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Susceptibility of ePTFE vascular grafts and bioengineered human acellular vessels to infection

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ABSTRACT

Background: Synthetic expanded polytetrafluorethylene (ePTFE) grafts are routinely used for vascular repair and reconstruction but prone to sustained bacterial infections. Investigational bioengineered human acellular vessels (HAVs) have shown clinical success and may confer lower susceptibility to infection. Here we directly compared the susceptibility of ePTFE grafts and HAV to bacterial contamination in a preclinical model of infection.

Materials and methods: Sections (1 cm^2) of ePTFE ($n = 42$) or HAV ($n = 42$) were inserted within bilateral subcutaneous pockets on the dorsum of rats and inoculated with *Staphylococcus aureus* (10^7 CFU/0.25 mL) or *Escherichia coli* (10^8 CFU/0.25 mL) before wound closure. Two weeks later, the implant sites were scored for abscess formation and explanted materials were halved for quantification of microbial recovery and histological analyses.

Results: The ePTFE implants had significantly higher abscess formation scores for both *S. aureus* and *E. coli* inoculations compared to that of HAV. In addition, significantly more bacteria were recovered from explanted ePTFE compared to HAV. Gram staining of explanted tissue sections revealed interstitial bacterial contamination within ePTFE, whereas no bacteria were identified in HAV tissue sections. Numerous $CD45^+$ leukocytes, predominantly neutrophils, were found surrounding the ePTFE implants but minimal intact neutrophils were observed within the ePTFE matrix. The host cells surrounding and infiltrating the HAV explants were primarily nonleukocytes ($CD45^-$).

Conclusions: In an established animal model of infection, HAV was significantly less susceptible to bacterial colonization and abscess formation than ePTFE. The preclinical findings presented in this manuscript, combined with previously published clinical observations, suggest that bioengineered HAV may exhibit low rates of infection.

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Introduction

Synthetic vascular grafts have provided life and limb–sustaining therapy for millions of patients as both arterial bypass conduits and dialysis access grafts. Although this technology has been useful for many years, one of its frequent failure modes in the clinic is that of bacterial colonization and infection.[1-3] Following implantation and hemodialysis access, it has been reported that as many as 28% of synthetic vascular grafts,[4] such as those constructed from expanded polytetrafluorethylene (ePTFE), will require revision or explant due to infection, which significantly impacts medical costs and patient morbidity.[5,6] It has been suggested that the microporous structure and synthetic composition of ePTFE grafts provide niches for bacterial accumulation[7] and interferes with the ability of host leukocytes to combat bacterial infections.[8,9]

Routine vascular access for hemodialysis presents an inherent infection risk due to potential introduction of bacteria during cannulation. This can lead to not only local infection of the vascular grafts and surrounding tissue but also bacteremia and sepsis. Consequently, bacterial infection is the second leading cause of death in hemodialysis patients with end-stage renal disease.[10] Gram-positive *Staphylococcus aureus* is responsible for the majority of vascular access infections[4,11] and subsequently linked to higher rates of patient mortality from serious medical complications including infective endocarditis and osteomyelitis.[12] *Staphylococci* have been shown to strongly adhere to the surface of implanted materials and then form a biofilm that often enables the bacterial infection to persist, despite prolonged antibiotic therapy.[13] It has been shown that biofilms produced by *S. aureus* readily form on contaminated ePTFE vascular grafts and survive for weeks despite active host immune responses and high blood flow shear stresses.[14]

We have developed an investigational tissue-engineered acellular blood vessel using primary human vascular cells that are seeded on a rapidly degrading polymer scaffold and cultured to form a tissue, which is then decellularized to remove the cells. The resulting engineered human acellular vessel (HAV) is a robust tube of human extracellular matrix (ECM).[15] These HAVs have been implanted into 60 patients within two phase II, single-arm trials and demonstrated functional capacity for use as a vascular conduit for hemodialysis.[16] Moreover, HAVs implanted in these patients have had a low infection rate (1.3% per patient-year), comparable to that of native arteriovenous fistulas used for chronic hemodialysis.[1,4]

In this study, we directly compare the susceptibility of HAV and ePTFE graft material to infection, using a well-controlled and established animal model of subdermal implantation with bacterial contamination.[17-19] Although repetitive post-operative needle punctures of the material would be more representative of dialysis cannulation events and potential infection, we chose to evaluate the response to one well-controlled intraoperative bacterial contamination event to determine each material's resistance to infection, as well as to limit animal distress and experimental variability. Upon implantation, the HAV and ePTFE materials were inoculated with controlled doses of either gram-positive *S. aureus*, or gram-negative *Escherichia coli* before wound closure. After 2 wk, the implant sites were scored for abscess formation and the explanted HAV or ePTFE material was processed for microbial recovery as well as for histological analyses to evaluate extent of bacterial contamination as well as host cellular response. The results of this preclinical study combined with initial clinical observations suggest that the HAV may have reduced potential for both acute and chronic infection in the setting of surgical implantation, as compared to ePTFE grafts.

