A novel bioengineered small-caliber vascular graft incorporating heparin and sirolimus: Excellent 6-month patency

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Objective: A bioengineered microporous polycarbonate-siloxane polyurethane graft has been developed for coronary artery bypass grafting. Biological agents can be impregnated into its absorbable collagen and hyaluronan microstructure and stable macrostructure to promote patency. The objective of this study was to examine the in vivo biological performance and biomechanical characteristics of this graft.

Methods: Three types of graft (3.6-mm internal diameter, 24-mm length) were manufactured: heparin alone (H) grafts, heparin and sirolimus (HS) grafts, and grafts without any drug impregnation (C). All H and HS grafts were impregnated with 54 U of heparin in the microstructure for early elution to prevent acute graft thrombosis and 56 U of heparin in the macrostructure to prevent late thrombosis. In addition to the heparin, the HS graft was impregnated with 2.1 mg of sirolimus in the macrostructure for prolonged elution to inhibit intimal hyperplasia. All grafts (3.6-mm internal diameter, 24-mm length) were implanted into the abdominal aortas of rabbits ($n = 55$). Expanded polytetrafluoroethylene grafts (4.0-mm internal diameter, 24-mm length; $n = 7$) were implanted as controls. At 1, 3, and 6 months after surgery, the grafts were removed for histologic, scanning electron microscopic, immunohistochemical, and biomechanical evaluations.

Results: The patency rate was 100% in the H, HS, and C grafts at each time point. Although the expanded polytetrafluoroethylene grafts were patent at 1 and 3 months after surgery, 1 of 2 grafts (50%) were occluded at 6 months. None of the H or HS grafts had any stenosis or thrombus. Scanning electron microscopic examination proved that endothelial cells propagated smoothly from the anastomotic sites after 6 months in the H and HS grafts in comparison with the expanded polytetrafluoroethylene grafts, which had rare endothelialization. Neointima formation was inhibited in the HS graft compared with the H or C graft at 6 months ($123 \pm 126 \mu\text{m}$ vs $206 \pm 158 \mu\text{m}$ or $202 \pm 67 \mu\text{m}$; $P < .05$). In addition, the H, HS, and C grafts had greater cellular infiltration inside the graft than the expanded polytetrafluoroethylene grafts. All grafts except the expanded polytetrafluoroethylene graft had marked neocapillary formation 6 months after surgery. The graft compliance between 80 and 120 mm Hg was $6.0\% \pm 2.5\%$ and $6.2\% \pm 0.9\%$ at 6 months in the H and HS grafts, respectively. The graft macrostructure was unchanged according to the biomechanical evaluation in the H and HS grafts.

Conclusion: A unique drug-eluting graft had excellent patency throughout the 6 months after implantation. The heparin-sirolimus graft encouraged luminal endothelialization without excessive intimal hyperplasia. This graft performed significantly better than the expanded polytetrafluoroethylene graft. This graft has the potential to become an implantable graft for coronary artery bypass grafting.

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
 ePTFE = expanded polytetrafluoroethylene
 SEM = scanning electron microscopy

Coronary artery bypass grafting (CABG) is performed in hundreds of thousands of patients each year in the United States.¹ The most widely used conduits are autologous internal thoracic arteries, radial arteries, and saphenous veins, which provide excellent mechanical stability and natural antithrombogenicity.^{2,3} However, these autologous bypass materials require surgical harvesting, vary in quality and size, and on occasion have limited availability. Saphenous vein conduits have the added disadvantage of relatively poor long-term patency because of degenerative alterations, such as atherosclerosis or aneurysm formation.⁴ It has been documented that endothelial cell density on the vein graft surface is immediately decreased because of arterial pressure-induced stretch and can damage the vessels.⁵

Because the patency of synthetic small-caliber grafts has been poor, they have not been practical for CABG. The major causes of graft failure have been thrombosis and intimal hyperplasia of the graft. If a synthetic small-caliber graft is resistant to thrombosis in addition to being biocompatible, it would have several significant advantages over traditional autologous grafts. A synthetic graft would have unlimited availability and consistent quality and patency. Moreover, the biomechanical uniformity of a synthetic graft could enable the development of an effective anastomotic device for minimally invasive surgery.

