Biodegradable Patches for Arterial Reconstruction Modified with RGD Peptides: Results of an Experimental Study

Viktoriia V. Sevostianova,* Larisa V. Antonova, Andrey V. Mironov, Arseniy E. Yuzhalin, Vladimir N. Silnikov, Tatiana V. Glushkova, Tatjana S. Godovikova, Evgeniya O. Krikina, Evgeniy Bolbasov, Tatiana N. Akentyeva, Mariam Yu. Khanova, Vera G. Matveeva, Elena A. Velikanova, Roman S. Tarasov, and Leonid S. Barbarash

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ABSTRACT: Modification by Arg-Gly-Asp (RGD) peptides is a promising approach to improve the biocompatibility of biodegradable vascular patches for arteriotomy. In this study, we evaluated the performance of vascular patches electrospun using a blend of polycaprolactone (PCL) and polyhydroxybutyrate/valerate (PHBV) and additionally modified with RGDK, AhRGD, and c[RGDFK] peptides using 1,6-hexamethylenediamine or 4,7,10-trioxa-1,13-tridecanediamine (TTDDA) linkers. We examined mechanical properties and hemocompatibility of resulting patches before implanting them in rat abdominal aortas to assess their performance in vivo. Patches were explanted 1, 3, 6, and 12 months postoperation followed by histological and immunofluorescence analyses. Patches manufactured from the human internal mammary artery or commercially available KemPeriplas-Neo xenopericardial patches were used as a control. The tensile strength and $F_{\text{max}}$ of KemPeriplas-Neo patches were 4- and 16.7-times higher than those made of human internal mammary artery, respectively. Both RGD-modified and unmodified PHBV/PCL patches demonstrated properties similar to a human internal mammary artery patch. Regardless of RGD modification, experimental PHBV/PCL patches displayed fewer lysed red blood cells and resulted in milder platelet aggregation than KemPeriplas-Neo patches. Xenopericardial patches failed to form an endothelial layer in vivo and were prone to calcification. By contrast, TTDDA/RGDK-modified biodegradable patches demonstrated a resistance to calcification. Modification by TTDDA/RGDK and TTDDA/c[RGDFK] facilitated the formation of neovasculature upon the implantation in vivo.

1. INTRODUCTION

Atherosclerosis is a leading cause of disability and mortality in developed countries.\(^1\) Internal carotid artery atherosclerosis is associated with poor survival and high risk of stroke. It is estimated that 10−15% of the overall stroke cases are attributable to carotid artery stenosis secondary to atherosclerosis. Carotid stenosis is commonly treated with medical therapy (e.g., lipid-lowering, antihypertensive, and anticalcific drugs), carotid endarterectomy (CEA), and stenting\(^2\) with the number of CEAs to restore carotid patency increasing annually.\(^1\) Despite recent advances in treatment and emergence of minimally invasive techniques, CEA remains the method of choice for treating patients with carotid stenosis. Conventional CEA is performed by a longitudinal arteriotomy extending from the common carotid into the internal carotid artery to remove the atherosclerotic plaque.\(^3\) Arteriotomy is then closed either primarily or with a patch. The choice between using a primary closure or patch still remains controversial. Recent randomized trials showed that patches reduce the risk of perioperative stroke by 1.5%, while primary closure is associated with a 4.5% increased stroke rate.\(^4\) In addition, the postoperative thrombosis was six times less frequent in patients with carotid patching as compared to those with routine primary closure (0.5% vs 3.1%, respectively).\(^4\)

However, vascular patches may lead to postoperative complications because of a difference in the compliance of the patch and intact native artery, resulting in abnormal blood flow in the anastomotic area leading to neointimal hyperplasia.\(^5\) Vascular patches made of biological and synthetic materials were compared in several studies. Fokin and Kuvatov

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reported similar rates of early postoperative complications in patients with CEA patches manufactured from either autologous veins or polytetrafluoroethylene (PTFE). However, the stroke-related death frequency was significantly higher in the PTFE group than in the autograft group in the long-term. Along similar lines, Rerkasem and Rothwell reported no differences in major cardiovascular event frequency between patients implanted with vein autograft or PTFE patches, yet pseudoaneurysms were more commonly detected in the autograft group. Finally, Bisdas et al. examined neurological pseudoaneurysms were more commonly detected in the patients implanted with vein autograft or PTFE patches, yet pseudoaneurysms were more commonly detected in the autograft group.7

2. RESULTS

2.1. Incorporation of RGD Peptides into the Surface of PHBV/PCL Patches. Peptides can be bound to the surface of polymer patches through linker groups such as amines. We first modified the surface of electrospun polyhydroxybutyrate/valerate (PHBV)/PCL grafts by two hydrophilic linkers of different lengths, short [1,6-hexamethylenediamine (HMD)] and long [4,7,10-trioxa-1,13-tridecanediamine (TTDDA)], assuming that the linker length may affect graft biocompatibility. The HMD-modified patches demonstrated an amino group density of 8.4 ± 0.3 × 10^{-9} mol/cm², whereas TTDDA-modified grafts had 8.2 ± 0.3 × 10^{-9} mol amino groups per cm². At the second step of patch modification, three different types of RGD-containing peptides (see Figure 8 for their complete sequence) were immobilized on the patch surface by treatment with glutaraldehyde and sodium cyanoborohydride. The presence of RGD peptides on the polymer surface was confirmed using the Sakaguchi test as evidenced by light-orange color characteristic of arginine (Figure 1). As a result, we produced a total of six different modifications of biodegradable vascular patches (Figure 1).

Figure 1. Sakaguchi test of RGD-functionalized PHBV/PCL patches. Orange staining of samples reveals the presence of the guanidino group characteristic of arginine. Unmodified PHBV/PCL patches were used as a control group. (Photograph courtesy of V.N.S. Copyright 2020.)

2.2. Mechanical Properties of RGD-Modified PHBV/PCL Patches. Preliminary testing revealed nearly identical tensile properties of biodegradable patches modified either with amines alone (HMD or TTDDA) or amines plus RGD-containing peptides (RGDK, AβRGD, or c[RGDFK]) (not shown). For this reason, all the patches modified with RGD peptides were assigned into one PHBV/PCL/REGD group when compared to other patch types.

