Development and in Vivo Evaluation of a Biodegradable Vascular Graft Reinforced with a Fused PCL Filament

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Abstract. Tissue-engineered vascular grafts developed for cardiovascular surgery must be fully functional after implantation, resistant to all types of kinks, compression and burst pressure. One of the approaches to improve mechanical properties includes the reinforcement with an outer polymeric sheath. This study was aimed at evaluating the mechanical properties of a tissue-engineered vascular graft reinforced with a fused PCL filament and in vivo testing in a carotid artery of sheep within one year.

1. Introduction

Tissue engineering combines the principles of bioengineering and cell biology for treating pathological conditions associated with damage or loss of tissues or organs [1]. Functional tissue-engineered small-diameter vascular grafts from biocompatible and biodegradable polymers have been being developed over the past decade [2]. In addition to the use of biocompatible polymers, biologically active substances, such as growth factors and various chemoattractants, are incorporated into the composition of polymer vascular grafts and are aimed at stimulating the attraction and differentiation of endogenous progenitor cells in situ [3, 4].

Nevertheless, despite the large number of experimental studies, the mechanical properties of tissue-engineered vascular grafts do not correspond to the ones that may ensure the integrity of the tissue-engineering constructs with kink resistance under arterial pressures [5]. Therefore, the reinforcement of vascular grafts with an outer sheath may provide them superior kink resistance and ensure their optimal functioning.

One of the approaches to reinforce the walls of the vascular graft is to form an outer sheath from biostable or biodegradable polymers [6].

2. Materials and methods

2.1. Fabrication of tissue-engineered vascular grafts

First, the samples of tubular grafts were fabricated to develop a biodegradable vascular graft reinforced with a fused PCL filament. The samples with the diameter of 4 mm were electrospun from a polymer blend containing polyhydroxybutyrate / valerate and polycaprolactone (PHBV/PCL) (1:2, Sigma Aldrich, USA) and incorporated with biologically active molecules (GFMix). Vascular
endothelial growth factor (VEGF) Sigma Aldrich, USA) was incorporated into the inner third of the graft wall. Basic fibroblast growth factor (bFGF; Sigma Aldrich, USA) and stromal cell-derived factor-1 alpha (SDF-1α; Sigma Aldrich, USA) were incorporated into the external 2/3 of the graft wall.

2.2. Graft reinforcement with an outer PCL-sheath
The PCL filament was printed with the fused deposition modeling (FDM) on the external surface of a 4 mm PHBV/PCL/GFmix graft. FDM allows extrusion-based printing from heated thermoplastic materials. The fused PCL filament was made from polycaprolactone (PCL) (Sigma Aldrich, USA).

An original FDM system consists of an extrusion head with a nozzle and a rotating shaft (figure 1a). The following parameters were applied to extrude the PCL filament: a shaft rotation speed of 1 r/s, an extrusion head speed of 1 mm/s, a feeding rate of 0.5 mm/sec (an extruder nozzle of 0.5 mm), a melting temperature of 160 °C. The choice of a wrapping mode was driven by the previous studies reporting its beneficial potential for optimal reinforcing with a polymeric filament that can preserve the residual lumen of the vessel under kink [7].

![Figure 1. FDM system containing an extruder head and a rotating shaft (a); A typical image of the graft reinforced with the outer sheath (b).](image)

2.3. Scanning electron microscopy of vascular grafts (SEM)
After extruding the PCL filament on vascular grafts (figure 1b), the surface of the manufactured grafts and the degree of fusion between the graft and the filament were examined on a S-3400N scanning electron microscope (Hitachi, Japan) under high-vacuum mode.

2.4. Assessment of physical and mechanical properties of vascular grafts
Each graft was divided into two parts to evaluate the impact of the reinforcing filament on the mechanical properties of vascular grafts: one graft segment was native and one contained the outer PCL filament (n = 5 in each group).

