An Infection-resistant PTFE Vascular Graft; Spiral Coiling of the Graft with Ofloxacin-bonded PTFE Thread*

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Objective: To develop an infection-resistant polytetrafluoroethylene (PTFE) vascular graft for potential clinical use in grafting in sites of bacterial contamination and in replacement of the infected grafts.

Setting: Experimental study in rabbits

Materials and methods: An antibiotic ofloxacin (OFLX) was bonded to a sheet of PTFE by impregnation, which was cut and twisted into fine threads. The in-vitro antibacterial activity of OFLX-PTFE thread was determined by measuring the zone of growth inhibition against Escherichia coli. The thread was spirally coiled around a ridged outerwall PTFE to make the OFLX-PTFE graft. OFLX-PTFE graft or control graft was interposed in the inferior vena cava (IVC) of rabbits and the entire graft was covered with fibrin containing a fixed number of E. coli. Three or 7 days after the grafting, the grafts with perigraft tissue were harvested and subjected to bacteriological studies.

Results: In spite of early phase rapid elution of OFLX, a significant antibacterial activity was retained for more than 2 weeks. The antibacterial activity of OFLX-PTFE threads implanted in the subcutaneous space of rabbits decreased to 48% after 24 h and to approximately 1% after a week. The swab culture of all the control grafts was positive, while only one of 13 PTFE-OFLX grafts was positive. The number of viable bacteria in the perigraft tissue of OFLX-PTFE grafts was remarkably low in comparison with that of control grafts. Thus, the OFLX-PTFE grafts exhibited a marked in-vivo antibacterial activity.

Conclusion: By a unique method, it was possible to furnish PTFE graft with an excellent infection-resistant property, without affecting the original biological behaviour.

Key Words: Graft infection; Polytetrafluoroethylene; Ofloxacin; Animal model.

Introduction

In view of the increasing number of vascular reconstructions using synthetic vascular prostheses, the vascular prosthetic graft infection remains the most serious complication in vascular surgery, and often results in loss of organ function, limb and life. To date, a large number of clinical¹ and experimental²³ studies have suggested that prophylactic intravenous antibiotics reduce the incidence of graft infections, and prophylactic administration has been widely practised. Nevertheless, the infection still occurs in a considerable number of patients and the infected graft may have to be completely removed in order to control the infection. Most of the infections are due to a contamination at the time of implantation,⁴ yet vascular prosthetic reconstruction may be required at possibly contaminated sites associated with in situ replacement of the infected graft. Moreover, with the increase in indications for vascular surgery, prosthetic reconstructions may be tried simultaneously with non-aseptic operations such as gastrointestinal or gynaecological surgery. Under these circumstances, considerable efforts have been made to develop an infection-resistant vascular prosthesis. As one of the approaches, bonding of antimicrobial agents to prosthetic materials has been attempted, by various techniques such as fixation with surfactants,⁵⁻⁹ synthetic or biological glue¹⁰,¹¹ collagen release,¹² silver containing antibiotics,¹³⁻¹⁵ passive method by preclothing,¹⁶ soaking into a protein-sealed textile graft¹⁷,¹⁸ and thermo fixation.¹⁹ The purpose of the present study was to furnish the polytetrafluoroethylene

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(PTFE) graft with an infection-resistant property by a novel modification with antibiotic bonded PTFE thread without impairing the original biological properties.

