Aus der Klinik für Herzchirurgie

der Heinrich-Heine-Universität Düsseldorf

Direktor: Univ.-Prof. Dr. Artur Lichtenberg

Kontrollierte autologe Rezellularisierung und inhibierte Degeneration von dezellularisierten Gefäßimplantaten durch seitenspezifische Beschichtung mit *stromal cell-derived factor 1α* und Fibronektin

Dissertation

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I. Zusammenfassung (Deutsch)

Eine optimierte Biokompatibilität ist entscheidend für die Haltbarkeit von Herz-Kreislauf-Implantaten. Bisher wurde gezeigt, dass eine kombinierte Beschichtung mit Fibronektin und aus Stromazellen stammendem Faktor 1α (SDF1α) die In-vivo-Zellularisierung von synthetischen Gefäßtransplantaten beschleunigt und die Verkalkung von biologischen Pulmonalarterienstammtransplantaten verringert. In dieser Studie untersuchen wir die Wirkung einer seitenspezifischen Beschichtung mit SDF1α und Fibronektin auf die In-vivo-Zellularisierung und -Degeneration von dezellularisierten Ratten-Aortenimplantaten. Spendergewebe bestehend aus Aortenklappe, Aorta ascendens und Aortenbogen sowie proximaler deszendierender Aorta wurden mit einem Detergenzien-basiertem Verfahren dezellularisiert. Die endoluminale Oberfläche wurde mit SDF1α beschichtet, während die Adventitialoberfläche mit Fibronektin beschichtet wurde. Mit SDF1α beschichtete und unbeschichtete Transplantate wurden Empfängerratten infrarenal implantiert (n = 20) und bis zu 8 Wochen nachuntersucht. Die Intima-Zellpopulation wurde nach 2 Wochen durch luminalen SDF1α-Beschichtung beschleunigt (92,4 ± 2,95% gegenüber 61,1 ± 6,51% bei den Kontrollen, p <0,001). Die SDF1α-Beschichtung hemmte die neo-intimale Hyperplasie, was nach 8 Wochen zu einem signifikant verringerten Verhältnis von Intima zu Medium führte (0,62 ± 0,15 gegenüber 1,35 ± 0,26 bei den Kontrollen, p <0,05). Darüber hinaus war nach 8 Wochen die Medienverkalkung in der SDF1α-Gruppe im Vergleich zur Kontrollgruppe signifikant verringert (Bereich der Verkalkung in der proximalen Bogenregion 1.092 ± 517 um^2 gegenüber 11.814 ± 1.883 um^2, p <0,01). Die Luminalbeschichtung mit SDF1α fördert die frühe autologe Intima-Rekellularisierung *in vivo* und mildert die Neo-Intima-Hyperplasie sowie die Verkalkung von dezellularisierten Gefäßtransplantaten.

II. Zusammenfassung (Englisch)

Optimized biocompatibility is crucial for the durability of cardiovascular implants. Previously, a combined coating with fibronectin and stromal cell-derived factor 1α (SDF1α) 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 coating with SDF1α and fibronectin 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 SDF1α, while the adventitial surface was coated with fibronectin. SDF1α-coated and uncoated grafts were infrarenally implanted (n=20) in rats and followed up for up to 8 weeks. Cellular intima population was accelerated by luminal SDF1α coating at 2 weeks (92.4 ± 2.95% vs. 61.1 ± 6.51% in controls, $p<0.001$). SDF1α coating inhibited neo-intimal hyperplasia, resulting in a significantly decreased intima-to-media ratio after 8 weeks (0.62 ± 0.15 vs. 1.35 ± 0.26 in controls, $p<0.05$). Furthermore, at 8 weeks, media calcification was significantly decreased in the SDF1α group as compared to the control group (area of calcification in proximal arch region 1,092 ± 517 μm2 vs. 11,814 ± 1,883 μm2, $p<0.01$). Luminal coating with SDF1α promotes early autologous intima recellularization in vivo and attenuates neo-intima hyperplasia as well as calcification of decellularized vascular grafts.

III. Abkürzungsverzeichnis

ACB	aortocoronarer bypass
AoG	Aortengefäßtransplantat
AoK	Aortenkonduit
CD34	*cluster of differentiation 34*
CO_2	Kohlenstoffdioxid
CXCR4	*chemokine receptor type 4*
EC	Endothelzellen
ECM	extrazelluläre Matrix
EPC	*endothelial progenitor cell*
FN	Fibronektin
IH	Intima-Hyperplasie
MMP	Matrix-Metalloproteinase
SDF1α	*stromal cell-derived factor 1α*
SDS	Natriumdodecylsulfat
SV	*Vena saphena magna*

IV. Inhaltsverzeichnis

1. Einleitung

1.1 Klinische Herausforderung in der kardiovaskulären Chirurgie

In den letzten Jahren steigt die Nachfrage nach Transplantaten mit einer dauerhaft hervorragenden Funktionalität in einer kardiovaskulären Chirurgie stetig an. Die am häufigsten verwendeten Transplantatmaterialien im Bereich der kardiovaskulären Chirurgie sind (a) künstliche Prothesen; (b) autologe Gewebe wie Perikard und SV; (c) Allotransplantate (also menschliche Spendergewebe); und (d) Xenotransplantate (also tierische Spendermaterialien) [1].

Die Biokompatibilität mit künstlichen Transplantaten ist jedoch immer noch unzureichend und es gibt viele Komplikationen wie Entzündungsreaktionen und Thrombosen. Venöse Autotransplantate, insbesondere die SV, stellen den Behandlungsstandard für die große Mehrheit der Patienten dar, die an peripherer arterieller Verschlusskrankheit leiden. Sie neigen jedoch dazu, einen funktionell ungünstigen Umbauprozess zu erfahren und Thrombosen zu entwickeln, insbesondere im Fall von „*small diameter vascular grafts*“ mit weniger als 6 mm Durchmesser. Auf der anderen Seite sind autologe Arteria mammaria interna, radiale Arterie und SV, die an den häufigsten verwendeten Transplantaten in der Bypasschirurgie der koronaren Herzkrankheit. Eine erhöhte Prävalenz von koronaren Herzkrankheiten, eine Zunahme von älteren Patienten mit Indikation für ACB sowie eine erweiterte Indikation zur Wiederholung dieses Verfahrens haben die Verfügbarkeit von geeigneten autologen Transplantaten für ACB eingeschränkt. Darüber hinaus kommt es bei etwa 15% der Venentransplantationen aufgrund von Transplantatprobleme oder technischen Fehlern innerhalb von 1 Monat zu einer frühen Venentransplantatthrombose. In der Zwischenzeit tritt das späte Venentransplantatversagen in bis zu 50% aller Venentransplantate auf, was in großen Beobachtungsstudien 10 Jahre postoperativ mit einem Gefäßverschluss einhergeht [2].

Allotransplantate wurden in klinischen Studien vielfach eingesetzt und weisen im Vergleich zu künstlichen Transplantaten eine gute Hämodynamik und ein geringes Thromboserisiko sowie eine gute Infektionsresistenz auf. Trotz dieser Vorteile bleibt das Problem der Haltbarkeit bestehen, wie z. B. die strukturelle Degeneration.