Mechanical properties of the samples were evaluated in the longitudinal and transverse directions with the universal testing machine series Z (Zwick/Roell, Germany) in accordance with the editorial rules of the ISO 7198-2013. The tensile strength of grafts was calculated as the maximally applied force representing the breaking load (Fmax, N). The calculation of strain was limited by the spiral shape of the PCL filament and inability to measure the exact cross-sectional area of the sample. Elastic deformation was estimated with the relative elongation adjusted to the elongation at break (%) and Young’s modulus (MPa).
2.5. In Vivo Implantation
Reinforced grafts with the diameter of 4 mm and the length of 40 mm were implanted in the sheep carotid artery (n = 5) for a period of 12 months to evaluate the impact of the fused PCL filament on its functioning and evaluate tissue response in a long-term implantation.

Serial echocardiography studies at 3, 6, 9 and 12 months after implantation were performed with the M7 Premium Colour Doppler Ultrasound System (Mindray, China) to evaluate the integrity of the implanted grafts.

2.6. Histological assessment
After 12 months of implantation, explanted vascular grafts with the fused PCL filament underwent histological assessment. The samples were stained with hematoxylin-eosin, Van Gieson, Alizarin Red S and DAPI to determine the presence of calcium in the sections of explanted grafts.

The explanted samples were fixed in formalin for 24 hours, then washed in running tap water to remove excess fixation solution, and dehydrated in IsoPrep (BioVitrum, Russian Federation). Impregnation of the samples with paraffin wax (3 portions) was performed at 56 °C for 60 minutes. Impregnated samples were poured into paraffin HISTOMIX (BioVitrum, Russia). The embedded samples were cut into 8-µm-thick using a conventional microtome (model HM 325, Thermo Scientific, USA). The samples were then placed in a thermostat and dried overnight at 37°C. After complete drying of the samples, they were dewaxed in xylene (3 portions) for 1-2 min and rehydrated in 96% alcohol (3 portions) for 1-2 min. The dewaxed sections were then stained according to the study protocol. The samples were studied with light and fluorescent microscopy using the AXIO Imager A1 microscope (Carl Zeiss, Germany) with the objective magnification of x50, x100 and x200.

3. Results and discussion

3.1. Scanning electron microscopy of vascular grafts with the outer PCL sheath
SEM showed that the fused PCL filament was well formed on the surface of PHBV/PCL/GFmix vascular graft at the selected manufacturing mode (figure 2c). Incorporated growth factors did not change their structure. The PCL filament completely fused with the outer surface of PHBV/PCL grafts. There were no signs of damaged graft wall at the site of the fused PCL filament (figure 2).

![Typical SEM images of PHBV/PCL/GFmix vascular grafts with the fused PCL filament.](image-url)
3.2. Physical and mechanical properties of reinforced vascular grafts
The physical and mechanical tests showed that the reinforcing filament did not affect the strength and elastic deformation in the longitudinal direction. However, the PCL reinforcing filament increased the tensile strength by 2.9 times (p = 0.028) and Young's modulus by 3.2 times (p = 0.04) and decreased elongation at break by 2.2 times (p = 0.04) in the transverse direction (table 1), possibly because of increased thickness of the graft. A decrease in the relative elongation, corresponding to Finax, may be explained by PCL properties. After reaching the tensile strength, a thinning of the filament with a substantial elongation at break >100% was observed.

