Prevention of perioperative vascular prosthetic infection with a novel triple antimicrobial-bonded arterial graft

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Objective: **Previously, we investigated a locally developed technique of bonding arterial grafts with three antimicrobials to protect against early (within 2 weeks) perioperative bacterial contamination encountered occasionally during aortic graft prosthetic reconstruction. Vascular graft infections are classified by their appearance time (early [<4 months] vs late [>4 months] after graft implantation), degree of incorporation into the surrounding vessel wall, connectivity to the postoperative wound, and extent of graft involvement. In the current phase of testing, we evaluated the ability of our novel triple antimicrobial-bonded graft to prevent infection in the first 8 weeks after implantation.**

Methods: **In nine Sinclair miniature pigs, we surgically implanted a 6-mm vascular Dacron patch graft in the infrarenal abdominal aorta. Five pigs received grafts chemically bonded with a 60-mg/mL solution of rifampin, minocycline, and chlorhexidine, and four pigs received unbonded grafts. Before implantation, the five bonded grafts and three of the unbonded grafts were immersed for 15 minutes in a 2-mL solution containing 1-2 × 10^7 colony-forming units (CFUs)/mL of *Staphylococcus aureus* (ATCC 29213); the fourth unbonded graft served as a control.**

Results: **At week 9, all of the grafts were explanted. All *S aureus*-inoculated bonded grafts (n = 5) showed no bacterial growth. The unbonded, uninoculated graft (n = 1) showed low-level bacterial growth (<1.2 × 10^3 CFUs); *S cohnii* spp *urealyticus*, but not *S aureus*, was isolated, which suggested accidental direct perioperative contamination. Two pigs that received *S aureus*-inoculated, unbonded grafts were euthanized because of severe *S aureus* infection (<6.56 × 10^8 CFUs per graft). Results of histopathologic analysis were concordant with the microbiologic findings. Most intergroup differences were observed in the inflammatory infiltrate in the aortic wall at the site of graft implantation. In all pigs that received bonded grafts, Gram staining showed no bacteria.**

Conclusions: **Our triple-bonded aortic graft prevented perioperative aortic graft infection for at least 8 weeks in a porcine model. The synergistic antimicrobial activity of this graft was sufficient to prevent and/or eradicate infection during that period. Further studies are needed to assess the graft's ability to combat early-onset vascular graft infection for up to 4 months. (J Vasc Surg 2015;■:1-10.)**

Clinical Relevance: **This novel vascular graft was developed to combat devastating periprosthetic aortic infections associated with major arterial reconstructive surgery. When the graft's ability to provide extended protection has been verified, the device could be recommended for in situ replacement of infected grafts and possibly for routine primary implantation—especially in immunocompromised patients and those with heavily contaminated fields such as perforating body cavity wounds, hostile abdomen, and redo procedures. The graft's preventive effects against *Staphylococcus aureus* could be extended for prevention of other, less common causes of perioperative infections, such as *Pseudomonas aeruginosa*, other members of the *Staphylococcus* family, and fungi.**

Aortic graft infections pose limb- and life-threatening complications in patients who undergo arterial reconstruction. The incidence of aortic graft infection is 0.6% to 3%,[1] with a mortality of ≤40%, reinfection rate of ≤18%, and amputation rate of approximately 25%.[2,3] These outcomes are associated with "open" repairs and endovascular techniques.[4]

The time of onset of arterial graft infection can be early or late. In early-onset infections (<4 months postoperative),

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Author conflict of interest: I.R. is a coinventor on two patents associated with antibiotic-coated devices. These patents are the property of the University of Texas MD Anderson Cancer Center and Baylor College of Medicine. Both patents were licensed to Cook Critical Care, American Medical Systems, Biomet, and TyRx with royalty rights to the institutions and inventors involved. One other patent has been licensed to Akorn. I.R. is also on the speakers' bureau for, and has received grants from, Cook Inc.

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the main features might be toxicity with fever and leukocytosis. Other clinical features might include septicemia, wound infection, unexpected thrombosis, and graft dehiscence with false aneurysm or profuse anastomotic bleeding.[4] In late-onset infections (>4 months postoperative), the clinical picture might be more subtle and the body temperature normal, even though complications such as false aneurysm, bleeding, fistulas, or remote infections might be present.[5-7]

Multiple pathogens, including Gram-positive and Gram-negative bacteria and (rarely) fungi, have been implicated in aortic graft infections. Early infection is usually monomicrobial, the most common cause being *Staphylococcus aureus.* Late infection is generally polymicrobial,[8] *S epidermidis* often being the responsible pathogen.[9] Methicillin-resistant *S aureus* (MRSA) is an emerging organism that causes aggressive infection and increases mortality.[4]

Regardless of the causative organism or route of infection, a common pathogenetic mechanism is involved. First, body fluids (blood, serum, lymph) surrounding the graft are inadequately perfused.[5] The poorly vascularized, perigraft fluid collections help bacteria proliferate; even in low numbers, these bacteria can trigger a graft infection. However, if there is no earlier perigraft contamination, the graft can link to adjacent tissues and obliterate the perigraft space, and become more resistant to infection.

