area, and histologic comparisons between Strattice and hybrid mesh in either *E. coli*–inoculated or saline-inoculated rats were performed using the paired *t* test or nonparametric Wilcoxon Signed Rank test if indicated. The *t* test was used to determine weight differences between saline- and *E. coli*–inoculated rats during the 4-week postoperative period and differences in bacterial counts. The Pearson Product-Moment Correlation was used to determine the relationship between bacterial recovery and inflammatory cell scores. Analysis of variance was used to determine differences in histologic parameters among the groups followed by pairwise multiple comparisons using the Holm-Sidak method to identify specific differences between groups. Statistical analyses were performed using SigmaPlot 11.2 software (Systat Software Inc., San Jose, Calif.) with $P < 0.05$ considered significant.

RESULTS

Viability of Endogenous Cells in Cryopreserved Human Placental Wound Matrices

As determined using a LIVE/DEAD cell viability assay (Life Technologies Corp) following the manufacturer's instructions, the presence of numerous live cells was observed in Grafix Prime samples that were assayed immediately and 1 hour after thawing. Poor viability of endogenous cells (< 10% estimated semiquantitatively) was revealed in the Stravix samples immediately and 2 hours after thawing (Fig. 1). These assay times were chosen because the directions for use of the cryopreserved matrices specify that Grafix Prime should be applied within 1 hour and Stravix should be applied within 2 hours of thawing.

Postoperative Course

All animals survived the 4-week postoperative period. Reduced weight gain in *E. coli*–inoculated rats was observed during the first postoperative week relative to saline-inoculated rats ($P < 0.001$), but differences in weight gain were not statistically significant at weeks 2, 3, and 4.

In *E. coli*–inoculated rats, wound complications included abscess formation, wound dehiscence, and skin ulceration, which did not differ between Strattice and hybrid meshes (15 and 19 total wound complications, respectively, $P = 0.37$). Abscesses were observed in 90% (9/10) Strattice meshes and 100% (10/10) hybrid meshes ($P = 0.02$). In saline-inoculated rats, seroma formation was observed in 100% (10/10) hybrid meshes but in 0% (0/10) Strattice meshes ($P < 0.001$). No other wound complications were observed in saline-inoculated animals.

Macroscopic Findings

At necropsy, all meshes were recovered from saline-inoculated rats, but in *E. coli*–inoculated rats, 6/10 Strattice and 6/10 hybrid meshes were completely degraded and not identified during necropsy. Strattice meshes in saline-inoculated rats demonstrated minimal incorporation into the surrounding host tissue and were easily removed with minimal adhesiolysis. Hybrid meshes inoculated with saline demonstrated somewhat greater adherence to surrounding tissue and required increased dissection to facilitate their removal. Remnants of Stravix could be identified grossly in hybrid meshes 4 weeks after implantation. In saline-inoculated animals, Strattice underwent an 8.2±2.7% reduction in surface area, whereas the hybrid meshes exhibited a 13.5±3.3% reduction in the Strattice component ($P = 0.31$).
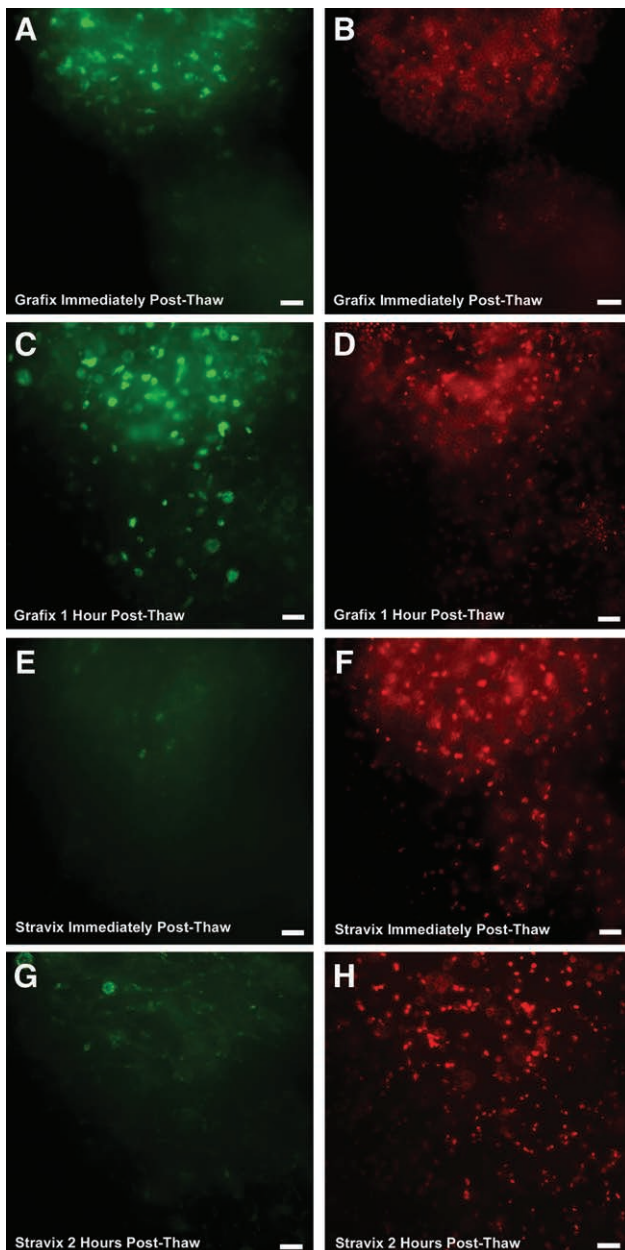
Fig. 1. Viability of endogenous cells post-thaw in human cryopre-served placental membrane (Grafix Prime; A-D) and human cryo-preserved umbilical tissue (Stravix; E-H). Representative images of a LIVE/DEAD cell viability assay on samples stained with calcein to view live cells (green; A, C, E, G) and ethidium to view dead cells (red; B, D, F, H). Note low cell viability in Stravix both immediately and 2 hours after thawing (E-H). Bar = 50 μm.