Unmodified PHBV/PCL patches and xenopericardial patches significantly differed from human internal mammary artery (IMA) in terms of their mechanical parameters (Table 1 and Figure 2). Both the tensile strength and F_{max} of KemPeriPlas-Neo patch exceeded those of IMA by 4- and 16.7-fold, respectively.

However, both RGD-modified and unmodified PHBV/PCL patches demonstrated tensile strength and F_{max} similar to human IMA. The Young’s modulus of KemPeriPlas-Neo patches was similar to that of IMA, whereas PHBV/PCL patches exceeded IMA’s Young’s modulus by nine times (p < 0.05) regardless of RGD modification. Furthermore, modification by RGD peptides resulted in a 3.25-fold reduction of tensile strength and a 2-fold decreased F_{max} while elongation at break and Young’s modulus remained unchanged.

Collectively, these data suggest that RGD modification results in a reduced tensile strength, while not affecting elongation properties of PHBV/PCL patches.

2.3. Modification of PHBV/PCL Patches by RGD Peptides Promotes Their Hydrophilicity. We then questioned if modification by amine linkers and RGD peptides alters the hydrophilicity of the polymer patches’ surface. Using the sessile drop technique, we found that unmodified PHBV/PCL patches displayed the highest water CA (Figure 3), which could be explained by low functional activity, high porosity, and heterogeneity of the PHBV/PCL blend. Modification by amines substantially reduced the water CA, while HMD conferred a more pronounced hydrophilicity than TTDDA.
Table 1. Mechanical Properties of PHBV/PCL Patches before and after RGD-Peptide Modification as Compared to the Xenopericardial KemPeriplas-Neo and Human IMA

| sample               | tensile strength, MPa median (25th and 75th quartiles) range | $F_{\text{max}}$, N median (25th and 75th quartiles) range | elongation at break, % median (25th and 75th quartiles) range | Young's modulus, MPa median (25th and 75th quartiles) range |
|----------------------|-------------------------------------------------------------|----------------------------------------------------------|------------------------------------------------------------|-----------------------------------------------------------|
| PHBV/PCL             | 3.9 (2.88–4.5)                                             | 3.0                                                      | 102.7                                                      | 21.8                                                      |
| PHBV/PCL/RGD         | 1.2 (1.12–1.3)                                             | 1.3                                                      | 27.5 (25–36.2)                                             | 1.12                                                      |
| IMA                  | 1.17 (1.3−6.52)                                            | 64.9                                                     | 1.11                                                      | 0.92                                                      |
| KemPeriplas-Neo      | 10.06 (9.12−21.38)                                         | 15.4                                                     | 27.5 (19.1−36.2)                                           | 1.02                                                      |
|                      | 6.26−21.69                                                | 8.51−26.96                                               | 1.02                                                      | 0.92                                                      |

$^a$p < 0.05 compared to IMA. $^b$p < 0.05 compared to PHBV/PCL patches. $^c$p < 0.05 compared to KemPeriplas-Neo.

Figure 2. The stress–strain curve of RGD-modified and -unmodified PHBV/PCL patches, KemPeriplas-Neo, and human IMA.

(Figure 3). RGD-modified patches demonstrated a variety of water CAs with the lowest value recorded for the c[RGDFK] peptide (113.8 ± 2.2°). Thus, RGD modification promoted hydrophilicity of PHBV/PCL patches.

2.4. RGD-Modified PHBV/PCL Patches Demonstrate High Hemocompatibility. We then sought to test the hemocompatibility of RGD-functionalyzed PHBV/PCL patches. The proportion of lysed red blood cells (RBCs) upon the contact with HMD-modified, TTDDA-modified, and unmodified patches was 0.36, 0.72, and 0%, respectively (Figure 4A). No significant differences were found between HMD- and TTDDA-modified PHBV/PCL patches. These data suggest a high hemocompatibility of the PHBV/PCL blend, as well as incorporation of amine linkers does not significantly affect polymer hemocompatibility.

The KemPeriplas-Neo xenopericardial patch demonstrated a RBC lysis of 2.12%, which is generally accepted for blood-contacting medical devices. It also exhibited the most pronounced platelet aggregation, which was significantly higher than that of PHBV/PCL patches regardless of RGD modification (Figure 4B). HMD- and TTDDA-modified patches and xenopericardial patches did not differ in terms of platelet aggregation. These data suggest that both RGD-modified and unmodified PHBV/PCL patches have higher hemocompatibility than KemPeriplas-Neo xenopericardial patches.

Upon the contact with platelet-rich plasma (PRP), all PHBV/PCL patches displayed the presence of fibrin on their surface, which complicated the platelet deformation analysis. Interestingly, platelet adhesion was twice as much frequent in KemPeriplas-Neo patches as compared to unmodified PHBV/PCL patches, yet the PDI did not differ between these groups (Figure 4C,D). This could be explained by the fact that type II platelets were predominantly found on the surface of unmodified PHBV/PCL patches (78.5%), while the surface of KemPeriplas-Neo patches mostly displayed type III platelets (73.1%). Patches modified by HMD/AhRGD and HMD/c[RGDFK] had 1.2- and 1.4-times higher PDI than that of KemPeriplas-Neo xenopericardial patches.
Figure 4. In vitro evaluation of hemocompatibility of experimental vascular patches: (A) hemolysis upon the contact with patches, whiskers indicate the interquartile range, *—p < 0.05 compared to KemPeriplas-Neo; (B) platelet aggregation, whiskers indicate the interquartile range, *—p < 0.05 compared to KemPeriplas-Neo, **—p < 0.05 compared to unmodified patches; (C) measurement of the platelet type ratios based on scanning electron microscopy (SEM) analysis; and (D) platelet deformation index (PDI), whiskers indicate the interquartile range, *—p < 0.05 compared to KemPeriplas-Neo, **—p < 0.05 compared to unmodified patches.

Figure 5. Representative SEM images of KemPeriplas-Neo patches, unmodified, and RGD-modified PHBV/PCL before (A, ×1000 magnification) and after (B, ×2000 magnification) direct contact with platelets.
unmodified PHBV/PCL patches. These samples had a higher number of adherent platelets mainly of the type III−IV as compared to the other RGD-modified patches (Figure 4D).

