# The Role of Antibiotic Bonding in the Prevention of Vascular Prosthetic Infections

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**Oxacillin, a negatively charged antibiotic, was bonded to polytetrafluoroethylene (PTFE) grafts using the cationic surfactant, benzalkonium chloride. Control PTFE grafts and bonded grafts prepared at room temperature and at 90 C were placed in the infrarenal aorta of dogs and challenged by local contamination with *Staphylococcus aureus*. Bonded grafts were superior to controls in negative cultures, patency, and survival. The possible role of antibiotic bonding in the prevention of vascular prosthetic infection is discussed.**

THE EXCESSIVE MORBIDITY AND MORTALITY associated with vascular prosthetic infections has led the overwhelming majority of surgeons to use prophylactic systemic antibiotics. Though the latter have been shown to be efficacious in a randomized clinical trial, they do not completely eliminate prosthetic infection and subject all patients to some risk.[1] In 1979, our laboratory described a graphite-benzalkonium-oxacillin surface that exhibited significant antibacterial activity *in vitro*.[2] In a later report we were able to delete the graphite and described a polytetrafluoroethylene-benzalkonium-oxacillin surface.[3] Recently, the reaction conditions and biochemical characteristics of the bonding process have been clarified.[4] In this report we describe our initial experience with a polytetrafluoroethylene-benzalkonium-oxacillin surface placed in the canine aorta and challenged by local contamination with *Staphylococcus aureus*.

## Materials and Methods

Vascular grafts were obtained from W. L. Gore and Associates, Inc. (Flagstaff, AZ). All grafts were 6 mm internal diameter polytetrafluoroethylene. Oxacillin was obtained from the Bristol Corporation (Syracuse, NY). Benzalkonium chloride was obtained from Matheson, Coleman and Bell (Norwood, OH).

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### *Binding of Benzalkonium and Oxacillin*

Grafts were cut into 2 cm lengths and submerged for one hour in 25% benzalkonium chloride at room temperature or at 90 C. The use of a group of grafts prepared at elevated temperatures was based on the observation that greater amounts of antibiotic are bound to polytetrafluoroethylene grafts under these conditions.[4] Grafts were drained and air dried. They were then washed by short vortexing in five changes of water and autoclaved (132 C at 27 psi for 3 minutes) in screw-cap glass test tubes. After cooling, grafts were soaked for 30 minutes in 1 mg of oxacillin per centimeter of graft. Thus, each 2 cm Gore-Tex® graft was soaked in 2 mg of oxacillin. Grafts were subsequently washed in five changes of water and drained.

### *Surgical Procedure*

Adult mongrel dogs (20–25 Kg) were anesthetized with thiamylal, nitrous oxide, and halothane. Group I animals received standard Gore-Tex grafts placed in the infrarenal aorta using 5-0 prolene suture. Group II animals received Gore-Tex grafts bonded to oxacillin with 25% benzalkonium chloride prepared at room temperature. Group III animals received Gore-Tex grafts bonded to oxacillin using 25% benzalkonium chloride prepared at 90 C. There were 15 animals in each of the three groups. At the completion of the second anastomosis, $1 \times 10^7$ *Staphylococcus aureus* was instilled over the graft in a 1 ml volume. Animals were then housed in individual cages and observed until death or sacrificed six weeks after implantation.

### *Evaluation of Grafts*

At autopsy or sacrifice, gross anatomic findings were noted. Grafts were incised longitudinally before cross-clamping to evaluate patency. The aorta and surround-

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ing tissue were then excised en block. Cultures of the graft bed and graft lumen were obtained. In addition, one half of the graft and adjacent aorta was cultured, and histologic evaluation was performed on the other half of each specimen. Trichrome, Brown and Brenn, hematoxylin and eosin, and fibrin stains were performed.

### *Concentration of Oxacillin Bonded to Grafts*

The concentration of oxacillin used in treatment of grafts was 1.0 mg per centimeter of graft. Since this was followed by liberal washing, the amount remaining on the graft could only be determined indirectly. Utilizing $^{14}$C-penicillin as a model singly charged anionic antibiotic, grafts bonded at room temperature contained $0.6 \pm 0.05$ μg/cm antibiotic, while those bonded at 90 C contained $1.2 \pm 0.1$ μg/cm antibiotic. When the grafts were used in an *in vitro* bioassay against *Staphylococcus aureus*, they caused zones of inhibition similar to those obtained with standard oxacillin discs containing 1.0 μg oxacillin (room temperature) and 1.5 μg oxacillin (90 C). The methodology for these determinations is reviewed elsewhere.[4]

## Results

### *Control Versus Bonded Grafts*

Results of all parameters in control and treatment groups are summarized in Table I. Results were evaluated by Fisher's exact test, and the data in each treatment group were compared separately with those of the control group. Room temperature grafts were superior to controls in survival ($p < 0.04$), patency ($p < 0.01$), and negative cultures of graft or retroperitoneum ($p < 0.01$ and $< 0.03$, respectively). However, when the presence or absence of bacteria in the graft matrix was evaluated histologically, these grafts failed by the barest of margins to demonstrate statistical significance when compared with those of the control group.

