levels. Thus, the differences in mechanism of tissue regeneration would offer a possible explanation for the incomplete endothelialization observed in the midportion of the bFGF/heparin impregnated graft. If so, coimmobilization of other growth factors with bFGF may be required for accelerated transmural capillary ingrowth. As described earlier, enhancement of neoarterial regeneration is important in the early phase of implantation. In the later phase, however, excessive and prolonged neoarterial regeneration results in pathologic development of graft stenosis, which is called intimal hyperplasia. Because the current implantation study was performed at 4 weeks after implantation, when neoarterial tissue regeneration was continuing, information on long term effects of the bFGF/heparin impregnation was not obtained. However, it has been reported that bFGF increased bFGF mRNA expression in rat aortic SMCs, and it has been suggested that such local bFGF autoinduction within a vessel wall could prolong the period of elevated bFGF synthesis.[13] Period limited sustained release of bFGF is highly desired. Therefore, incorporation of both time dependent progressive and suppressive functions into a design concept for artificial grafts is expected to increase patency rates of small caliber artificial grafts.

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# Penetrating Micropores Increase Patency and Achieve Extensive Endothelialization in Small Diameter Polymer Skin Coated Vascular Grafts

TAKAFUMI OKOSHI,* GIORGIO SOLDANI,† MOSES GODDARD,‡ AND PIERRE M. GALLETTI‡

**This article points to the importance of penetrating micropores through the graft wall to minimize thrombosis and to enhance endothelialization in small diameter polymer skin coated vascular grafts. Four types of spongy polyurethane-polydimethylsiloxane vascular grafts (PUG) fabricated by a spray, phase-inversion technique, 1.5 mm inner diameter, 1.5–1.9 cm in length, were implanted end-to-end in the infrarenal aorta of 26 adult rats. Some had a continuous inner skin and a hydraulic permeability (HP) of 0 ml/min/cm²/120 mmHg (PUG-S-O). Some had an inner skin with varying amounts of isolated penetrating micropores and a mean hydraulic permeability of 11 (PUG-S-11), 37 (PUG-S-37), or 58 ml/min/cm²/120 mmHg (PUG-S-58). Twelve PUG-S-O, 6 PUG-S-11, 4 PUG-S-11, and 4 PUG-S-58 were evaluated between 2 hr and 3 months after implantation. All PUG-S-O occluded soon after implantation. The PUG that had a HP of more than 11 ml/min/cm² showed acceptable patency. However, endothelialization was limited to anastomoses in**

**patent PUG-S-11. In contrast, the patent PUG-S-37 and PUG-S-58 were largely endothelialized. In all patent grafts at 3 months, numerous host cells had migrated, and newly formed capillaries were seen in the voids of the graft wall, which appeared moderately to highly cellular. In conclusion, it appears that penetrating micropores through the graft wall increase patency and that a highly porous structure is needed to achieve extensive endothelialization in small diameter polymer skin coated vascular grafts. *ASAIO Journal* 1996;42:M398–M401.**

It has been said that small diameter vascular grafts with a smooth inner surface, such as continuous polymer skin, are more refractory to early mural thrombosis than are microporous grafts with a textured inner surface, and thus have a better patency.[1,2] However, our experience points to the importance of penetrating micropores through the graft wall to minimize thrombosis and enhance endothelialization in small diameter polymer skin coated vascular grafts. This study addressed the effects of penetrating micropores through the graft wall on patency and endothelialization.

## Materials and Methods

### *Preparation of Grafts*

Spongy polyurethane-polydimethylsiloxane (Cardiothane 51, Kontron Instruments, Inc., Everett, MA) vascular grafts were fabricated by a spray, phase-inversion technique described elsewhere[3] and according to well established principles.[4]

Microporous tubes were formed from a thermodynamically unstable polymer solution in a 2:1 tetrahydrofuran-dioxane mixture directed onto a rotating mandrel by a longitudinally sliding spray gun. Another spray gun directed a stream of nonsolvent (water) at the point of impact of the polymer solution jet on the mandrel, leading to the precipitation of a filamentous mat. The porosity of the tube could be adjusted by varying the flow rate and concentration of polymer in the casting solution and the relative positions of the mandrel and spray guns. An inner skin layer was created by heating of a small amount of precipitated material around the mandrel at an early stage of the fabrication process. Once a deposit of the desired thickness was obtained, the fabrication process was stopped and the microporous tube separated from the mandrel.

Four types of spongy vascular grafts with a continuous inner skin or with a skin accompanied by varying size and amount of isolated micropores in the inner surface were prepared with an internal diameter (ID) of 1.5 mm and a wall thickness of 0.45 mm. Their hydraulic permeability was characterized by measuring the volume of degassed distilled water collected in the first minute by filtration through the graft wall at the standard transmural pressure of 120 mmHg.

