Biomedical Materials

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Controlled autologous recellularization and inhibited degeneration of decellularized vascular implants by side-specific coating with stromal cell-derived factor $1\alpha$ and fibronectin

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Abstract

Optimized biocompatibility is crucial for the durability of cardiovascular implants. Previously, a combined coating with fibronectin (FN) and stromal cell-derived factor $1\alpha$ (SDF1$\alpha$) has been shown to accelerate the in vivo cellularization of synthetic vascular grafts and to reduce the calcification of biological pulmonary root grafts. In this study, we evaluate the effect of side-specific luminal SDF1$\alpha$ coating and adventitial FN coating on the in vivo cellularization and degeneration of decellularized rat aortic implants. Aortic arch vascular donor grafts were detergent-decellularized. The luminal graft surface was coated with SDF1$\alpha$, while the adventitial surface was coated with FN. SDF1$\alpha$-coated and uncoated grafts were infrarenally implanted ($n = 20$) in rats and followed up for up to eight weeks. Cellular intima population was accelerated by luminal SDF1$\alpha$ coating at two weeks (92.4 ± 2.95% versus 61.1 ± 6.51% in controls, $p < 0.001$). SDF1$\alpha$ coating inhibited neo-intimal hyperplasia, resulting in a significantly decreased intima-to-media ratio after eight weeks (0.62 ± 0.15 versus 1.35 ± 0.26 in controls, $p < 0.05$). Furthermore, at eight weeks, media calcification was significantly decreased in the SDF1$\alpha$ group as compared to the control group (area of calcification in proximal arch region 1092 ± 517 $\mu m^2$ versus 11 814 ± 1883 $\mu m^2$, $p < 0.01$). Luminal coating with SDF1$\alpha$ promotes early autologous intima recellularization in vivo and attenuates neo-intima hyperplasia as well as calcification of decellularized vascular grafts.

1. Introduction

Due to epidemiological changes in the aging society and lifestyle habits, the number of patients who require vascular or valvular implants is increasing. Biological heart valve grafts represent the standard of care for the vast majority of patients, particularly at an advanced age, and have been shown to exhibit excellent hemodynamics, low risk of thrombosis and good resistance to infection compared with artificial grafts. However, immune-mediated structural deterioration limits the durability of these biological xenografts and allografts.

To overcome these limitations, tissue engineering strategies aim at improving the graft biocompatibility [1–3]. Decellularization is a method of removing cells from donor tissue or organs so that only extracellular matrix remains. Such a construct allows low immunogenicity and allows for in vivo migration of host cells into the scaffolds [1, 2].

However, since an approach of rapid and complete in vivo recellularization of decellularized vascular grafts has not been established yet, the performance of various kinds of biomaterials has been studied [3, 4]. Fibronectin (FN) is an extracellular matrix protein. Since FN promotes cell adhesion, growth, migration...
and differentiation [5], our group has previously examined the effect of FN coating of decellularized grafts on their autologous in vivo recellularization in a heterotopic rat implantation model [6, 7]. These studies showed that FN coating on the adventitial side enhances media recellularization, while FN coating on the luminal side not only accelerates the formation of neo-endothelium but also stimulates intimal hyperplasia.

As a further candidate for biofunctionalization of cardiovascular grafts, stromal cell-derived factor 1α (SDF1α) has become of interest. SDF1α is a chemokine with diverse activities, e.g. guiding epithelial progenitor cells (EPCs) to target sites. This mode of action is mediated by binding to and activation of its selective receptor, C-X-C chemokine receptor type 4 (CXCR4), that is abundantly expressed on EPCs [4]. Other important actions of SDF1α include the involvement in the differentiation of T helper cells which are guided towards an anti-inflammatory subtype, thereby creating a rather anti-inflammatory environment in the healing tissue milieu. These characteristics of SDF1α make this chemokine a promising candidate also for research areas outside the field of regenerative medicine, e.g. in the setting of oncological therapy of lung or breast cancer [8–10]. Moreover, post-myocardial infarction remodeling has been shown to be significantly improved under SDF1α action, and some beneficial effect has also been demonstrated for ischemic cardiomyopathy [11, 12].