The surface morphology of a material may substantially alter its hemocompatibility; thus, we analyzed our experimental grafts using SEM. The serosal surface of KemPeriplas-Neo patches displayed the native pericardial architecture characterized by tortuous collagen fibers, nonporous relief, and the absence of endothelial lining (Figure 5). In contrast, both unmodified and RGD-modified PHBV/PCL patches had a highly porous structure with a wall thickness of 170−250 μm, composed of homogeneous, randomly arranged polymer fibers with a diameter of 350 nm to 4.0 μm. Pores of 5.1−27.6 μm were formed as a result of fiber interweaving. The SEM analysis demonstrated that RGD modification did not affect the surface of PHBV/PCL grafts (Figure 5A). Consistent with the PDI results (Figure 4D), HMD/AhRGD- and HMD/c[RGDFK] patches had massive accumulations of adhered blood proteins on their surface (Figure 5B).

2.5. In vivo Implantation of RGD-Modified PHBV/PCL Patches. Based on in vitro experiments mentioned above, the most favorable biocompatibility was documented for TTDDA/RGDK, TTDDA/AhRGD, and TTDDA/c[RGDFK] patches. Previous studies also reported that these combinations improved adhesion, viability, and proliferation of both endothelial colony-forming cells and human umbilical vein endothelial cells on the surface of electrospun PHBV/PCL scaffolds.25,26 Thus, we tested the performance of TTDDA/RGDK-, TTDDA/AhRGD-, and TTDDA/c[RGDFK]-modified patches upon the implantation into the rat abdominal aorta. Unmodified PHBV/PCL patches and commercially available KemPeriplas-Neo patches were used as controls. Explanted aortas were 100% patent in all the rats implanted with PHBV/PCL patches regardless of RGD modification. No evidence of thrombosis or neointima was observed at the site of implantation.

The implanted KemPeriplas-Neo patch revealed the presence of calcium deposits already 1 month following surgery, as shown by Alizarin Red S staining. Three months postimplantation, a rapid growth of calcium deposits resulted in a partial delamination of the patch (Figure 6). One year following surgery, 83.3% of the implanted KemPeriplas-Neo patches displayed a substantial delamination as well as structural degradation. All KemPeriplas-Neo patches were severely calcified at the study endpoint. A parietal thrombus was detected in one of the six rats implanted with
KemPeriplas-Neo xenopericardial patch, yet the aorta was 100% patent in this animal. Neointimal hyperplasia was observed in 50% of KemPeriplas-Neo patches. The neointima was 3-fold thicker than normal aortic wall of the rat. Cellularity of KemPeriplas-Neo patches was poor.

Both unmodified and RGD-functionalized PHBV/PCL patches exhibited calcium deposits 3 months following surgery as evidenced by Alizarin Red S staining (Figure 6). Unlike KemPeriplas-Neo patches, all the polymer grafts remained free from structural degeneration during the entire 12 months of the study (Figure 6). Calcification of RGD-modified patches varied from mild to moderate. The TTDDA/RGDK group had the lowest calcification rate 12 months after the implantation (50% of cases; three out of six). All the PHBV/PCL, TTDDA/AhRGD, and TTDDA/c[RGDFK] patches demonstrated crystalline calcific deposits by the end of the experiment (12 months). The TTDDA/AhRGD group had the most severe calcification compared to the experimental patches. We focused on the formation of neovascularure at the vessel sites where porous polymer patches had been implanted. The neovascularure can be formed if cellular elements migrate into its thickness. Six months postimplantation, TTDDA/c-[RGDFK] patches demonstrated high cellularity with a slight decrease 12 months postimplantation. In TTDDA/RGDK patches, most cells migrated in the outer 2/3 of the wall within the 12-month period without any decrease in their proportion. TTDDA/AhRGD patches and unmodified PHBV/PCL had poor cellularity with most cells residing in the outer part of the patch walls. Significant cellular infiltration, tissue remodeling, and ECM deposition were observed in patches modified with TTDDA/RGDK and TTDDA/c[RGDFK] in comparison with intact and TTDDA/AhRGD-incorporated samples. Thus, the arterial wall was thickened in rats implanted with TTDDA/RGDK and TTDDA/c[RGDFK] patches. Moderate chronic granulomatous inflammation was detected in several rats in each group of polymer patches without a tendency to neointimal hyperplasia. Both intact and RGD-modified PHBV/PCL patches had a thin neointima lining the vessel lumen with thickenings ensheathing the zones of calcific deposits (Figure 6).

Migration of mature endothelial cells from the aorta adjacent to the patch to the anastomotic areas suggested endothelialization of RGD-modified patches. One month postimplantation, all the RGD-modified polymer patches had a thin neointima along their luminal surface with sporadic endothelial cells located at the central segment of the patch and endothelial cell foci near anastomotic zones (Figure 7). The entire inner surface of biodegradable patches contained type IV collagen. Patches functionalized with c[RGDFK] and RGDK peptides through the TTDDA linker had better endothelization of the vessel lumen. At 3 and 12 month time points, a continuous endothelial monolayer of mature endothelial cells (defined as CD31⁺CD34⁻vWF⁺) was more frequently detected in these groups (Figure 7). In addition, a layer of type IV collagen was found at the inner surface of the implanted patches. Newly formed capillaries with endothelial lining inside the wall of biodegradable patches confirmed patch endothelialization. By contrast, KemPeriplas-Neo patches did not exhibit any signs of endothelialization at all time points. One month postimplantation, sporadic endothelial cells were detected near

Figure 7. Representative images of indicated patches implanted into rat abdominal aorta wall 3 and 12 months following surgery, stained for CD31 (mature endothelial cells), CD34 (progenitor cells, scale bar = 20 μm), vWF (von Willebrand factor, scale bar = 50 μm), and type IV collagen (scale bar = 50 μm). All slides were counterstained with 4′,6-diamidino-2-phenylindole (blue).
the anastomotic zones without further proliferation or migration to the central part of the xenopericardial patch. No endothelial cells and type IV collagen staining were detected at its surface (Figure 7).

