Tissue-Engineered Acellular Small Diameter Long-bypass Grafts with neointima-inducing activity

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Abstract

Researchers have attempted to develop efficient antithrombogenic surfaces, and yet small-caliber artificial vascular grafts are still unavailable. Here, we demonstrate the excellent patency of tissue-engineered small-caliber long-bypass grafts measuring 20–30 cm in length and having a 2-mm inner diameter. The inner surface of an acellular ostrich carotid artery was modified with a novel heterobifunctional peptide composed of a collagen-binding region and the integrin $\alpha 4\beta 1$ ligand, REDV. Six grafts were transplanted in the femoral-femoral artery crossover bypass method. Animals were observed for 20 days and received no anticoagulant medication. No thrombogenesis was observed on the luminal surface and five cases were patent. In contrast, all unmodified grafts became occluded, and severe thrombosis was observed. The vascular grafts reported here are the first successful demonstrations of short-term patency at clinically applicable sizes.

Keywords

small-diameter vascular graft, long bypass, acellular, tissue engineering, neointima

Running headline

Acellular Small Diameter Long-bypass Grafts

1. Introduction

Synthetic artificial vascular grafts with inner diameters (IDs) larger than 6 mm are frequently used for bypass grafts or replacements, and they provide good, long-term clinical patency [1]. However, small-caliber vascular grafts with IDs smaller than 4 mm cannot achieve patency due to acute thrombogenicity on the luminal surface and occlusion [2,3]. Anastomotic intimal hyperplasia, aneurysm formation, infarction, and atherosclerotic disease progression are major health problems worldwide and may be addressed by using small-caliber vascular grafts [2-6]. During the last half century, researchers have investigated the potential of clinical applications of different types of grafts, such as endothelial cell-seeded synthetic materials [7-9], biodegradable polymers [10-15], cell sheets [16,17], and biopolymers [18-20]. The majority of these studies used 1–2-mm ID very short grafts transplanted into the abdominal aorta in rats or 3-mm ID grafts measuring approximately 5 cm in length transplanted for carotid artery replacement in dogs [10, 12, 14, 21-23]. Although these studies provided a large amount of fundamental information regarding tissue response, antithrombogenicity, and patency of small-diameter artificial blood vessels, clinically usable small-diameter vascular grafts have yet to be developed. One explanation for this may stem from the discrepancies in required diameters and lengths between animal models and humans. For a coronary artery or limb distal bypass graft to be clinically relevant, small-diameter vascular grafts with IDs of less than 2 mm and lengths greater than 10 cm are required. Indeed, the majority of previous efforts have focused on obtaining a much more effective antithrombogenic surface, and the development of appropriate grafts yielding good patency has not been achieved.

Rapid endothelialization on the luminal surface of the artificial graft must inhibit the initial thrombosis and lead to long-term patency. In this study, we focused on four important features for designing small-caliber vascular grafts: (i) compliance matching and high tensile strength to facilitate easy suturing and to prohibit rupture; (ii) achieving high endothelial cell affinity of the modified luminal surfaces [24]; (iii) ability of the engineered graft to be replaced with host tissue in order to achieve life-time usage; and (iv) creating a graft with adequate size for clinical use, such as for a coronary artery bypass or distal limb bypass. Our final design was a 2-mm ID decellularized vascular graft with a length of 20–30 cm.

To this end, decellularized ostrich carotid arteries were employed as the graft material due to their straight and branchless structure. We have been studying larger sized decellularized cardiovascular tissue using non-detergent technology [25, 26] and

1 reported their good patency and performance. However we did not achieve high patency
2 in this thin and long vascular graft. The purpose of this study was to achieve the patency
3 of the small diameter long-bypass decellularized graft by modifying the luminal surface
4 with the integrin $\alpha 4\beta 1$ ligand peptide, REDV. Increasing of the endothelial cell binding
5 affinity through peptide-modification was indicated in vitro, and the mechanical
6 property of the graft was also evaluated. The patency and neointima-inducing activity
7 were assessed by transplanting to pig in the femoral-femoral artery crossover bypass
8 method.