Um diese Aufgaben zu überwinden ist eine wissenschaftliche Forschung zur Verbesserung kardiovaskulärer Transplantate unverlässlich. Hierbei ist die Entwicklung von Herzklappen oder auch von kleinkalibrigen Blutgefäßen mit hoher Biokompatibilität und guter Langzeithaltbarkeit durch den Einsatz des *Tissue-Engineerings* sehr zu erwarten [1, 3-6].

1.2 Stand des *Tissue-Engineerings*

In den letzten Jahrzehnten ist ein bemerkenswerter Fortschritt des *Tissue-Engineerings* auf medizinischem Gebiet zu verzeichnen.

So besteht heute die Hoffnung, dass durch Methoden der regenerativen Therapie Zellen eines erkrankten Organs stimuliert und zu einem positiven Umbau des erkrankten Gewebes induziert werden können [3]. Die für in vitro Modifikation von autologen Zellen notwendige Zellkulturtechnik ist jedoch sehr zeitaufwendig und stellt auch heute ein technisches Problem. Hierbei ist insbesondere das Infektionsrisiko während der Zellmanipulation entscheidend. Bei einigen Verfahren, die eine Induktion von Stammzelleigenschaften aus adulten differenzierten Zellen beinhalten, können ferner ethische Probleme entstehen, die eine breite Applikation des Verfahrens einschränken. Daher befindet sich *Tissue Engineering* bis auf einen kleinen Kreis von klinischen Anwendungen größtenteils in der präklinischen Forschung.

Das klassische Konzept des *Tissue-Engineerings* beinhaltet drei traditionelle Komponenten, die als Zellen, extrazelluläres Gerüst und bioaktive Faktoren bezeichnet werden. Dies bedeutet, dass wir ein Gerüst in Form eines Biomaterials als Grundlage für das klassische *Tissue-Engineering* verwenden und nach Hinzugabe von Zellen eine Ansiedelung der Zellen erreichen könnten, indem wir den bioaktiven Faktor hinzufügen, um die Histogenese zu induzieren [1]. Diese Theorie ist möglicherweise realistischer für klinische Anwendung als die derzeitige Zellkulturtechnik, da sie leicht reproduzierbar, schnell herstellbar und je nach Bedarfszweck flexibel ist. In diesem Sinn wird die Entwicklung eines Allotransplantats unter Verwendung von *Tissue-Engineering*, insbesondere unter Verwendung von biologisch abgeleiteten Biomaterialien, z.B. durch Dezelluralisierung, erwartet.

1.3 Bedeutung der Dezelluralisierung

Die Dezellularisierung befreit ein Spendergewebe weitgehend von den Spenderzellen, die die Hauptursache für die Immunogenität von allogenen oder auch xenogenen Spendergewebe sind und stellt sozusagen die zellfreie Trägermatrix für eine erneute Repopulation durch Empfängerzellen zur Verfügung, bestehend aus diversen Proteinen der ECM mit der überwiegenden Dominanz der Kollagene, . Im Idealfall ähnelt das so gewonnene Gewebe wie ein natives Gewebe und kann so als Transplantat verwendet werden. Aktuell werden verschiedene dezellularisierte Gewebe bereits in der klinischen Anwendung untersucht. Beispielsweise ist SynerGraft® der amerikanischen Firma CryoLife als vom Menschen stammende dezellularisierte Herzklappe bekannt. SynerGraft® wird hergestellt, indem zelluläre Komponenten durch osmotischen Druck abgebaut werden, dann zelluläre Komponenten mit Enzymen zersetzt werden, und Rückstände durch Spülvorgänge entfernt werden. In den letzten Jahren wurde eine Reihe von Forschungsberichten über die Entfernung von Zellbestandteilen aus ganzen Organen und die anschließende Wiederbesiedlung von Gewebezellen veröffentlicht. Das Erscheinungsbild von kardiovaskulärem dezellularisierten Gewebe ist äußerst gut, und die Erwartungen an die Dezellularisierungsforschung ist dadurch insbesondere im Bereich der kardiovaskulären Medizin erheblich gestiegen.

Unsere experimentelle Forschungsgruppe hat nicht nur die *in vitro*, sondern auch die *in vivo* Leistung von dezellularisierten Implantaten ausgewertet, was in einer Reihe von Originalarbeiten seit 2012 mündete [7-12].

1.4 Ein Rattenmodell für die *in vivo* Bewertung biologischer und mittels *Tissue-Engineerings* hergestellter Klappen- und Gefäßtransplantate

Zur *in vivo* Beurteilung von mittels *Tissue-Engineerings* hergestellten Transplantaten führten wir, wie zuvor beschrieben, ein heterotopes Aortenklappenimplantationsmodell der Ratte durch [13]. Kurz gesagt, die Spendertiere wurden durch CO_2-Insufflation eingeschläfert. Nach der Thorakotomie wurde die Aorta aus dem umgebenden Gewebe herausgeschnitten und mit einem Mikroskop (Nikon, Düsseldorf, Deutschland) ein U-förmiges Spendertransplantat

bestehend aus Aorten-klappe, Aorta ascendens, Aortenbogen und deszendierender Aorta (AoG) präpariert.

Nach dem *Tissue-Engineering* von Transplantaten wurde die Implantation zur funktionellen Evaluation durchgeführt. Empfänger-Ratten wurden mit 2,0–2,5% Isofluran anästhesiert, und die zentrale Halsvene wurde freigelegt. Nach der medianen Laparotomie wurde die infrarenale Aorta von der Vena cava inferior abgetrennt, wobei die Lendenarterien so weit wie möglich geschont wurden. Heparin (100 IE / kg) wurde systemisch durch die Vena jugularis centralis verabreicht, die infrarenale Aorta wurde geklemmt und die proximale Seite des U-förmigen Transplantats wurde unter Verwendung einer monofilen Naht der Stärke 10-0 (nicht resorbierbare Polypropylennaht; Ethicon, Norderstedt, Deutschland) in End-zu-Seit-Konfiguration anastomosiert . Nach einer temporären Reperfusion der unteren Extremität durch kurzfristige Freigabe der Aortenklemme zur Verhinderung einer Querschnittslähmung wurde die distale Seite des Transplantats auf ähnliche Weise anastomosiert. Anschließend wurde die ausreichende Durchgängigkeit des Transplantats getestet und die native Aorta zwischen den beiden Anastomosen ligiert, um einen kontinuierlichen Blutfluss durch das Implantat aufrechtzuerhalten. Schließlich wurde die Laparotomie mehrschichtig verschlossen. Nach Beobachtungswartezeit wurden alle Implantate explantiert und mit verschiedenen Methoden ausgewertet.