3. DISCUSSION

Mechanical properties define the performance of vascular patches, which ideally should have a high tensile strength and wear resistance, and at the same time, be elastic and compliant, thus mimicking the native artery.27 Even though our novel experimental patches displayed tensile strength similar to that of the human IMA, they were highly rigid. The stiffness difference between the arterial wall and implant can result in blood flow alterations in the anastomotic zone, eventually leading to intimal hyperplasia and subsequent vascular stenosis.5,28 Compared to native vessels, PHBV/PCL patches are stiffer and more prone to elongation, which may negatively affect their hemocompatibility and hemodynamics under cyclic loads. Under physiologic loads, intact arteries demonstrate low Young’s modulus owing to straightening of tortuous collagen fibers, while the stress–strain curve is contributed entirely by elastin fibers. With increase in pressure, collagen fibers start to straighten progressively, and because they are more rigid than elastin fibers, the stress–strain curve shifts toward an increase in stress with low elongation.29 In our study, polymer samples, despite their highly porous structure, exhibited high stress without significant elongation upon loading, which indicates their high rigidity. It is possible that during patch manufacture by electrospinning, each subsequent layer of polymer stabilizes the underlying layer, forming a porous structure. In this scenario, the initial loading breaks the rigid bond at the intersection of polymer fibers, as a result of which the initial “loops” of polymer strands are straightened under the action of subsequent stretching.

Upon the implantation, gradual patch remodeling and infiltration with host cells lead to a shift in its mechanical properties toward those of a native artery, thereby improving the hydrodynamics of the reconstructed vessel. Previously, we showed that PHBV/PCL vascular grafts are populated by ECM-producing cells within 6 months following implantation, which results in a decrease in their relative elongation and Young’s modulus.30

Hemocompatibility is another key determinant of vascular graft’s performance.27 RGD-modified biomaterials can pose a certain risk of thrombosis because the RGD sequence can bind to the GP IIb/IIIa receptor found in activated platelets.31,32 However, the GP IIb/IIIa receptor is inactive in intact platelets and is characterized by low affinity. Upon platelet activation by thrombin, a conformational change occurs in the GP IIb/IIIa receptor, leading to an exposure of the high-affinity site to bind soluble fibrinogen. The binding of fibrinogen to GP IIb/IIIa leads to platelet aggregation. However, there is no published evidence that RGD peptides cause platelet activation per se. Furthermore, Zheng et al. have shown that incorporation of RGD into PCL vascular grafts improves their hemocompatibility.53 In our studies, we observed an increase in platelet aggregation upon the contact with RGD-modified patches, but the maximum platelet aggregation did not differ from that of KemPeriplas-Neo patches. In addition, the use of the HMD linker for RGD modification substantially increased the PDI in comparison with unmodified patches, yet no differences in the PDI were found for other study groups. Furthermore, our SEM findings suggest that the porous surface of PHBV/PCL patches has no effect on platelet activation and aggregation. Along similar lines, higher hydrophilicity of HMD-modified patches did not positively affect their hemocompatibility. Unmodified PHBV/PCL samples and TTDDA-modified patches demonstrated the optimal hemocompatibility as compared to all other study groups.

In terms of RBS lysis, we observed that the proportion of lysed RBCs for both control and RGD-modified PHBV/PCL patches did not exceed 2%, suggesting negligible hemolysis. Commercially available KemPeriplas-Neo xenopericardial patches demonstrated a more pronounced RBC lysis, yet it did not exceed the generally accepted threshold of 5% used for blood-contacting materials.34

In situ tissue engineering has demonstrated significant advances in development of artificial blood vessel shunts as well as CEA vascular patches. Biodegradable patches made from various biologically active compounds have previously demonstrated a partial restoration of vascular wall at the implantation site.35–37 One of the aims of this study was to evaluate the in situ regeneration potential of our experimental tissue-engineered patches. The formation of the endothelial layer on TTDDA-modified patches was faster than that on unmodified PHBV/PCL samples. The surface of RGD-modified patches had been ensheathed with endothelium within the first month of the implantation, and the endothelial monolayer completely formed by 12 months of the experiment. By contrast, no continuous endothelial layer was observed in KemPeriplas-Neo patches even 1 year following implantation.

Viable cell populations in the implanted biodegradable patch are the key factor for vascular wall regeneration. In our studies, TTDDA/RGDK- and TTDDA/c[RGDFK]-modified patches demonstrated rapid endothelialization as well as infiltration of ECM-producing macrophages and fibroblasts. However, the number of live cells substantially decreased in unmodified patches and TTDDA/AhRGD-modified patches at 1 year postimplantation. The porous structure of PHBV/PCL patches potentially facilitated cell migration into grafts because KemPeriplas-Neo xenopericardial patches exhibited significantly fewer infiltrating cell as compared to polymer grafts. It is known that 6 months following implantation of the porous biocompatible materials, the number of cells migrating in the first months gradually decreases. De Valence et al. suggested poor tissue response to the implant and the gradual disappearance of macrophages, leading to capillary regression, lower access of oxygen and nutrients to fibroblasts located in the thickness of the material.38 Therefore, it may explain the significant decreased number of cells in the unmodified patches. Moreover, the TTDDA/RGDK- and TTDDA/ c[RGDFK]-modified patches maintained their cellularity, probably because of the ability of RGD peptides to ensure cell adhesion.59

Calcification of vascular grafts including patches remains a challenge for cardiovascular surgery,60,61 with blood-contacting biodegradable materials are especially prone to calcification.58 Our histology studies showed that TTDDA/RGDK-modified patches resulted in a less-pronounced calcification, whereas the KemPeriplas-Neo patches demonstrated the most severe calcification. In addition, KemPeriplas-Neo patches had evident signs of structural degeneration 1 year following implantation.

In our study, PHBV/PCL patches modified by TTDDA/RGDK and TTDDA/c[RGDFK] resulted in improved
biocompatibility. Our findings are consistent with previous studies reporting high biocompatibility of cyclic RGD peptides facilitating cell attachment to the artificial matrix following endothelialization.\textsuperscript{39,42} It should be noted, however, that high biocompatibility of the linear RGDK peptide was partly owing to the TTDDA linker. The substantial length of the TTDDA arm compared to the HMD counterpart may have contributed to a better accessibility of RGD peptides to the cells within the graft microenvironment.