Methods

Animals

All animal experiments were performed at WuXi AppTec (St. Paul, MN) using procedures approved by WuXi AppTec's Institutional Animal Care and Use Committee. Adult (~3- to 5-month old) male Sprague–Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). Animal health was evaluated upon arrival, and they were allowed to acclimatize for 5 d in individual cages before surgery. All animals selected for the study weighed at least 250 g and had no signs of clinical disease. Before and after surgery, animals received food and water *ad libitum*, daily general health evaluations, and periodic measurements of body temperature and weight.

Surgical implantation and bacterial inoculation

To evaluate the susceptibility of implanted ePTFE and HAV to bacterial infection and assess the biological host response, we chose an established animal model of material implantation and infection.[17-19] Specifically, randomly assigned adult male rats were anesthetized with isoflurane and then a ~1 cm-long subcutaneous sterile incision was made on each side of and parallel to the midline of the back to create two offset subcutaneous pockets for bilateral implantation of 1 cm^2 samples of either ePTFE (Advanta VXT ePTFE Vascular Graft, Atrium Medical Corporation) or HAV (Fig. 1). When possible, bilateral HAV and ePTFE samples were implanted within each animal. After insertion of the ePTFE or HAV material into the subcutaneous pocket, 0.25 mL of solution containing either 10^7 colony-forming units (CFUs) of gram-positive *S. aureus* (*S. aureus* [ATCC #25923]) or 10^8 CFU of gram-negative *E. coli* (*E. coli* [ATCC #25922]) bacteria were directly pipetted onto the implanted material. The inoculum dosages for each bacterium were determined in a previous pilot study to establish the lowest nonlethal bacterial concentration that generated a sustained infection after 14 d in >50% of the control (ePTFE) implants as determined by abscess formation and microbial recovery. After inoculation, incisions were closed and the animals were monitored for 14 d until explant. The 2-week implant duration was within the time frame used by other groups (10-21 d)[17,18] that used a similar rodent model. A total of 42 ePTFE and 42 HAV implantations were performed (21 *S. aureus* and 21 *E. coli* in each group).

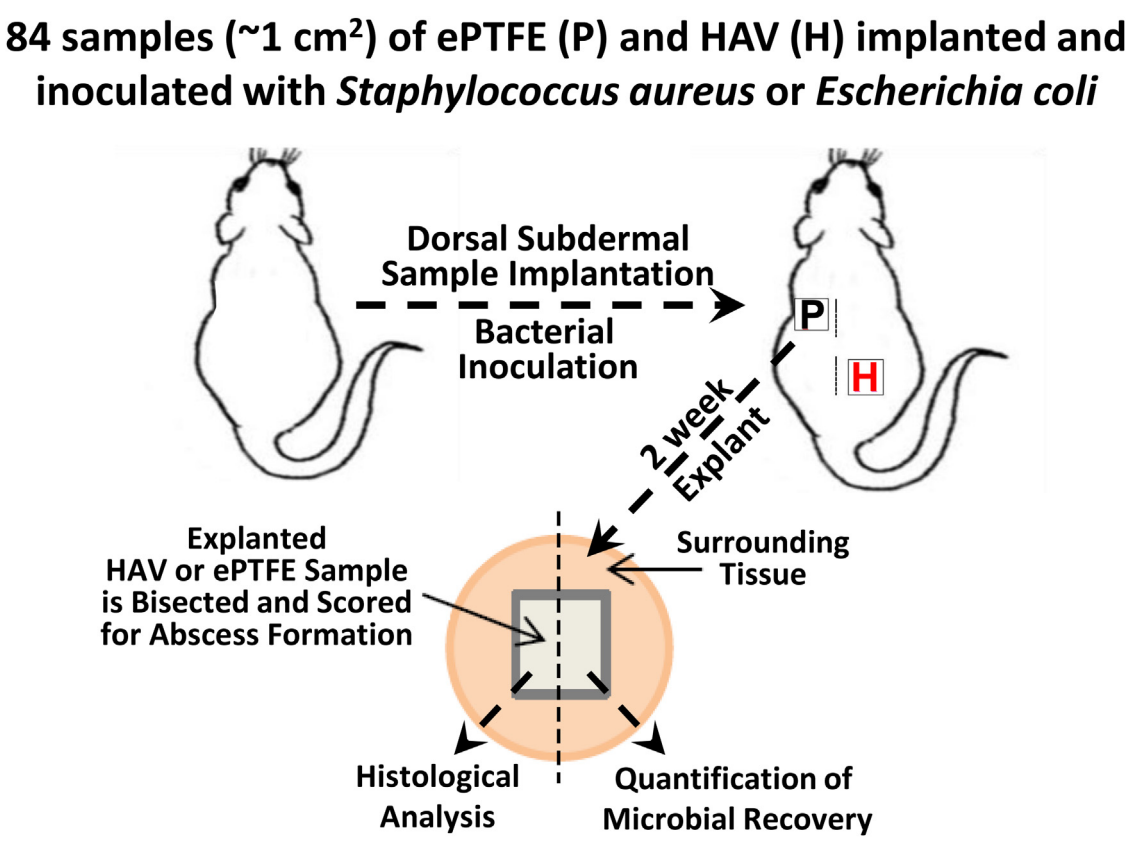

Fig. 1 – Experimental design of animal model for material implant, inoculation, and analysis at explant. (Color version of figure is available online.)

