Gelatin-Sealed Dacron Graft is not more Susceptible to MRSA Infection than PTFE Graft

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Objectives. The purpose of this experimental study was to compare the susceptibility of gelatin-sealed Dacron and PTFE prostheses to infection by MRSA.

Design. Prospective, randomized, controlled animal study.

Materials and Methods. Graft infections were established in the subcutaneous tissues of 60 female Spraque-Dawley rats by the implantation of gelatin-sealed Dacron or PTFE prostheses followed by topical inoculation with methicillin-resistant Staphylococcus aureus. The study groups were as follows: (1A) uncontaminated gelatin-sealed Dacron group, (1B) untreated contaminated gelatin-sealed Dacron group, (1C) contaminated gelatin-sealed Dacron group with intraperitoneal teicoplanin treatment, (2A) uncontaminated PTFE group, (2B) untreated contaminated PTFE group, and (2C) contaminated PTFE group with intraperitoneal teicoplanin treatment. The grafts were removed after 7 days and evaluated for infection by counting the number of adherent bacteria on the graft material after rinsing and sonication. The perigraft tissue was harvested for histopathological study. To investigate the existence of any infection, blood samples were collected by cardiopuncture for a culture analysis.

Results. No significant difference in bacteria counts was observed between gelatin-sealed Dacron and PTFE grafts. In groups 1A and 2A, there was no infection detected. The bacterial counts for MRSA were $7.4 \times 10^5$ in group 1B and $8.6 \times 10^5$ in group 2B. There was also no infection in groups 1C and 2C. While the difference between group 1B and 2B was not significant ($p > .05$), bacterial counts in group 1B or 2B were significantly higher than those in other groups. Blood cultures were only positive in four rats in group 1B and in two rats in group 2B. The severities of the inflammation of the perigraft tissues was low in groups 1A and 2A, high in groups 1C and 2C, and between the range from low to moderate in groups 1B and 2B.

Conclusion. The susceptibility of gelatin-sealed Dacron to bacterial infection was not higher than that of PTFE.

Keywords: Dacron; PTFE; Vascular graft infection; Susceptibility to infection.

Introduction

The use of vascular prostheses has dramatically changed the surgical outcomes in vascular patients. However, vascular prostheses are associated with a number of complications, including bacterial infection. Vascular graft infections are relatively uncommon and have an incidence of 0.5–6%.[1,2] Despite their infrequency, vascular graft infections are associated with significant morbidity and mortality and exist as a dreaded complication of vascular surgery.[1–3]

Currently, the commonly used prosthetic grafts in vascular surgery are polyethylene terephthalate (Dacron) and polytetrafluoroethylene (PTFE). They have been made from polymers and used in clinical settings for about 40 years. There is general agreement that Dacron grafts are associated with a greater risk of thrombosis and infection. This belief is based on an earlier in vitro experimental study which suggests that PTFE grafts are less susceptible to infection than Dacron grafts.[4] However, the preference of PTFE over Dacron is not due to clinical but rather experimental evidence. Most clinical studies have found no significant difference in these grafts.[5–8] Moreover, it has been reported that new generation Dacron prostheses seem to be superior to PTFE prostheses.[9]

In addition, no in vivo experimental studies comparing the use of Dacron with that of PTFE were found by means of a review of the literature, and it
was not determined whether Dacron is more prone
to develop infection when compared to PTFE in an
in vivo experiments. The present study was planned
to test and compare the susceptibilities of Dacron
and PTFE grafts to MRSA infections using a standard-
ized well characterized experimental rat model.10

Material and Methods

Organism

The strain of Methicillin-resistant Staphylococcus
aureus (MRSA) used in this study was isolated from
a clinical specimen submitted for routine bacteriolog-
ical investigation to the Department of Microbiology,
Faculty of Medicine, Kahramanmaras Sutcu Imam
University, Turkey. Commercially available Staphylo-
coccus aureus ATCC 25923 was used as the control
strain of methicillin susceptibility test. The organism
was incubated overnight on sheep blood agar. The
numbers of the bacteria were determined by turbi-
dimetry and confirmed by the culture results.