4. CONCLUSIONS

RGD-modification of PHBV/PCL patches reduces their tensile strength without affecting elongation at break. However, both RGD-modified and unmodified PHBV/PCL patches had tensile strength and $F_{\text{max}}$ nonsignificantly reduced as compared to the human IMA. In vitro tests suggested a hemocompatibility of RGD-modified patches. One year postimplantation in rats, only TTDDA/c[RGDFK] and TTDDA/RGDK-modified PHBV/PCL patches were completely populated by host cells and exhibited neointima formation along with continuous endothelial lining on their surface. Implantated xenopericardial patches were unable to form an endothelial layer and were also prone to calcification. Mild and moderate calcium deposits were found 3 months after the implantation of RGD-modified patches, with TTDDA/RGDK combination showing the highest resistance to calcification in comparison with xenopericardial patches, unmodified PHBV/PCL modified patches, and TTDDA/AhRGD and TTDDA/c[RGDFK]-modified samples. A linear peptide alanine–glycine–aspartic acid–lysine (RGDK) and a cyclic alanine–glycine–aspartic acid–phenylalanine–lysine (c[RGDFK]) peptide modified through the TTDDA arm linker promoted the formation of neovascularure and reduced calcification when implanted in vivo.

5. EXPERIMENTAL SECTION

5.1. Fabrication of Biodegradable Patches. Polymer matrices were electrospun using a polymer blend containing 5\% w/v PHBV (Sigma) and 10\% w/v PCL (Sigma) dissolved in trichloromethane using a Nanon-01A instrument (MECC) at a voltage of 20 kV, a solution feeding rate of 0.5 mL/h, a collector rotation speed of 200 rpm, and a tip-to-collector distance of 150 mm. A metal drum with a diameter of 8.0 mm was used as a collector. The polymer was cut lengthwise and peeled off before being removed from the drum.

5.2. Modification of Biodegradable Patches with Amine Linkers. Amine linkers HMD and TTDDA were purchased from Sigma. PHBV/PCL patches were immersed in a solution of HMD or TTDDA prepared in a mixture of isopropanol–water (1:1) and incubated at 37 °C for 60 or 30
min, respectively. Samples were rinsed with ddH2O and air dried. A schematic illustrating the modification pipeline is summarized in Figure 8.

To confirm the successful modification of samples with amine linkers, 1 cm² samples were placed in 2 mL tubes following the addition of 1% ethanol solution of ninhydrin (Sigma) in the presence of 0.05% ascorbic acid (Sigma). Tubes were incubated for 30 min at 80 °C. Samples were rinsed twice with ethanol, thoroughly air dried, and dissolved in 0.5 mL chloroform. Finally, 0.5 mL isopropanol was added to the resulting solution and the optical density was measured at 568 nm to determine the number of amine groups. We generated calibration curves for the ascending concentration of free HMD and TTDDA. To determine the number of amines in the experimental samples, the following formula was used

\[ C = \frac{DV}{\varepsilon LS} \]

where \( C \) is the amount (mol) of amino groups per 1 cm²; \( D \) is the average optical density of the sample, obtained from three independent experiments; \( V \) is the volume of the analyzed solution \((10^{-3} \text{ L})\); \( \varepsilon \) is the molar absorption coefficient calculated from calibration curves and is equal to 9810 L/mol/cm, \( L \) is the thickness of the spectrophotometer cuvette \((1 \text{ cm})\), and \( S \) is the sample area \((1 \text{ cm}^2)\).

5.5. Tensile Testing. Mechanical properties of samples and polymer patches resulted in the cross-sectional mismatch. Therefore, we calculated the ultimate tensile strength, which represented the breaking load \((F_{\text{max}} \text{ N})\). Elastic deformation was estimated with the relative elongation adjusted to the elongation at break \((\% \) and Young’s modulus \((\text{MPa})\) determined in the range of physiological load \((80–120 \text{ mmHg})\).

5.6. Hydrophobicity Testing. Hydrophobic assessment was performed using the sessile drop technique. A 3 μL drop of deionized water was placed on the sample surface. After 2 min incubation, measurements were recorded using an EasyDrop optical goniometer (Kruss, Germany). Six measurements of the CA at different surface areas were performed for each experimental sample. Unmodified PHBV/PCL samples were used as a control.

5.7. Hemolysis Testing. The blood withdrawn from healthy donors was mixed with 3.8% sodium citrate at a ratio of 1:9 (citrate/blood). Patch samples of 25 cm² \((n = 5\) for each group\) were placed in buckets followed by the addition of 10 mL saline. Buckets were incubated at 37 °C for 120 min. Positive and negative controls were citrated blood mixed with distilled water and saline, respectively. A volume of 200 mL citrated blood was added to each bucket followed by incubation at 37 °C for 4 h. After incubation, solutions were transferred from buckets into test tubes, followed by centrifugation at 2800 rpm for 10 min to precipitate erythrocytes. The absorbance of supernatants was measured using the GENESYS 6 spectrophotometer (Thermo, Waltham, MA, USA) at a wavelength of 545 nm.

The percent of hemolysis \((H)\) was calculated using the following formula

\[ H(\%) = \frac{D_{\text{pe}} - D_{\text{nc}}}{D_{\text{pe}} - D_{\text{nc}}} \times 100\% \]

where, \( D_{\text{pe}} \) is the absorbance of the experimental samples; \( D_{\text{nc}} \) is the absorbance of the negative control (citrated blood mixed with saline); \( D_{\text{pe}} \) is the absorbance of a completely hemolyzed sample (citrated blood mixed with distilled water).

5.8. Platelet Aggregation Testing. To evaluate platelet aggregation, 3.8% sodium citrate was added to the blood withdrawn from healthy volunteers at a ratio of 1:9 (citrate/blood). The citrated blood was centrifuged at 1000 rpm for 10 min to obtain the PRP. Alternatively, PRP was reconstituted at 4000 rpm for 20 min to generate the platelet-poor plasma (PPP). Intact pure PRP was used as a positive control.

Spontaneous platelet activation was measured without any aggregation inducers. A volume of 25 μL 0.025 M CaCl₂ was added to 250 μL PRP to restore the level of Ca²⁺ in citrated blood. Samples were exposed to CaCl₂-supplemented PRP for 3 min. Platelet aggregation was assessed using a semi-automatic 4-channel platelet aggregation analyzer APECT 4004 (LABiTeC, Germany).
5.9. Scanning Electron Microscopy. To examine the surface structure of experimental patches, a S-3400N scanning electron microscope (Hitachi, Chiyoda, Japan) was used. The surface of xenopericardial patches was used as a positive control. Samples were mounted using conductive tape and a gold/palladium (Au/Pd) coating of 15 nm thickness was then applied under high vacuum conditions. Imaging was performed at 5 kV.