Our group has developed a small-caliber graft that incorporates soluble collagen and hyaluronic acid into the graft pores. Biological agents can be impregnated into the stable macrostructure and resorbable microstructure of the graft,

creating a 2-tiered drug-release system to promote patency. The biostability of the graft 1 month after implantation has been demonstrated.⁶ The objective of this study was to examine the in vivo long-term biological performance and biomechanical characteristics of this graft, and compare them with those of the present gold-standard expanded polytetrafluoroethylene (ePTFE) graft.

Materials and Methods**Graft**

Experimental grafts with an internal diameter of 3.6 mm were fabricated from a nonresorbable polycarbonate-siloxane polyurethane using Kensey Nash Corporation's (Exton, Pa) proprietary foaming technology (Porous Tissue Matrix technology) (Figure 1). The material was formed using a tube mold and a controlled thermal cycle to induce rapid solvent evaporation. This resulted in a highly porous interconnected scaffold with pore sizes of approximately 25 μ m. The main function of the resultant macrostructure is to provide most of the mechanical properties for the graft, including compliance, burst strength, and suturability. The microstructure incorporated into the pores of the graft was resorbable bovine hide-derived collagen (Kensey Nash) and hyaluronic acid (LifeCore Biomedical, Chaska, Minn). The purposes of the microstructure are to provide the biological properties for proper cell attachment and proliferation, deliver a biological additive over a prolonged time period, and prevent leakage of fluid through the porous macrostructure. Moreover, drugs were incorporated into the graft wall during the manufacturing process. Heparin (Celsus Laboratories, Cincinnati, OH) was added to both the macrostructure and microstructure raw materials before device fabrication, and sirolimus (Chemwerth, Woodbridge, Conn) was also added to the macrostructure raw material before fabrication. Biological agents were incorporated into the vascular grafts during manufacturing to prevent short and long-term thrombosis, as well as chronic smooth muscle cell hyperplasia.

In this study, 3 types of grafts (3.6-mm internal diameter, 24-mm length) were manufactured: heparin alone (H) grafts, heparin and sirolimus (HS) grafts, and grafts without any drug incorporation as a control (C). All H and HS grafts were manufactured with 40

Figure 1. Explanted H and HS grafts at 6 months. There was no stenosis at the anastomotic sites and center of the grafts. *H*, Heparin alone; *HS*, heparin + sirolimus.

U of heparin (Celsus Laboratories, Cincinnati, OH) in the microstructure for early elution to prevent acute graft thrombosis and 100 U of heparin in the macrostructure for prevention of late thrombosis. In addition to the heparin incorporation, the HS graft was manufactured with 2.1 mg of sirolimus (Chemwerth) in the macrostructure for prolonged elution to inhibit intimal hyperplasia. All grafts were terminally sterilized by exposure to gamma radiation at a minimum dose of 25 kGy. The data were compared with the ePTFE graft (Gore-Tex Vascular Graft Configured for Pediatric Shunt; WL Gore & Associates, Inc, Flagstaff, Ariz) with an internal diameter of 4.0 mm and a length of 24 mm.

Surgical Procedure

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 Care and Use of Laboratory Animals," prepared by the National Academy of Science and published by the National Institutes of Health (Publication 86-23, revised 1985). In addition, the Animal Studies Committee of the Washington University School of Medicine approved this study protocol.

Sixty-two New Zealand White rabbits weighing between 3 and 4 kg were used randomly in this study. All animals were anesthetized with ketamine (70 mg/kg) intramuscularly, intubated with a 3-mm cuffed endotracheal tube, and mechanically ventilated with a pressure-controlled ventilator. An adequate level of anesthesia was maintained by inhaled isoflurane (1%–3%). A limb-lead electrocardiogram was monitored. A central ear artery catheter was inserted to continuously monitor systemic arterial pressure. Arterial blood samples were drawn every 30 minutes to determine arterial oxygen tension, acid-base balance, and electrolyte levels. Ringer's lactate solution was infused continuously, and sodium bicarbonate, potassium chloride, and calcium chloride were supplemented to maintain pH and electrolytes within normal values. Enrofloxacin (5 mg/kg) was administered preoperatively to reduce the risk of infection.