Drugs

Teicoplanin (Targocid) used in this study was
obtained from Aventis Pharma (Istanbul, Turkey). Tei-
coplanin was dissolved in sterile distilled water at
a concentration of 4 mg/ml. Solutions were made
fresh on the day of the experiments.

Susceptibility testing

The antimicrobial susceptibility of the strains were
determined by using the microbroth dilution method,
according to the procedures outlined by the National
Committee for Clinical Laboratory Standards. The min-
imum inhibition concentration was taken to be the low-
est antibiotic concentration at which observable growth
was inhibited. Experiments were performed in triplicate.

Rat model

The study was approved by the Ethics Committee of
Faculty of Medicine, Kahramanmaras Sutcu Imam
University, Turkey. Sixty adult female Sprague-
Dawley rats (weight range, 200 to 250 g) were studied.
All rats had free access to standard rat chow and tap
water. The rats were divided into two main groups.
Group 1 received gelatin-sealed Dacron graft and
Group 2 received PTFE graft. Both group included
a control group with no graft contamination and no
antibiotic prophylaxis (group 1A and 2A), one
contaminated group that did not receive antibiotic
prophylaxis (group 1B and 2B), and one contaminated
group that received perioperative 10 mg/kg intraperi-
toneal teicoplanin treatment (group 1C and 2C). Teico-
planin administration began from the initiation of
surgery and continued twice a day in the following
72 h. All operations were performed under sterile con-
ditions. Each group included 10 animals. The rats were
anesthetized with intraperitoneal ketamine (10 mg/kg)
and xylazine (3 mg/kg). The hair on the backs was
shaved, and the skin was cleaned with 10% povidone-
iodine solution. One subcutaneous pocket was made
on the right side of the median line by a 1.5 cm incision.
Aseptically, 1 cm² sterile gelatin-sealed Dacron grafts
(Gelseal; Sulzer Vascutek Ltd, UK) or PTFE grafts
(Gore-Tex; W.L. Gore & Associates Inc, USA) were
implanted into the pockets. The pockets were closed
by 5/0 polypropylene sutures (Dogsan, Turkey), and
a sterile saline solution (1 mL) containing the MRSA
strain at a concentration of 2 × 10⁷ CFU/mL was inoc-
ulated onto the graft surface using a tuberculin syringe
to create a subcutaneous fluid-filled pocket (group 1B,
2B, 1C and 2C). The animals were returned to individ-
ual cages and thoroughly examined daily. They were
euthanized by an overdose of anesthesia after seven
days following implantation. Under sterile conditions,
all grafts were explanted for bacteriological study.
The perigraft tissue was harvested for histological study.
Additionally, blood samples were collected via cardio-
puncture for culture analysis in order to assess the
existence of any infection.

Assessment of the infection

The explanted grafts were placed in sterile tubes and
washed in sterile saline solution. Thereafter, they were
placed in tubes containing 10 mL of phosphate-
buffered saline solution and sonicated for 5 min to
remove the adherent bacteria from the grafts. Quantifi-
cation of viable bacteria was performed by preparing
serial 10-fold dilutions (0.1 ml) of the bacterial suspensions
in 10 mM buffer to minimize the carryover effect
and by culturing each dilution on blood agar plates.
All plates were incubated at 37 °C for 48 h and evalu-
ated for the presence of the MRSA strain. The organ-
isms were quantified by counting the number of CFU
per plate. The threshold of detection for this method
was approximately 5 × 10⁴ CFU/cm² per graft tissue.

Analysis of blood culture

The blood samples were drawn by cardiopuncture
after cleaning the skin with 10% povidone-iodine.
The blood samples were inoculated at a volume of 4 ml into BACT/Alert Plus + Aerobic/F blood culture bottles and were placed in the blood culture instrument (BACT/ALERT PF Pediatric BIOMERIEUX INC., Durham.). All bottles were incubated for a period of 7 days. Whenever there was a sign of microbial growth, the detection time was documented. The bottles that had a positive signal were smeared and stained with Gram stain. Subcultures on blood agar, chocolate agar and MacConkey agar plates were also used. Subcultures were incubated at 35 °C for duration of 48 h. When growth was detected, identification of S. aureus was performed by Mini API and conventional methods. The time of detection was measured in hours, beginning with the placement of the bottles in the instrument and ending with the positive signal of the instrument.