To evaluate platelet aggregation upon the contact with PRP, vascular patches (0.5 cm²) were incubated in 300 μL PRP at 37 °C for 2 h. Samples were then washed with PBS (pH = 7.4) to remove the unabsorbed plasma. Samples were fixed in a 2% glutaraldehyde solution, thoroughly washed with PBS, dehydrated in ascending concentrations of ethanol (30–100%, 15 min each), and finally dried at room temperature. Samples were mounted, sputtered, and imaged as described above. Nine representative fields of view were randomly selected and platelet adhesion was evaluated using the PDI.

\[
PDI = \frac{N_I \times 1 + N_{II} \times 2 + N_{III} \times 3 + N_{IV} \times 4 + N_V \times 5}{N_{total}}
\]

where, \(N_I\) is the number of type I platelets; \(N_{II}\) is the number of type II platelets; \(N_{III}\) is the number of type III platelets; \(N_{IV}\) is the number of type IV platelets; \(N_{V}\) is the number of type V platelets; and \(N_{total}\) is the total platelet count (Figure 9).

5.10. In vivo Implantation of Vascular Patches. All rats were bred at the Animal Core Facility of the Research Institute for Complex Issues of Cardiovascular Diseases. Animal procedures were carried out in accordance with the principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986). The Local Ethics Committee of the Research Institute for Complex Issues of Cardiovascular Diseases approved the study protocol.

Experimental TTDDA/RGDK, TTDDA/AhRGD, and TTDDA/c[RGDFK] patches were implanted into the abdominal aorta of 6 month old male Wistar rats weighing 400–450 g (n = 96). Xenopericardial KemPeriplas-Neo patches were used as a control (n = 24). All animals were anesthetized by 3% isoflurane, which was maintained at 1.5% during surgery. The KemPeriplas-Neo patch as well as experimental patches with a diameter of 1.5 or 2 mm and a length of 10 mm were sterilized by irradiation. All surgical procedures were performed using a strict aseptic technique. The abdominal aorta was mobilized from renal arteries till the bifurcation through a midline by laparotomy at ×10 to 40 magnification. Stay sutures (8–0 prolene) were placed on the aorta below renal arteries and above the bifurcation. The aorta was clamped followed by a 0.5 mm longitudinal aortotomy. Patches (0.5 × 0.5 mm) were sutured with four interrupted 8-0 prolene sutures (Ethicon). The clamps were taken off and the vascular system was reperfused. The subcutaneous tissue was sutured in a layer-to-layer manner using 3-0 polyethylene terephthalate sutures. Animals did not receive any antiplatelet drugs. Following surgery, the rats were kept in the vivarium and given access to water and food ad libitum. Animals were sacrificed 1, 3, 6, and 12 months after surgery (a total of 120 animals; n = 6 for each time point per group). Patches were explanted together with the adjacent aortic tissue and were either snap frozen at −140 °C or fixed in 10% neutral phosphate buffered formalin (Electron Microscopy Sciences) at 4 °C for 24 h.

5.11. Histological Examination. After being fixed with formalin, the patches were embedded in paraffin following staining with hematoxylin and eosin and Alizarin Red S as described previously. Slides were visualized using the Axios Imager A1 light microscope (Carl Zeiss).

5.12. Immunofluorescence Examination. Snap-frozen tissue blocks were sectioned and stained for CD31 (ab119339, Abcam), CD34 (ab185732, Abcam), collagen type IV (ab6586, Abcam), and vWF (ab8822, Abcam) as described previously. Slides were visualized using the LSM 700 confocal laser scanning microscope (Carl Zeiss) as described previously.

5.13. Statistical Analysis. The normality of distribution was tested using the Kolmogorov–Smirnov test. For normally distributed variables in three or more independent groups, the Kruskal–Wallis test [analysis of variance (ANOVA)] followed by Tukey’s post hoc test was used. For non-normally distributed variables in paired samples, the Kruskal–Wallis one-way ANOVA followed by Dunn’s test was used. p-values <0.05 were considered statistically significant. Data were presented as a mean and standard deviation (M ± SD) or
median and interquartile range [25th and 75th percentiles] where appropriate.

### AUTHORITY INFORMATION

**Corresponding Author**

Viktoria V. Sevostianova — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia; orcid.org/0000-0003-0195-8803; Phone: +7-3842-64-3802; Email: sevostv@gmail.com

**Authors**

Larisa V. Antonova — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Andrey V. Mironov — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Arseniy E. Yuzhalin — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Vladimir N. Silnikov — Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk 630090, Russia

Tatiana V. Glushkova — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Tatyana S. Godovikova — Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk 630090, Russia

Evgeniya O. Krivkina — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Evgeniy Bolbasov — National Research Tomsk Polytechnic University, Tomsk 634050, Russia; orcid.org/0000-0002-9789-2185

Tatiana N. Akentyeva — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Mariam Yu. Khanova — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Vera G. Matveeva — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Elena A. Velikanova — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Roman S. Tarasov — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Leonid S. Barbarash — Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo 650002, Russia

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c02593

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**Notes**

The authors declare no competing financial interest.

**ABBREVIATIONS**

CEA, carotid endarterectomy; PTFE, polytetrafluoroethylene; PCL, polycaprolactone; PHBV, polyhydroxybutyrate/valerate; HMD, 6-hexamethylenediamine; TTDDA, 4,7,10-trioxa-1,13-tridecanediamine; ECM, extracellular matrix; IMA, internal mammary artery; PRP, platelet-rich plasma; PPP, platelet-poor plasma; PDI, platelet deformation index; vWF, Willebrand factor

**REFERENCES**

(1) Bonati, L. H.; Dobson, J.; Featherstone, R. L.; Ederle, J.; van der Worp, H. B.; de Borst, G. J.; Mali, W. P. T. M.; Beard, J. D.; Cleveland, T.; Engels, S. T.; Lyner, P. A.; Ford, G. A.; Dormann, P. J.; Brown, M. M. Long-term Outcomes after Stenting Versus Endarterectomy for Treatment of Symptomatic Carotid Stenosis: The International Carotid Stenting Study (ICSS) Randomised Trial. *Lancet* 2015, 385, 529–538.