After a midline abdominal incision, the intestines were displaced to the right side and covered with moistened gauze. The infrarenal aorta was carefully dissected from the surrounding tissue. The lumbar arterial branches were spared to avoid spinal cord ischemia. Intravenous heparin (200 U/kg) was administered. The abdominal aorta was clamped with microapproximator clamps between the lumbar branches and transected. The H, HS, C, or ePTFE grafts were anastomosed to the aorta in an end-to-end fashion with a continuous 7-0 polypropylene suture. The total anastomotic time was less than 30 minutes in every animal (22 ± 4 minutes). Blood flow was measured with an ultrasonic flow probe (Transonic System Inc, Ithaca, NY) proximally and distally. The abdominal incision was closed. The animals received analgesia (buprenorphine 0.3–0.5 mg/kg) and antibiotic (enrofloxacin 5 mg/kg) treatments subcutaneously twice daily for 2 days after surgery. Postoperative antiplatelet therapy (aspirin 15 mg/kg) was administered daily.

At 1, 3, and 6 months after surgery, the animals were anesthetized again with intramuscular ketamine (70 mg/kg) (H grafts: N = 9, 9, and 9; HS grafts: N = 7, 9, and 9; C grafts: N = 0, 0, and 3; and ePTFE grafts: N = 2, 3, and 2 at 1, 3, and 6 months, respectively). The abdominal incision was reopened. The surgical site was examined for adhesions, fibrosis, hematoma, or arteriove-

nous fistula. Blood flow at the proximal and distal anastomoses was measured with an ultrasonic flow probe. The animal was euthanized, and the graft was carefully removed for biomechanical, histologic, and immunohistochemical evaluations.

Biomechanical Characterization

Graft Compliance Test. The graft and approximately 1 cm of intact aorta proximally and distally were dissected. The graft was secured to a customized fixture designed to pressurize the sample while holding it at a set length. The setup contained a pressure gauge (Digimano 1000, Netech, NY) inserted in-line downstream from the graft. The system was pressurized by injecting saline in 0.02-mL boluses using a calibrated repeat pipettor (Repeater Plus, Eppendorf, NY) placed upstream from the graft. The internal radius was calculated from the volume assuming an incompressible fluid and a length that remained constant. Radial compliance was reported as the percent change in calculated radius per change in measured pressure.

Graft Tensile Strength Test. The graft and approximately 1 cm of intact aorta proximally and distally were dissected. Two 5-mm-wide "rings" were cut from the middle of the graft for tensile testing. Graft outer diameter, length, and thickness were measured. Two dowel pins were inserted into the ring and secured with a holding fixture to a mechanical test stand (Model TCD200, Chatillon, Largo, Fla) with a 2-lb load cell (Model DFGS 2, Chatillon). The pins were then pulled apart at a rate of 50 mm/min while measuring tensile force versus displacement. Ultimate tensile strength was calculated as ultimate tensile strength = max load/(2 × thickness × length).

Graft Anastomotic Strength Test. After the "rings" were removed from the center of the graft for tensile strength testing, the remaining ends of the graft/aorta interface were tested to determine the strength of the anastomosis. Samples were clamped on each end so that 2 mm of graft and 2 mm of aorta were exposed. The grips were secured to a mechanical test stand (Model TCD200, Chatillon) with a 2-lb load cell (Model DFGS 2, Chatillon). Samples were pulled apart at 2 inches per minute, and the peak tensile force was recorded.

Histologic and Immunohistochemical Evaluations

Histology. The graft and approximately 1 cm of intact aorta proximally and distally were dissected. The specimen was fixed in 10% buffered formalin, dehydrated in a graded series of ethanol, and embedded in paraffin. Longitudinal and transverse sections were obtained along the length of the graft for tissue processing, and the paraffin blocks were sectioned at 4 to 6 μ m and mounted on a charged glass slide. Slides were stained with hematoxylin-eosin, and Movat pentachrome. Graft patency, neointima formation, endothelialization of the graft, and tissue ingrowth and angiogenesis in the graft wall were examined histologically.

