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Appropriate density of PCL nano-fiber sheath promoted muscular remodeling of PGS/PCL grafts in arterial circulation

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ABSTRACT

Cell-free approach represents a philosophical shift from the prevailing focus on cells in vascular tissue engineering. Porous elastomeric grafts made of poly(glycerol sebacate) (PGS) enforced with polycaprolactone (PCL) nano-fibers degrade rapidly and yield neoarteries nearly free of foreign materials in rat abdominal aorta. However, considering the larger variation of blood pressure and slower host remodeling in human body than in rat, it is important to investigate the *in vivo* performance of PGS-PCL graft with enhanced mechanical properties, so that optimized arterial grafts could be developed for clinical translation. We acquired increasingly compacted sheath by prolonging the electrospinning period of PCL appropriately, which significantly enforced whole grafts. The rational design of sheath density significantly decreased the risk of dilation, rupture as well as enabling the long-term muscular remodeling. Since 3–12 months after implantation, the PGS grafts with rationally strengthened sheath were remodeled into neoarteries resembled native arteries in the following aspects: high patency rate and even vessel wall thickness; a confluent endothelium and contractile smooth muscle layers; expression of elastin, collagen and glycosaminoglycan; tough and compliant mechanical properties. Although loose sheath may result in rupture of vessel wall, adequate porosity was proved to be essential for sheath structure and directly determined muscular remodeling through M2 macrophage involved constructive remodeling. Therefore, this study confirmed that adequate density of PCL sheath in PGS grafts could initiate stable and high-quality muscular remodeling, which contributes to long-term success in arterial circulation before clinical translation.

1. Introduction

Continued advances in the tissue engineering of vascular grafts have enabled a conceptual shift from the desire to design for adequate suture retention, burst pressure and thrombo-resistance to the goal of achieving grafts with growth potential and vascular regeneration [1–3]. Achieving this far more ambitious outcome will require the identification of optimal, not just adequate, scaffold structure and material properties [4,5]. Biodegradable, synthetic vascular grafts provide promising alternative to vein or non-degradable prosthetic grafts [6]. Neointima formation in cell-free vascular graft suggests that the host remodeling is more efficient than *in vitro* cell seeding based "tissue-engineering" strategy [7,8]. Elastomeric instead tough, biodegradable instead bio-inert, opening instead dense vascular scaffolds have been proved to be more favorable to functional remodeling, thus represents the new generation of vascular graft [9,10].

Porous structure of arterial grafts favored cell infiltration whereas is easy to dilate or rupture when bearing arterial pressure [3], conversely, vascular grafts with dense structure present stable resistance while result in limited cell infiltration and regeneration of very thin neointima [11,12]. Based on "remodeling favorable" concept, we have fabricated PGS-PCL bi-layered vascular grafts with opening porous inner part and electrospun PCL nanofibers wrapped outside [2]. *In vivo* results showed PGS-PCL grafts is fast degradable, which fully harnessed the host remodeling capacity to regenerate artery. PCL nano-fiber sheath outside PGS endowed scaffold with adequate mechanical support such as suture retention, pressure resistance and blood sealing. Rapid cell recruitment

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and production of ECM compensate the degradation of PGS scaffold and vitalized the grafts. However, we found PGS-PCL grafts always regenerate vessel wall with uneven thickness at the early stage, which may cause aneurysmal dilatation or graft rupture in the context of clinical application of arterial grafts, as is the most catastrophic risks of graft implantation. Moreover, we found less muscular remodeling in some grafts implanted for 1 year [13], which may cause stiffening of vessel wall, weakening function and probably long-term failure. Owing to the rapid degradation of PGS itself, PCL sheath is the only factor which could influence the mid-later term remodeling. It is important to continue to investigate the temporal changes of PGS-PCL graft with variant sheath microstructure, so that further optimized arterial grafts could be developed for clinical translation.

In order to evaluate the influence of sheath structure in graft mediated host remodeling and offer rational design of sheath parameter, in this study, we fabricated PGS-PCL vascular grafts with increasing amount of PCL nano-fibers, which could provide PGS grafts with significantly enforced strength, thus increased clinical safety. We further evaluated cell infiltration and alignment in the scaffolds and assessed the muscular remodeling *in vivo* by implanting bi-layered grafts into rat abdominal aorta, so that the rational design of PCL sheath could be acquired for clinical translation.

2. Materials and methods

2.1. Fabrication of grafts with different sheath microstructure

As reported previously [14], the PGS core of the composite graft was fabricated by casting a PGS prepolymer solution into a fused salt template that we used a 1.0-mm mandrel and a 1.5-mm outer mold. To cross-link the PGS prepolymer, constructs were heated at 150 °C under vacuum for 24 h to produce a PGS-salt template. To fabricate the different microstructure PCL sheath, PCL (Mn 80 kDa; Aldrich, MO, USA) was dissolved in 2,2,2-trifluoroethanol at 14% weight/volume and electrospun onto a rotating PGS-salt template at 120 RPM for 1, 2 and 3 min respectively. So that PCL sheath with about 12-μm thickness but with varying bulk density was formed. Salt was removed from PGS-PCL-salt composites by immersing them in deionized water. Composite grafts were lyophilized (Labconco, Virtis, Advantage, USA) and stored in a desiccator at ambient temperature until use. Grafts were sterilized with ethylene oxide prior to implant.