Abscess scoring and explant procedures

Fourteen days after implantation, the animals were euthanized and aseptically prepared for necropsy. The skin over each implant site was resected, and each implant site was inspected for evidence of white matter (abscess formation). Abscess formation was qualitatively scored as none (0), mild (1), moderate (2), or marked (3). The HAV or ePTFE implants were aseptically explanted encased in their surrounding tissue, placed on a sterile, nonabsorbable surface and aseptically bisected for either microbial recovery or histology.

Microbial recovery and identification

Explanted halves of HAV or ePTFE materials designated for microbial recovery were placed into preweighed containers of sterile saline with 0.5% Tween-80 followed by 15 s of vortexing and then 5 min of sonication. Resulting sonicants were transferred to a sterile container. An equal amount of Tween-80 solution was added to the original sample container and the vortex/sonication procedures repeated. Postsonication solutions were combined and serially diluted 10^{-1}, 10^{-2}, and 10^{-3} (additional dilutions were plated, when needed). Each dilution, along with the undiluted sonicant solution (0.2 mL), was then plated onto tryptic soy agar plates and incubated at $37 \pm 2^{\circ}C$ for up to 72 h. Resulting growth was quantitated as CFU/mL sonicant, and the resulting bacterial colonies were also identified via gram stain, colony morphology, and analytical profile index strips.

Statistical analysis of abscess formation and microbial recovery

Abscess formation was evaluated using an ordinal scoring system. Therefore, abscess scores are presented as median and interquartile range, and statistically significant differences ($P < 0.05$) between the ePTFE and HAV data sets were analyzed using nonparametric Mann–Whitney tests. Data for quantification of microbial recovery are presented as mean ± standard error, and unpaired two-tailed Student's t-tests were performed to evaluate statistically significant differences ($P < 0.05$) between the HAV and ePTFE data sets. Statistical analyses were performed using GraphPad Prism (GraphPad Software Inc, La Jolla, California).

Histology

Explanted halves of HAV or ePTFE for histological processing were fixed in 10% neutral-buffered formalin and embedded into paraffin for sectioning (5 μm) and staining (Vet Path Services, Mason, OH and Humacyte Inc, Research Triangle Park, NC). Hematoxylin and eosin (H&E) or bacterial gram staining was performed using standard techniques. Immunohistochemistry was performed after 20 min of heat-mediated antigen retrieval (R&D Systems Acidic AR buffer) using

antibodies for CD45 (Abcam #ab10558, diluted 1:200) or neutrophil elastase (Abcam #ab68672, diluted 1:100) applied overnight at 4°C. An anti-rabbit secondary probe and polymer HRP kit (Biocare Medical #M3R531H) followed by 3,3′-diaminobenzidine staining (Biocare Medical #BDB2004 L) was used for detection. Images were taken on an Olympus BX41 microscope equipped with an Olympus DP25 and CellSens image capture software.

Results

None of the animals exhibited signs of systemic infection, died prematurely, or were removed from the study based on regular health evaluations. Qualitative abscess scoring was performed on all 84 implant sites at the 2-week explant time point. Quantification of microbial recovery was assessed in 79 of the 84 samples because two explanted samples (one HAV and one ePTFE in the *S. aureus* groups) were inadvertently fixed in formalin, and three HAV samples within the *E. coli* group were not found within the bisected half of the explanted tissue designated for microbial recovery processing. We subsequently examined the other halves of the tissue from these three HAV explants that were fixed for histological analysis. After sectioning and H&E staining, we were able to locate the HAV in one of the three samples but it was folded over in half. This may explain why it could not be found in the other half of the bisected tissue used for microbial recovery. The remaining two HAV samples were not identified in the sections evaluated histologically but may have also been folded or not captured within the tissue regions explanted. No evidence of substantial resorption or change in thickness was observed in any of the HAV explants examined histologically.

Gross examination of the implant sites, after 2 wk, generally revealed purulent exudate surrounding control ePTFE samples (Fig. 2A and B), whereas HAV implants within the same animal had little to no abscess formation (Fig. 2C and D). Correspondingly, the median abscess formation score at explant for the HAV samples was significantly lower ($P < 0.0001$) than that of the ePTFE samples for both the *S. aureus* and *E. coli* inoculation groups (Fig. 2E). The median abscess formation scores for ePTFE and HAV samples dosed with *S. aureus* were 2 and 0, respectively, while ePTFE and HAV samples dosed with *E. coli* were 3 and 1, respectively (Fig. 2E, horizontal red lines).

Gram staining of the explanted samples revealed numerous areas of gram-positive *S. aureus* or gram-negative *E. coli* inhabiting the interstices of the ePTFE implants (Fig. 3A and C, respectively). Conversely, no bacteria were able to be identified in the HAV samples based on gram staining (Fig. 3B and D). These histological observations were further supported by the quantification of viable bacteria recovered from each explant. Explanted ePTFE samples contained significantly more *S. aureus* ($P = 0.008$) and *E. coli* ($P = 0.001$) bacteria than the HAV samples (Fig. 3E). The average CFU/mL (mean ± s.e.m.) of *S. aureus* recovered were $13.1\times10^5 \pm 4.03\times10^5$ *versus* $1.49\times10^5 \pm 9.31\times10^4$ and in ePTFE and HAV samples, respectively. Similarly, the explanted ePTFE samples inoculated with *E. coli* yielded $13.5\times10^5 \pm 3.06 \times 10^5$ CFU/mL compared to $1.36\times10^5 \pm 5.99 \times 10^4$ CFU/mL from the *E. coli*–dosed HAV samples at explant. Microbial identification confirmed that the only bacterial strains recovered from each explant were the specific strain introduced during inoculation at implantation.