From *Division of Cardiovascular Surgery, Second Department of Surgery, Teikyo University School of Medicine, Tokyo, Japan, †Istituto di Fisiologia Clinica del C.N.R., Pisa, Italy, and ‡Artificial Organ Laboratory, Brown University, Providence, Rhode Isalnd.

Reprint requests: Dr. Takafumi Okoshi, Division of Cardiovascular Surgery, Second Department of Surgery, Teikyo University School of Medicine: 2-11-1, Kaga, Itabashi-ku, Tokyo, 173 Japan.

The grafts with a continuous inner skin identified as PUG-S-O had a hydraulic permeability (HP) of 0 ml/min/cm$^2$/120 mmHg. The other three grafts with a discontinuous inner skin and varying density of isolated penetrating micropores showed mean HP of 11, 37, or 58 ml/min/cm$^2$/120 mmHg, respectively. The grafts are identified for this study as PUG-S-0, PUG-S-11, PUG-S-37, and PUG-S-58. "PUG" stands for polyurethane graft; "S" means that a skin layer is formed at the luminal surface; and the numbers indicate the values of hydraulic permeabilities. The materials were characterized by scanning electron microscopic study (SEM; Hitachi, S-2700 or HS-800, Tokyo, Japan). The grafts were sterilized by sequential exposure to 1) sterilized isotonic saline for 10 min, 2) 0.1 M hydrochloric acid for 30 min, 3) sterilized isotonic saline for 10 min and were stored in the sterilized isotonic saline.

### *Implantation of Grafts*

Twelve PUG-S-0, 6 PUG-S-11, 4 PUG-S-37, and 4 PUG-S-58 were implanted by the same surgeon end-to-end in the infrarenal aorta of 26 male Sprague-Dawley rats weighing 250–350 g. Pentobarbital sodium intraperitoneal anesthesia and standard microsurgical techniques were used. Two segments of the aorta at the level of the left renal vein for the proximal anastomosis and proximal to the iliac bifurcation for the distal anastomosis were independently dissected. The longest possible graft that could be accommodated anatomically and surgically was implanted in each case. The bypassed segment of the native aorta was ligated, divided at both stumps, and left behind the implanted graft. Six to seven 10-0 nylon sutures were used for each anastomosis. No antithrombogenic agent was administered before or after surgery.

### *Retrieval of Grafts*

Specimens were retrieved between 2 hr and 3 months after implantation. Under deep intraperitoneal pentobarbital anesthesia, the rat was perfused through the left ventricle and simultaneously drained from the right atrium, first with 300–400 ml of heparinized saline and then with 150–200 ml of fixative (3% paraformaldehyde + 2.5% glutaraldehyde). Thereafter, the graft specimen was resected with the surrounding tissues and margins of the native aorta at both ends. The graft was opened longitudinally, carefully examined, and photographed. All animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Institute of Laboratory Animal Resources* published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

### *Preparation of Specimens*

For light microscopic study, the specimens were embedded in resin (Historesin, Reichert-Jung Optische Werke AG, Wien, Austria), sectioned by a microtome (Microtome 2050 Supercut, Reichert-Jung Optische Werke AG), and stained with hematoxylin and eosin. Samples for SEM were dehydrated in graded alcohols (50–100%), critical-point dried with $CO_2$, sputter coated with gold and palladium, and ex-

