Influence of Microporous Structures on Mural Thrombosis and Endothelialization at Blood-Contacting Surfaces

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Summary. The influence of microporous structures in the walls of small-diameter arterial prostheses was investigated with the aim of minimizing thrombosis and enhancing endothelialization of blood-contacting surfaces. Six types of spongy polyurethane–polydimethylsiloxane grafts (PUG), 1.5-mm in an internal diameter and 1.5–2 cm in length, were implanted end-to-end in the infrarenal aorta of 66 adult rats. Some had a continuous inner skin and a hydraulic permeability (HP) of 0 ml/min/cm^2 at the standard transmural pressure of 120 mmHg (PUG-S-0). Some had a discontinuous inner skin with some isolated windows connecting penetrating micropores though the graft wall and a mean HP ranging from 11 (PUG-S-11) to 37 (PUG-S-37) or 58 (PUG-S-58) ml/min/cm^2. The rest had a microporous inner surface with penetrating micropores through the graft wall and a mean HP of 2.7 (PUG-2.7) or 39 (PUG-39) ml/min/cm^2. PUG which had a HP of less than 2.7 ml/min/cm^2 showed poor patency. PUG with a HP of more than 11 ml/min/cm^2 had acceptable patency, but endothelialization was limited to their anastomoses. In contrast, the patent PUG-S-37 and PUG-S-58 were largely endothelialized and all but one of the patent PUG-39 implants were completely endothelialized. In conclusion, penetrating micropores through the graft wall appear to inhibit critical mural thrombosis. A microporous inner surface seems to be superior to a skinned inner surface in achieving a high degree of endothelialization.

Key words: Polyurethane–polydimethylsiloxane — Spray phase-inversion technique — Penetrating micropores — Microporous structure — Endothelialization

Introduction

Blood-contacting surfaces for cardiovascular devices have mainly been made of smooth, nonthrombogenic surface materials [1,2]. However, mural thrombosis and thromboembolism are not completely avoided [3]. Based on observation of the rat abdominal aorta replacement model with small-diameter vascular grafts, we recently suggested that materials with a microporous surface and wall structure may be superior to smooth surfaces for inhibiting mural thrombosis and enhancing endothelialization at blood contacting surfaces [4,5]. The present paper reports further investigation of that hypothesis.

Materials and Methods

Preparation

Polyurethane–polydimethylsiloxane (Cardiothane 51, Kontron Instruments, Everett, MA, USA) vascular grafts with an internal diameter of 1.5 mm and a wall thickness of 0.45 mm were fabricated by a spray, phase-inversion technique described elsewhere [6] and according to well-established material-processing principles [7].

Six types of spongy vascular grafts with a continuous inner skin, a discontinuous inner skin featuring windows of varying sizes and amounts, or a totally microporous inner surface, were prepared. Their hydraulic permeability was characterized by measuring the volume of degassed distilled water collected during the first minute by filtration through the graft wall under the standard transmural pressure of 120 mmHg.

The grafts with a continuous inner skin (identified as PUG-S-0) had a hydraulic permeability (HP) of 0 ml/min/cm^2. Three types of grafts with a discontinuous inner skin and varying density of isolated windows showed mean HP of 11, 37, or 58 ml/min/cm^2 (identified as PUG-S-11, PUG-S-37, and PUG-S-58, respectively). The other two types had a microporous inner surface and an average HP of 2.7 or 39 ml/min/cm^2 (identified as PUG-2.7 and PUG-39). Thus, the graft types with a discontinuous inner skin and a microporous inner surface had penetrating micropores though the graft wall. PUG stands for polyurethane graft, S means that a skin layer is formed at the inner surface, and the numbers indicate the values of hydraulic permeabilities. The material structures were

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characterized by scanning electron microscopy (SEM, Hitachi, S-2700 or HS-800 Tokyo, Japan).

Implantation

Twelve PUG-S-0, 6 PUG-S-11, 4 PUG-S-37, 4 PUG-S-58, 23 PUG-2.7, and 17 PUG-39, 1.5–2 cm in length, were implanted by the same surgeon end-to-end in the infrarenal aorta of 66 male Sprague-Dawley rats weighing 250–350 g. Pentobarbital sodium intraperitoneal anesthesia and standard microsurgical techniques were employed. 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 separately dissected. In each case, the longest possible graft which could be accommodated anatomically and surgically was implanted. 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 needed for each anastomosis. No antithrombogenic agent was administered pre- or postoperatively.

Retrieval

Specimens were retrieved between 2 h and 3 months after implantation. Under deep intraperitoneal pentobarbital anesthesia, the rat cardiovascular system 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% (w/v) paraformaldehyde + 2.5% (w/v) glutaraldehyde). Thereafter, the graft specimen was resected together with the surrounding tissues and margins of the native aorta at both ends. The graft was opened longitudinally, carefully examined, and photographed.

Study of Specimens

For light microscopy, the specimens were embedded in resin (Historesin, Reichert-Jung Optische Werke, Vienna, Austria), sectioned by a microtome (Microtome 2050 Supercut, Reichert-Jung Optische Werke), 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 examined with a Hitachi S-2700 or HS-800 SEM.

Statistical Analysis

The Fisher test was used to determine the significance of differences in patency between the PUG-2.7 group and PUG-39 group. Differences were considered significant if the P value was less than 0.05.

Results

In PUG-S-0 grafts with 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. 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. 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 open and the outer surface features were the same as those of PUG-S-0. 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 open and the outer surface displayed features similar to those of PUG-S-0. In the microporous surface grafts, the inner surface of PUG-2.7 showed pores measuring 30 to 70 μm in their largest dimension. The graft wall and the outer surface features were the same as those of PUG-S-0. PUG-39 showed a filamentous inner surface with interfiber spaces ranging from 90 to 130 μm. The structure of the graft wall was widely open and the outer surface displayed features similar to those of PUG-S-0.

All six types of grafts displayed good surgical handling properties and suturability. After the aortic clamp was released and blood started passing through the graft, no blood leakage was recognized through the wall of PUG-S-0. In PUG-S-11, however, a few to several reddish spots appeared on the external surface, spread, and fused with each other. Eventually, the entire external surface looked red and blood oozed for a few minutes. The polymer wall of PUG-2.7 showed a reddish tint, and occasional red spots were observed on the external surface. In contrast, blood oozed through the entire wall of PUG-S-37, PUG-S-58, and PUG-39 immediately after release of the aortic clamp; oozing continued for several minutes and the grafts looked uniformly red.

With regard to patency, in the skinned surface graft group, 2 out of 3 PUG-S-0 retrieved at 1–3 days were patent. However, all eight grafts of that type retrieved at 1 to 2 weeks were occluded. The sole graft retrieved at 3 months was also occluded with an organized tissue, which suggested that the occlusion had occurred at an early stage. In contrast, the patency of PUG-S-11 was 80% (4/5) at 3 months (one graft was acutely thrombo-occluded at 2 h after implantation). The overall patency rate, thus, was 67% (4/6) up to 3 months, below 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 with a HP of more than 11 ml/min/cm^2 showed a better patency than less permeable grafts. In the