**Histopathological study**

The perigraft tissue was taken. The tissues were fixed in a formalin solution for a maximum of 24–48 h. Samples were washed with water and were soaked in a graded series of ethanol (60, 70, 80, 90, and 100%). Then they were held in a solution of xylene for 90 min and were embedded in paraffin at 60 °C. Cross sections (5 mm thick) were cut. Hematoxylin and eosin (H&E) staining was used for the histological examination.

All perigraft tissues were examined for signs of inflammation and infection and classified semi-quantitatively as follows: grade 0; no neutrophils, grade I; a low number of neutrophils, grade II; a moderate number of neutrophils, and grade III; a high number of neutrophils as proposed by Hernandez-Richter et al.11

**Statistical analysis**

Quantitative culture results for all groups are presented as mean ± standard deviation, and the statistical comparisons between groups were made using Kruskal-Wallis analysis of variance followed by post-hoc Mann–Whitney U test. Statistical significance was defined as a P value of <.05. Statistical analyses were performed using SPSS 9.0 for Windows (SPSS Inc., Chicago, IL).

**Results**

**The assessment of graft infection**

Depending on the microbiological study, none of the animals included in groups 1A and 2A (uncontaminated groups) showed any signs of graft infection. On the contrary, there were severe signs of infection on microbiological examination in all rats in groups 1B and 2B (untreated contaminated groups) with quantitative culture data showing $7.4 \times 10^5 \pm 2.0 \times 10^5$ cfu/cm² grafts and $8.6 \times 10^5 \pm 2.1 \times 10^5$ cfu/cm² grafts, respectively. Between groups 1B and 2B, there was no significant difference in the results from quantitative bacterial graft cultures ($P > .05$). In addition, groups 1C and 2C, contaminated and treated with intraperitoneal teicoplanin, showed no evidence of staphylococcal infection on microbiologic examination with quantitative cultures below the limit of detection. Data concerning the quantitative results and statistical comparisons of the groups are summarized in Table 1.

### Table 1. Quantitative microbiological results of in vivo experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>Methods of study</th>
<th>Quantitative graft culture (CFU/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>uncontaminated Dacron group</td>
<td>0±[a,b]</td>
</tr>
<tr>
<td>1B</td>
<td>untreated contaminated Dacron group</td>
<td>7.4 × 10^5 ± 2.0 × 10^5 [a,c,d,f]</td>
</tr>
<tr>
<td>1C</td>
<td>contaminated Dacron group with intraperitoneal teicoplanin treatment</td>
<td>0±[a,b]</td>
</tr>
<tr>
<td>2A</td>
<td>uncontaminated PTFE group</td>
<td>0±[a,b]</td>
</tr>
<tr>
<td>2B</td>
<td>untreated contaminated PTFE group</td>
<td>8.6 × 10^5 ± 2.1 × 10^5 [a,c,d,f]</td>
</tr>
<tr>
<td>2C</td>
<td>contaminated PTFE group with intraperitoneal teicoplanin treatment</td>
<td>0±[a,b]</td>
</tr>
</tbody>
</table>

Each group consisted of 10 animals and statistical significance was evaluated by using Kruskal-Wallis analysis of variance followed by post-hoc Mann-Whitney test.

- a statistically significant versus group 1A.
- b statistically significant versus group 1B.
- c statistically significant versus group 1C.
- d statistically significant versus group 2A.
- e statistically significant versus group 2B.
- f statistically significant versus group 2C.
was low and there was no significant difference between the two groups ($P > .05$). On the contrary, in contaminated untreated groups (groups 1B and 2B) the severity was high. While the difference between groups 1B and 2B was not significant ($P > .05$), the differences between groups 1A and 1B and between 2A and 2B were significant ($P < .01$). In groups 1C and 2C, the severity of the inflammation of perigraft tissue was between the range from low to moderate. There was not any significant difference between groups 1C and 2C ($P > .05$). Similarly, no difference between groups 1A and 1C and between groups 2A and 2C was observed ($P > .05$), in spite of the higher degree of the inflammation seen in groups 1C and 2C. Moreover, despite the higher degree of the inflammation in groups 1B and 2B when compared to that of groups 1C and 2C, these differences were also not significant ($P > .05$).