1.5 Ziele der Arbeit

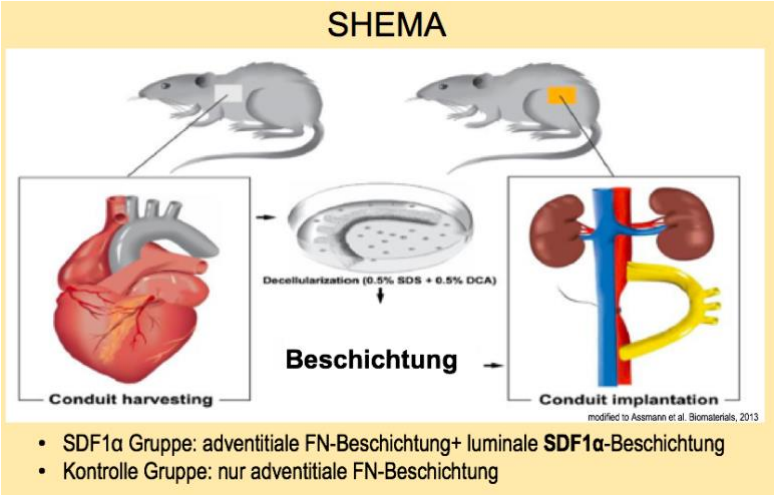
Um einen Beitrag zur Weiterentwicklung der aktuell klinisch *angewandten* dezellularisierten Transplantaten zu leisten, gibt es wichtige Komponenten, die die Haltbarkeit des Transplantats nach der Implantation beeinflussen; Endothelialization, IH, Degeneration und Rezellularisierung. Die Endothelialisation des Transplantats nach der Implantation ist wichtig, um nachteilige Auswirkungen wie Thrombosen zu vermeiden. Dadurch kann es jedoch in der Folge auch zu einer IH kommen, die das Gefäßlumen verengt. Darüber hinaus kann eine Degeneration, z.B. Verkalkung als Folge einer immunologisch getriggerten entzündlichen Reaktion auf Transplantate auftreten und zu einem Versagen des Transplantats führen. Daher ist es für die Haltbarkeit von Transplantaten erforderlich, ein Verfahren zu finden, um die Rezellularisierung *in vivo* angemessen schnellmöglich zu induzieren

und die o.g. negativen Umbauprozesse zu minimieren. Da die zelluläre Repopulation ein langsamer Prozess ist, wurden in der Vergangenheit verschiedene Strategien untersucht, um die autologe Rezellularisierung von mittels *Tissue-Engineerings* hergestellten Gewebe zu beschleunigen.

FN ist ein extrazelluläres Matrixprotein mit multiplen Signalwirkungen [14]. Unsere Arbeitsgruppe hat zuvor die Wirkung der FN-Beschichtung dezellularisierter Transplantate auf ihre autologe *in vivo*-Rezellularisierung in einem heterotopen Rattenimplantationsmodell untersucht [11]. Diese Studien zeigten, dass die FN-Beschichtung auf der adventitialen Seite die Medienrezellularisierung verstärkt, während die FN-Beschichtung auf der luminalen Seite nicht nur die Entstehung der Endothelialisation beschleunigt, sondern auch eine unerwünschte IH stimuliert.

Bezogen auf das Niederdrucksystem der Pulmonalstrombahn wurde in einer kürzlich veröffentlichten Studie eine kombinierte Beschichtung mit FN- und SDF1α-Beschichtung angewandt, was zu einer erhöhten Endothelialisation von dezellularisierten Pulmonalprothesen führte, wobei die IH und Verkalkung in einem Schafmodell auch verbessert wurden [15].

Daher stellten wir die Hypothese auf, dass eine seitenspezifische Beschichtung mit SDF1α an der luminalen Seite und FN an der adventitialen Seite potenziell auch im systemischen Hochdrucksystem vorteilhafte Auswirkungen haben könnte. Insbesondere sollte die luminale SDF1α-Beschichtung die in früheren Arbeiten als hartnäckig beobachtete IH und die anschließende Verkalkung der Implantate abschwächen.



2. Publizierte Originalarbeit

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

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

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ABSTRACT

Optimized biocompatibility is crucial for the durability of cardiovascular implants. Previously, a combined coating with fibronectin and stromal cell-derived factor 1α (SDF1α) 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 SDF1α coating and adventitial fibronectin 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 SDF1α, while the adventitial surface was coated with fibronectin. SDF1α-coated and uncoated grafts were infrarenally implanted (n=20) in rats and followed up for up to 8 weeks. Cellular intima population was accelerated by luminal SDF1α coating at 2 weeks (92.4 ± 2.95% vs. 61.1 ± 6.51% in controls, $p<0.001$). SDF1α coating inhibited neo-intimal hyperplasia, resulting in a significantly decreased intima-to-media ratio after 8 weeks (0.62 ± 0.15 vs. 1.35 ± 0.26 in controls, $p<0.05$). Furthermore, at 8 weeks, media calcification was significantly decreased in the SDF1α group as compared to the control group (area of calcification in proximal arch region 1,092 ± 517 μm^2 vs. 11,814 ± 1,883 μm^2, $p<0.01$). Luminal coating with SDF1α promotes early autologous intima recellularization *in vivo* and attenuates neo-intima hyperplasia as well as calcification of decellularized vascular grafts.

KEY WORDS: tissue engineering; decellularization; vascular graft; biofunctionalization; stromal cell-derived factor 1α

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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 the 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 xeno- 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 organ so that only extracellular matrix remains. Such 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 does not only accelerate 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 CXCR4, that is

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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 turn this chemokine to a promising candidate also for research areas out of 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 also some beneficial effect has 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.

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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 receiving chow *ad libitum* and were exposed to constant temperature, humidity, and circadian daylight rhythm were used for 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 CO_2 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 was defined here as mass/volume.

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2.3. Graft coating with SDF1α and fibronectin

For side-specific coating of the luminal graft surface, we have modified our way 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, 0.5µg/ml; Sigma-Aldrich, Taufkirchen, Germany) **(Fig. 1)**, and adventitially incubated in a FN (Sigma-Aldrich, Taufkirchen, Germany) solution (50 µg/ml in PBS) after clipping of 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, adventitially FN-coated grafts, serving as a control group, were treated accordingly, however, using no SDF1α in any step.

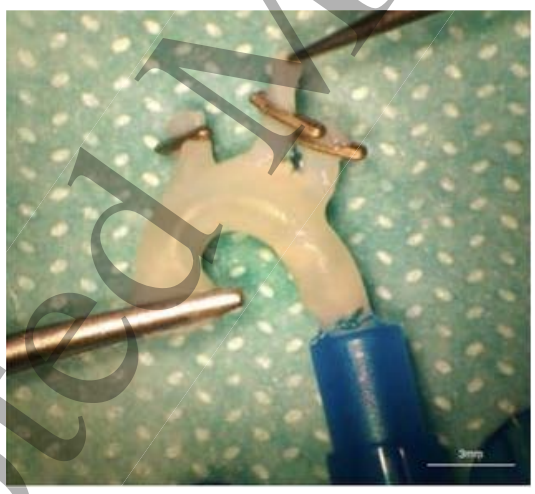

Fig. 1 Side-specific luminal SDF1α coating of decellularized AGs. *A 22 G catheter was inserted into the distal end of the decellularized graft, while clamping the proximal region of the AG and clipping the cervical aortic branches with titanium hemostatic clips. Then, the graft was filled with SDF1α for luminal SDF1α coating and incubated in FN solution for adventitial FN coating. AG, aortic graft; FN, fibronectin; SDF1α, stromal cell-derived factor 1α.*

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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, nonabsorbable 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 multilayered fashion, and the recipient animals were allowed to recover from anesthesia using subcutaneous carprofen 5 mg/kg for postoperative analgesia. The recipient animals were randomly assigned to two experimental groups: The SDF1α group (n=10) received decellularized AGs coated with SDF1α 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 2 (n=5 per group) or 8 (n=5 per group) weeks, respectively.