H&E staining showed that the ePTFE samples were surrounded by more host inflammatory and immune cells as

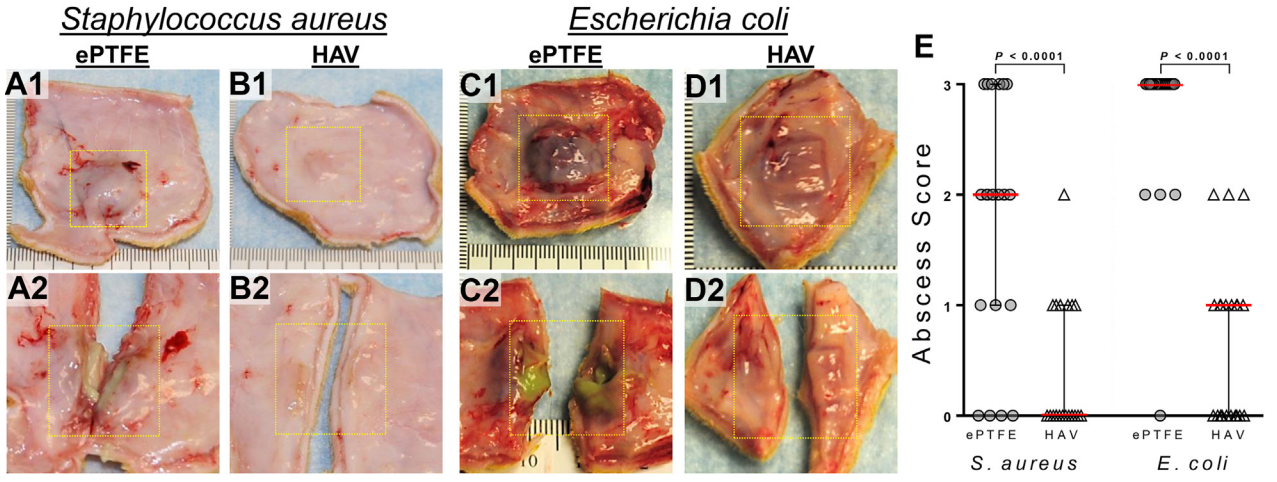

Fig. 2 – Representative images of implant material and abscess formation at 2-week explant from rat dorsal subcutaneous pockets. ePTFE (A and C) and HAV (B and D) implant sites inoculated with *S. aureus* (A and B) and *E. coli* (C and D) were resected after 2 wk within the dorsal subdermal rat infection model. Implant material was then exposed, and abscess formation was qualitatively scored for all groups (abscess scores for samples shown were 2, 0, 3, and 0 for A, B, C, and D, respectively). Samples shown from the same bacterial inoculation were explanted from the same animal (bilateral ePTFE and HAV implants). Yellow-dashed squares are 1 cm × 1 cm for reference. (E) Scatter plot of abscess formation scores showing median values (horizontal red lines) and interquartile ranges at the 2-week explant for ePTFE (gray circles, $n = 21$ each) and HAV (white triangles, $n = 21$ each) implants inoculated with *S. aureus* or *E. coli*.