Immunohistochemistry. Dewaxed paraffin sections were treated with 0.3% hydrogen peroxide to inactivate endogenous peroxidases. The sections were then immersed in protein-free block (Dako, Carpinteria, Calif) to block nonspecific binding of primary antibodies. Sections were incubated for 1 hour at room temperature with primary antibodies against human smooth muscle beta-actin (clone HHF35, dilution 1:20, Enzo, Farmingdale, NY), the macrophage marker Ram-11 (dilution 1:200, Dako), and a purified polyclonal antibody to von Willebrand factor (dilution 1:2000, Strategic Biosolutions, Newark, Del).

Primary antibodies were labeled with anti-mouse biotinylated link antibody from a peroxidase-based kit (LSAB, Dako). Positive staining (rose reaction product) was visualized using a 3-amino-9-ethylcarbazole substrate-chromogen system; the sections were counterstained with Gill's hematoxylin.

Scoring. Luminal surface fibrin/platelet aggregation, endothelialization, and cellular infiltration of the grafts were scored from 0 to 4. Grade 0 was defined as no appreciable fibrin/platelet aggregation or cellular infiltration present. Grade 1 (minimal) denoted fibrin/platelet aggregation, the presence of cellular infiltration less than one fourth the thickness of the graft conduit wall, or endothelialization of one quarter of the cross-sectional luminal surface. Grade 2 (mild) indicated fibrin/platelet aggregation or the presence of cellular infiltration up to or equal to one half of the thickness of the graft conduit wall or endothelialization of one half of the cross-sectional luminal surface. Grade 3 (moderate) signified fibrin/platelet aggregation or the presence of cellular infiltration up to or equal to three quarters of the thickness of the graft conduit wall or endothelialization of three quarters of the cross-sectional luminal surface. Grade 4 (severe/marked) was identified by fibrin/platelet aggregation, the presence of cellular infiltration throughout the full thickness of the graft conduit wall, or complete endothelialization of the cross-sectional luminal surface. The histologic index was calculated by dividing the sum of the grade by the number of sample grafts (grade/N) at the anastomotic sites and center of the grafts.

Scanning Electron Microscopy Evaluation

For 1 of the grafts in each group at each time point, scanning electron microscopy (SEM) imaging was performed. SEM was used to evaluate the presence of thrombi, endothelial coverage, and endothelial maturity. Before processing, the specimens were bisected longitudinally to expose the luminal surface and photographed. Specimens were rinsed in 0.1 mmol/L sodium cacodylate buffer (pH 7.2) and then post-fixed in 1% osmium tetroxide for 30 minutes. Specimens were dehydrated in a graded series of ethanol. After critical point drying, the tissue was mounted and sputter-coated with gold, and specimens were visualized using a Hitachi scanning electron microscope (Hitachi Medical, Tokyo Japan). The percentage of endothelium was based on a visual estimate.

Statistical Analysis

Continuous values are expressed as mean $\pm$ 1 standard deviation. Comparisons were made by Student *t* test assuming unpaired data.

Results

All rabbits in groups H, HS, C, and ePTFE survived after surgery. Paraplegia was not observed in any animal during the postoperative period.

Rheologic Data and Graft Morphology

The patency rate was 100% in the H, HS, and C grafts at each time point. Although the ePTFE graft was patent at 1 and 3 months after surgery, 50% of the grafts were occluded at 6 months. There were no significant differences in blood flow at the proximal and distal anastomoses after the initial implantation and at the end of study in any group (Table 1).