Materials and Methods

Bonding of ofloxacin (OFLX) to PTFE

OFLX was kindly provided by Dai-ichi Pharmaceutical Company, Tokyo. OFLX (200–600 mg) was suspended in 4 ml of a 2.5% solution of polylactic acid (50 Kd, Polyscience Co., Warrington, PA, U.S.A.) in dioxane (Wako Pure Chemical Co., Osaka, Japan). The solution was impregnated onto two kinds of 10 × 25 cm PTFE sheets (LUP-300 and UP-005) obtained from Sumitomo Electric Industries. The thickness was identical (40 μm), but the pore size of LUP-300 and UP-005 was 3 μm and 0.05 μm, respectively. After air-drying completely, the PTFE sheet was cut into strips (0.5 cm wide) and lightly twisted into a form of thread. Then, using dioxane as adhesive, 10 cm of the OFLX-PTFE thread or control thread was spirally coiled around a 3 cm long ridged outerwall PTFE tube graft (same structure as Technograft®, a product of Sumitomo Electric Industries) with specifications as follows. The inner diameter was 3 mm and the internodal distance was 30 μm. The wall thickness changed every 0.5 mm and the thickness of the valley and ridge portion was 0.3 mm and 0.5 mm, respectively. The OFLX-PTFE graft is shown in Fig. 1b. For in vitro study and also for subcutaneous implantation, the OFLX-PTFE thread (7.5 cm) was coiled around a 6 × 6 mm PTFE sheet with identical specification to the PTFE tube graft (Technograft®), as shown in Fig. 1a.

Determination of antibacterial activity

Escherichia coli, JM109, a stock culture in the Department of Microbiology, Osaka University Medical School was used as a representative bacterial strain in the entire experiment. In vitro and in vivo antibacterial activity of OFLX-PTFE thread coiled around the PTFE sheet was determined by measuring the zone of growth inhibition against the E. coli and interpolating the value from a standard curve described below. Ten microlitres of known concentration of OFLX were adsorbed onto a filter paper with a diameter of 6 mm. The paper, containing various doses of OFLX, was placed on a nutrient agar plate (consisting of 1% Bacto Tryptone, 0.5% Bacto Yeast Extract, 0.5% NaCl and 1.5% Bacto Agar, Difco, Detroit, MI, U.S.A.) on which 3 × 10⁷ of the bacteria was inoculated. After incubation at 37°C for 12 h, the zone of growth inhibition, (diameter of the inhibitory zone –6) / 2 mm, was determined (Fig. 2).

In vitro experiments

Influence of various sterilisation procedures of OFLX-PTFE on the antibacterial activity

Non-treated OFLX-PTFE threads and those sterilised by autoclave (at 121°C for 15 min and drying at 60°C),

![Fig. 1. Biomaterial used for the study. (a) OFLX-bonded PTFE thread coiled around PTFE sheet for subcutaneous implantation. (b) PTFE tube graft spirally coiled by OFLX-bonded PTFE thread. The detailed specifications are described in Materials and Methods.](image-url)

![Fig. 2. Correlation between the inhibitory zone on disc and amount of OFLX added on the agar containing 3 × 10⁷ E. coli. Vertical bars indicate s.d. (n = 3).](image-url)
immersion into 70% ethanol for 5 min, or ethylene oxide gas (EO 20%/CO₂ 80%, 1 kg/cm² for 6 h) were placed on the nutrient agar plates onto which 3 × 10⁷ of E. coli had been inoculated and incubated for 12 h at 37°C. The size of the inhibitory zone of the treated samples was compared with that of the non-treated ones.

Release of OFLX from OFLX-PTFE in saline
Two kinds of OFLX-PTFE threads (UP-005 and LUP-300, initial OFLX dose; 50mg/ml) were coiled around the PTFE sheet as described above. Eight of each of the threads were incubated in saline (10ml, 37°C). Two threads from each group were removed from the saline at days 1, 3, 5, 9, 14. The residual antibacterial activity was determined by comparing zone of growth inhibition with the standard curve. Three different doses of OFLX (50, 100 and 150 mg/ml) were bonded to PTFE sheets (LUP-300) as described above to obtain OFLX-PTFE threads containing different amounts of OFLX. Also, OFLX-PTFE threads with different lengths were prepared (1.9, 3.75, 7.5 cm). These threads were subjected to immersion in saline and to determination of the residual activity. The OFLX-PTFE thread (initial OFLX dose; 50mg/ml) was autoclaved as described above and the sterilised thread was likewise subjected to the determination of residual activity upon immersing in saline.