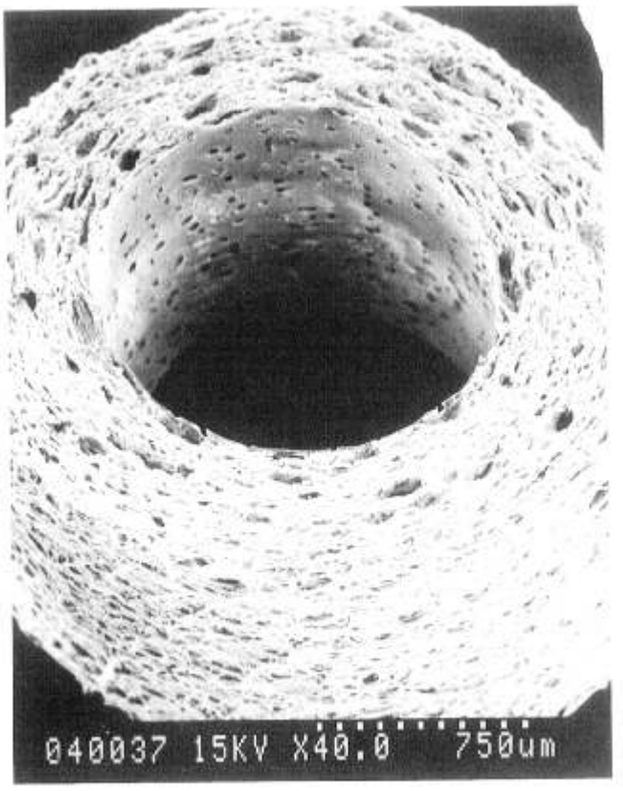


**Figure 1.** Scanning electron micrograph of PUG-S-58. Isolated micropores ranging from 10 to 50 μm are seen on the inner surface. The outer surface displays micropores with the range of 70 to 130 μm (magnification ×40).

amined with a Hitachi S-2700 or HS-800 scanning electron microscope.

## Results

The PUG-S-0 had a continuous inner skin (2.2 μm in thickness); the wall section was compact, and the outer surface showed a filamentous appearance with interfiber intervals ranging from 70 to 130 μm. The PUG-S-11 had an inner skin (6.7 μm in thickness) with isolated pores ranging from 10 to 60 μm. The graft wall was open, and the outer surface features were similar to those of PUG-S-0. The PUG-S-37 had an inner skin (6.5 μm in thickness) with isolated pores measuring 10 to 80 μm in their largest dimension. The graft wall was porous and the outer surface features were the same as those of PUG-S-0. The PUG-S-58 had an inner skin (6.6 μm in thickness) with isolated pores measuring 10 to 50 μm. The structure of the graft wall was porous and the outer surface displayed features similar to those of PUG-S-0 **(Figure 1)**.

All four types of the grafts displayed good handling properties and suturability. After the aortic clamps were released and blood started passing through the graft, no blood leakage through the graft wall of PUG-S-0 was recognized. However, in PUG-S-11 a few to several red spots appeared on the external surface, spread, and fused with each other. Finally, the entire external surface looked red, and blood oozed for a few minutes. In contrast, blood oozed through the entire wall of PUG-S-37 and PUG-S-58 immediately after release of the aortic clamps, continued oozing for several minutes, and the grafts looked uniformly red.

In the polymer skin coated surface graft group, two of three PUG-S-0 retrieved at 1–3 days were patent; however, all eight PUG-S-0 showed occlusion with thrombus at 1 to 2 weeks. The sole graft retrieved at 3 months also was occluded, with an organized tissue that suggested the occlusion occurred at an early stage. In contrast, the patency of PUG-S-11 was 80% (4/5) at 3 months, although 1 graft was acutely thrombo-occluded at 2 hr after implantation. Thus, the overall patency rate was 67% (4/6) to 3 months, which was inferior to that of PUG-S-37 or PUG-S-58. The patency of PUG-S-37 and PUG-S-58 was 100% (4/4) and 75% (3/4) at 3 months, respectively. Thus, PUG, which had a HP of more than 11 ml/min/cm$^2$, showed much better patency than did less permeable grafts **(Table 1)**.

In the patent specimens of PUG-S-0 retrieved in the first few days after implantation, a red thrombus layer or a mosaic of multiple tiny red thrombi in a fibrin network was observed. In the patent PUG-S-11 at 3 months, those grafts displayed endothelialization limited to 1–2 mm from proximal and distal anastomoses. A proteinaceous layer covered most of the graft luminal surface and exhibited tiny red thrombi. In contrast, the patent PUG-S-37 and PUG-S-58 were largely endothelialized. In all patent grafts at 3 months, numerous host cells had migrated, and newly formed capillaries were seen in the voids of the graft wall, which appeared moderately to highly cellular. Thick mural thrombus, anastomotic hyperplasia, or aneurysm formation was not observed in patent PUG-S-37 or PUG-S-58. Mural thrombosis was the least in PUG-S-37 or PUG-S-58, followed in order by PUG-S-11 and PUG-S-0.

## Discussion

In this study, four kinds of polyurethane vascular grafts were prepared to investigate the effects of penetrating micropores in small diameter polymer skin coated vascular grafts. The material of the polyurethane vascular grafts was Cardiothane 51, which has well known hemocompatibility and has been used for catheters and intra-aortic balloon materials.