Previous work has suggested SDF1α as a promising candidate for coating of vascular aortic grafts (AGs) or aortic valve conduits (AVCs) [4]. Furthermore, a combined coating of valve-bearing conduits with FN and SDF1α has led to increased endothelialization after implantation into the right ventricular outflow tract, with further improvement of intimal hyperplasia and calcification in a sheep model [13].

Based on previous results of isolated FN coating of aortic conduit grafts implanted in a functional rat model, we sought to evaluate the potentially beneficial effect of a side-specific coating with SDF1α and FN in a chronic model with the implantation of the graft in the systemic circulation. In particular, luminal SDF1α coating was supposed to attenuate intima hyperplasia and subsequent calcification of the implants.

2. Materials and methods

All animal experiments were conducted according to the national Animal Welfare Act and approved by the state animal care committee (reference number 84-02.04.2012.A391).

2.1. Animals

For in vivo evaluation of the functional performance of SDF1α- and FN-coated AGs, we used a heterotopic aortic transplantation model, as previously described [14]. Standard Wistar rats (male, 200–250 g, n = 40) from an in-house breed of the local animal care facility received chow ad libitum and were exposed to constant temperature, humidity and circadian daylight rhythm during the experiments.

2.2. Preparation of donor aorta and graft decellularization

AGs were harvested from donor rats (n = 20) and detergent-decellularized, as previously described [15]. Briefly, the animals were euthanized by CO₂ insufflation. After thoracotomy, the thoracic aorta was dissected from surrounding tissue, and a U-shaped AG was explanted en bloc using a stereomicroscope. Directly after harvesting, AGs were rinsed with heparinized phosphate buffered saline (PBS). Then, the decellularization protocol was initiated by four repetitive 12 h cycles with 10 h 0.5% sodium dodecyl sulfate (SDS) + 0.5% deoxycholate + 0.05% sodium azide and 2 h deoxyribonuclease (DNase) for the elimination of residual cell remnants, followed by three repetitive 24 h washing cycles with PBS containing 1% penicillin/streptomycin. All chemicals required were obtained from Sigma-Aldrich (Taufkirchen, Germany) and Merck (Darmstadt, Germany). The percentage is defined here as mass/volume.

2.3. Graft coating with SDF1α and FN

For side-specific coating of the luminal graft surface, we have modified the method that was reported by our research group previously [16]. A 22 G catheter (Vasofix Safety, B. BRAUN, Melsungen, Germany) was inserted into the distal end of the decellularized AG, while clamping the proximal region of the AG and clipping the cervical aortic branches with titanium hemostatic clips (Vitalitec Inc., Plymouth, MA, USA). Then, the AG was filled with SDF1α (0.3 ml, 0.5 μg ml⁻¹; Sigma-Aldrich, Taufkirchen, Germany) (figure 1), and adventitiously incubated in an FN (Sigma-Aldrich, Taufkirchen, Germany) solution (50 μg ml⁻¹ in PBS) after clipping both ends of the graft. After incubation at 37 °C for 24 h, the whole graft was rinsed with PBS at room temperature and was then implanted directly into the recipient animal. Non-SDF1α-coated, adventitiously FN-coated grafts, serving as a control group, were treated accordingly, however, using no SDF1α in any step.