In vivo experiments
Residual antibacterial activity of OFLX-PTFE threads after implantation into the subcutaneous space
Albino rabbits weighing approximately 2.5 kg, obtained from Kitayama Labes Co., Ltd. (Nagano, Japan), were used in this study. They were fed with a standard diet for at least one week before use. The animals were anaesthetised with an intravenous administration of sodium pentobarbiturate (30 mg/kg) via the ear vein. An additional dose was given to prevent them from suffering any further discomfort. To study in vivo release of OFLX, OFLX-PTFE threads coiled around the PTFE sheet were implanted in the subcutaneous pockets (1 cm) on the back of rabbits (four pockets in each rabbit). The wound was closed in one layer with 4-0 silk. At 1, 3 and 7 days after implantation, the threads were harvested under sterile conditions (n = 4 each). The specimens were placed on E. coli (3 × 10⁷) inoculated agar plates and incubated at 37°C for 12 h. The residual antibacterial activity of the specimen was determined as described above.

In vivo antibacterial activity of OFLX-PTFE and control PTFE graft
Prior to the implantation of OFLX-PTFE or control graft into IVC of rabbits, the bacterial solution for perigraft inoculation was prepared. Bacterial cultures of 2 × 10⁸ CFU growing logarithmically were precipitated by centrifugation at 15000 rpm for 10 min, and mixed with 0.4 ml sterile fibrinogen solution (Beriplast P, Hoechst Japan, Tokyo). The technique of graft implantation was basically similar to that used in our previous study. Briefly, through a median laparotomy, the infrarenal IVC was gently exposed. After systemic heparinisation (sodium heparin, 100 U/kg), two small atraumatic clamps were placed on the IVC just below the renal veins and just above the iliac bifurcation. A 1 cm segment of IVC was resected and then replaced with a 3 cm long OFLX-PTFE or control graft. The anastomoses were carefully constructed using an interrupted suture of 7-0 polypropylene. After haemostasis was secured, the fibrinogen solution containing E. coli (2 × 10⁸, 0.4 ml) and freshly prepared 100 U/ml thrombin calcium solution (0.4 ml) were simultaneously instilled around the entire surface of the graft. After the complete gelation of fibrinogen, the retroperitoneum was tightly closed with 7-0 polypropylene and the abdomen was closed in one layer. At days 3 and 7 after the implantation, the rabbits were anaesthetised and the abdomen was reopened. The abdominal cavity was thoroughly examined with respect to manifestation of infection. To examine for the presence or absence of viable bacteria around the grafts, swab cultures of the grafts were made as follows. The external surfaces of the graft were rubbed on nutrient agar plates and the grafts were also put on agar plates. These plates were incubated at 37°C for 12 h. To examine the number of viable bacteria in perigraft tissues semi-quantitatively, the perigraft tissues were weighed aseptically and homogenised with an equal volume (ml/g) of nutrient broth by a homogeniser (pellet mixer from Toho Co. Ltd., Tokyo, Japan). The homogenates were diluted with nutrient broth serially and plated on nutrient agar plates.

All animal care and procedures were performed at the Animal Experimental Center of Osaka University Medical School. Animal protocols were approved by the Osaka University Medical School Animal Committee and conformed to the guidelines set forth by the Osaka University Medical School.
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Statistical analysis

Data of in vivo positivity of abscess formation and swab culture were compared using Fisher's extract probability test. Since distribution of bacterial number was grossly heterogeneous, the non-parametric Mann-Whitney U-test was used for comparing the CFU value in perigraft tissue.

Results

In vitro study

Influence of sterilisation

The antibacterial activity of non-sterilised OFLX–PTFE threads and those sterilised by three different methods was determined as described in Materials and Methods. The inhibitory zone of the non-sterilised OFLX–PTFE thread was 35 mm / 7.5 cm. The inhibitory zones of the sterilised threads were as follows; autoclave 36 mm, ethanol immersion 33 mm, ethylene oxide gas exposure 35 mm. Thus, the antibacterial activity of OFLX–PTFE threads was not affected by any of the sterilisation methods used.