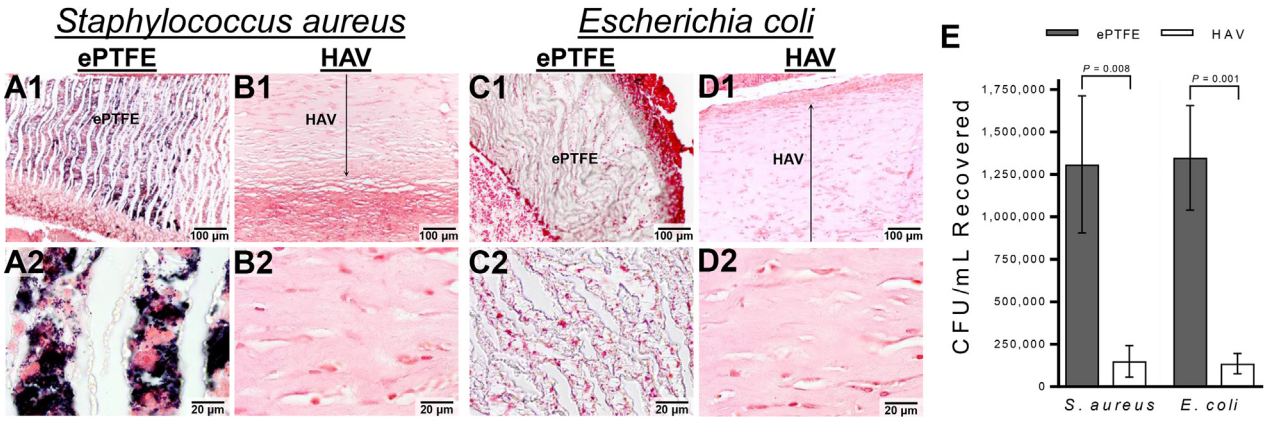

Fig. 3 – Representative gram stain images of histological sections from ePTFE and HAV explants 2 wk after implantation and bacterial inoculation with *S. aureus* (A and B) or *E. coli* (C and D) bacteria. Gram-positive *S. aureus* (dark violet circles) and gram-negative *E. coli* (dark pink rods) were identified within interstices of ePTFE (A and C) but not seen within or around HAV explants (B and D). Surrounding tissue and host cells as well as HAV proteins are stained light pink due to application of safranin counterstain (A-D). (E), Average microbial recovery at 2-week explant for ePTFE (dark gray bars, $n = 20$ and 21) and HAV (white bars, $n = 20$ and 18) material inoculated with *S. aureus* and *E. coli*, respectively.

compared to the HAV samples (Fig. 4A1 and B1). A majority of the cells adjacent to the ePTFE implants had a morphology consistent with neutrophils or polymorphonuclear (PMN) leukocytes (Fig. 4A2), whereas the majority of the host cells surrounding the HAV implant had an appearance (i.e., nuclear morphology and larger size) consistent with nonleukocytes (e.g., fibroblasts or myocytes) as shown in Figure 4B2. Importantly, we found that the HAV also supported migration of these nonleukocyte host cells from the surrounding tissue and infiltration into the HAV (Fig. 4B2). It appeared that host cells were either inhibited from infiltrating ePTFE or nonviable once they migrated into the ePTFE because despite some eosin-positive cytoplasmic staining within the interstices of the ePTFE implants (Fig. 4A2), relatively few intact nuclei could be identified within the ePTFE implants. This may suggest that some of the host cells (e.g., leukocytes or other nonimmune cells) were able to penetrate into the ePTFE matrix but then underwent cell death and karyolysis.

Immunohistochemical staining for CD45 (also known as leukocyte common antigen) confirmed that most of the cells surrounding the ePTFE material at the time of explant were indeed $CD45^+$ leukocytes (stained brown, Fig. 5A). In contrast, very few $CD45^+$ cells were found surrounding or within the HAV implants (Fig. 5B). The majority of the $CD45^+$ surrounding the ePTFE implant, many of which appeared to be neutrophils based upon a PMN appearance after H&E staining, were confirmed to indeed be neutrophils using immunohistochemical staining for neutrophil elastase (Fig. 6). Dense populations of neutrophils (stained brown, Fig. 6A) were identified within the tissue and purulent exudate surrounding the ePTFE implants; however, very few or no intact neutrophils were observed within the interstices of the ePTFE itself (Fig. 6A2). Very few neutrophils were seen in the tissue surrounding or within the explanted HAV tissue sections (Fig. 6B2). This may suggest that bacteria inoculated within the HAV implants were cleared successfully (as supported by gram staining) and efficiently by infiltrating and actively responsive neutrophils, and that host leukocytes had largely evacuated the HAV and surrounding tissues by the time of the 2-week explant.

Discussion

Synthetic ePTFE vascular grafts have been essential for modern vascular surgical practice and life-sustaining hemodialysis; however, ePTFE has also been shown to be more prone to bacterial infection than native arteriovenous fistulas,[1,20] human allografts,[21] as well as nonhuman biological materials for surgery including vascular bioprostheses,[22] biological mesh,[23] and small-intestinal submucosa.[7] Sustained infection of ePTFE grafts not only increases a patient's risk of bacteremia and sepsis but has also been shown to increase the risk of intimal hyperplasia and graft failure because of stenosis even at subclinical levels of bacterial contamination.[24,25] When infected, ePTFE vascular grafts usually do not recover and thus require surgical intervention to remove the infected and often biofilm-covered ePTFE material.[5,26] It has been suggested that as many as 35% of all synthetic arteriovenous grafts are eventually abandoned due to infection.[26] The resulting impact of infected ePTFE grafts on higher incidence of patient mortality, patient morbidity, and health care costs is substantial.[5,6,12]

The significantly higher infection rates of synthetic vascular graft material such as ePTFE may be attributed to how its composition and structure interact with both bacteria and normal host cells. Specifically, the interstitial porosity, surface tension, and electronegativity of synthetic vascular graft materials have previously been shown to attract and support bacterial adherence.[27,28] The ability of bacteria within contaminated ePTFE to persist without adequate clearance by host immune cells, resist antibiotic therapy, and produce sustained infections and biofilms[14,25,29] may be connected to