TABLE 1. Blood flow at the proximal and distal anastomosis

Graft	Graft implantation			End of study		
	Proximal	Distal	<i>P</i> value	Proximal	Distal	<i>P</i> value
H	51 $\pm$ 23	48 $\pm$ 22	.6206	56 $\pm$ 24	50 $\pm$ 21	.3402
HS	48 $\pm$ 25	43 $\pm$ 21	.4664	64 $\pm$ 20	59 $\pm$ 18	.3075
C	35 $\pm$ 4	32 $\pm$ 9	.6367	56 $\pm$ 27	55 $\pm$ 27	.9660
ePTFE	41 $\pm$ 16	35 $\pm$ 16	.5138	46 $\pm$ 24	33 $\pm$ 19	.2831

ePTFE, Expanded polytetrafluoroethylene.

Although none of the H, HS, and C grafts had any thrombus or stenosis inside the graft, 2 of 3 ePTFE grafts at 3 months had thrombus, which was not occlusive. One of 2 ePTFE grafts at 6 months was completely occluded by thrombus. Although all grafts were covered by an adhesion capsule, there was no stenosis observed histologically at the proximal or distal anastomoses (Figure 1). There were no apparent changes in the graft, including dilatation, collapse, dehiscence, or aneurysm formation, during the 6-month postoperative period. There was no bleeding from the grafts and no arteriovenous fistulae formation.

Biomechanical Characterization

Graft Compliance. The graft compliance data are shown in Figure 2, A. The preoperative graft compliances between 80 and 120 mm Hg of the H and HS grafts were $7.4\% \pm 2.4\%$ and $8.3\% \pm 0.4\%$, respectively ($P = .6932$). The postoperative compliances of the H graft were $8.5\% \pm 1.6\%$, $4.4\% \pm 1.0\%$, and $6.0\% \pm 2.5\%$ at 1, 3, and 6 months after surgery, respectively. The postoperative compliances of the HS graft were $11.6\% \pm 0.2\%$, $5.1\% \pm 1.3\%$, and $6.2\% \pm 0.9\%$ at 1, 3, and 6 months after surgery, respectively. There were no significant differences in the graft compliance over time.

Graft Tensile Strength. The tensile strengths of the H and HS grafts were 967 ± 200 kPa and 794 ± 70 kPa preoperatively ($P = .0371$, Figure 2, B). The postoperative tensile strength of the HS graft was significantly greater than that of the H graft at 1 and 3 months after surgery ($P = .0013$ and $.0053$, respectively). At 6 months, there was no significant difference in the tensile strength between H and HS grafts ($P = .1671$). The tensile strength of the rabbit descending thoracic aorta was measured to be 1410 ± 310 kPa. Although there was no significant difference in the tensile strength between the HS graft at 6 months and the descending thoracic aorta ($P = .1290$), the H graft strength at 6 months was significantly lower than that of the descending thoracic aorta ($P = .0092$).

Anastomotic Strength. The proximal and distal anastomotic strengths of the H graft were significantly greater than those of the HS graft at 1 month after surgery ($P = .0002$ and $.0033$, respectively; Figure 3). However,

Figure 2. A, Preoperative and postoperative graft compliance. B, Preoperative and postoperative graft tensile strength. * $P < .05$. ** $P < .01$. H, Heparin alone; HS, heparin + sirolimus.

there was no significant difference in anastomotic strength between the H and HS grafts at later time periods. The proximal anastomotic strengths of the HS grafts increased as time advanced.

Histologic Characterization

Although 1 ePTFE graft was occluded at 6 months after surgery, all H, HS, and C grafts were patent and had no stenoses histologically during the 6-month follow-up (Figure 4). In the early postoperative period (1–3 months), all grafts were mildly covered with luminal surface fibrin (Figure 5, A). The graft wall was predominantly infiltrated by red blood cells and aggregates of platelets with some leukocytes. Less luminal fibrin/platelet formation was found in the H, HS, and C grafts at 6 months after surgery when compared with the ePTFE grafts.