2.5. Graft explantation

Rats were anesthetized as described above at 2 or 8 weeks after implantation, respectively. After median laparotomy, the implanted aortic grafts 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

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(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 are 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 fibres: 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) **(Fig. 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-

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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 semiquantitative 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 8 separated sections in each region and we defined 4 regions of explants as described before. Furthermore, the area of calcification was quantified in all regions evaluating representative cross-sections.

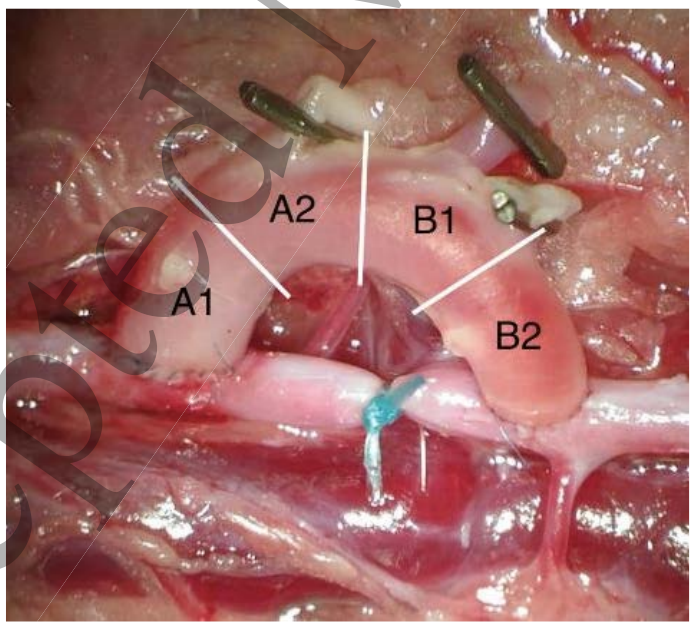

Fig.2 Functional aortic transplantation rat model. *Explanted grafts were divided into four regions: ascending aorta (region A1), proximal aortic arch (region A2), distal aortic arch (region B1), and descending aorta (region B2).*

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2.7. Immunohistological graft analysis

Immunohistology was performed as described above. Five µm cryo-sections 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 von SDF1α (Sigma-Aldrich, Taufkirchen, Germany), 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 DM2000 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 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

At the beginning, we want to describe the efficiency of detergent-decellularization of

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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 theses 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 sign of SDF1α coating was confirmed on the adventitial side. However, the signal of SDF1α was not clarified in explants at 2 weeks. (Fig.3)

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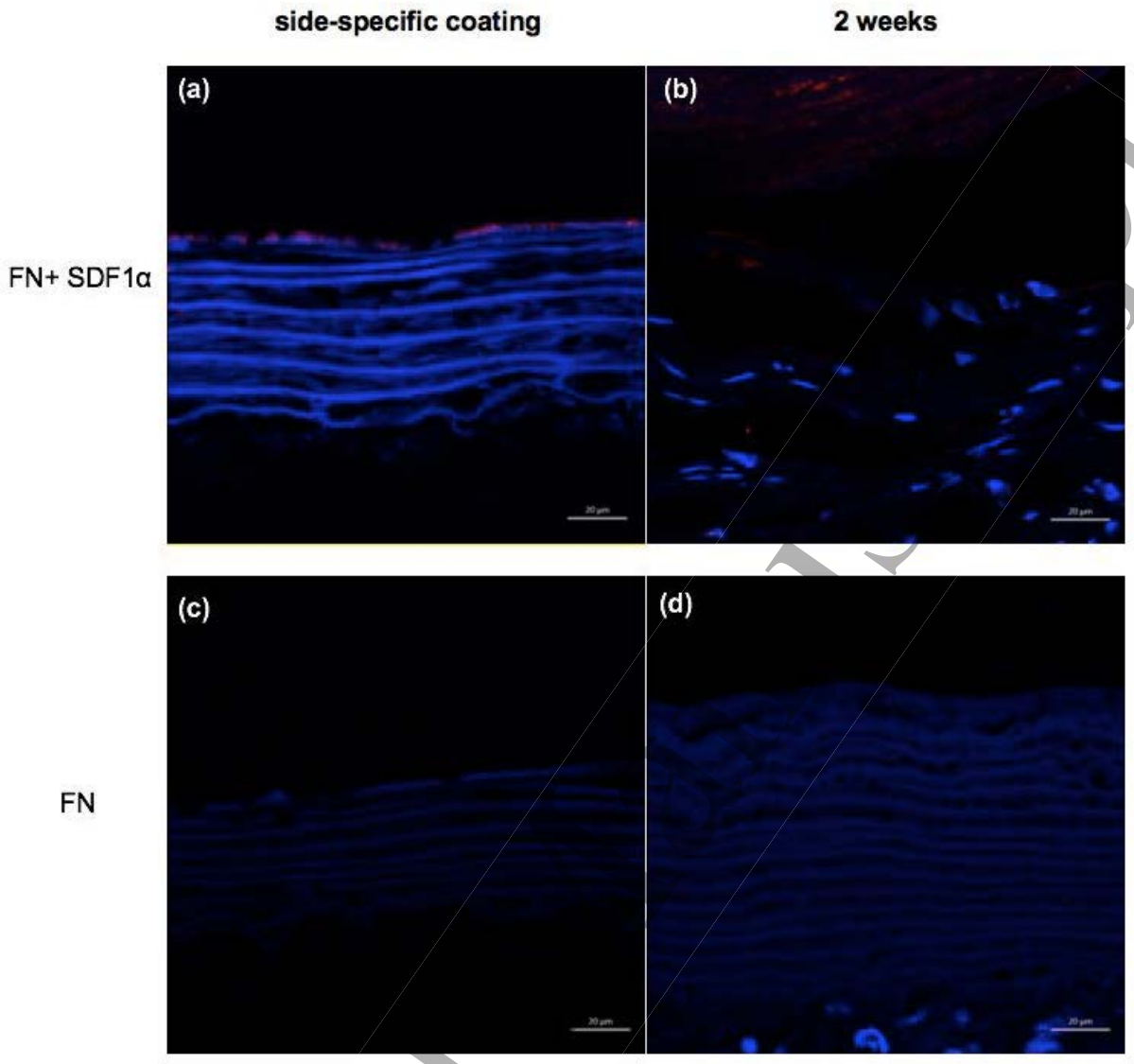

Fig.3 Immunofluorescence of SDF1α coating.

After coating, aortic graft showed a superficial layer of Alexa635-conjugated SDF1α (a). However, SDF1α was not present at 14 days in explants after circulation in rats (b). No sign was observed in control group (b, d). Red, Alexa635-conjugated SDF1α. Scale bars 20 μm.