2. Materials and methods

2.1 Decellularization of the graft.

Carotid arteries were isolated from African black ostriches weighing 90–130 kg (Shimizu-Laboratory Supplies Co., Ltd, Kyoto, Japan). Fat tissue was removed from the arteries, and trimmed arteries were washed and packed with saline. They were then decellularized using a modified the ultrahigh hydrostatic pressure (UHP) method. Arteries were treated with the high-hydrostatic pressure with a cold isostatic pressurization machine (Dr. Chef; Kobelco, Kobe, Japan) containing pressure-transmission fluid which consists of ethylene-glycol and water [25, 26]. The pressure was increased up to 980 MPa at a rate of 65.3 MPa/min and then maintained within the chamber for an additional 10 min. After decreasing the pressure until atmospheric pressure was reached, the specimen was washed with saline. The sample was then immersed with saline containing 40 U/mL of DNase I (Roche Applied Science, Indianapolis, IN, USA), 20 mM $MgCl_2$, and antibiotics for 3 days under 37°C. After washing with saline, the specimen was immersed in saline containing 20 mM $MgCl_2$ and antibiotics for 3 days at 37°C to remove the remaining DNase I. Finally, the sample was washed with saline and preserved in the same medium until experimental use. The quantification of the remaining DNA was carried out using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA), and fluorescence DNA quantification kit (Bio-Rad, Richmond, CA)

The physical and morphological features of the graft were evaluated. To evaluate histological staining, tissues were fixed with 10% formalin (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and samples were embedded in paraffin. Paraffin-embedded tissues were then sectioned and stained with hematoxylin-eosin, Elastica van Gieson, or von Willebrand factor. Staining was carried out by the Applied Medical Research Laboratory (Osaka, Japan). The mechanical properties were evaluated by stress-strain curve measurements. The specimen was fastened to the stage

1 of a tensile strength tester (AGS-H autograph, Shimadzu Co., Kyoto, Japan). The
2 specimen was stretched with the speed of 2 N/min, and the stress and strain curves were
3 analyzed.

2.2 Peptide-modification

4
5 Two peptides, having the sequences
6 (Pro-Hyp-Gly)₇-Gly-Gly-Gly-Arg-Glu-Asp-Val (POG7G3REDV) and
7 (Hyp-Pro-Gly)₇-Gly-Gly-Gly-Arg-Glu-Asp-Val (OPG7G3REDV)
8 (Hyp=hydroxyproline), were purchased from Sigma-Aldrich Japan (Tokyo, Japan) as
9 custom-made synthesized peptides. Decellularized carotid arteries were immersed in 10
10 µM peptide solution in saline, and samples were then incubated at 60°C for 1 h. The
11 solution containing the specimen was then cooled to room temperature. Before
12 experimental use, the decellularized tissue was washed with saline.

13
14 Peptide modification on the luminal surface was verified using Alexa Fluor
15 633-labeled peptides under a confocal laser scanning microscope (CLSM). Alexa
16 Fluor 633 NHS esters (Life Technologies, Gaithersburg, MD, USA) were added to the
17 Gly-Gly-Gly-(Pro-Hyp-Gly)₇ peptide solution and incubated overnight at room
18 temperature. After the incubation, labeled peptide was purified with a PD-10 column.
19 Peptide modification was carried out using the same procedure. Cross sections of the
20 modified graft were observed using a FV1000-D CLSM system (Olympus, Tokyo,
21 Japan).