There was a mild amount of neointima formation at 3 to 6 months after surgery in the H and C grafts (Figure 5, A). In the HS graft, neointima formation was inhibited more than the H or C graft for the first 6 months (Figure 6). There was no excessive intimal hyperplasia in any HS grafts. The average

Figure 3. Proximal and distal anastomotic strengths. * $P < .01$. N, Newton; H, heparin alone; HS, heparin + sirolimus.

neointimal thickness at the region of anastomotic sites at 6 months was $206 \pm 158 \mu\text{m}$ in the H grafts and $123 \pm 126 \mu\text{m}$ in the HS grafts ($P = .0288$). Although the neointima thickness serially increased in the HS graft after surgery during 3 months, neointima hyperplasia was inhibited in the HS graft after 3 months. There was less neointima in the ePTFE graft at all time points. The neointimal thickness of the ePTFE was $28 \pm 14 \mu\text{m}$ at 6 months.

Serial changes in the endothelialization of the grafts are shown in Figure 5, B. At 1 month, the H graft had a layer of spindle-shaped, immature mesenchymal cells covering the anastomotic site from the native aorta (grade 1). Although the HS grafts showed no endothelialization at the anastomotic sites and the center of the graft at 1 month, the HS grafts showed considerable endothelialization at the 3-month time point. Despite the increased endothelialization score, the scores were slightly lower than that of the H graft. The H, HS, and C grafts had significant endothelialization at the anastomotic sites and center of the graft at 6 months. The ePTFE graft barely had any endothelialization at 6 months.

The H and ePTFE grafts had significant cellular infiltration inside the grafts 1 month after surgery (Figure 5, B). The resorbable microstructure in the H and HS grafts consisting of bovine-derived collagen was completely replaced by the autologous tissue. In the HS graft, although autologous tissue infiltrated mildly at 1 month, the score of the cellular

Figure 4. Histology and immunohistochemical findings of the grafts at 6 months. Normal-appearing endothelial cells were aligned with the blood flow. The endothelial cell layer is smooth from the native aorta to the graft in H, HS, and C grafts. There were less endothelial cells in the ePTFE graft. Moderate cellular infiltration was observed in the graft wall in the H and HS grafts (upper: Movat stain 40 $\times$). A layer of endothelial cells is shown on inside of the H, HS, and C grafts. Moderate neocapillary formation is shown in the graft wall in the H, HS, and C grafts (lower: von Willebrand factor stain 100 $\times$, red dots in the graft wall). *ePTFE*, Expanded polytetrafluoroethylene; *H*, heparin alone; *HS*, heparin + sirolimus.

infiltration was serially increasing at anastomotic sites and center of the graft after 3 months. A great deal of neocapillary formation was observed in the graft wall in the H, HS, and C grafts. Although there were blood cells inside the neocapillaries in the H, HS, and C grafts, the ePTFE graft had no neocapillary formation inside the graft during 6 months.

Scanning Electron Microscopy Findings

The lumens of the H and HS grafts were completely covered by a layer of endothelial cells at 6 months (Figure 7). The HS graft had a smoother and tighter endothelial cell layer compared with the other grafts. Although the endothelial cells covered the entire inside of the C graft, the layer of endothelial cells of the C graft was not smooth or regular. In the ePTFE graft, there was minimal endothelialization at 6 months. A luminal fibrin/platelet sheet covered the ePTFE graft wall.

Discussion

This study examined the in vivo biological and mechanical performance of a novel bioengineered small-caliber vascular graft during a period of 6 months. The graft enabled the controlled release of biological agents to theoretically inhibit acute and chronic thrombus formation, as well as intimal hyperplasia. Heparin was embedded in the microstructure of the graft for early elution. Heparin and sirolimus were incorporated into the macrostructure of the graft for late

elution. Although both the H and HS grafts had a 100% patency rate during the 6 months after surgery, the HS graft showed less neointima formation than the H graft. A thin neointima, which included elongated endothelial cells, extended smoothly into the graft wall without any intimal hyperplasia in the HS graft 6 months after implantation.