3.3. Intima formation

After 2 weeks, the luminal recellularization, defined as the percentage of the luminal circumference covered by single- or multi-layer cells was significantly accelerated in

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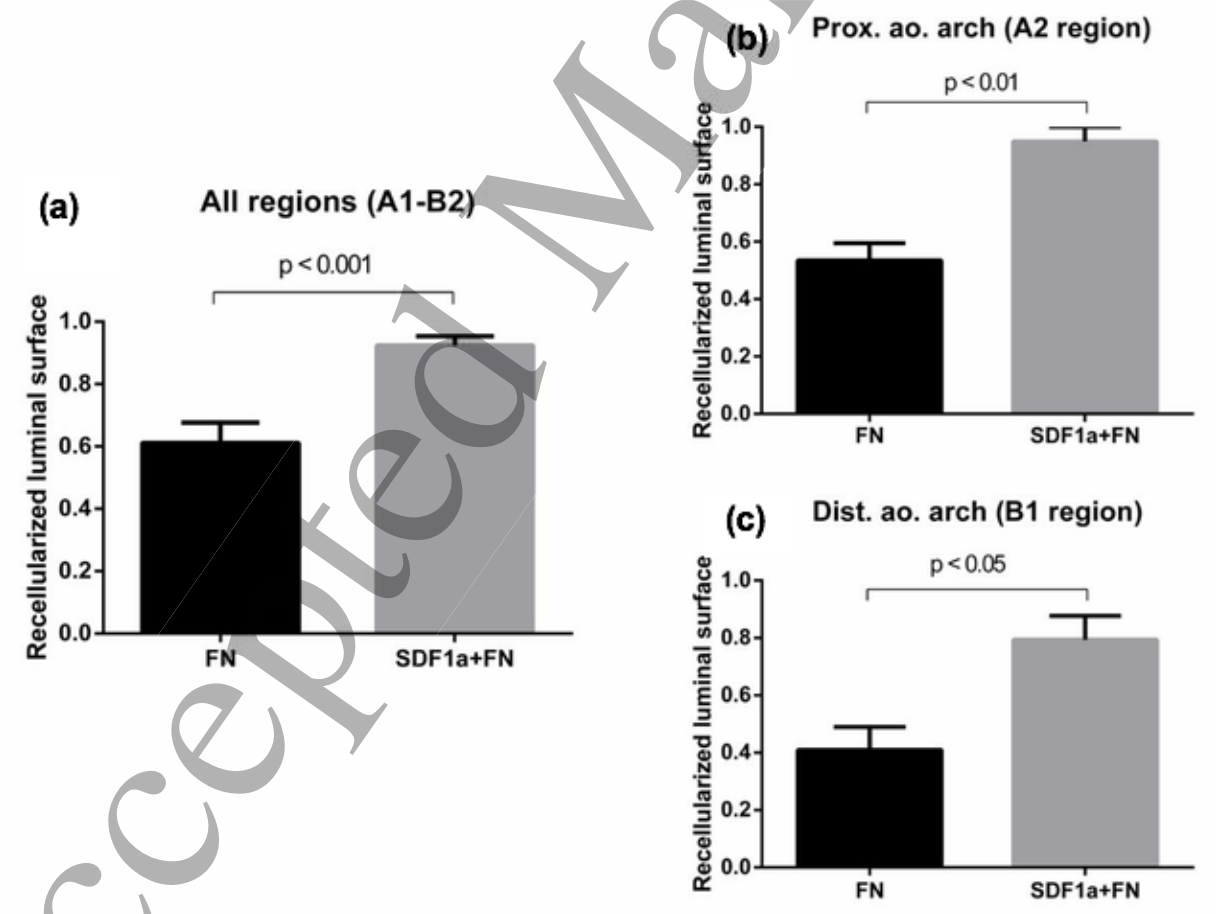
SDF1α group as compared to controls (92.4 ± 2.95% vs. 61.1 ± 6.51%, p<0.0001) **(Fig. 4a) (Suppl. 1)**. To identify differences in local luminal recellularization, evaluation of each region was also performed. Interestingly, in the A2 and B1 regions, AG parts distant from the anastomotic sites, the percentage 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) **(Fig. 4b, 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% vs. 70.8 ± 0.17%, p=0.29; in B2: 96.6 ± 0.03% vs. 79.3 ± 0.12%, p=0.18; SDF1α vs. Control). By week 8, both groups have demonstrated complete recellularization over the entire circumference of the luminal surface and no difference was found anymore.



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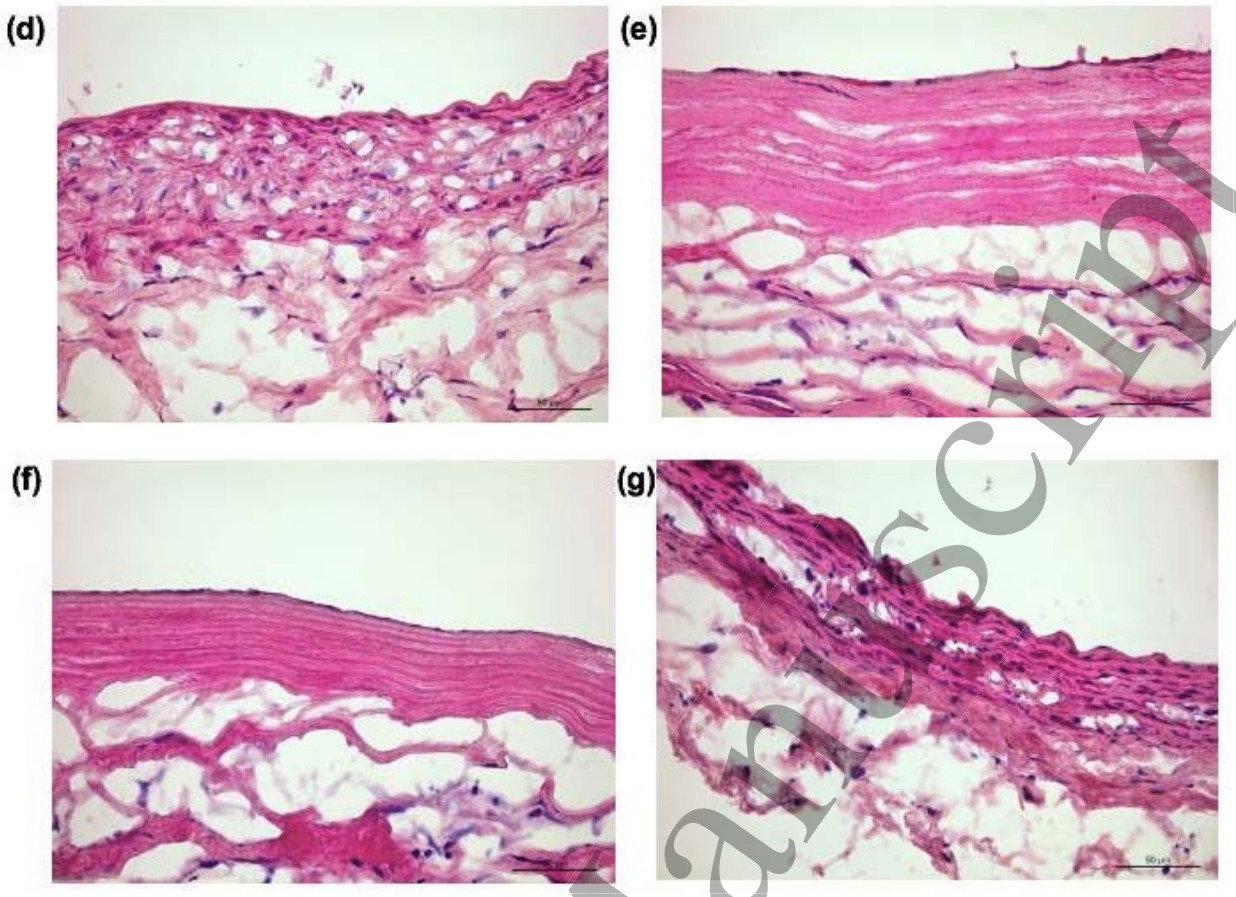