In contrast, severe or complete mesh degradation concurrent with abscess formation was observed in 90% (9/10) Strattice meshes and in 100% (10/10) hybrid meshes in *E. coli*–inoculated animals (78.2±10.0% and 83.7±9.9% reduction in surface area, respectively, $P = 0.63$; Figs. 2, 3). In some cases, abscesses were still present at the time of harvest, but in all rats, whose abscesses resolved by 4 weeks, meshes of both types could not be identified.

Microbiologic Findings

Only 4 Strattice and 4 hybrid meshes inoculated with *E. coli* were obtained for microbiologic analysis at 4 weeks. Quantitative cultures revealed the presence of viable *E. coli* on 75% (3/4) recovered Strattice meshes (mean bacterial load, $1.78±1.1×10^7$ cfu/mesh) and 100% (4/4) recovered hybrid meshes (mean bacterial load, $4.29±2.0×10^7$ cfu/mesh) with no difference between mesh types ($P = 0.32$). These results equate to 25% bacterial clearance for recovered Strattice meshes and 0% bacterial clearance for recovered hybrid meshes inoculated with *E. coli*. None of the saline-inoculated meshes and none of the blood cultures were positive.

Microscopic Findings

Representative images and histologic scores are presented in Figs. 4, 5, respectively. In saline-inoculated rats, Strattice meshes were well tolerated and exhibited minimal acute inflammation, whereas hybrid meshes demonstrated an increased inflammatory response and enhanced neovascularization in the Stravix component but not in the Strattice. No statistically significant differences in histological parameters were observed between mesh types inoculated with saline when evaluating only the Strattice component of the hybrid meshes. In rats inoculated with *E. coli*, severe acute inflammation was observed in recovered Strattice and hybrid meshes. Scores for cell types (inflammatory cells) correlated with bacterial recovery ($r = 0.71$; $P < 0.001$; Pearson Product-Moment Correlation). Meshes inoculated with *E. coli* (especially the hybrid meshes) had lower scores (worse remodeling characteristics) for cellular infiltration, inflammatory cell types, and extracellular matrix deposition compared with saline-inoculated meshes of either type ($P = 0.03$; $P < 0.001$; and $P = 0.01$, respectively).