The H and HS grafts incorporated either heparin or heparin and sirolimus in the graft wall. In a previous study from our laboratory, it was shown that heparin had eluted from the graft microstructure during the first month postoperatively.⁶ In this study, the microstructure of the graft was replaced by autologous tissue after 1 month. It has been proposed that heparin eluted from the graft wall could prevent acute thrombus.^{7,8} Although our study showed similar histology between the grafts with (H) and without (C) heparin, the SEM evaluation revealed that there was a considerable difference in the degree of endothelialization between the H and C grafts. The C graft had rougher endothelial layers than the H graft at 6 months. Heparin and sirolimus impregnated in the macrostructure played a more important role in endothelialization and neointima thickening than heparin alone. Sirolimus has been reported to inhibit the proliferation of smooth muscle cells and prevent neointima thickening.⁹⁻¹² Our study supported this hypothesis. The HS graft had a complete and smooth layer of endothelial cells and less neointima formation compared with the H or C graft in this study.

Figure 5. Serial changes in neointima formation, luminal fibrin/platelet formation, endothelialization, and cellular infiltration at the anastomotic sites and center of the grafts. The histologic index was calculated by dividing the sum of the grade by the number of sample grafts (grade/n). *ePTFE*, Expanded polytetrafluoroethylene; *H*, heparin alone; *HS*, heparin + sirolimus.

Synthetic grafts have been used for revascularization in patients who have limited autologous graft materials available. Satisfactory synthetic materials have not been developed for CABG because of their poor long-term patency rates. Although Dacron and ePTFE grafts have been used successfully in peripheral revascularization cases, these small-caliber vascular grafts have failed for coronary revascularization.¹³ Dacron grafts suffer from thrombosis and neointimal proliferation. ePTFE grafts also have had poor

patency rates because of surface thrombogenicity.¹⁴ It has been described that endothelial cell-seeded grafts could decrease thrombogenicity and intimal hyperplasia.¹⁵⁻¹⁷ However, cell-seeded grafts are not practical for CABG cases because of the complex, time-consuming, and costly manufacturing process.

In this study, the ePTFE graft had less endothelialization and moderate luminal fibrin/platelet formation at 6 months. The SEM evaluation showed that fibrin and platelets covered

Figure 6. Neointima thickness at the proximal and distal anastomoses. *ePTFE*, Expanded polytetrafluoroethylene; *H*, heparin alone; *HS*, heparin + sirolimus.

the inside of the ePTFE graft wall instead of endothelial cells at 6 months. Less endothelialization likely caused 1 graft occlusion of the graft 6 months after surgery. Smooth endothelial layers are prerequisite for good long-term patency.¹⁸ The HS graft had the smoothest endothelial layers inside the graft in all grafts.

The biomechanical tests established the biostability of the grafts under arterial blood flow conditions. None of the H, HS, and C grafts showed any aneurysm formation, dilatation, or structural collapse during the 6-month postoperative period. Fresh porcine carotid artery compliance was $9.4\% \pm 2.2\%$.⁶ Human saphenous vein and ePTFE graft compliances over a pulse pressure of 40 mm Hg are known to be 2.0% and 1.0%, respectively.^{19,20} Significant changes in the graft compliance in the H and HS grafts were not seen during 6 months. The graft compliances of the H and HS graft were always greater than that of human saphenous vein or ePTFE grafts. The tensile strength of the native descending thoracic aorta of rabbits was 1436 ± 450 kPa, as reported in our previous study.⁶ There were no significant changes in tensile strength of the H and HS grafts over time and at 6 months. They were not statistically different from each other. In general, the tensile strength, particularly of the HS graft, approximated that of the native aorta.

By histologic and immunohistochemical evaluations, there was significant neocapillary formation in the walls of the H, HS, and C grafts 1 month after surgery. However, the

Figure 7. SEM findings. Endothelial cells covered the inside of the H and HS grafts. The layer of endothelial cells of the HS graft was smoother than that of the H graft. The C graft had rough endothelial cells. The ePTFE graft had less endothelial cells and marked fibrin/platelet deposition. *ePTFE*, Expanded polytetrafluoroethylene; *H*, heparin alone; *HS*, heparin + sirolimus.

ePTFE graft had no neocapillary formation even 6 months after surgery. If synthetic vascular grafts are used for CABG, the length of the graft may need to be long. Endothelial cells can migrate into the graft from the (1) anastomotic sites, (2) circulating blood, and (3) neocapillaries inside the graft.²¹ It would take a long time to extend endothelial cells from the anastomotic sites in a long graft for clinical application. In addition to the extension from anastomotic sites and the contribution from the circulating blood, migration from the neocapillaries could carry endothelial cells to the center of the graft. The neocapillary formation may be an advantage to encourage endothelialization for synthetic vascular grafts.