2.3 Cell binding assay

22
23 Human umbilical vein endothelial cells (HUVECs, Kurabo, Osaka Japan) were
24 cultured on a tissue culture polystyrene surface (Iwaki, Tokyo, Japan) using endothelial
25 basal medium (EBM-2; Lonza, Switzerland) supplemented with EGM Single Quots
26 supplements and a growth factor kit (Lonza, Switzerland). Human endothelial
27 progenitor cells (EPC; Biocat GmbH, Heidelberg, Germany) were also cultured on a
28 collagen-coated polystyrene surface using endothelial basal medium (EBM-2; Lonza,
29 Switzerland) supplemented with EGM-2-MV supplements and a growth factor kit
30 (Lonza, Switzerland). The cells were grown to confluence. The cultures were placed in
31 a humidified atmosphere containing 95% air and 5% CO₂ at 37°C. The culture medium
32 was changed every two days, and cells typically reached confluence in 6–8 days. After
33 the cells reached confluence, they were washed with room-temperature HEPES buffer
34 and then immersed in 0.025% trypsin/HEPES solution containing 0.01% EDTA (Lonza,
35 Switzerland). After 2–5 min, the cells became round, indicating detachment from the
36

1 surface. After neutralization of the trypsin, 5×10^5 cells were seeded on 10-cm culture
2 dishes and cultured until reaching confluence. NIH/3T3 cells were cultured using the
3 same procedure used on HUVECs, except that Dulbecco's modified Eagle's medium
4 with low glucose was used instead. All disposable materials and subculture media were
5 the same as those used in HUVEC cultures.

6 To evaluate the cell-binding efficiency for peptide-modified decellularized tissue,
7 porcine aortic tissue was selected. The tissue was decellularized using UHP treatment,
8 and cells were labeled with a Q-dot 625 cell labeling kit (Life Technologies, Grand
9 Island, NY, USA). The labeling procedure was performed according to the
10 manufacturer's instructions. Peptide-modified and unmodified 8×8 mm sections of
11 decellularized tissue were placed into cell culture multiplates (Iwaki, Tokyo, Japan), and
12 the cell suspension containing 2×10^5 cells was added to the chamber. The plates were
13 incubated in a humidified atmosphere containing 95% air and 5% CO₂ at 37°C for 24 h.
14 The tissues were then washed three times with PBS, and adherent cells were eluted
15 using cell lysis buffer (Promega, Madison, WI, USA). The fluorescence intensities of
16 the lysed solutions were measured, and adherent cell numbers were calculated from a
17 standard curve. Additionally, 2×10^5 EPCs were seeded on peptide-modified and
18 unmodified 8×8 mm sections of decellularized tissue. After 24-h incubation, the tissue
19 was washed with PBS. The tissues were fixed with 3.7% formaldehyde solution for 10
20 min at room temperature, and then the cells were immersed in 0.1% Triton X in PBS for
21 5 min. The fixed cells were stained with rhodamine phalloidin (Life technologies,
22 Grand Island, NY, USA) and DAPI solution (Dojin chemical Co., Kumamoto, Japan).
23 After staining, specimens were observed using the FV1000-D CLSM system (Olympus,
24 Tokyo, Japan).

2.4 Transplantation of grafts

25 All animal experiments were conducted in accordance with the Guidelines for
26 Animal Experiments established by the Ministry of Health, Labor, and Welfare of Japan
27 and by the National Cerebral and Cardiovascular Center Research Institute in Japan.
28 The protocol was approved by the Committee on the Ethics of Animal Experiments of
29 the National Cerebral and Cardiovascular Center Research Institute (Permit Number:
30 009017). Goettingen minipigs, purchased from Ellegaard Göttingen Minipigs A/S
31 (Dalmoose, Denmark), were anesthetized with 100 mg/h intravenous injection of 1%
32 propofol (Diprivan; AstrazZeneca, Wilmington, DE). Activated coagulation time,
33 monitored by ACT (Medtronic, Inc, Minneapolis, MN), was controlled at around
34 150–200 s during transplantation surgery by a single injection of heparin

(Novo-heparin; Novo Nordisk, Copenhagen, Denmark). Six peptide-modified decellularized carotid arteries and three unmodified decellularized carotid arteries were transplanted into each pig using 8-0 proline sutures (Ethicon, Somerville, NJ). Under an operating microscope, the left and right femoral arteries were exposed and clamped with single-use microvessel clamps (Bear Medic Co., Ibaraki, Japan). The graft was connected to left femoral artery in a side-to-end fashion and to the right femoral artery in an end-to-end fashion. To check the blood flow condition just after the transplantation, blood velocity (a.u.) was recorded using laser Doppler flowmetry (Omega Flow FLO-C1; Omega wave, Tokyo, Japan). After the operation, additional anticoagulation medicine was not used.