2.4. Graft implantation

Recipient rats received heterotopic implantation of the engineered AGs as previously described [14]. Briefly, rats were anesthetized with 2.0%–3.0% isoflurane in 100% oxygen and a 22 G catheter (Vasofix Safety, B. BRAUN, Melsungen, Germany) was inserted into the right jugular vein as a central venous catheter. After median laparotomy, the infra-renal aorta was separated from the inferior vena cava, sparing the lumbar arteries. Heparin (300 IU/kg) was systemically administered through the central venous catheter, the
infra-renal aorta was clamped, and the proximal side of the U-shaped graft was anastomosed in an end-to-side manner, using a 10-0 monofilament, non-absorbable polypropylene suture (Ethicon, Norderstedt, Germany). After temporal reperfusion through the lower limb to prevent paraplegia, the distal side of the graft was anastomosed similarly. Then, the native abdominal aorta was ligated with 5-0 silk sutures (Ethicon, Norderstedt, Germany). Finally, the laparotomy was closed in a multi-layered fashion, and the recipient animals were allowed to recover from anesthesia using subcutaneous carprofen 5 mg kg$^{-1}$ for postoperative analgesia.

The recipient animals were randomly assigned to two experimental groups: the SDF1$\alpha$ group ($n = 10$) received decellularized AGs coated with SDF1$\alpha$ on the luminal side and FN on the adventitial side, while the control group ($n = 10$) received decellularized AGs only with FN on the adventitial side. Each group was followed up for two ($n = 5$ per group) or eight ($n = 5$ per group) weeks, respectively.

2.5. Graft explantation

Rats were anesthetized as described above at two or eight weeks after implantation, respectively. After median laparotomy, the implanted AGs were rinsed with heparin solution in PBS (12.5 IU/ml), excised, embedded in KP-Cryo-Compound medium (Klinipath BV, Duiven, Netherlands) and processed via cryostat sectioning (CM 1950; Leica, Wetzlar, Germany) using standard protocols.

2.6. Histological graft analysis

For histological analysis, frozen sections of 5 μm thickness were stained with hematoxylin/eosin (HE), von Kossa and Movat’s pentachrome staining according to standard protocols and then visualized using a transmission light microscope (DM 2000; Leica, Wetzlar, Germany) equipped with a digital camera (DFC 425C; Leica, Wetzlar, Germany) and the Leica Application Suite v3.7 software.

Calcium deposits in the tissue were visible in the form of black-brown color upon von Kossa staining. In Movat’s pentachrome staining, various tissue qualities are highlighted as shown by the following colors: cores and elastic fibers: black; ground substance and musculature: blue; muscle fibers: red; collagen and reticular tissue: yellow; glycosaminoglycans: green; fibrin and fibrinoid: intense red.

The following histomorphological analyses were conducted as previously published, supported by means of Image J v1.46 software (National Institutes of Health, Bethesda, MD, USA) [17].

In order to analyze the biocompatibility of the AGs, the explants were divided into four regions: ascending aorta (region A1), proximal aortic arch (region A2), distal aortic arch (region B1), and descending aorta (region B2) (figure 2). In all regions, each cross-section was separated into eight segments by lines (angles: 45°) commencing from the center of the aortic lumen. For the assessment of luminal cellularization, the percentage of the recellularized luminal surface in HE staining in each segment was determined, and for each graft region, the mean values were calculated. Additionally, the repopulation of the media of the AGs was examined by calculating the number of cells per area migrated into the media.

Intimal hyperplasia was evaluated by measuring the thickness of the luminal neo-intima as well as the thickness of the media in HE staining and calculating the intima-to-media ratio of all regions with three
representative cross-sections (quotient of neo-intimal thickness and medial thickness of the graft). Finally, the average intima-to-media ratio of each region was calculated.

Moreover, concerning mineralization, a semi-quantitative scoring system based on von Kossa staining of the grafts was applied. Intima scoring ranged from 0 to 3: 0 = no calcification; 1 = micro-calcification; 2 = macro-calcification <50% of the tissue area; 3 = macro-calcification >50% of the tissue area. Media scoring ranged from 0 to 5: 0 = no calcification; 1 = micro-calcification; 2 = macro-calcification <25% of the tissue area; 3 = macro-calcification <50% of the tissue area; 4 = macro-calcification <75% of the tissue area; 5 = macro-calcification >75% of the tissue area. We evaluated eight separate sections in each region and we defined four regions of explants as described before. Furthermore, the area of calcification was quantified in all regions evaluating representative cross-sections.