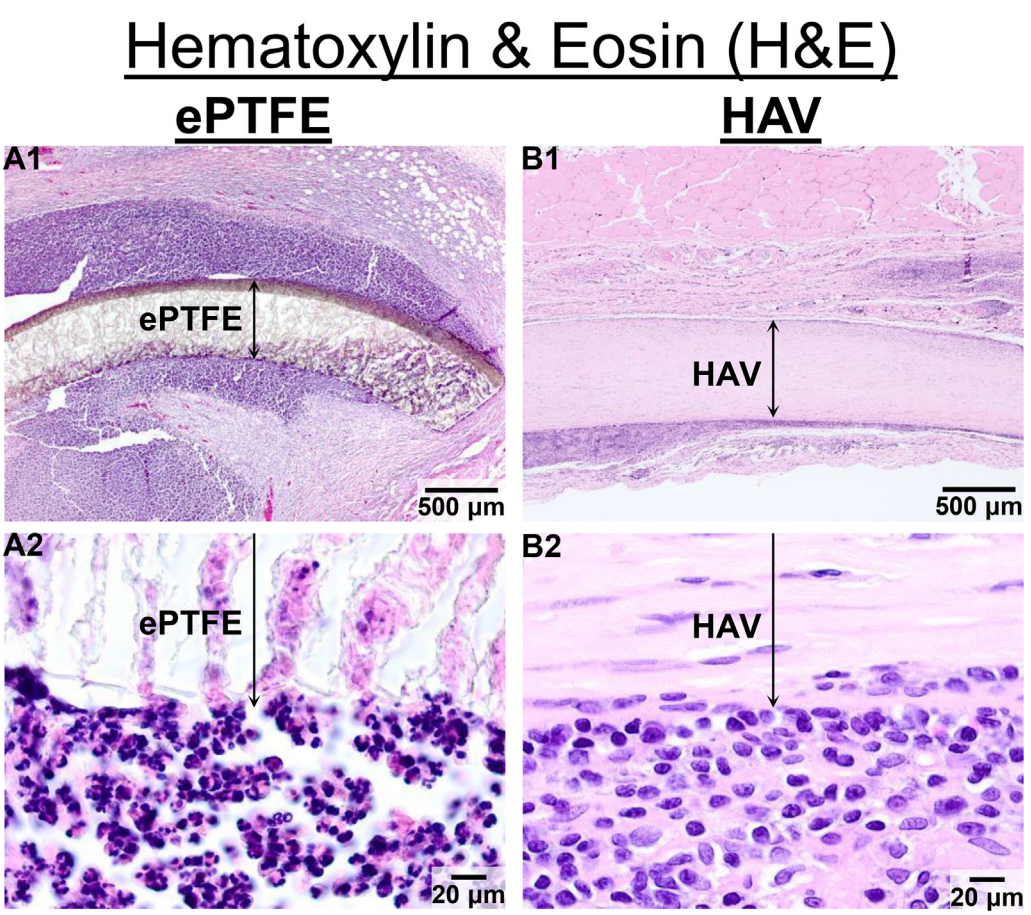

Fig. 4 – Representative images of H&E-stained histological sections from ePTFE and HAV explants 2 wk after implantation and bacterial inoculation. H&E staining revealed greater cellular response and purulent exudate in the tissue surrounding ePTFE implants (A1) compared to that of the HAV implants (B1). In addition, differences were also observed in the morphology of host cells surrounding and infiltrating into the ePTFE and HAV implants (A2 *versus* B2). Dark purple color (hematoxylin) indicates cell nuclei, while pink (eosin) labels cytoplasm and HAV collagen matrix.

interstitial colonization and subsequent inaccessibility of bacteria within the ePTFE matrix. Interstitial bacterial accumulation within the ePTFE matrix has previously been shown *in vitro*[30] as well as *in vivo* here in our study and by others.[7,25,31] Shell *et al.*[7] demonstrated that the interstitial matrix of ePTFE grafts provided a favorable environment for bacteria, including *S. aureus*, to survive and proliferate for up to 6 wk (study end-point) in a porcine iliac interposition graft model.[7]

Bacteria residing within contaminated ePTFE *in vivo* likely further evade host immune defenses because ePTFE has been shown to disrupt the chemo-stimulated migratory capabilities of neutrophils,[8] leading to poor infiltration of neutrophils into ePTFE. Moreover, it has also been shown that ePTFE binding induces rapid nonapoptotic cell death in neutrophils.[9,27] Specifically, Nadzam *et al.*[9] demonstrated that adhesion to ePTFE triggers the production of reactive oxygen species in neutrophils that led to increased neutrophil membrane permeability, decondensation of chromatin, and further ultrastructural changes to neutrophil nuclei and cytoplasm during a nonapoptotic cell death response. The scarce number of host cells with intact nuclei that we observed within the interstices of the explanted and H&E stained ePTFE tissue sections despite the presence of eosin-stained cytoplasm supports these findings. Thus, it is likely that the microporous structure and material composition of ePTFE provides a safe haven for bacterial accumulation as well as interferes with host neutrophil migration into ePTFE, thereby diminishing neutrophil-mediated clearance of bacteria. Our immunostaining for neutrophil elastase, a serine proteinase found embedded within neutrophil extracellular traps[32] that ensnare and degrade bacteria, showed that dense populations of neutrophils were recruited and remained in the tissue surrounding the contaminated implants of ePTFE, but few of these neutrophils were identified intact within the ePTFE matrix near the accumulated bacteria.