2.5 Blood compatibility of grafts and neointima formation

To evaluate the very early stage thrombogenesis, the grafts were exposed to blood flow for 60 min by connecting them to a pump oxygenator during the surgery. The specimen was dehydrated with ethanol and immersed in t-butyl alcohol, and dried by vacuum. The luminal surface was coated with a gold layer in an ion coater (IB-3 ion-coater, Eiko Engineering, Ibaraki, Japan) and observed under a scanning electron microscopic (SEM) (JCM 5700 microscope, JEOL, Tokyo, Japan).

The peptide-modified graft was extirpated at 1 week to evaluate the endothelialization. These experiments were separately carried out from the graft patency. Histological staining (hematoxylin-eosin, Elastica van Gieson, or von Willebrand factor) of the peptide-modified tissue was carried out by the Applied Medical Research Laboratory (Osaka, Japan).

2.6 Patency of the grafts

The six transplanted peptide-modified grafts were exposed, and the patency was checked with ultrasonic diagnosis (Prosound II, SSD-6500 SV, Hitachi Aloka Medical, Ltd., Tokyo, Japan), laser Doppler flowmetry, and microscopic angioscopy (3 CCD imaging system FT-203F, FiberTech Co., Ltd., Tokyo, Japan) at 3 weeks post-transplantation. The luminal surfaces of the grafts were observed by gross appearance. As control experiments, three mini pigs were transplanted with the unmodified grafts and sacrificed at 7 (two pigs) and 27 (one pig) days.

3. Results

The small diameter vascular grafts were prepared by the ultrahigh hydrostatic pressure (UHP) decellularization and peptide modification of the ostrich carotid arteries

(Figure 1). Peptide modification was accomplished by the incubation with peptide solution at 60 °C for 1 hour. Histological feature, mechanical property, and graft patency were studied.

3.1 Decellularization of ostrich carotid artery

A decellularized ostrich carotid artery is shown in Figure 2a. The entire length of the ostrich carotid artery was 80–90 cm, and the ID was 2 (distal) to 4 (proximal) mm. Cell components were observed on native and UHP treatment tissues (Figure 2b). On the other hand, the components were not observed after UHP treatment and washing. The remaining DNA in the tissue was not detected on the DNA quantification assay (Figure 2d). Therefore, cell components were completely washed from the tissue (Figure 2b-d). The histological staining of the tissue after UHP treatment and washing was shown in Figure 2c. Vimentin and vWF positive cells were not detected on the luminal surface of the graft. That is, the endothelial cell layer on the luminal surface was completely eliminated (Figure 2c). A typical J curve was observed in the stress-strain curve of the decellularized graft (Figure 2e). The elastic moduli at low-strain region of porcine femoral artery and the prepared graft were 0.39 and 0.31, respectively. Those at the high-strain region were 3.63 and 3.20, respectively. In terms of compliance matching, the artery's elasticity was similar to that of the native femoral blood vessel.

3.2 Heterobifunctional peptide modification for rapid endothelialization

The (Pro-Hyp-Gly)₇ (POG7) peptide was conjugated to Arg-Glu-Asp-Val (REDV), the specific integrin $\alpha 4\beta 1$ ligand expressed on endothelial cells and circulating endothelial progenitor cells, through the Gly-Gly-Gly (G3) spacer domain; [24] this was termed POG7G3REDV. An inverted (Hyp-Pro-Gly)₇ peptide (OPG7G3REDV) was used as the control sequence.

