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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).