(2) Abbott, A. L.; Paraskevas, K. I.; Kakkos, S. K.; Golleji, J.; Eckstein, H.-H.; Diaz-Sandoval, L. J.; Cao, L.; Fu, Q.; Wijeratne, T.; Leung, T. W.; Montero-Baker, M.; Lee, B.-C.; Pircher, S.; Bosch, M.; Dennenkamp, M.; Ringleb, P. Systematic Review of Guidelines for the Management of Asymptomatic and Symptomatic Carotid Stenosis. *Stroke* 2015, 46, 3288–3301.

(3) Pechenkin, A. A.; Lyzikov, A. A. Carotid Endarterectomy: Outcomes and Prospects. *Novosti Khirurgii* 2014, 22, 231–238.

(4) Naylor, A. R.; Rico, J.-B.; de Borst, G. J.; Deb, S.; de Haro, J.; Halliday, A.; Hamilton, G.; Kakisis, J.; Kakkos, S.; Lepidi, S.; Markus, H. S.; McCabe, D. J.; Roy, J.; Sillesen, H.; van den Berg, J. C.; Vermassen, F.; Kolh, P.; Chakfe, N.; Hinchi, R. J.; Koncar, I.; Lindholt, J. S.; Vega de Ceniga, M.; Verzini, F.; Archie, J.; Bellmunt, S.; Chaudhuri, A.; Koelmay, M.; Lindahl, A. K.; Padberg, F.; Venermo, M. Editor’s Choice—Management of Atherosclerotic Carotid and Vertebral Artery Disease: 2017 Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). *Eur. J. Vasc. Endovasc. Surg.* 2018, 55, 3–81.

(5) Harrison, G. J.; How, T. V.; Poole, R. J.; Brennan, J. A.; Naik, J. B.; Vallabhaneni, S. R.; Fisher, R. K. Closure Technique after Carotid Endarterectomy Influences Local Hemodynamics. *J. Vasc. Surg.* 2014, 60, 418–427.

(6) Fokin, A. A.; Kuvatov, A. V. Long-term Outcomes of Carotid Reconstructions with Patch Angioplasty. *J. Exp. Clin. Surg.* 2013, 6, 239–243.

(7) Rerkasem, K.; Rothwell, P. M. Systematic Review of Randomized Controlled Trials of Patch Angioplasty Versus Primary Closure and Different Types of Patch Materials During Carotid Endarterectomy. *Asian. J. Surg.* 2011, 34, 32–40.

(8) Bisadas, T.; Pichmaier, M.; Bisadas, S.; Haverich, A.; Teebken, O. E. Early Neurologic Outcome after Bovine Pericardium Versus Venous Patch Angioplasty in 599 Patients Undergoing Carotid Endarterectomy. *Vascular* 2010, 18, 147–153.

(9) Radke, D.; Jia, W.; Sharma, D.; Fena, K.; Wang, G.; Goldman, J.; Zhao, F. Tissue Engineering at the Blood-Contacting Surface: a Review of Challenges and Strategies in Vascular Graft Development. *Adv. Healthcare Mater.* 2018, 7, 1701461.

(10) Cho, S.-W.; Jeon, O.; Lim, J. E.; Gwak, S.-J.; Kim, S.-M.; Choi, C. Y.; Kim, D.-J.; Kim, B.-S. Preliminary Experience with Tissue Engineering of a Venous Vascular Patch by Using Bone Marrow–Derived Cells and a Hybrid Biodegradable Polymer Scaffold. *J. Vasc. Surg.* 2006, 44, 1329–1340.

(11) Antonova, L. V.; Mukhamadiyarov, R. A.; Mironov, A. V.; Burago, A. Yu.; Velikanova, E. A.; Sidorova, O. D.; Kudryavtseva, Yu.A.; Barbarash, O. L.; Barbarash, L. S. A Morphological Investigation of the Polyhydroxybutyrate/valerate and Polycaprolactone Biodegradable Small-Diameter Vascular Graft Biocompatibility. *Genes Cells* 2015, 10, 71–77.

(12) Mettler, B. A.; Sales, V. L.; Stucken, C. L.; Anttila, V.; Mendelson, K.; Bischoff, J.; Mayer, J. E., Jr. Stem Cell–Derived, Tissue-Engineered Pulmonary Artery Augmentation Patches In Vivo. *Ann. Thorac. Surg.* 2008, 86, 132–141.

(13) Sevostyanova, V. V.; Golovkin, A. S.; Antonova, L. V.; Glushkova, T. V.; Barbarash, O. L.; Barbarash, L. S. Modification of Polycaprolactone Scaffolds with Vascular Endothelial Growth Factors
(14) Kudryavtseva, V. E.; Stankovich, K. S.; Gudima, A.; Kibler, E.; Zhukov, Y.; Bolbasov, E.; Malashicheva, A.; Zhuravlev, M.; Riabov, V.; Liu, T.; Filimonov, V.; Remnev, G.; Klüter, H.; Zhlyshkhowskaja, J.; Tverdokhlebov, S. Atmospheric Pressure Plasma Assisted Immobilization of Hyaluronic Acid on Tissue Engineering PLA-Based Scaffolds and Its Effect on Primary Human Macrophages. Mater. Des. 2017, 127, 261–271.

(15) Antonova, L. V.; Sevostyanova, V. V.; Mironov, A. V.; Krivkina, E. O.; Velikanova, E. A.; Matveeva, V. G.; Glushkova, T. V.; Elgudin, Y. L.; Barbarash, L. S. In Situ Vascular Tissue Remodeling Using Biodegradable Tubular Scaffolds with Incorporated Growth Factors and Chemotaxtactant Molecules. Complex Issues of Cardiovasc. Dis. 2018, 7, 25–36.

(16) Wang, F.; Li, Y.; Shen, Y.; Wang, A.; Wang, S.; Xie, T. The Functions and Applications of RGD in Tumor Therapy and Tissue Engineering. Int. J. Mol. Sci. 2013, 14, 13447–13462.

(17) Harburger, D. S.; Calderwood, D. A. Integrin Signalling at a Glance. J. Cell Sci. 2009, 122, 159–163.