Grafts bonded at 90 C showed greater differences when compared with controls. Survival was superior ($p < 0.009$), and patency was highly significant ($p < 0.001$). Results of graft and retroperitoneal cultures were also highly significant ($p < 0.0001$ and $p < 0.01$, respectively). Finally, grafts bonded at 90 C were also superior to controls in the absence of bacteria histologically ($p < 0.01$).

### *Relationship of Infection, Culture, and Patency*

Since one half of each graft was evaluated histologically for the presence of bacteria and the other half cultured, the effect of treatment on both of these parameters of infection was evaluated, and results were analyzed statistically by Fisher's exact test. When all animals, regardless of treatment, were evaluated, there was a strong correlation between a positive culture and the presence of bacteria histologically. Thus, of 17 grafts in which bacteria were present histologically, only one had negative cultures. Conversely, of the 28 grafts in which bacteria were absent histologically, only five had positive cultures ($p < 0.0001$). The same pattern pertained when each group was examined separately, though p values were less significant because the number of animals was smaller.

The relationship between bacteria, culture, and patency was also examined. In the control group, 12 animals had positive cultures, and only one of these grafts was patent. Nine animals had bacteria in the graft histologically, and all of these grafts were thrombosed. Three animals in the control group had negative cultures and negative histology. Only one of these grafts was thrombosed. These relationships were altered by treatment. Thus, in the room temperature grafts, there were five animals with positive cultures who had patent grafts and four animals with bacteria present histologically and patent grafts. Nine animals had negative histology, but four of these grafts were thrombosed. On the other hand, in the grafts prepared at 90 C, four with

TABLE 1. *The Effect of Antibiotic Bonding on Infection, Patency, and Survival*

| | | Control | Bonded (RT) | P Value vs Control | Bonded 90C | P Value vs Control |
|---|---|---|---|---|---|---|
| Culture | | | | | | |
| Graft | +/Total | 12/15 | 5/15 | <0.01 | 0/15 | <0.0001 |
| Site | +/Total | 11/14 | 5/15 | <0.03 | 4/15 | <0.01 |
| Histology (Bacteria) | | | | | | |
| | +/Total | 9/15 | 6/15 | <0.07 | 2/15 | <0.01 |
| Patency | | | | | | |
| | P/Total | 3/15 | 10/15 | <0.01 | 12/15 | <0.001 |
| Survival | | | | | | |
| | A/Total | 9/15 | 14/15 | <0.04 | 15/15 | <0.009 |

RT = Room Temperature; P = Patent; A = Alive.

positive cultures had patent grafts, and two grafts with positive histology were patent. Conversely, of the 11 grafts with negative cultures and the 13 grafts with negative histology, three were thrombosed.

Finally, in this study a control group treated only with benzalkonium was not utilized. Previous studies from this laboratory have demonstrated little effect of benzalkonium alone against *Staphylococcus aureus* either by *in vitro* or *in vivo* analyses.[3,4] The *Staphylococcus aureus* used in these experiments was obtained from the Microbiology Department of Rutgers Medical School. The original source of the bacterium was a wound culture. The minimum inhibitory concentration for oxacillin was 0.1 μg/ml. The minimum inhibitory concentration for benzalkonium chloride was 0.8 μg/ml. A checkerboard susceptibility study was performed to evaluate the effect of the combination of oxacillin and benzalkonium. This resulted in a minimum inhibitory concentration of 0.075 μg oxacillin and 0.2 μg benzalkonium, suggesting an additive, but not synergistic, effect. When bonded grafts, control grafts, benzalkonium-treated grafts, and oxacillin-soaked grafts were evaluated in a microbiologic assay against *Staphylococcus aureus*, these data were corroborated. Thus, oxacillin-bonded grafts produced zones of inhibition far superior to those of controls and to grafts treated with benzalkonium or oxacillin alone. Finally, when benzalkonium-treated grafts were contaminated by local contamination with *Staphylococcus aureus* in a rat animal model, the benzalkonium-treated grafts were similar to controls in their inability to prevent infection, while oxacillin-bonded grafts were statistically superior.