Fig. 4 Intima formation on decellularized AGs after 2 weeks. *SDF1α stimulated autologous recellularization significantly as compared to the control group (all regions in (a)), especially for the parts distant from the anastomoses (region A2 in (b), region B1 in (c)). Representative high magnification images in the SDF1α group for region A1 (d), A2 (e), B1 (f) and B2 (g). Scale bars = 50 μm. AG, aortic graft; ao, aortic; Dist, distal; FN, fibronectin; Prox, proximal; SDF1α, stromal cell-derived factor 1α. Fraction 1.0 corresponds 100% in the graphs.*

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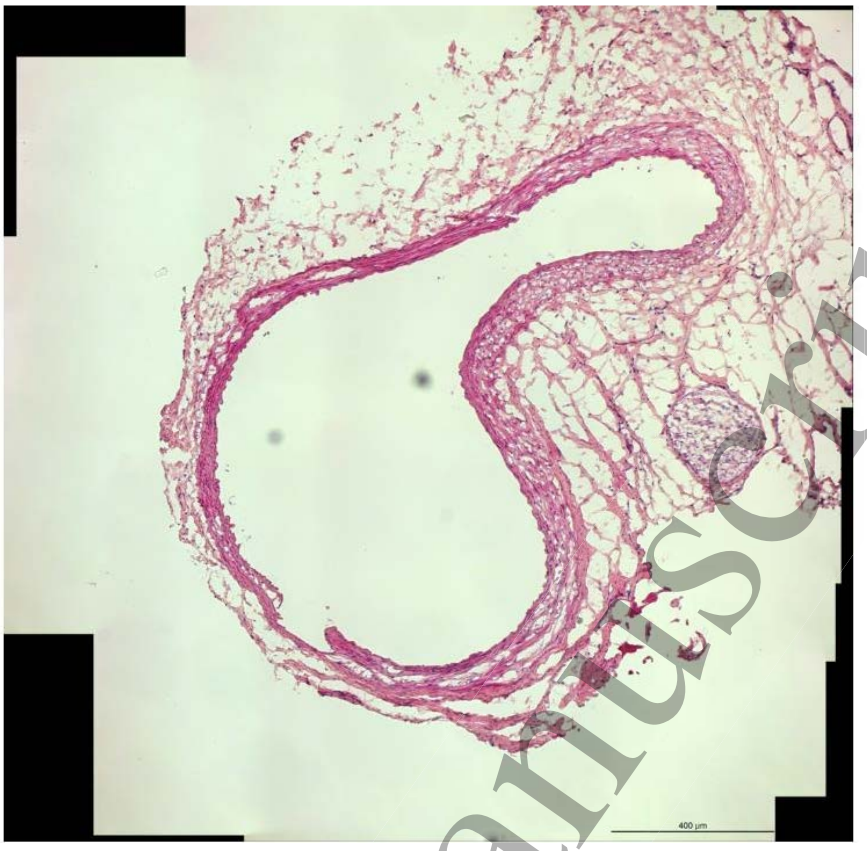
(a)



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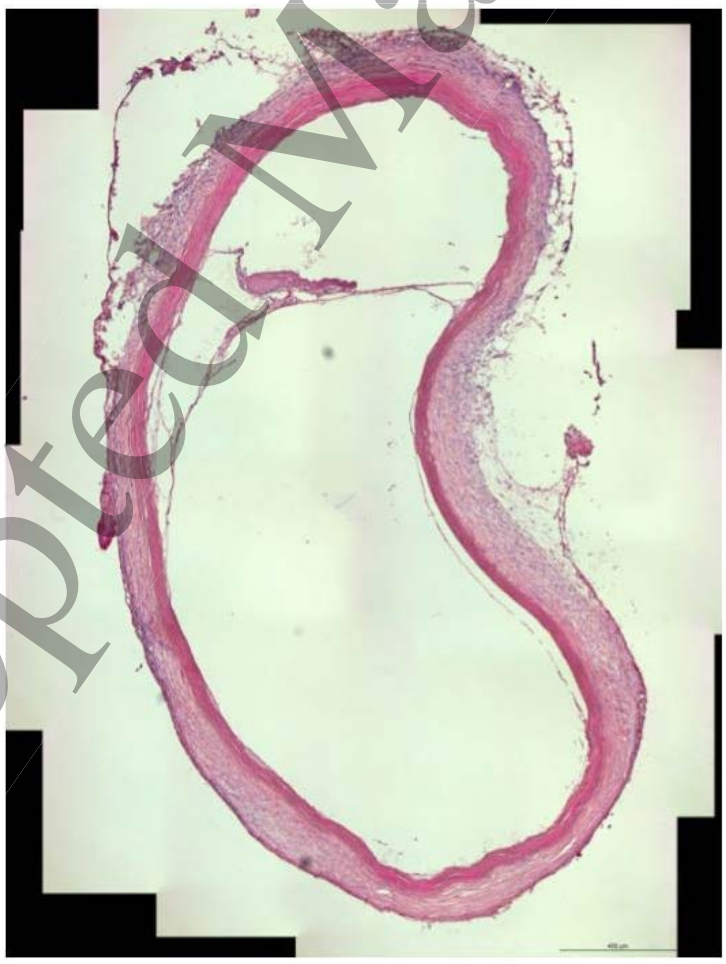