Peptide modification, shown in Figure 1, was confirmed using a confocal laser scanning microscope analysis of fluorescence-labeled peptides (Figure 3a). The peptide was immobilized to the graft wall to a depth of approximately 45 μ m in the tunica intima. To confirm REDV-dependent cell-binding affinity on the peptide-modified surface, human umbilical vein endothelial cells (HUVECs) expressing $\alpha 4\beta 1$ integrin were used. When HUVECs were seeded on the Peptide-modified surface, 86.6% of the cells attached. In contrast, few cells attached to the unmodified and OPG7G3REDV-modified decellularized tissues (Figure 3b). Additionally, fibroblasts (integrin $\alpha 4\beta 1$ -negative) were not able to efficiently adhere to either of these surfaces.

When the endothelial progenitor cells (EPCs) were seeded onto the peptide-modified surface, actin fiber formation was observed (Figure 3c). In contrast, the cells did not exhibit spreading on the unmodified surface. These results indicated that POG7G3REDV was bound to the decellularized tissue and promoted the binding of integrin $\alpha 4 \beta 1$ -positive cells in a peptide sequence- and cell type-specific manner.

3.3 Blood compatibility and neointima formation

The very short term evaluation of thrombus formation under SEM were shown in Figure 4. Although the luminal surface of the POG7G3REDV-modified graft was clean, severe thrombogenesis was observed on the unmodified graft.

To evaluate the endothelium formation at an early period of time, the peptide-modified grafts were transplanted in femoral-femoral bypass manner as shown in Figure 5. The graft length was adjusted to 20–30 cm, depending on the anatomical features of the porcine recipients. Just after transplantation, blood flow in the graft and the proximal (LFA; left femoral artery) and distal (RFA; right femoral artery) positions was measured by laser Doppler flow analysis (Figure 5b). The pulsatile blood flows were clearly observed and their blood velocities were almost same, indicating that the blood flow was stably bypassed by the graft from LFA to RFA. Histological staining of the peptide-modified graft at 7 days after transplantation was carried out. (Figure 6). The von Wilbrand-positive and Vimentin-positive layer which was formed during 7 days was clearly observed. The cells were aligned on the luminal surface of the graft. No von Kossa positive staining was observed in medium and outer layer of the graft. The EVG staining result indicated the elastic fiber structure in the graft.

3.4 Evaluation of patency in the transplanted grafts

The patency of the grafts was tested using a porcine femoral-femoral crossover bypass (FF bypass) model. All unmodified grafts quickly occluded. Two unmodified grafts were confirmed by taking out in one week and another one was in 27 days. In contrast, surprisingly, after three weeks transplantation, five POG7G3REDV-modified grafts were completely patent. Only one graft was occluded due to an unstable suturing at proxymal anastomotic site. The transplanted grafts were beating and entrained by pulsatile blood flow such that it was as large as the native aorta (Supplementary Movie 1).

Figure 7 shows the luminal surface of the unmodified and peptide-modified grafts. The unmodified graft was covered by the severe coagulation and completely occluded (Figure 7a). In the case of peptide-modified graft, the luminal surface was clear, and

thrombogenic formation was not observed (Figure 7b). Figure 8 shows laser Doppler results, endoscopic images of luminal surface, and the echocardiograph for peptide-modified grafts. The clear pulsatile signals demonstrated the adequate patency of the arteries (Figure 8a). Endoscopic image indicated a clear luminal surface of the graft without any thrombogenic formation 20 days after transplantation (Figure 8b, Supplementary movie 2). Echocardiography has also shown the graft patency without any stenosis (Figure 8c).

4. Discussion

We have developed here the small-caliber long bypass graft. As shown in the Supplementary Movie 2, microscopic angioscopy observations at 20 days after transplantation revealed a surprisingly white luminal surface and no thrombus formation. These results clearly show that we succeeded in completely inhibiting thrombus formation using the novel POG7G3REDV peptide and achieved full patency without any anticoagulant medication. This is the first report of the successful use in terms of a small-diameter long bypass graft under short-term patency in large animal model.