DISCUSSION

The ideal mesh material is one that enables optimal integration into host tissue, provides long-term structural integrity, and resists bacterial colonization; however, the search for the ideal mesh continues.[1] Although acellular bioprosthetic materials have been developed in an effort to improve biocompatibility and resistance to infection compared with synthetic meshes, experimental, and clinical data suggest that in actuality they may not resist infection or improve outcomes when used in contaminated surgical fields.[23,31,36]

The consequences of early, aberrant mesh degradation, and subsequent mesh failure in the setting of a reconstructive procedure are difficult and costly to remediate. Experimental studies have shown that acellular bioprosthetic materials including porcine dermis, bovine pericardium, human dermis, and porcine small intestinal submucosa are not resistant to infection, particularly in the presence of Gram-negative pathogens.[23,31–33] This may be a result of enzymatic degradation by the invasive pathogens themselves or caused by collagenases and matrix metalloproteinases produced by infiltrating activated leukocytes during an increased inflammatory response. It is also possible that bioprosthetic materials are not adequately vascularized to enable clearance of the bacteria.
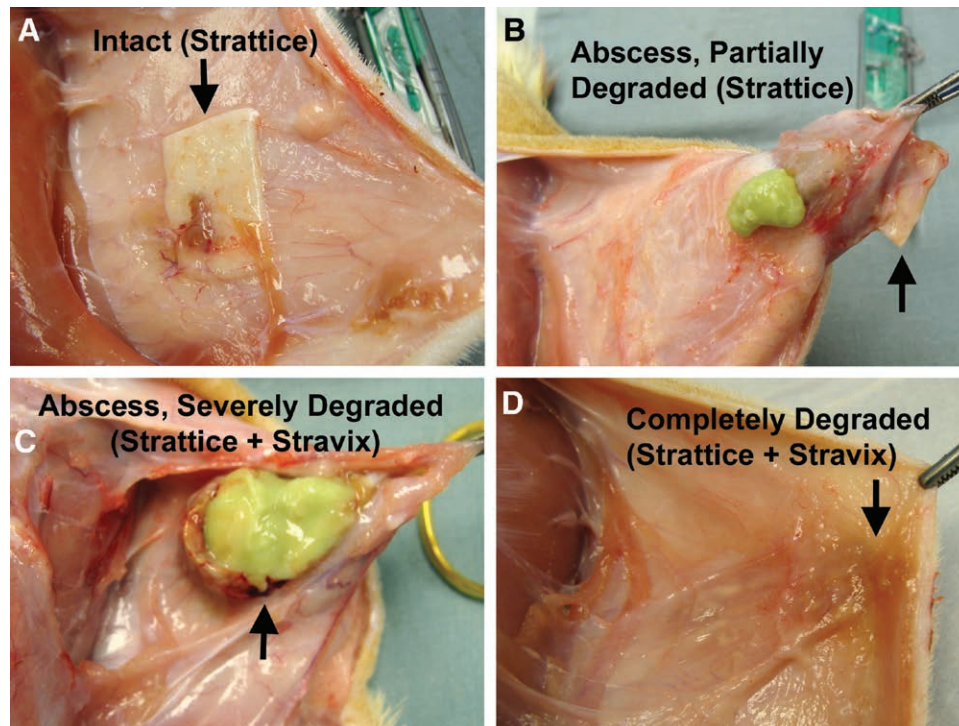
Fig. 2. Fate of *E. coli*–infected Strattice and "hybrid" (Strattice + Stravix) meshes 4 weeks after implantation. In animals inoculated with *E. coli*, a single Strattice implant remained intact (A), but all other implants regardless of mesh type demonstrated abscess formation with severe degradation (B, C) or complete degradation at 4 weeks after abscess resolution (D).
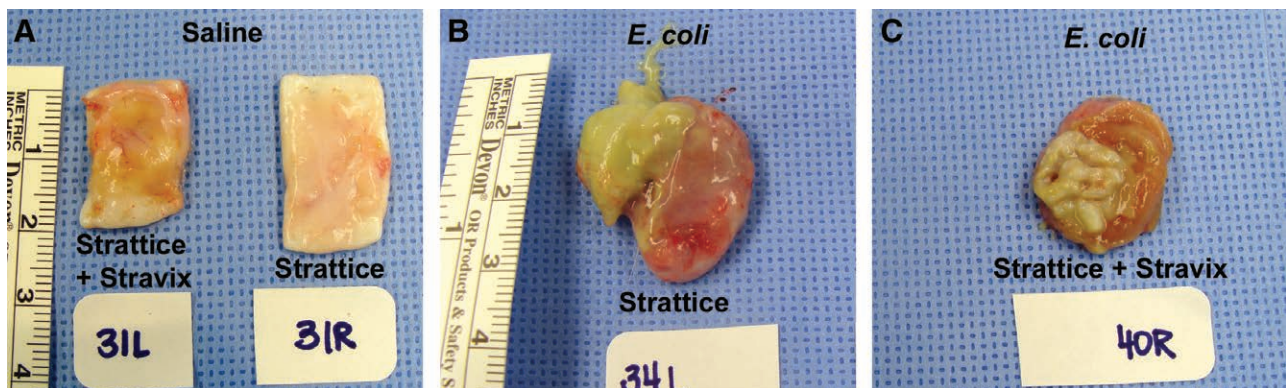