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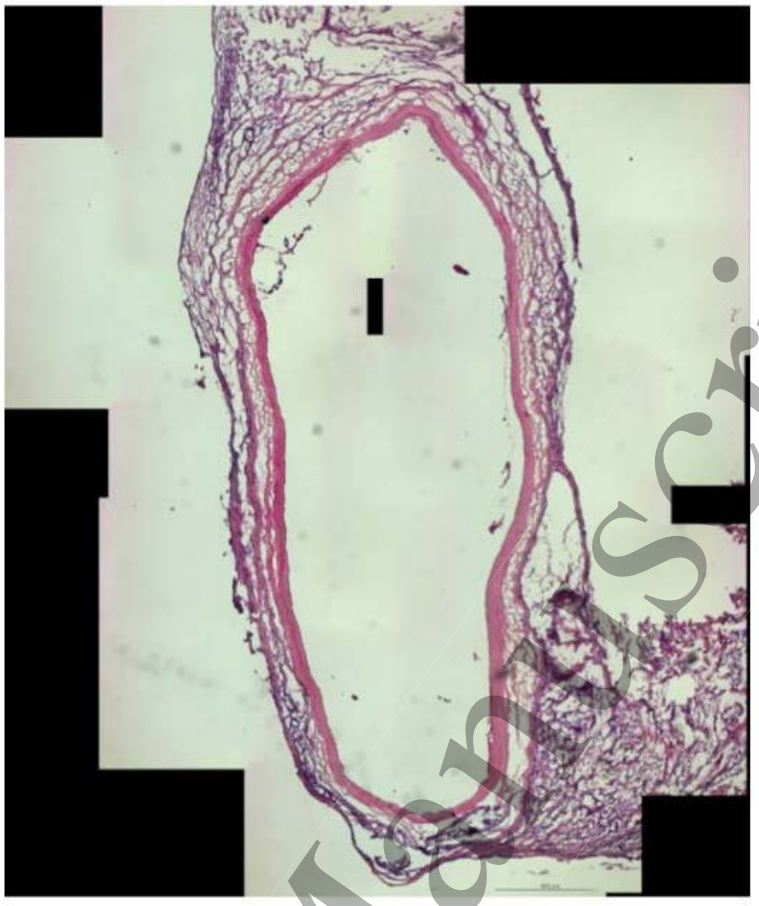
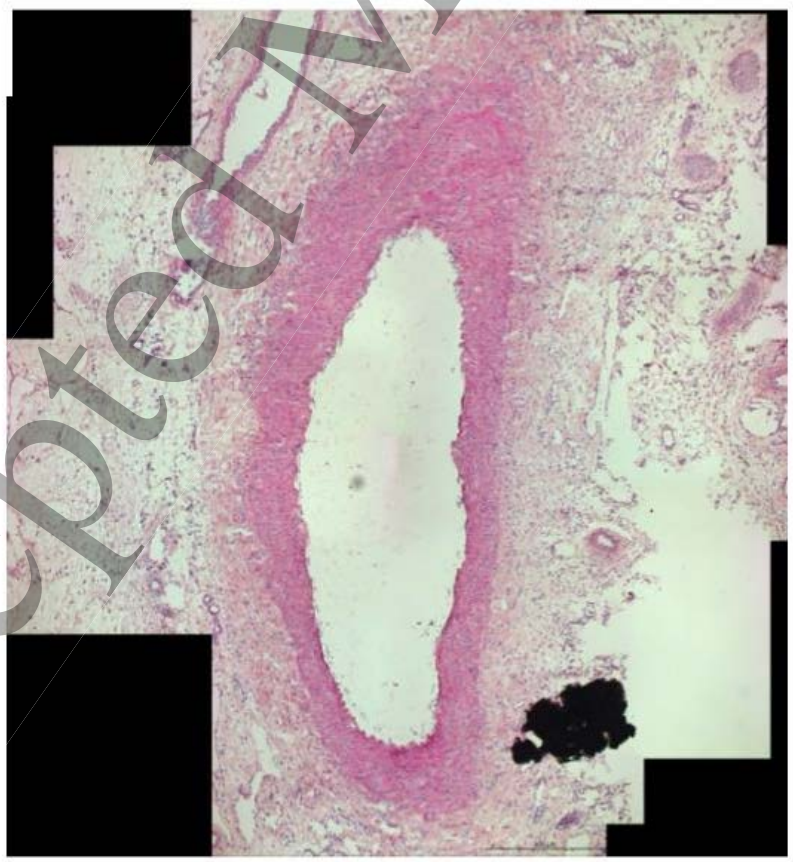
(c)



(d)



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Suppl. 1 Representative low magnification images of decellularized AGs after 2 weeks. *the SDF1α group for region A1 (a), A2 (b), B1 (c) and B2 (d). SDF1α, stromal cell-derived factor 1α. Scale bars = 400 µm.*

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 SDF1α group as compared to controls at 8 weeks (37.7 ± 1.00 µm vs. 85.0 ± 15.1 µm, p<0.05) **(Fig. 5a)**. SDF1α coating resulted in decreased intima-to-media ratio (0.62 ± 0.15 vs. 1.35 ± 0.26 in controls, p<0.05) **(Fig. 5b, Fig. 6a, b) (Suppl. 2a-d)**.

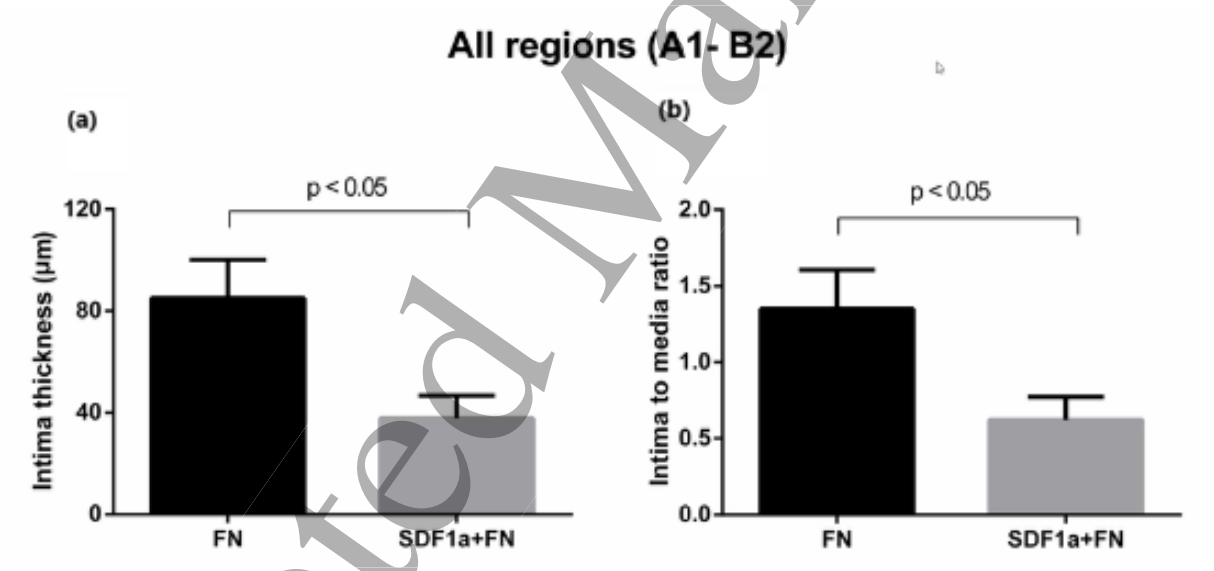

Fig. 5 Neo-intima hyperplasia on decellularized AGs after 8 weeks. *Intima thickness (**a**) and intima-to-media ratio (**b**) decreased in group SDF1α. AG, aortic graft; FN, fibronectin; SDF1α, stromal cell-derived factor 1α.*

3.5. Morphological analysis of intima formation

By means of Movat's pentachrome staining, glycosaminoglycans and traces of

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collagen accompanied with spindle-shaped cells were shown in hyperplastic intima areas **(Fig. 6c, d)**, which were observed in both groups. In some hyperplastic areas, cells with a chondroid phenotype occurred **(Fig. 7a, b, c) (Suppl. 2e)**. 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 **(Fig. 7d)**.