## Discussion

The eradication of prosthetic infections is clearly an important goal of vascular surgical research. Though Moore's group has established the bacteremic model experimentally, local contamination at the time of operation appears to be the most common cause of graft sepsis.[5] Prophylactic systemic antibiotics to be effective must be given in large doses, since only a small percentage will be available at the site of surgical dissection. In addition, the graft matrix, a foreign body with no intrinsic blood supply, is the likeliest site to harbor bacteria and is unlikely to be easily impregnated with systemic antibiotics. Thus, attempts to place the antibiotics at the site of contamination are logical models of infection prophylaxis. Unfortunately, irrigated or soaked antibiotics elute too rapidly from the graft and operative field to be uniformly effective.[6] Previous work in our laboratory has shown the polytetrafluoroethylene-benzalkonium-oxacillin surface to exhibit superior antibacterial activity *in vitro* when compared with grafts untreated with antibiotics and those simply soaked in oxacillin or benzalkonium. The data presented herein extend that experience to an animal model in which grafts were placed in the canine aorta and challenged by local contamination with *Staphylococcus aureus*.

The concentration of antibiotic utilized in the pretreatment of grafts is very small. Only one milligram per centimeter of graft was employed to correlate closely with parallel *in vitro* studies that have been reported elsewhere.[4] Small concentrations allow more precise radiochemical evaluation of bound and unbound antibiotic. Recent studies have shown that tenfold increases in the pretreatment concentration of antibiotics result in greater bonding.[3] However, precise radiochemical measurement of tightly bound antibiotic is only achieved when grafts are thoroughly washed after pretreatment. Unwashed samples contain a large amount of antibiotic, which rapidly elutes from the surface. The concentration of antibiotic present on these grafts after washing was evaluated by comparing zones of inhibition produced by grafts with those obtained with oxacillin discs in an *in vitro* bioassay. In addition, corroboration was sought in comparison to radiochemical studies using $^{14}$C-penicillin measured by liquid scintillation counting. We believe this analogy is sound, since penicillin and oxacillin are both singly negatively charged compounds. However, the data is clearly inferential and requires corroboration with radiolabeled oxacillin and other anionic antibiotics.

The method of contamination used in these studies consisted of local contamination at the completion of the anastomoses. Admittedly, the procedure may be criticized by those who favor the bacteremic model or those who would argue for contamination prior to completion of the anastomosis. Nevertheless, the size of the inoculum of *Staphylococcus aureus* represents, in our opinion, a valid experimental challenge to the efficacy of these grafts in preventing infection. Finally, the use of antibiotics bound to an implantable prosthesis challenges studies that demonstrate the necessity of administering parenteral antibiotics preoperatively to achieve adequate blood levels at the time of surgical dissection. On the other hand, antibiotic bonding places the antibiotic at the site of dissection and may well turn out not to require the serum levels necessary with parenteral administration.

Evaluation of graft infection was accomplished by histology and culture. There was a very strong statistical relationship between these parameters. This was true when all animals, regardless of treatment, were evaluated and when each group was evaluated separately. Histologic evaluation of the grafts for the presence of bacteria is fraught with error caused by dead bacteria

and sampling. These data are included because of our interest in the ability of the bonding process to eliminate bacteria from the graft matrix. Though this may be adequately evaluated by culture techniques, it is conceivable that a graft harboring small numbers of bacteria may yield negative results when only cultures are performed. When treatment groups were compared with controls, both bonded grafts demonstrated superior performance. Those bonded at room temperature were statistically significant in their effect on survival, patency, and culture when compared with those of the control group. However, the difference in histologic absence of bacteria fell just short of statistical significance. On the other hand, grafts bonded at elevated temperature, which contained greater concentration of surfactant and antibiotic, are statistically significant when compared with controls in all parameters.

There were some noteworthy discrepancies in the treatment groups between infection and patency. In the room temperature group, five of the six grafts considered infected were patent, and four of the nine grafts considered sterile were thrombosed. In the group bonded at elevated temperature, all four grafts characterized as infected were patent, and three of 11 sterile grafts were thrombosed. Whether these results suggest that treated grafts remain patent despite infection or that treated grafts are thrombogenic is uncertain. Obviously, the latter is of greater concern, since the cationic surfactant remains on the graft for longer periods than does the antibiotic.

The short period of implantation used in these studies does not allow complete evaluation of the effect of antibiotic bonding on healing. Light microscopy of the specimens retrieved from these animals shows little difference between controls and antibiotic-bonded grafts in the development of neo-intima. A more recent study using light and electron microscopy in uninfected grafts shows no significant effect on healing in general and neo-intimazation. These data will be the subject of a future report.

Our studies support the efficacy of antibiotic bonding in the prevention of vascular prosthetic infections. The bonding of oxacillin to polytetrafluoroethylene grafts using benzalkonium chloride does not, however, completely eliminate graft infection, at least in this model. Admittedly, small amounts of antibiotics were used in the pretreatment process, and there is reason to believe that increasing the concentration used in pretreatment will be uniformly effective. Should that not be the case, it remains likely that the combination of systemic or locally irrigated antibiotics with antibiotic bonding will produce an infection-resistant vascular prosthesis.

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