### Discussion

In the present study, the susceptibility of distinct vascular grafts, gelatin-sealed Dacron and PTFE, to MRSA infection was investigated. There was no difference in bacterial counts between two vascular grafts after inoculation of MRSA strain, and teicoplanin administration prevented the occurrence of vascular graft infections. In groups with MRSA infection, the severities of perigraft inflammations in both grafts were greater than those in other experimental groups.

Despite the use of aseptic vascular surgical techniques and prophylactic antibiotics, vascular graft infection remains as an uncommon, but devastating complication in vascular surgery. The basis of this consideration is Schmitt’s study, in where PTFE was found to have lower quantitative bacterial adhesion compared to Dacron fabrics. They suggested that this difference in bacterial affinity is the result of the hydrophilic porosity of the Dacron graft. This observation is opposite to our data. The reason of this difference between the above mentioned study and ours may be that Schmitt used an in vitro model and we used an in vivo model. In addition, they used old generation woven Dacron and velour knitted Dacron material and we used new generation gelatin-sealed Dacron. Gelatin sealing lowers the incidence of early bacterial infection. This effect is probably due to a decrease in the porosity of polyester with gelatin-sealing. The PTFE material has a microporous structure and is relatively non-porous compared to the old generation materials.

A study by Camiade et al. compared the resistance of allograft and synthetic prostheses and showed that bacterial infection of Dacron prostheses was generally higher than PTFE prostheses and gelatin-sealed Dacron prostheses. But they suggested that differential resistances of infection in these prostheses were due to distinct strains of bacteria. Methodological differences of studies may also play an important role in this difference (in vivo versus in vitro).

There are some limitations in our study. First, the animal model used in the present study is not directly

### Table 2. Bacteriologic evaluation of blood cultures

<table>
<thead>
<tr>
<th>Groups</th>
<th>Positive blood culture</th>
</tr>
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<tbody>
<tr>
<td>1A</td>
<td>0/10</td>
</tr>
<tr>
<td>1B</td>
<td>4/10</td>
</tr>
<tr>
<td>1C</td>
<td>0/10</td>
</tr>
<tr>
<td>2A</td>
<td>0/10</td>
</tr>
<tr>
<td>2B</td>
<td>2/10</td>
</tr>
<tr>
<td>2C</td>
<td>0/10</td>
</tr>
</tbody>
</table>

### Table 3. Inflammation intensity results of in vivo experiments

<table>
<thead>
<tr>
<th>Grade</th>
<th>Group 1A</th>
<th>Group 1B</th>
<th>Group 1C</th>
<th>Group 2A</th>
<th>Group 2B</th>
<th>Group 2C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>1</td>
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<td>++</td>
<td>+++</td>
<td>+++</td>
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<tr>
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<td>+++</td>
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</tr>
<tr>
<td>3</td>
<td>+++</td>
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+ indicates the number of the perigraft tissues involved in each grade category.
comparable with graft implantation into a blood vessel, and caution is needed in relating these results to patients. Further investigations using big animal models are required. Nevertheless, we feel that our in vivo results are more relevant than previous in vitro studies. In this study, we examined only the early graft infection on the postoperative 7 days, but not late appearing vascular prosthetic vascular graft infection. Finally we examined only infection with MRSA and not other mixed bacterial populations (Figs. 1 and 2).

PTFE and Dacron vascular graft possess many of the properties of the ideal vascular prostheses, but are highly hydrophobic surfaces that limit endothelial surface adhesion. Modifications of these prostheses surfaces that stimulate endothelialization could resist bacterial infection. Vascular prostheses are potential sources of future infections. Dacron may be more likely to develop at least a partial ‘pseudointima’, rendering it less susceptible to late bacteraemic seeding. Early Dacron grafts were not ideal substitutes for vascular surgery. Subsequently, efforts to increase the quality of Dacron grafts led to marked improvements in those prostheses. The operative experience and short-term results with newer Dacron grafts may be profitable.

In conclusion, in our model there is no differences in the incidence of infection of Dacron or PTFE graft.

References


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