Fig. 3. Representative explants 4 weeks after surgery. While saline-inoculated meshes remained intact (A), meshes inoculated with *E. coli* exhibited abscess formation and mesh degradation (B, C). No mesh material was identified in center panel (B).

Cellularized meshes provide a promising option for use in a contaminated environment. Recently, augmentation of a bioprosthetic mesh with bone marrow–derived MSC was shown to markedly improve bacterial clearance in vivo and preserve mesh integrity when inoculated with *E. coli*.[23] Additional benefits of MSC-seeded implants include enhanced incorporation into surrounding host tissue, improved mechanical properties, and increased neovascularization.[17–22] The presence of MSCs on the surface of a bioprosthetic may protect against degradation by promoting effective bacterial clearance and downregulation of the inflammatory cascade.[25–29] Several studies have begun to investigate the immunomodulatory and antimicrobial properties of MSCs.[27–29,37,38] Human MSCs have been shown to cause direct bacterial killing by secreting antimicrobial peptides such as the human cathelicidin hCAP-18/LL-37, which is effective against both Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria.[29] Other antimicrobial proteins such as beta-defensin-2, lipocalin 2, and keratinocyte growth factor also have been shown to mediate antibacterial effects of MSCs.[28,37,38] MSCs appear to have the ability to modulate the acute phase response while enhancing phagocytosis and upregulating expression of antimicrobial peptides. This is likely responsible for reducing the negative consequences of unchecked inflammation while directly enhancing pathogen clearance.[24,27]
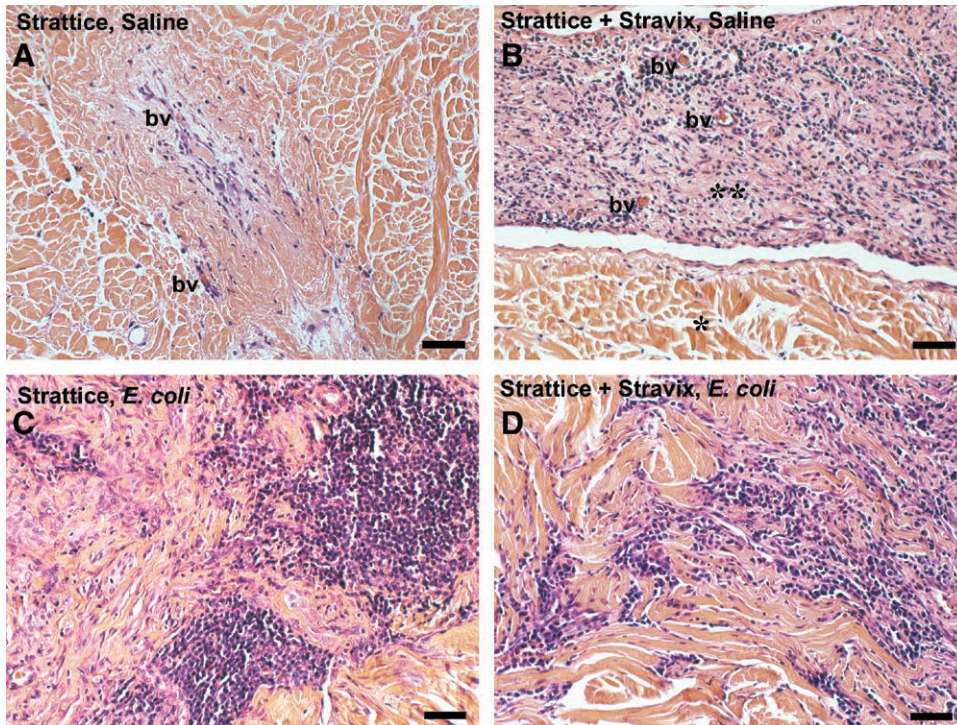
Fig. 4. Representative hematoxylin-eosin–stained sections of Strattice and hybrid mesh inoculated with saline (A, B) and *E. coli* (C, D). In uninfected animals, Strattice elicited mild inflammation, with visualization of cellular infiltration and new blood vessel (bv) formation (A). In hybrid meshes, the Stravix (**) component elicited increased cellularization and neovascularization compared with the Strattice (*) component (B). In *E. coli*–inoculated animals, both mesh types exhibited severe acute inflammation (C, D). Bar = 50 μm.
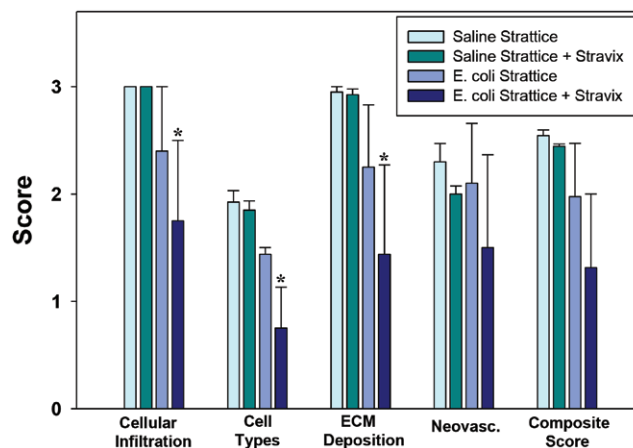
Fig. 5. Histologic scoring of explanted meshes 4 weeks after implantation. A composite histologic score was calculated by taking the average of the scores in each of the subcategories. Higher scores represent more favorable remodeling characteristics. Values are reported as mean ± SEM. *P < 0.05, *E. coli*–inoculated hybrid mesh vs the 2 saline-inoculated groups. ECM, extracellular matrix; Neovasc., neovascularization.