Decellularized tissues originating from porcine valves and horse pericardium are widely used in clinical operations. From previous histological evaluation and clinical experience, studies have shown that the decellularization process is important in reducing immunogenicity of the grafts. Unfortunately, porcine, equine, and bovine blood vessels are not small in diameter, not long in length, nor branchless. An ostrich carotid artery (ID, 2–4 mm; length, up to 90 cm) was employed as the graft material, because it is an appropriate size and exhibits a straight, branchless structure. The carotid arteries were decellularized by UHP treatment, which was originally developed in our laboratory, and a washing procedure; the arteries were used for the long-term replacement of the descending aorta in pigs [25-28]. The UHP treatment completely inactivates the xenogeneic cells [29] and is also well known to suppress even viral activity. We are the first to show the feasibility of an ostrich carotid artery as an ideal vessel for small-caliber, long-bypass decellularized vascular grafts, but the decellularized vessels rapidly occluded.

To improve the affinity of the luminal surface for endothelial cells, we used a novel peptide modifier containing a collagen-binding peptide sequence and an endothelial cell-binding sequence. Both of the surface modification reaction and our decellularization method, UHP treatment, are completely chemical-free and likely safe and desirable for preparing the medical implants. Collagen mimetic peptide, (Pro-Hyp-Gly)_n has been reported to bind preferentially to collagen fibers in a

strand-inversion manner [30]. We utilized this mechanism for surface modification of the decellularized tissue, because the main component of acellular tissue is collagen; this binding occurs under mild conditions by stabilization with interchain hydrogen bonds [31].

Cell binding and spreading of endothelial and endothelial progenitor cells expressing integrin $\alpha 4\beta 1$, were observed only on peptide-modified surface, indicating this slight modification under the mild condition was very effective for the acellular tissues. The initial thrombogenic formation was also suppressed by this modification. Anticoagulation medicine is normally adopted during transplantation surgery for controlling an activated clotting time. In our experiments, the heparin was used only during the operation. Even in this situation, microthrombosis was induced on the naked decellularized surface whereas thrombosis was not observed on the peptide-modified surface. This may be due to the collagen being exposed to the blood stream, which is well known as a strong coagulant. Although such coagulation occurs even in acellular, large-diameter blood vessels, it does not lead to occlusion. However, small-diameter vessels cannot handle early coagulation. The high blood compatibility of our designed grafts is probably achieved by the shielding effect of the exposed collagen matrix by the POG7G3REDV peptide. This must also contributed to the good patency of the modified grafts.

The decellularized carotid artery modified with the peptide demonstrated the good patency and stable pulsatile blood flow in femoral-femoral bypass model. Since the laser-Doppler signal largely depends on the distance between the probe and blood stream, the relative blood velocity was slightly weakened due to tissue formation on the graft's outer surface. In our experimental results, the thrombogenic formation on the luminal surface of the unmodified graft occurred during an exposing blood flow for one hour resulting in the occlusion. Therefore, the twenty days duration with no-heparin condition was enough to evaluate the initial graft patency. The rapid endothelial layer formation after transplantation revealed the antithrombogenic property and maintained the stable blood flow, indicating the neointima-inducing technology would be the most important for the patency of small diameter vascular grafts.

We hypothesize that it may be difficult for endothelial cells to be recruited to the middle region at around 15 cm from the native intima at the suturing edge. Moreover, we are currently investigating whether circulating endothelial progenitor cells play a major role in the rapid neointima formation [32]. Minimal clotting at the suturing point was observed. For future work, we plan to investigate the response and patency of our grafts over longer periods. Immunogenicity of the graft in long-term

period is also important issue for evaluating of clinical application. Fundamentally, decellularization is good approach for xenograft preparation for reducing the immunogenicity. On the other hand, extracellular tissue components such as a glycosaminoglycan also induce the immunoreaction [33]. Therefore, we should investigate not only long-term patency but also the immunogenicity under long-term transplantation model in future work.

By combining UHP-decellularized ostrich carotid arteries with the peptide modifier, we succeeded in developing the first antithrombogenic, small-caliber vascular grafts that can maintain patency. Clinically, autovascular grafts are used for bypass in peripheral and coronary artery bypass surgery, but physicians cannot guarantee adequate size, length, and quality of the grafts for every patient. To our knowledge, our results are the first report of small-diameter regenerative vascular grafts with a clinically applicable size.