2.7. Immunohistological graft analysis
Immunohistology was performed as described above. Cryo-sections of 5 μm were treated at room temperature for 10 min with 0.25% Triton-X-100 and 1 h with 5% bovine serum albumin (Sigma-Aldrich) with 0.1% Tween-20 (Merck Millipore Calbiochem, Darmstadt, Germany) in PBS. The sections were then stained with primary antibodies against SDF1α (Sigma-Aldrich, Taufkirchen, Germany), von Willebrand factor (vWF; DAKO, Hamburg, Germany), α-smooth muscle actin (αSMA; Sigma-Aldrich, Taufkirchen, Germany), CD3 (Sigma-Aldrich, Taufkirchen, Germany) and CD68 (Abcam, Cambridge, UK) for 1 h at 37°C, and then with secondary Alexa448-, Alexa546- and Alexa635-conjugated antibodies (Invitrogen, Carlsbad, CA, USA) for 45 min in a humid chamber in the dark at 37°C; all antibodies were diluted in 1% bovine serum albumin and 0.1% Tween-20 in PBS. Sections were covered with 4′,6-diamidino-2-phenylindole (DAPI)-containing Vectashield mounting medium (Vector Labs, Peterborough, UK), and images were conducted using a DM 2000 microscope equipped with a digital camera DFC 425C and the Leica Application Suite v3.7 software (Leica, Wetzlar, Germany).

2.8. Statistical analysis
All statistical analyses were conducted with GraphPad Prism v5.04 software (GraphPad Software, San Diego, USA). The data are presented as the mean ± standard errors of the mean. Unpaired t-tests for parametric testing and Mann–Whitney tests for non-parametric testing were used to compare the means of the two groups. P-values less than 0.05 were considered statistically significant.

3. Results
First, we want to describe the efficiency of detergent-decellularization of AGs. According to previous reports, only SDS-based detergent-decellularization results in an appropriate combination of decellularization and extracellular matrix preservation [18]. On the other hand, the detergent-decellularization approach would demonstrate incomplete removal of cellular remnants from tissues with a dense extracellular matrix structure. To eliminate these remnants, the addition of DNase should be considered [19]. From our past reports, our small animal as well as large animal studies have confirmed the acceptable acellularity and in vivo functionality of detergent-decellularized AGs based on SDS [6, 15–17, 20–22].

3.1. Operative outcome
The 20 recipient rats showed unimpaired graft perfusion with no clinical or Doppler-sonographic signs of lower body malperfusion up to the explantation time point. The mean operative time amounted to 86.1 ± 1.56 min.

3.2. Existence of SDF1α coating
SDF1α coating persistence was examined by immunofluorescence. The signal of SDF1α was observed on the luminal side of decellularized AGs after side-specific coating. No signal of SDF1α coating was confirmed on the adventitial side.

However, the signal of SDF1α was not clarified in explants at two weeks (figure 3).

3.3. Intima formation
After two weeks, the luminal recellularization, defined as the percentage of the luminal circumference covered by single- or multi-layer cells was significantly accelerated in the SDF1α group as compared to controls (92.4 ± 2.95% versus 61.1 ± 6.51%, p < 0.0001) (figure 4(a)) (suppl. 1). To identify differences in local luminal recellularization, evaluation of each region was also performed. Interestingly, in the A2 and B1 regions, AG parts distant from the anastomotic sites, the percentage of the recellularized luminal surface area was significantly increased by SDF1α as compared to controls (p < 0.01 for A2, p < 0.05 for B1) (figures 4(b), (c)) (suppl. 1). In the A1 and B2 regions, there was a trend towards enhanced recellularization in the SDF1α group, but no statistically significant difference (in A1: 98.6 ± 0.01% versus 70.8 ± 0.17%, p = 0.29; in B2: 96.6 ± 0.03% versus 79.3 ± 0.12%, p = 0.18; SDF1α versus control). By week 8, both groups demonstrated complete recellularization over the entire circumference of the luminal surface and no difference could be observed anymore.
3.4. Intimal hyperplasia
For analysis of the neo-intima formation, the intima thickness was measured in both groups, resulting in significantly thinner layers in the SDF1α group as compared to controls at eight weeks (37.7 ± 1.00 μm versus 85.0 ± 15.1 μm, p < 0.05) (figure 5(a)). The SDF1α coating resulted in a decreased intima-to-media ratio (0.62 ± 0.15 versus 1.35 ± 0.26 in controls, p < 0.05) (figures 5(b), 6(a), (b)) (suppl. 2(a)–(d)).