Release of OFLX from OFLX–PTFE threads

As shown in Fig. 3, the initial antibacterial activity was higher in the threads made of LUP-300 than those of UP-005. The residual antibacterial activity after incubation in saline was also higher in LUP-300. Therefore, in the subsequent experiments, LUP-300 was exclusively used to prepare OFLX-bonded or control threads. The initial antibacterial activity of OFLX–PTFE thread was not proportional to the initial concentration of OFLX in the reaction mixture (data not shown). As there was no significant difference in the residual antibacterial activity of the three groups in saline up to 14 days (data not shown), 50 mg/ml OFLX solution was used for the subsequent bonding. The antibacterial activity of OFLX–PTFE thread increased proportionally with the length (data not shown). The antibacterial activity of the autoclaved OFLX–PTFE threads incubated in saline for 6 h and for 24 h were 28.4% and 13.6% of the initial amount, respectively, indicating rapid liberation of the bonded OFLX at an early stage (Fig. 4). The liberation after day 2 up to day 14 was very gradual and an activity equivalent to 5.8 μg of OFLX was recovered from the PTFE even after 2 weeks (Fig. 4).

In vivo experiments

Residual antibacterial activity of OFLX–PTFE threads after implantation into the subcutaneous space of rabbits

The antibacterial activity of OFLX–PTFE threads kept in the subcutaneous space for 24 h decreased to approximately 50% and by day 3 rapidly decreased to 1% of the initial activity as shown in Fig. 5. However, the activity equivalent to 1.4–2.2 μg of OFLX was maintained for at least 7 days. Even with this amount of OFLX, a significant value of inhibitory zone was obtained, as shown in Fig. 5.

Fig. 3. Antibacterial activity of OFLX–PTFE threads made of PTFE with different pore sizes; 3 μm LUP-300 (●), 0.05 μm UP-005 (○). Results were expressed as the mean values (n = 2) comparable to the amount of OFLX.

Fig. 4. Antibacterial activity of autoclaved OFLX–PTFE threads incubated in saline. The results were expressed in two parameters; left vertical axis, mean values of the inhibitory zones (○), right vertical axis, mean values comparable to the amount of OFLX (●). Vertical bars indicate s.d. (n = 3).

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In vivo antibacterial activity of OFLX-bonded PTFE grafts

There was no sign of dissemination of infection into the peritoneal cavity in any of the rabbits studied. All the grafts were patent at the time of harvest. As summarised in Table 1, at day 3, the OFLX-bonded PTFE grafts exhibited marked infection-resistant properties, judging from the incidence of abscess formation, result of swab culture and semi-quantitative assay of bacteria in the perigraft tissues. As shown in Fig. 6, the actual number of bacteria in the perigraft tissue of OFLX-PTFE grafts was approximately 5000 times less than that in control grafts at day 3. The OFLX-PTFE grafts at day 7 still exhibited significant infection-resistant properties, as evidenced by the incidence of abscess formation, result of swab culture and semi-quantitative assay of bacteria in the perigraft tissues. Remarkably, the bacteria recovered from the OFLX-PTFE grafts at day 7 were below the detection limit (less than \(10^2\) CFU/g perigraft tissue) as shown in Fig. 6.

Discussion

In most cases, synthetic vascular graft infection is provoked by bacterial seeding on the outer wall of the graft at the time of implantation.22 This fact may well explain the reason why intravenous administration of various antibiotics has its limits in preventing or treating graft infection.9,25,24 Topical application of antibiotics directly to the graft has been attempted, but the beneficial effect has not been proven because of the rapid elution of the antibiotic.12,25 The topical application of antibiotic would be effective if it could be kept at a significant concentration for a longer period.10,26 A large number of investigators have attempted to develop an antibiotic-bound vascular prosthesis capable of exhibiting an antibacterial activity over a long period of time.5-19 In most of the methods, the antibiotics and carrier agent or glue are fixed not only to the outer surface but also in the wall and on the inner surface of the vascular prosthesis. This condition may impair the innate histocompatibility and antithrombogenicity.27 Furthermore, the healing of vascular prosthesis may be hampered when the porous wall structure is filled with the carriers. Considering these problems, we made an attempt to furnish the PTFE graft with an infection-resistant property only in the perigraft area where bacteria grow. This was achieved by winding antibiotic-bound PTFE thread around a PTFE tube graft. To date, such an invention used to furnish an infection-resistant property to vascular prosthesis has not been reported. Among the various new quinolone antibiotics, OFLX was selected for bonding in this study, as this agent exerts potent antimicrobial activity against methicillin-resistant Staphylococcus aureus as well as other Gram-positive and Gram-negative bacteria including E. coli.29 Instead of a conventional smooth-surface PTFE graft, a ridged outer wall PTFE was used in the experiment, because the precise coiling on the tube graft may be achieved with excellent antikinking and incollapsible properties, without any external support.28