Table 1. Mechanical properties of native and reinforced PHBV/PCL/GFmix grafts

|                          | Longitudinal (Along the graft) | Transverse (Across the graft) |
|--------------------------|--------------------------------|-------------------------------|
|                          | Graft                          | Reinforced graft              | Graft                        | Reinforced graft |
| The force applied to the | 4.34 (3.63-8.25)                | 5.3 (4.47-7.82)               | 3.33 (1.45-4.41)             | 9.96 (8.54-14.3)*|
| sample before the onset  |                                |                               |                              |                 |
| of destruction, N       |                                |                               |                              |                 |
| Relative extension, %    | 129.83 (69.57-140.77)          | 119.37 (116.87-135.41)        | 127.18 (78.6-131.01)         | 56.77 (45.11-58.78)*|
| Young's modulus, MPa     | 3.1 (2.31-3.41)                | 2.17 (1.93-2.45)              | 2.03 (1.7-2.43)              | 6.55 (5.02-6.84)*|
| Outer vessel diameter /  | 4.6 (4.54-4.89)                | 5.0 (5.0-5.22)*               | 0.66 (0.34-0.83)             | 0.92 (0.9-1.32) |
| Sample thickness, mm     |                                |                               |                              |                 |
| The area of the sample,  | 4.05 (3.62-6.21)                | 7.07 (7.07-8.83)*             | 3.3 (1.7-4.15)               | 4.6 (4.5-6.6)* |
| mm²                     |                                |                               |                              |                 |

*p <0.05 between native grafts and reinforced grafts in one direction

3.3. Results of histological assessment

Figure 3. An explanted vascular graft and typical histological images of an explanted graft reinforced with PCL filament after long-term implantation. The arrows indicate the location of the reinforcing filament. An explanted reinforced graft (a); Van Gieson staining at the objective magnification of x50 (b); Hematoxylin-eosin staining at the objective magnification of x50 (c); Alizarin Red C and DAPI staining at the objective magnification of x100 (d).
4. Conclusion
The results of the study showed the benefits of the layer-by-layer extrusion of PCL filament for reinforcing a biodegradable vascular graft.

The mechanical properties of reinforced vascular grafts did not change in the longitudinal direction. An increase in the tensile strength and Young’s modulus in the transverse direction was observed due to the spiral arrangement of the reinforcing filament on the surface of the vascular graft.

Histological assessment after long-term implantation showed the integrity of the tissue-engineered construct. It did not reveal any significant resorption of the filament, inflammation and calcification in the surrounding tissues.

Acknowledgements
This study was supported by the Complex Program of Basic Research under the Siberian Branch of the Russian Academy of Sciences within the Basic Research Topic of Research Institute for Complex Issues of Cardiovascular Diseases № 0546-2019-0002 “Pathogenetic basis for the development of cardiovascular implants from biocompatible materials using patient-oriented approach, mathematical modeling, tissue engineering, and genomic predictors”.

References
[1] Fisher M B and Mauck R L 2013 Tissue engineering and regenerative medicine: recent innovations and the transition to translation Tissue Engineering Part B: Reviews 13 1 pp 1-13
[2] Antonova L V, Sevostyanova V V, Mironov A V et al 2018 In situ vascular tissue remodeling using biodegradable tubular scaffolds with incorporated growth factors and chemoattractant molecules Complex Issues of Cardiovascular Diseases 7 2 pp 25-36
[3] Woods I and Flanagan T C 2014 Electrospinning of biomimetic scaffolds for tissue-engineered vascular grafts: threading the path Expert review of cardiovascular therapy 12 7 pp 815-832
[4] Ingavle G C, Gehrke S H and Detamore M S 2014 The bioactivity of agarose–PEGDA interpenetrating network hydrogels with covalently immobilized RGD peptides and physically entrapped aggrecan Biomaterials 35 11 pp 3558-3570
[5] Spadaccio C, Nappi F, De Marco F et al 2016 Preliminary in Vivo Evaluation of a Hybrid Armored Vascular Graft Combining Electrospinning and Additive Manufacturing Techniques: Supplementary Issue: Current Developments in Drug Eluting Devices Drug Target Insights 10
[6] Li G, Liu J, Zheng Z et al 2015 Structural Mimetic Silk Fiber-Reinforced Composite Scaffolds Using Multi-Angle Fibers Macromolecular bioscience 15 8 pp 1125-1133
[7] Ovcharenko E A, Klyshnikov K U, Rezvova M A et al 2019 Analysis of the Flexural Rigidity of Vascular Grafts by Numerical Simulation Methods Biophysics 64 3 pp 485-492