In contrast, no bacteria were able to be identified by gram staining in the explanted HAV tissue sections examined, either within or surrounding the HAV. Microbial recovery studies at 2 wk after implant did show that viable bacteria were able to be cultured from some explanted HAV samples, but all at a significantly lower bacterial load than that from ePTFE implants inoculated with either gram-positive *S. aureus*

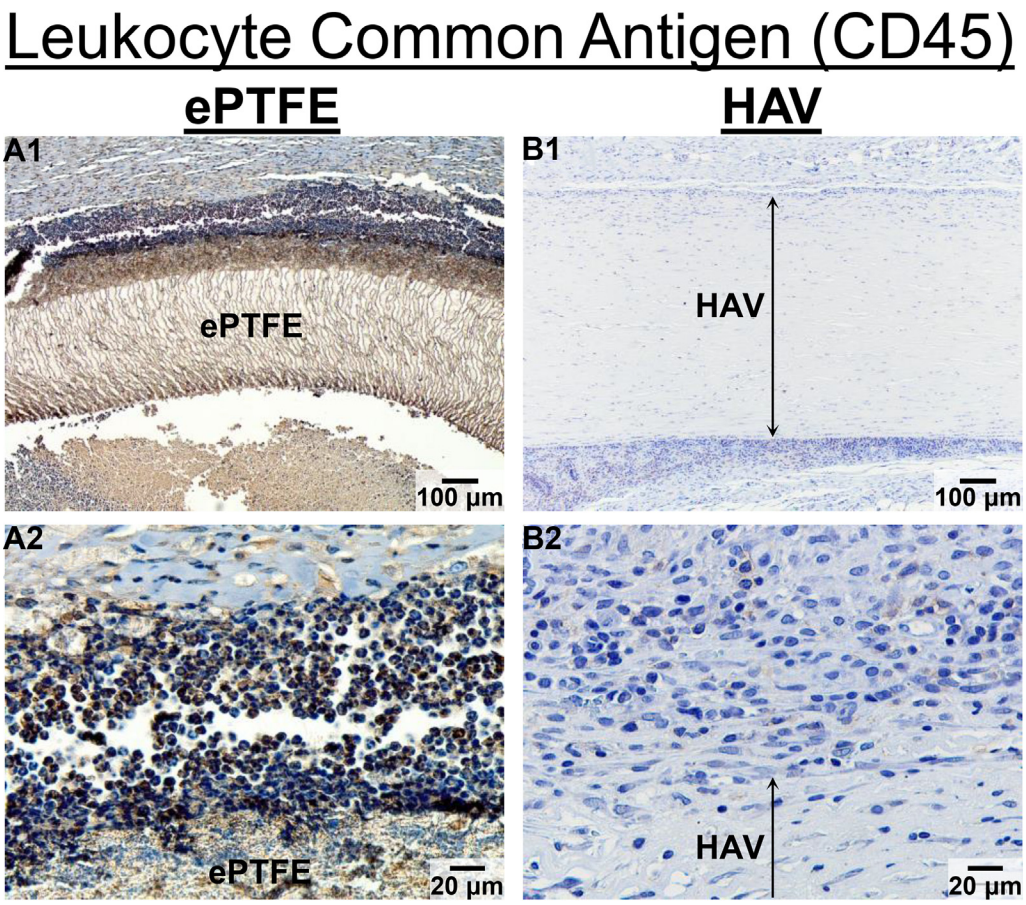

Fig. 5 – Representative images of ePTFE and HAV explants immunostained for leukocyte common antigen (CD45). ePTFE and HAV samples within surrounding host tissue were explanted 2 wk after implantation and bacterial inoculation. Histological sections were immunostained for CD45 and then counterstained with CAT hematoxylin (blue nuclei and light blue background). The tissue surrounding the ePTFE implants (A1 and A2) had a much higher density of CD45$^+$ leukocytes (cells labeled brown) compared to that of the HAV implants (B1 and B2). Only a very small fraction of the surrounding leukocytes were observed to have infiltrated or remained intact within the ePTFE matrix.

or gram-negative *E. coli*. Consequently, the HAV implants also had significantly lower average abscess formation scores than ePTFE when examined at explant. Compared to the ePTFE explants, histological staining of the HAV tissue sections identified relatively few leukocytes but numerous non-leukocyte host cells in both the tissue surrounding and within the HAV matrix at the time of explant. These results are consistent with previous preclinical[15] and clinical[16] studies of explanted HAV that demonstrate active host cell infiltration and migration through the HAV matrix. In contrast to the synthetic ePTFE polymer, the HAV is composed of a dense meshwork of human ECM proteins including tightly packed collagen fibers, fibronectin, vitronectin, and other ECM proteins.[15] We believe that the biological, native-like composition and organization of these human proteins in the tissue-engineered HAV provides a favorable environment for recellularization by host cells. The results from this study uphold that theory and further demonstrate the ability of the HAV matrix to support other natural biological processes such as neutrophil-mediated bacterial clearance during the innate immune response.