To overcome the prohibitive logistical barriers of traditional stem cell therapy, this study was undertaken to evaluate a practical alternative to MSC-seeded bioprosthetics with potential to take cellular materials from bench to bedside. We wished to determine whether the favorable outcomes of MSC-seeded mesh when confronted with *E. coli* colonization[23] could be replicated by pairing "off the shelf" cryopreserved, viable, MSC-containing umbilical tissue (Stravix) with a stronger bioprosthetic mesh commonly used in contaminated hernia repair (Strattice).

Neonatal MSC derived from placental and umbilical tissue are thought to be an ideal source of MSC for allogeneic regenerative medicine applications because they are known to possess low immunogenicity, they have a high efficiency of MSC recovery and a high proliferation rate, there are minimal ethical concerns with their acquisition and use, and they are from healthy, young donors.[39]

Results from this study determined that the presence of Stravix did not improve infection resistance and did not preserve integrity of Strattice after inoculation with *E. coli* in a rat model of mesh infection. In addition, Stravix augmentation was not protective for the development of wound complications either in a contaminated or sterile environment. Given the poor cell viability of Stravix samples assayed at the time of surgery, 1 possible explanation for the unfavorable findings is that insufficient numbers of viable MSC were present in the Stravix to exert their beneficial effects. Previous studies demonstrated efficacy of MSC-coated biologic materials when seeding the meshes at a density of 2.5×10^4 and 4×10^5 cells per cm^2.[22,23] These densities are consistent with the number of MSC present in umbilical tissue in which isolation efficiency has been reported of up to 5×10^4 MSC per cm of umbilical cord.[39] It is possible that the nonviable and xenogeneic properties of the MSC in the Stravix may have elicited an enhanced inflammatory response rather than exerting immunomodulatory and antibacterial effects.

Limitations of this study exist. Given this is an animal model, results may not be generalizable to patients. This investigation did not quantify clinical outcomes other than infection and was restricted to a monomicrobial inoculum using a single bioprosthetic material. Preclinical studies examining the benefits of various MSC-seeded bioprosthetics for reconstructive procedures such as abdominal wall repair in the setting of polymicrobial contamination are warranted.

CONCLUSIONS

Although previous studies have shown that augmenting a bioprosthetic mesh with MSCs enhances its resistance to infection, preserves mesh integrity, and facilitates incorporation into surrounding host tissue, these favorable outcomes were not replicated when augmenting a bioprosthetic mesh with commercially available, cryopreserved, viable human placental tissue. The search for a readily available, affordable, and mechanically durable MSC-containing bioprosthetic for the repair of contaminated abdominal wall defects continues.

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