5. Conclusions

In this study, we firstly achieved the good patency of the tissue-engineered acellular small diameter long-bypass grafts with clinically usable size and aspect ratio prepared from ostrich carotid artery in the clinically applicable operation system. The neointima-inducing activity of the grafts was the key feature to lead to the stable blood flow in small diameter vessels. Longer-term follow-up of the grafts including the degradation and the replacement with the host tissue should be studied for their clinical use in the future.

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FIGURES

Figure 1.

Figure 2.

Figure 3.

(a)

(b)

(c)

Figure 4

Figure 5.

Figure 6.

Figure 7.

(a)

(b)

Figure 8.

Figure caption

Figure 1. Concept schematic of surface modification with the peptide modifier. Binding was accomplished by strand inversion.

Figure 2. Decellularized ostrich carotid artery. (a) The entire length of the ostrich carotid artery. The length of the scale bar is 300 mm. (b) HE staining of the carotid artery before and after the decellularization. The scale bar is 50 μm . Asterisk indicates luminal side. (c) Histological staining images of the decellularized vascular graft with Hematoxylin and eosin (HE), von Kossa, Elastica van Gieson (EVG), von Willebrand factor (vWF), and Vimentin (Vim) staining. The scale bar is 50 μm . Asterisk indicates luminal side. (d) DNA contents in native tissue and decellularized graft. (e) Stress-strain curve analysis of the minipig native femoral artery and decellularized graft.

Figure 3. Surface modification of decellularized graft by peptide-modifier. (a) Peptide modification on the luminal surface of the graft. Alexa 655 labeled peptide was monitored by confocal laser scanning microscopy. The scale bar is 50 μm . (b) Cell binding affinities of HUVECs (open bar) and NIH/3T3 (filled bar) cells on peptide-modified and unmodified decellularized surfaces. Each data point is the average $\pm$ standard error of the mean from three independent experiments. The statistical significance of the data was analyzed by the Student's t test. (c) Adherence patterns of endothelial progenitor cells on unmodified and Peptide-modified decellularized tissues. Actin fibers and nuclei were stained with rhodamine phalloidin and DAPI, respectively. The scale bar is 100 μm .

Figure 4. Scanning electron microscopic images of the luminal surface of the unmodified and POG7G3REDV-modified graft after one hour of contact to the blood flow. The scale bar is 20 μm .

Figure 5. Transplantation of the decellularized vascular graft to minipigs by femoral-femoral crossover bypass (a) Schematic images and the picture of the transplantation of peptide-modified decellularized long-bypass grafts into the minipig femoral arteries (FA). Blood flow of right FA was connected from the left FA through the bypass graft. The left FA was connected with the graft. (b) Blood flow at left FA (LFA), right FA (RFA), and graft position was analyzed after the transplantation by laser Doppler flow meter.

Figure 6. In vivo Neointima-inducing activity on POG7G3REDV-modified surface after the transplantation. Histological staining of the graft one week after transplantation. The transplanted tissues were stained with Hematoxylin and eosin (HE), von Kossa, Elastica van Gieson (EVG), von Willebrand factor (vWF), and Vimentin (Vim) staining. The scale bar is 50 μ m. Asterisk indicates luminal side.

Figure 7. Gross anatomy of the luminal surface (a) Luminal surface of the occluded unmodified grafts. (b) Luminal surface of the POG7G3REDV-modified grafts at 20 days after transplantation. Scale bar is 10 mm.

Figure 8. Evaluation of the patency of bypass grafts transplanted into the femoral arteries of minipigs. (a) Blood velocity (a.u.) measured by laser Doppler flow meter (Omega Flow FLO-C1) at the middle area of graft after 20 days. (b) Endoscopic observation of the luminal surface of the graft 20 days after transplantation. (c) Echocardiograph of the graft after 20 days. The scale bar is 2 mm.