3.5. Morphological analysis of intima formation
By means of Movat’s pentachrome staining, glycosaminoglycans and traces of collagen accompanied with spindle-shaped cells were shown in hyperplastic intima areas (figures 6(c), (d)), which were observed in both groups. In some hyperplastic areas, cells with a chondroid phenotype occurred (figures 7(a)–(c)) (suppl. 2(e)). Immunohistological analysis revealed single-layer cells that stained vWF-positive, identifying them as newly formed endothelium, while the underlying multi-layered hyperplastic regions stained positive for αSMA (figure 7(d)).

3.6. Media repopulation
The numbers of autologous cells migrating into the media did not differ between the groups at eight weeks (2326 ± 658/nm² versus 2923 ± 274/nm², p = 0.43; SDF1α versus control). Furthermore, there was no inter-group difference in any graft region (in A1: 6109 ± 832/nm² versus 6143 ± 787/nm², p = 0.98; in A2: 46.0 ± 13.5/nm² versus 36.4 ± 10.7/nm², p = 0.59; in B1: 80.3 ± 30.9/nm² versus 192 ± 111/nm², p = 0.36; in B2: 5457 ± 630/nm² versus 5203 ± 846/nm², p = 0.81; SDF1α versus control). Remarkably, in the anastomotic regions, autologous cells completely populated the media as early as two weeks after implantation.

3.7. Mineralization
3.7.1. Intima calcification
As described above, the extent of calcification was quantified by von Kossa staining. There was no statistical
difference between the two groups at two or eight weeks (two weeks: 5.60 ± 1.66 versus 5.40 ± 1.69, p = 0.93; eight weeks: 9.60 ± 1.75 versus 20.8 ± 6.22, p = 0.18; SDF1α versus control). However, in the anastomotic regions, no intima calcification was observed in group SDF1α at eight weeks, while the control group exhibited calcified areas in these regions.

3.7.2. Media calcification

After two weeks, the semiquantitative von Kossa scoring system revealed a significant difference between the two groups in the non-anastomotic regions (1.40 ± 0.87 versus 7.80 ± 0.86, p < 0.001 for A2; 2.00 ± 0.84 versus 8.00 ± 2.17, p < 0.05 for B1; SDF1α versus control) (figures 8(a), (b)). Similarly,
significant differences were shown after eight weeks (2.95 ± 0.87 versus 9.05 ± 1.60, p < 0.01 for all regions; 3.80 ± 1.43 versus 9.40 ± 0.98, p < 0.05 for A2; 8.00 ± 1.14 versus 16.0 ± 2.67, p < 0.05 for B1; SDF1α versus control) (Figures 9(a)–(e)). Interestingly, no media calcification was found in the anastomotic regions in SDF1α-coated grafts at eight weeks (Figures 9(f), (g)). Furthermore, the area of calcification in the media was significantly decreased in the SDF1α group as compared to controls (p < 0.01 for two and eight weeks) (Figure 10).

3.8. Inflammatory response
Immunohistological staining revealed the complete absence of T-cells (CD3+ cells) and cells of the macrophage lineage (CD68+ cells) at all time points (Figures 11(a), (b)).