Conclusions

A unique bioengineered microporous drug-eluting graft had excellent patency throughout the 6 months after implantation. Biological agents, heparin and sirolimus, were impregnated into the graft and encouraged luminal endothelialization and neointimal formation. This graft has the potential to become an implantable graft for CABG.

This study was a 6-month evaluation of a novel bioengineered synthetic small-caliber graft involving 62 rabbits. Because grafts were examined for histology and biomechanical properties at several different time points, the number of implanted grafts at each time point was small. However, data of each time point were similar and consistent. The C grafts were evaluated at only 6 months after surgery to be compared with the H, HS, and ePTFE grafts. This is a preliminary study in a rabbit aortic model. A longer term study will be needed to evaluate the chronic efficacy of the eluted drugs. A large animal coronary bypass graft model is needed to establish clinical feasibility.

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Discussion

Dr Robert Poston (Baltimore, Md). Why does sirolimus affect endothelialization in stents but not in your grafts? What is the difference there? It is pretty well known that sirolimus reduces re-endothelialization on top of an intracoronary stent, but it did appear to have the same affect on the conduits in your research.

Dr Ishii. Regarding the endothelialization, in the SEM variations we can see it occurring beautifully inside the graft wall. When compared with a non-sirolimus graft, sirolimus might therefore facilitate good endothelialization. However, although both heparin and heparin-sirolimus grafts had a 100% patency rate during the 6 months after surgery, the heparin-sirolimus grafts showed less neointima formation than the heparin grafts.

Dr Poston. Maybe it is not just understood how that works. The second question is related to your model. The main concern that people have regarding the use of small prosthetic grafts as bypass conduit for the heart would be acute thrombosis. Your control grafts in your model didn't acutely thrombose, even the PTFE graft without heparin. So do you think that this model is adequately testing the key limitation of these grafts?

Dr Ishii. Actually you are right. This is a good question, Dr Poston. We didn't see any acute thrombosis in either the control grafts without drugs or in the heparin and sirolimus grafts. However, half of the ePTFE grafts in this study occluded during the 6 months after surgery. When compared with our microporous polyurethane grafts, the ePTFE grafts showed less endothelialization. As I showed you with the SEM data, out of all the grafts the heparin-sirolimus grafts had the most beautiful layer of endothelial cells.

Dr Beat H. Walpoth (*Geneva, Switzerland*). I think what are you doing is a bit controversial. Our series on rapamycin-treated grafts was published in the *European Journal of Cardiothoracic Surgery* in 2000, and we showed that there is a significant reduction of intimal hyperplasia. I think your data were not significant. In addition, we had no endothelialization; we had much more thrombus formation. So this goes along with what is now seen in the drug-eluting stents. In medicine, everything is a question of dosage. I am not sure if eventually your dosage was not potent

enough to achieve this goal. Anyhow, they are some interesting results.

Dr Valavanur Subramanian (*New York, NY*). Just a brief comment to bring you up to date. There are drug-eluting wraps around the grafts that have already been in clinical use in arteriovenous fistula. Our colleagues in endovascular cardiology have now taken a different form of wrap around the distal anastomosis with a PTFE or saphenous vein graft. The first feasibility trial will soon be done in coronary bypass grafts pretty, and the 6-month patency in the arteriovenous fistula has been excellent. So there is already a clinical movement on this approach.

Dr Cliff Choong (*Cambridge, United Kingdom*). Do you have any data on longer-term results, and are you planning to do any long-term experimental studies?

Dr Ishii. Actually this is just a preliminary rabbit model. We don't have any longer-term data yet. As you mentioned, we should do a larger animal study and longer-term study before attempting any clinical applications. We are planning that now.