In clinical observations of 60 patients (totaling 85 patient-years of follow-up) enrolled in two phase II multicenter trials using an HAV for hemodialysis access, there have been no reported incidences of perioperative infection and only three cases of vascular access site infections after cannulation reported.[16] Of the three cases of vascular access site infections after cannulation reported, one involved an infected hematoma that was successfully treated with intravenous antibiotics. The second case involved an infected ePTFE segment that had been used to reconstruct the HAV venous anastomosis, but the infection of the ePTFE did not spread to the HAV material. In the third case, a perigraft cannulation–associated hematoma became cellulitic with positive blood cultures 18 mo after implantation and was treated by local resection and reconstruction of the adjacent HAV segment (followed by successful dialysis). To date, no HAV in the phase II clinical trials has required total explant due to infection. A phase III clinical trial is currently underway that directly compares HAV to ePTFE grafts in the setting of hemodialysis access. The findings from this trial may help elucidate the relative infection rate of the

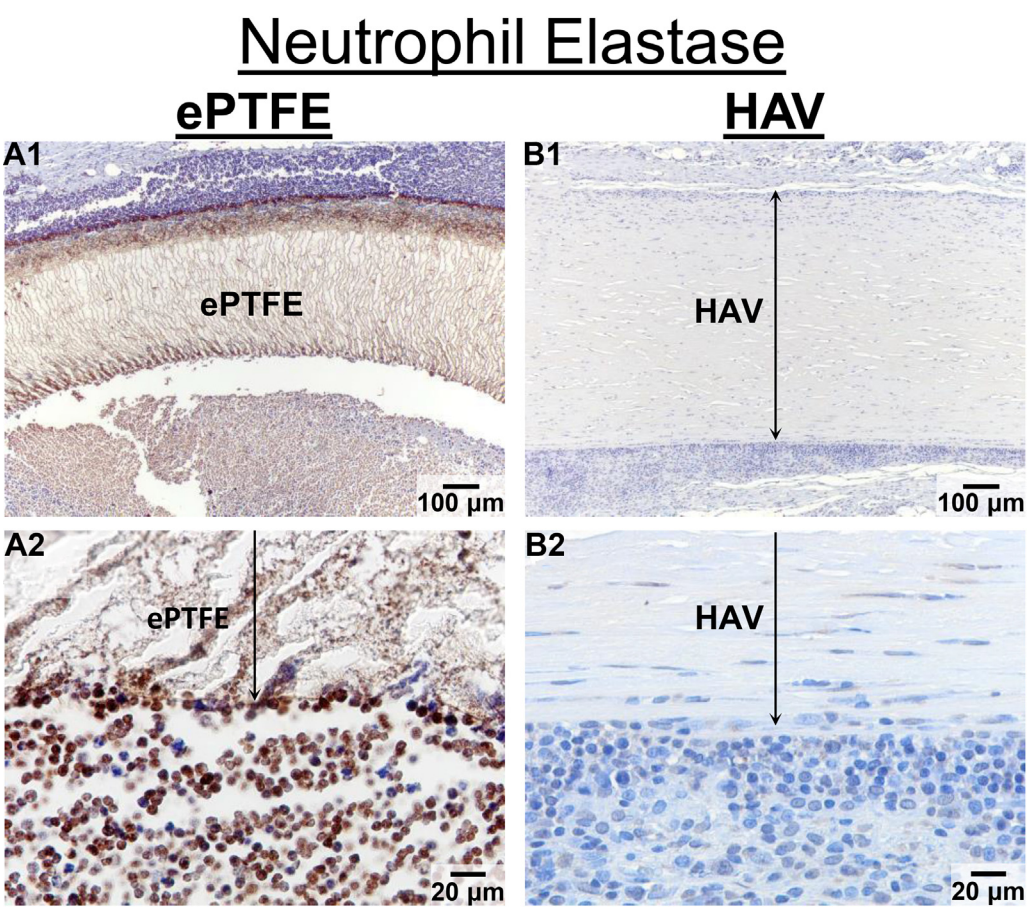

Fig. 6 – Representative images of ePTFE and HAV explants immunostained for neutrophil elastase. ePTFE and HAV samples within surrounding host tissue were explanted 2 wk after implantation and bacterial inoculation. Histological sections were immunostained for neutrophil elastase to label PMN neutrophils and counterstained with CAT hematoxylin (blue nuclei and light blue background). The tissue surrounding the ePTFE implants (A1 and A2) had a much higher density of neutrophil elastase–positive PMNs (neutrophils labeled brown) compared to that of the HAV implants (B1 and B2). Similar to that seen with CD45 staining, only a very small fraction of the surrounding neutrophils were observed to have infiltrated or remained intact within the ePTFE matrix. HAV implants and surrounding tissue contained only a few neutrophils when observed at time of explant.

bioengineered HAV compared to that of synthetic ePTFE grafts in patients.

Conclusion

This study demonstrated that the HAV shows superior resistance to bacterial infection compared to ePTFE in an *in vivo* animal infection model likely due to its native ECM composition that better supports natural host cell and tissue processes (e.g., the innate immune response). In addition to its off-the-shelf availability, mechanical integrity, and capacity for remodeling by host cells after implantation,[15,16] the low susceptibility to bacterial infection observed in the HAV from this preclinical study, as well as that from previously published clinical trials,[16] may also establish a rationale for its preferred use in surgical locations that require high resistivity to bacterial infection, such as vascular access for dialysis, patients with intraabdominal contamination or traumatic vascular injuries.

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Authors' contributions: R.D.K., H.L.P., L.E.N, J.H.L, and S.L.M.D. designed experiments, performed data analysis, wrote, and revised the manuscript; R.D.K. and M.S.M. conducted histological experiments and compiled data; and S.L.M.D., J.H.L, and L.E.N. obtained funding.

Disclosure

The authors have relationships with Humacyte, Inc that include board membership, ownership of stock, or stock options, employment, and/or consultant agreements.

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