4. Discussion
4.1. Existence and persistency of SDF1α on decellularized AGs
In the present study, we performed simple loading or adsorption of SDF1α on decellularized AGs. After coating, it was confirmed that the luminal site of AGs was covered with SDF1α. However, no SDF1α was observed on AGs at two weeks. Generally, the half time of injected SDF1α is very short in the bloodstream [23]. Furthermore, it has been confirmed in vitro that 60% of membrane-loaded SDF1α is burst-released in the first 4 h and then gradually released to approximately 80% until 35 days [24]. Thus, some strategies in order to sustain SDF1α for a longer time on scaffolds in vivo have been discussed [25]. On the other hand, we confirmed the numerous effects of the simple
loading of SDF1α on decellularized AGs in this study. We could not conclude how long the SDF1α coating worked in vivo functionally. However, we can hypothesize that SDF1α has a strong influence in the acute phase, at least within 14 days, in vivo, which can be assumed to have influenced the long-term results that we observed.

4.2. Functional autologous recellularization

4.2.1. Impact of SDF1α on endothelialization

Functional autologous endothelialization is of crucial importance for the in vivo performance of vascular implants, particularly in case of acellular grafts. Here, we demonstrate that luminal SDF1α coating accelerates early autologous intima formation.

Interestingly, the endothelialization in the SDF1α group was predominantly accelerated in graft areas distant from the anastomotic sites, where cellular migration from the anastomosed aorta does not play a major role in the early implant population. In these areas, the significance of cellular recruitment from the blood stream is generally higher. This finding hints at a cell-recruiting effect of luminal SDF1α coating on the blood stream, particularly as previous studies on decellularized implants in our rat model have shown slow repopulation in areas distant from the anastomotic sites [6].

In order to identify the mechanism of early endothelialization on non-anastomotic sites in our study, we need to think about the function of SDF1α. SDF1α plays a significant role in the recruitment of EPCs via the blood stream to home to sites of injury. Consequently, SDF1α serum levels and the number of recruited EPCs have been observed to increase after vascular injury [4]. Thus, we assumed that the SDF1α coating induced-presence of more EPCs on the luminal surface of AGs may be an underlying reason for the observed acceleration of endothelialization. In fact, it has been previously proven that FN/SDF1α coating recruits EPCs to bovine pericardium-based cardiovascular grafts [26]. Furthermore, it has been reported that the coating with heparin and SDF1α stimulated the migration of bone marrow stem cells to decellularized AVCs in vitro and increased endothelialization in vivo [27]. These reports support our assumption of these study results about the impact of the luminal coating of SDF1α on early endothelialization.

4.2.2. Impact of SDF1α on intima hyperplasia

For the biofunctionality of the decellularized AGs, controlling the development of neo-intimal hyperplasia...
is still critical. In our study, the luminal SDF1α coating inhibited neo-intimal hyperplasia, resulting in a significantly decreased intima-to-media ratio after eight weeks. We think that this fact correlates with the early endothelialization due to the luminal SDF1α coating. It means that the early endothelialization owing to the SDF1α might suppress the neo-intima hyperplasia. In fact, it has also been demonstrated that coating with
heparin and SDF1α increased endothelialization and reduced myofibroblast-associated intima hyperplasia in vivo [27]. Visscher et al applied FN and SDF1α coating on synthetic knitted polyester transplanted as carotid interposition grafts in sheep [28]. As a result, a higher number of CD34-positive cells were detected, endothelialization was increased, and intimal hyperplasia decreased. These reports are in line with our results.

In summary, the impacts of the luminal SDF1α coating, i.e. promoting early endothelialization and inhibiting neo-intimal hyperplasia formation, seem to be two consecutive steps in the mechanism.

4.2.3. Impact of SDF1α on media repopulation
In our study, luminal coating with SDF1α did not influence the amount of media repopulation in AGs at eight weeks. As described previously, cell migration originating from the blood stream does not play a major role with regard to media population, since cells regularly fail to cross the luminal basal lamina of detergent-decellularized grafts [6]. The majority of media-populating cells invade from the adventitial graft side, predominantly beginning at the anastomotic sites. Thus, luminal SDF1α coating does not significantly affect media repopulation.