2.2. Characterization of scaffold (SEM, micro-CT and tensile test)

Scaffolds were cut into 3 mm long segments and their luminal surfaces and cross-sections were examined. All sections were mounted onto an aluminum stub with carbon tape, sputter-coated with gold, and observed by scanning electronic microscopy (Hitachi, s-4800, Japan). Sheath thickness of each scaffold was measured from cross-sectional SEM images by included software of scanning electronic microscopy. Porosity and bulk density of the PCL sheath of the graft was calculated with the following equations:

$$\text{Sheath porosity} = (V_{sheath} - V_{PCL})/V_{sheath};$$

$$V_{sheath} = \left(\pi r_{graft}^2 - \pi r_{PGS\ \ core}^2\right) \times L_{graft}; V_{PCL} = g_{PCL}/\rho_{PCL}; g_{PCL} = g_{gross} - g_{collection}$$

V_{sheath} means the gross volume of the sheath, which equals the volume of PCL in the sheath and the gap between PCL nanofibers. V_{PCL} is the exact volume of PCL material in the sheath. The r_{core} means radius of PGS tube (0.75 mm). The r_{graft} equals r_{core} plus the thickness of the sheath of the each group. L_{graft} is the length of the graft. The g_{gross} is the amount of spraying PCL in 1 min, 2 min and 3 min respectively, and The $g_{collection}$ is the amount of spraying PCL on collecting plate. The g_{PCL} is the amount of spraying PCL on grafts. The ρ_{PCL} is the density of PCL (1.145 g/mm^3). Bulk density of the PCL sheath was calculated with the following equations:

$$\text{Bulk density} = g_{PCL}/V_{sheath.}$$

Tensile strength, elastic modulus, and suture retention strength of the scaffolds were determined by the force test instrument (BOSE, Electroforce 3200 seriesII, USA). Suture retention strength was measured on samples ($n = 5$) by placing a 9-0 prolene suture approximately 1 mm from the end of the scaffold. The suture was fixed into the upper hook with the scaffold immobilized in the lower hook. The force needed to pull the suture apart from the scaffold was measured using a speed of 2 mm/min. As control, break points of 9-0 sutures were measured to evaluate whether the suture retention meet the microsurgical need. For the measurement of tensile strength and elastic modulus, scaffolds were cut into 2-mm long segments. All segments were fixed to two identical hooks connected to the load cell and the bottom plate of machine. Uniaxial tensile force was applied to each segment at a rate of 2 mm/min with initial force of 0.05 N until rupture.

2.3. Animal grouping and surgery

Animal experiment was approved by the Animal Experiments Ethical Committee of Fourth Military Medical University and complied with the Guide for Care and Use of Laboratory Animals. Totally 45 male Sprague Dawley rats (body weight: 250–300 g, Fourth military medical university laboratory animal center) were assigned randomly into 1 min, 2 min, and 3 min group with 15 rats in each group. We performed interpositional implantation in rat abdominal aortas as described before. In brief, rats were anesthetized by isoflurane inhalation (5% for induction, then 2% for maintenance). A midline abdominal incision was made to expose the abdominal aorta. After aorta was separated from the inferior vena cava, the infrarenal abdominal aorta was cross-clamped, then a 4-mm aortic segment was transected. The composite graft (8–10 mm long) was placed in the gap and connected to the native aorta by end-to-end anastomosis with 9-0 suture. No anticoagulation or antiplatelet treatment was administrated postoperatively. At the planned time points (the 3rd and 12th months), rats were anesthetized to explant grafts for ex-vivo analysis, then were sacrificed by injecting overdosage of pentobarbital sodium (Sigma, USA). The adventitia of neoartery was preserved during sample dissection, so that we can evaluate the thickness of the media layer and the vascularization of adventitia. The patency and vessel morphology were determined by Computed Tomographic angiography (CTA) and necropsy. The “dilation” was evaluated according to morphological appearance by using necropsy and CTA, which is characterized by some protuberances (>2) on vessel wall, accompanies with tortuous or twisted outline; or significantly enlarged vessel diameter (>1.5 times of PGS-PCL graft diameter).

2.4. Computed Tomographic Angiography (CTA)

Rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (Sigma, USA) according to 45 mg/kg. After injecting contrast agent (Xenetix, Guerbet, France) through caudal veins, CT scanning (General Electric Bright Speed Ultra 8, Salt Lake, USA) was performed for whole body then 3 D images were