Judging from the data on the in vitro decay of antibacterial activity, the elution of OFLX from the thread may be divided into two phases; rapid release within 24 h followed by gradual release up to 2 weeks. Decay patterns comparable to ours have been also documented in other reports.7,29 The rapid release (more than 80% within 24 h) may be partly due to the presence of loosely-bound OFLX in the outer surface of the thread and the subsequent slow release might be due to the elution of OFLX from the void space on the inner structure of the thread. It was not possible to compare the release rate directly with those in other reports due to differences in methodology. However, the elution of OFLX from PTFE was much slower than that of oxacillin bound to PTFE by benzalkonium5 and chloramphenicol or amikacin bonded to Dacron by collagen.29 Our data may be comparable with that of penicillin G bonded to PTFE by tridodecylmethylammonium.6 As the liberation of non-covalently bonded agents from biomaterials may be affected not only by the carrier but also by the property of the agent itself, such as its chemical structure and hydrophobicity, the release rate may differ significantly.
Table 1. *In vivo* effects of OFLX-PTFE grafts on abscess formation, swab culture and number of bacteria.

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<th>Abscess formation</th>
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<td>4/4***</td>
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<td>9.2x10^3††</td>
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<tr>
<td>OFLX-PTFE</td>
<td>4</td>
<td>2/4***</td>
<td>0/4****</td>
<td>&gt; 1x10^2††</td>
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Fisher’s extract probability test (asterisks), Mann-Whitney U-test (daggers).

*.*, **, †: p < 0.01.

****, ‡: p < 0.05.

***: NS.

depending on the agent bonded to the prosthesis. Elution of OFLX from OFLX-PTFE threads kept in living tissue was rather slow for the initial 24 h in comparison with the *in vitro* elution. The subsequent elution, however, was much faster than the elution *in vitro*. The elution of the agents bound to biomaterials may be affected by a number of factors, such as the presence of body fluid containing various lipids acting as surfactant and cellular infiltration and uptake. The relatively slow elution within 24 h in our study cannot be fully explained, but the subsequent rapid decay of the activity may be due to the cellular intake of OFLX. The elution rate in our study, however, was much slower than that of penicillin- or cefazolin-bound Dacron. The *in vivo* elution of silver antibiotic PTFE has been reported to be very slow (approximately 20% at 1 week), but this result was based on the radioactivity of 110Ag and may not actually reflect the actual amount of antibiotic. Despite the rapid *in vitro* elution of the bonded agents, most of them, graft in animals, exhibited excellent *in vivo* infection-resistant properties. The present OFLX-PTFE grafts also showed excellent infection-resistant properties at days 3 and 7. The findings at day 3 seemed to be more remarkable than those at day 7 in terms of difference in bacterial number and gross appearance of infection. A precise comparison between the two, however, may not be possible, because the bacterial growth at day 7 of the control group was apparently suppressed by the endogenous immune system. The present results may also suggest that the initial suppression of infection may be beneficial to the control of subsequent development of graft infection as has been suggested. However, it was also possible that a small but significant amount of OFLX remaining in the graft was sufficient to control the bacterial growth in our model. From these observations in the present study, it was concluded that OFLX-PTFE grafts, made by a unique but simple method, exhibit an excellent *in vivo* antibacterial activity. Considering its possible clinical application, the OFLX-PTFE graft may have various advantages, such as sterlissability, adjustability and availability. Infection-resistant grafts made by soaking rifampicin into protein-sealed Dacron graft have been already utilised in clinical patients at high risk of developing infection. The outcome is promising but this ingenious approach cannot apparently be applied to vascular prosthesis made of PTFE. The next step to clinical application would be the use of OFLX-PTFE threads as an infection-resistant suture, which may be required in various contaminated surgical procedures.

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