4.3. Graft degeneration
With regard to intima calcification, we found a tendency of inhibition of the calcification by luminal SDF1α coating in each graft region at eight weeks, and proved that the calcification in the media of AGs decreased significantly in the SDF1α group as compared to the control group at both the two and eight-week time points.

In a previous study, the combined coating with FN and SDF1α eliminated the calcification in the wall portion of ovine pulmonary roots in vivo [13]. Moreover, we previously reported that FN single coating might also inhibit the calcific degeneration in valvular interstitial cells [29]. The addition of FN to the SDF1α coating may support the anti-degenerative function of SDF1α by inducing polarization including redistribution of the SDF1α receptor [30].

In the case of the intima, the decreased amount of hyperplasia in the SDF1α group may be causal. Regarding the media, this study reported that SDF1α significantly inhibited media mineralization of decellularized grafts in vivo. As a cause of media calcification, there are some hypotheses, such as on inflammation, oxidative stress, mechanical stress and advanced glycation end-products. As a result, elastin
fragmentation in the media is induced, and contributes to calcification by vascular remodeling [31]. We believe that some of these potential causes of calcification have been alleviated by the early intima cellularization induced by SDF1α. Actually, in the anastomotic regions where early intima cellularization occurred, irrespective of the presence or absence of SDF1α coating, the degree of media calcification was remarkably decreased, and no significant difference was observed between the two groups at two weeks. In the non-anastomatic regions, the media calcification significantly decreased in the SDF1α group where early intima cellularization was enhanced. Further, no media calcification was observed in the anastomatic regions of the SDF1α group at eight weeks. These findings indicate a close correlation between the early intima cellularization and the regulation of media calcification.

4.4. Inflammatory response
We found no inflammatory cells such as CD3+ and CD68+ cells in any explant. Therefore, we can conclude that the repopulating cells were not part of an inflammatory response against the implants, which is predominantly due to the inherent anti-inflammatory properties of decellularized cardiovascular grafts [6]. In general, SDF1α is a migration factor for lymphocytes which has the function of converting helper T-cells to the anti-inflammatory subtype, which may prevent graft calcification [13]. Our observation of no inflammatory cells at two weeks does not exclude an early immune-modulating SDF1α effect, which may be rather accessible to examination at earlier time points. Thus, future experiments with shorter follow-up times may be conducted to evaluate potential anti-inflammatory effects of SDF1α. Indeed, it has been demonstrated in vitro that lymphocyte activation marker CD25-positive cells and other inflammatory proteins such as tumor necrosis factor and monocyte hemactotic protein-1 adhered significantly less in electrospun scaffolds with SDF1α peptides compared with control scaffolds under pulsatile flow conditions using a mesosilicid device for 14 h [32]. It seems that this fact supports our hypothesis that the effect of SDF1α in the acute phase creates long-term differences in our AG implantation model.

4.5. Limitations
There are several limitations of this study. First, the follow-up period to examine the long-term degenerative effects in the grafts was rather short. This may be a reason why we could not demonstrate a significant difference in the intima hyperplasia in each region, but only a trend. Furthermore, full media recellularization in regions distant from the anastomotic sites was not accomplished at eight weeks, so longer observation periods may be indicated. On the other hand, investigation of time points at earlier than two weeks may reveal early immune-modulating properties of SDF1α that underlie the anti-degenerative mid-term effects.

4.6. Conclusion
In conclusion, the present study demonstrated that luminal SDF1α coating of decellularized AGs significantly accelerates early autologous endothelialization and inhibits intima hyperplasia as well as graft calcification in a functional rat implantation model. As a luminal coating material, SDF1α exhibits good potential to improve the biofunctionality and hemocompatibility of decellularized vascular grafts.

Conflict of interest
The authors all declare that they have no conflict of interest.

Ethical approval
All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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