(18) Gabriel, M.; Nazmi, K.; Dahn, M.; Zentner, A.; Vahl, C.-F.; Strand, D. Covalent RGD Modification of the Inner Pore Surface of Polycaprolactone Scaffolds. J. Biomater. Sci., Polym. Ed. 2012, 23, 941–953.

(19) Gabriel, M.; van Nieuw Amerongen, G. P.; van Hinsbergh, V. W. M.; van Nieuw Amerongen, A. V.; Zentner, A. Direct Grafting of RGD-motif-containing Peptide on the Surface of Polycaprolactone Films. J. Biomater. Sci., Polym. Ed. 2006, 17, 567–577.

(20) Lin, H.-B.; Sun, W.; Mosher, D. F.; García-Echeverría, C.; Schaufelberger, K.; Kelkes, P. I.; Cooper, S. L. Synthesis, Surface, and Cell Adhesion Properties of Polyurethanes Containing Covalently Grafted RGD-peptides. J. Biomed. Mater. Res. 1994, 28, 329–342.

(21) Parniak, M. A.; Lange, G.; Viswanatha, T. Quantitative Determination of Monosubstituted Guanidines: A Comparative Study of Different Procedures. J. Biochem. Biophys. Methods 1983, 7, 267–276.

(22) Bolbasov, E. N.; Antonova, L. V.; Stankovich, K. S.; Ashrafov, A.; Matveeva, V. G.; Velikanova, E. A.; Khodyrevskaya, Y. L.; Kudryavtseva, Y. A.; Anisimov, Y. G.; Tverdokhlebov, S. I.; Barbarash, L. S. The Use of Magnetron Sputtering for the Deposition of Thin Titanium Coatings on the Surface of Bioreorbable Electrospun Fibrous Scaffolds for Vascular Tissue Engineering: A Pilot Study. Appl. Surf. Sci. 2017, 398, 63–72.

(23) Ganjalinia, A.; Akbari, S.; Solouk, A. PLLA Scaffolds Surface-Engineered via Poly (Propylene Imine) Dendrimers for Improvement on its Biocompatibility-Controlled pH Biodegradability. Appl. Surf. Sci. 2017, 394, 446–456.

(24) American Society for Testing and Materials A. Standard Practices for Assessment of Hemolytic Properties of Materials: Philadelphia. ASTM F, 2000; 756-00.

(25) Antonova, L.; Silnikov, V.; Sevostyanova, V.; Yuzhalin, A.; Koroleva, L.; Velikanova, E.; Mironov, A.; Godovikova, T.; Kutikhin, A.; Glushkova, T.; Serpokrylova, I.; Senokosova, E.; Matveeva, V.; Khanova, M.; Akentyeva, T.; Krivkina, E.; Kibler, M.; Wallpoth, B. H. Long Term Performance of Polycaprolactone Vascular Grafts in a Rat Abdominal Aorta Replacement Model. Biomater. 2012, 33, 38–47.

(26) Bellis, S. L. Advantages of RGD Peptides for Directing Cell Association with Biomaterials. Biomaterials 2011, 32, 4205–4210.

(27) Mehta, R. I.; Mukherjee, A. K.; Patterson, T. D.; Fishbein, M. C. Pathology of Explanted Polytetrafluoroethylene Vascular Grafts. Cardiovasc. Pathol. 2011, 20, 213–221.

(28) Li, X.; Guo, Y.; Ziegler, K. R.; Model, L. S.; Eghbali, S. D. D.; Brenes, R. A.; Kim, S. T.; Shu, C.; Dardik, A. Current Usage and Future Directions for the Bovine Pericardial Patch. Ann. Vasc. Surg. 2011, 25, 561–568.

(29) Kämmerer, P. W.; Heller, M.; Brieger, J.; Klein, M. O.; Al-Nawas, B.; Gabriel, M. Immobilisation of Linear and Cyclic RGD-peptides on Titanium Surfaces and Their Impact on Endothelial Cell Adhesion and Proliferation. Eur. Cells Mater. 2011, 21, 364–372.

(30) Arimura, S.-i.; Kawahara, K.-i.; Biswas, K. K.; Abeyama, K.; Tabata, M.; Shimoda, T.; Ogomi, D.; Matsusaki, M.; Kato, S.; Ito, T.; Sugihara, K.; Akashi, M.; Hashiguchi, T.; Murayama, I. Hydroxyapatite Formed on/in Agarose Gel Induces Activation of Blood Coagulation and Platelets Aggregation. Interact. Cardio. Vasc. Thorac. Surg. 2011, 20, 2321–2322.

(31) Li, X.; Guo, Y.; Ziegler, K. R.; Model, L. S.; Eghbali, S. D. D.; Brenes, R. A.; Kim, S. T.; Shu, C.; Dardik, A. Current Usage and Future Directions for the Bovine Pericardial Patch. Ann. Surg. 2011, 25, 561–568.

(32) Kämmerer, P. W.; Heller, M.; Brieger, J.; Klein, M. O.; Al-Nawas, B.; Gabriel, M. Immobilisation of Linear and Cyclic RGD-peptides on Titanium Surfaces and Their Impact on Endothelial Cell Adhesion and Proliferation. Eur. Cells Mater. 2011, 21, 364–372.

(33) Arimura, S.-i.; Kawahara, K.-i.; Biswas, K. K.; Abeyama, K.; Tabata, M.; Shimoda, T.; Ogomi, D.; Matsusaki, M.; Kato, S.; Ito, T.; Sugihara, K.; Akashi, M.; Hashiguchi, T.; Murayama, I. Hydroxyapatite Formed on/in Agarose Gel Induces Activation of Blood Coagulation and Platelets Aggregation. J. Biomed. Mater. Res., Part A 2007, 81, 456–461.

(34) Shen, X.; Su, F.; Dong, J.; Fan, Z.; Duan, Y.; Li, S. In Vitro Biocompatibility Evaluation of Bioreorbable Copolymers Prepared from L-Lactide, 1,3-trimethylene Carbonate, and Glycolide for Cardiovascular Applications. J. Biomater. Sci., Polym. Ed. 2015, 26, 497–514.

(35) Singha, C.; Song, C.; Wang, X. Medical Textiles as Vascular Implants and Their Success to Mimic Natural Arteries. J. Funct. Biomater. 2015, 6, 500–525.