The classic management strategy is total excision of the graft, extensive debridement of infected material, and extra-anatomic bypass to a distal vessel.[10] More recently, surgeons have adopted in situ reconstruction, using several types of conduits, including autogenous veins, coated synthetic material, and cryopreserved allografts.[11] Our group has often performed in situ replacement of the thoracic aorta with a Dacron graft wrapped with a greater omental flap, which necessitates lifelong antibiotic therapy.[12] This maneuver avoids aortic stump "blow-out" if the thoracic or abdominal aorta is sewn shut. Despite elaborate planning and execution, complications of graft infection are devastating. Hence, the primary goal should be prevention of such infection.

In an earlier 2-week pilot study, we investigated Dacron grafts that were coated with a combination of chlorhexidine, rifampin, and minocycline (M/RCHX), inoculated with *S aureus,* and implanted in the infrarenal aorta of Sinclair miniature pigs.[13] In the current study, we hypothesized that bonding three antimicrobial agents to the graft would prevent or minimize perioperative graft infections in a pig model for at least 8 weeks.

METHODS

These studies followed the guidelines of the Animal Welfare Committee at The University of Texas Health Science Center, Houston.

Animal model. Nine 8-month-old, 30 to 35 kg Sinclair miniature swine (Sinclair Bio Resources, Columbia, Mo) were divided into three groups (Fig 1):

- Group one (n = 1): This single pig (control animal) received an unbonded, uninoculated graft.
- Group two (n = 3): These pigs received unbonded, inoculated grafts. Before implantation, the grafts were immersed for 15 minutes in a 2-mL bacterial solution containing 1-2 × 10^7 colony-forming units (CFUs)/mL of *S aureus* (ATCC 29213). We immersed the graft before implantation to mimic accidental graft contamination during surgery and provide a uniform, reproducible model as opposed to the local contamination method. The bacterial count in the inoculum was high enough to ensure that the soaked graft would be heavily inoculated with bacteria and that the bonded graft would be tested under conditions similar to severe aortic graft infection.
- Group three (n = 5): These pigs received grafts bonded with triple antibiotics (M/RCHX) and inoculated with bacteria similar to those in group 2.

Surgical protocol. Each pig was given an intramuscular injection of a 1-mL mixture of zolamine (5 mg/mL), butorphanol (1 mg/mL), ketamine (10 mg/mL), and xylazine (2 mg/mL). The animal was then intubated, and anesthesia was maintained with isoflurane inhalation (0.5%-3%) throughout the procedure. For analgesia, we used 0.25% bupivacaine (1 mL/kg) before the incision was made and buprenorphine (0.01-0.02 mg/kg, subcutaneously or intramuscularly) postoperatively. A fentanyl transdermal patch (75-100 μg/kg/h) was placed immediately after surgery.

Surgical details were described in the pilot phase of this study.[13] Briefly, the infrarenal abdominal aorta was exposed via a retroperitoneal incision in the left flank and was cross-clamped. A 20- × 6-mm Dacron roof patch was sutured to the aortotomy incision with a continuous fine monofilament polypropylene suture. Routine sterile surgical technique was used. At the end of the procedure, the animals were extubated and followed up in our large-animal veterinary care unit for 9 weeks. They were monitored for clinical and laboratory signs of infection. No antibiotic agents or blood transfusions were administered. Autopsy examination was done for pigs 2 and 3 at postoperative weeks 1 and 2, respectively, and during week 9 for the rest of the animals. The aorta was excised, and the grafts were processed for microbiologic and histopathologic studies.

Grafts. Knitted Dacron vascular grafts (Terumo Cardiovascular Systems Corp, Ann Arbor, Mich) were impregnated with M/RCHX using a modified proprietary method that involved precoating the grafts with a chlorhexidine solution and soaking them in a solvent solution of minocycline and rifampin. The doses of rifampin and minocycline (30 and 15 mg/mL, respectively) were selected to ensure that the largest graft contained less than the normal daily dose of both drugs used clinically. Before implantation, each graft was soaked for 15 minutes in a bacterial solution of *S aureus* (ATTC strain 29213).[14,15]