The graft wall has a spongy structure that is complicated and maze-like and in which some channels reach the outside of the graft and some may lead to dead-ends within the graft wall. The penetrating micropores consist of micropores opening in the inner skin layer and microchannels within the graft wall that reach the outside of the graft. The density of penetrating micropores is reflected by hydraulic permeabil-

**Table 1. Patency of Polyurethane-Polydimethylsiloxane Vascular Grafts**

| | Within 3 Days | 1–2 Weeks | 3 Months |
|---|---|---|---|
| PUG-S-0 | 67% (2/3) | 0% (0/8) | 0% (0/1) |
| PUG-S-11 | 0% (0/1) | — | 80% (4/5) |
| PUG-S-37 | — | — | 100% (4/4) |
| PUG-S-58 | — | — | 75% (3/4) |

ity or blood permeability: namely, the bleeding rate through the graft wall. This wall structure changes immediately after exposure to blood pressure but remains compliant and responsive to pulsatile blood flow through the graft lumen. The continuous inner skin totally shields this maze-like structure, whereas isolated micropores in the inner skin open a passageway to the maze-like structure of the wall.

In grafts with a continuous inner skin, where the blood contacts only the surface of the material and does not enter the wall, thrombosis developed rapidly, and occlusion was observed at an early stage. In grafts with a porous inner skin, which allows blood to infiltrate the wall, blood also enters the microchannels in the graft wall. The results of the current study demonstrate that the differences in morphology of blood contacting surfaces and in the amount of blood flow passing through the penetrating micropores seem to determine whether or not each graft will be thrombo-occluded after implantation.

Why do these differences determine the degree of mural thrombosis? Thrombi having formed on the continuous inner skin of PUG-S-0 may partially detach because the inner skin has a low anchoring property for thrombi because of its smoothness. Blood turbulence may occur, and thrombi may accumulate at that portion, leading to thrombo-occlusion of the graft. In another scenario, thrombus may completely detach and be trapped in the distal portion of the graft. New thrombi may develop around that thrombus and the graft finally may be occluded by the developed thrombi. However, in PUG-S-11, PUG-S-37, and PUG-S-58, blood passes through the microchannels and coagulates within the graft wall in a short time. A thrombus layer also formed on the inner surface of the graft. The thrombi within the graft wall and on the inner surface can connect with each other because of the presence of micropores opening in the inner skin. As a result, the mural thrombi may be more resistant to shear stress caused by the blood stream than are those attaching to the continuous inner skin of PUG-S-0. Consequently, PUG-S-11, PUG-S-37, and PUG-S-58 promise better anchoring for mural thrombi than does PUG-S-0.

However, the differences of thrombus formation in each class of grafts cannot be explained only by the anchoring effect for mural thrombus. In our previous experiments, PUG-2.7, which has a low hydraulic permeability of 2.7 ml/min/cm$^2$ but presents a complete microporous inner surface, was thought to have better anchoring effects for mural thrombus and better thrombo-resistance than does PUG-S-11. Nevertheless, mural thrombi in PUG-2.7 were relatively thick at 2 weeks after implantation, but they developed with time and finally occluded almost all of the PUG-2.7 at 3 months. However, 76% (13/17) of the grafts did not show thrombo-occlusion at 3 months in PUG-39, with a microporous inner surface similar to that of PUG-2.7.[5,6] This means that although those grafts have a similar anchoring effect, a higher hydraulic permeability, which implies the presence of more penetrating micropores throughout the wall is a significant factor in controlling critical mural thrombosis in the acute and early stage.

In summary, differences in the process of development of initial thrombus and in the relationship between mural thrombus and intramural thrombus seem to determine whether the mural thrombus may stay in a thin layer, enlarge, or detach from the inner surface. Additional investigation is needed to establish the precise mechanism that explains these phenomena.

With regard to endothelialization on the inner surface of the graft, all PUG-S-0 clotted before endothelialization occurred. In PUG-S-11 at 3 months, pannus accompanied by endothelial cells were localized in the anastomotic region. A proteinaceous layer covered most of the graft inner surface and exhibited tiny red thrombi. Some portions of the inner surface appeared almost bare. The proteinaceous layer on the inner surface seemed to be unstable and to result from repeated thrombus formation and fibrinolysis or detachment. The proteinaceous layer contains cell-adhesive proteins and favors endothelial cell adhesion. It rarely occurs on an inner surface, such as in the PUG-S-11 series. In contrast, PUG-S-37 and PUG-S-58 showed endothelialization on more than half of the tube's inner surface, whereas the portions without endothelialization were almost bare. We think that endothelialization developed on stable proteinaceous layers, which were anchored by penetrating micropores. Other portions of unstable proteinaceous layers were sporadically associated with defects of endothelialization on the inner surface.

## Conclusions

In conclusion, it seems to be important to provide the graft with an appropriate amount of penetrating micropores, as reflected by hydraulic permeability, to promote formation of stable proteinaceous layers on the inner surface of the grafts, leading to prohibition of early thrombotic occlusion and enhancement of endothelialization.

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