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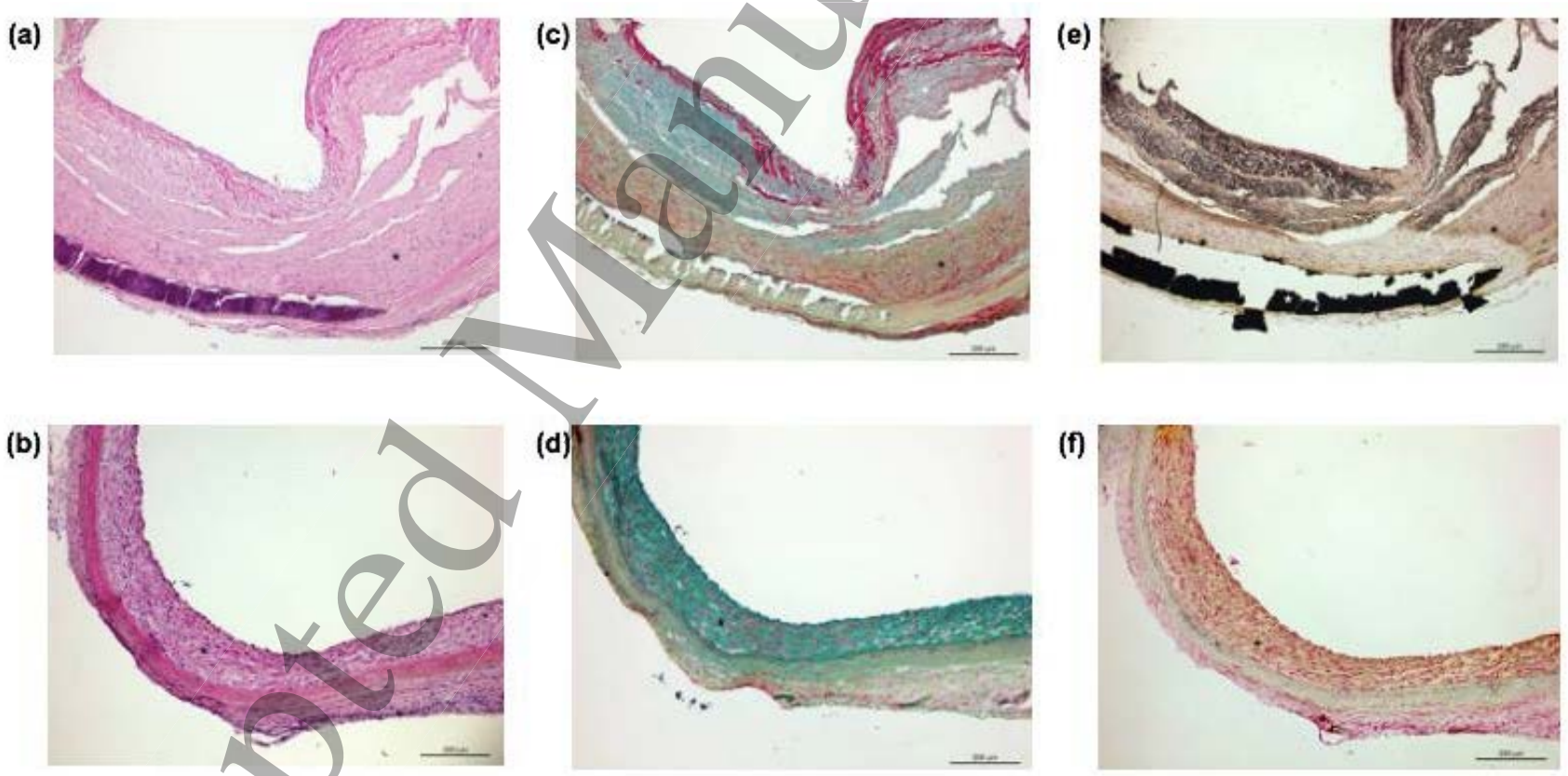

Fig. 6 Neo-intima morphology in decellularized AGs after 8 weeks in vivo. *Representative images of hematoxylin/eosin (a, b), Movat's pentachrome (c, d), and von Kossa (e, f) staining of explanted AGs. Cross sections of tissue from the B1 region explanted after 8 weeks are depicted. Multi-layered neo-intima hyperplasia (single black asterisks in a-f) and macro-calcification in the media (single white asterisks in a, c, e). (a), (c), (e): Control group; (b), (d), (f): SDF1α group. SDF1α, stromal cell-derived factor 1α. Scale bars = 200 µm*

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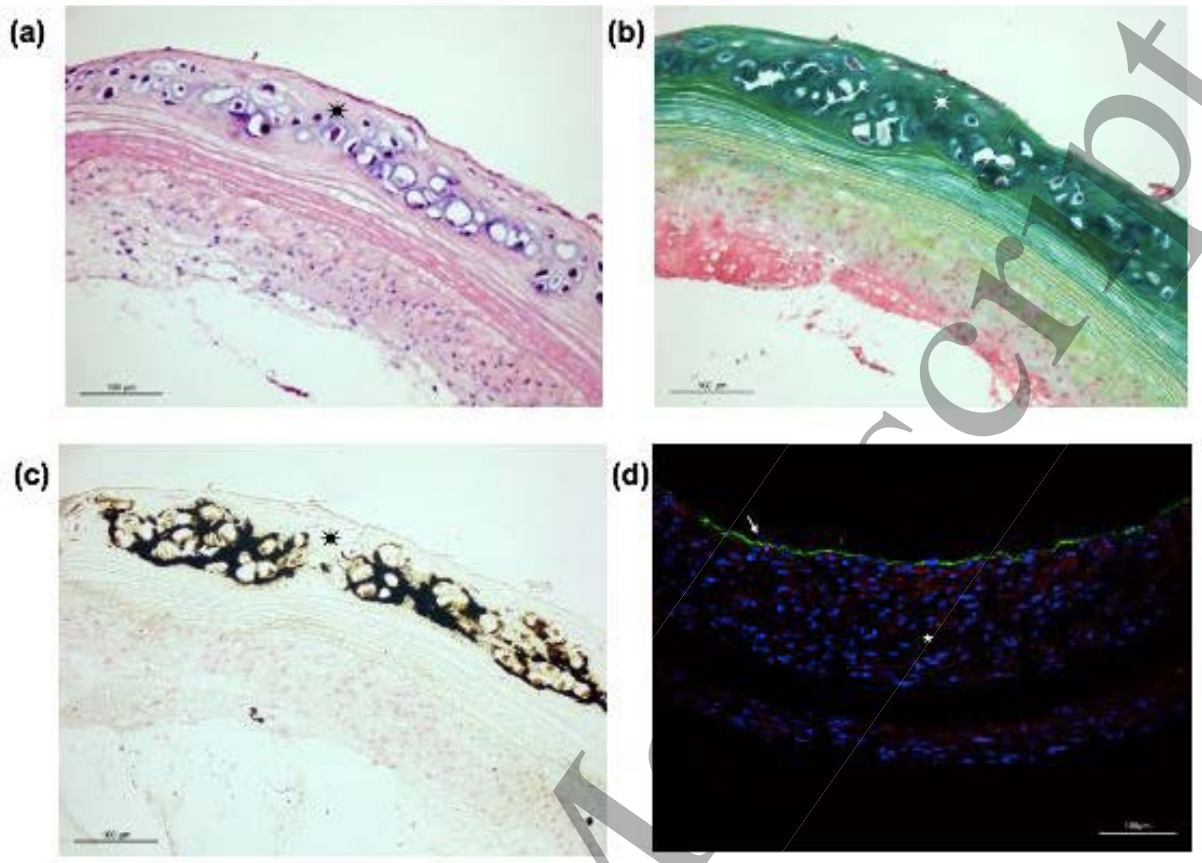

Fig. 7 Chondrogenic cell transformation and luminal endothelialization in hyperplastic regions of the control group at 8 weeks. *Cross sections of tissue from the B1 region of the control group explanted after 8 weeks are depicted. Cells with chondroid phenotype (asterisks in a, b) and pronounced calcification (asterisks in c) were occasionally observed in the hyperplastic neo-intima regions (hematoxylin/eosin (a), Movat's pentachrome (b), von Kossa (c) staining). Representative image of single-layered endothelium on the luminal surface staining positive for von Willebrand factor (vWF; green; arrow in d), while multi-layered hyperplastic regions stained positive for α-smooth muscle actin (αSMA; red; asterisks in d). Blue, 4',6-diamidino-2-phenylindole (DAPI). Scale bars = 100 µm.*

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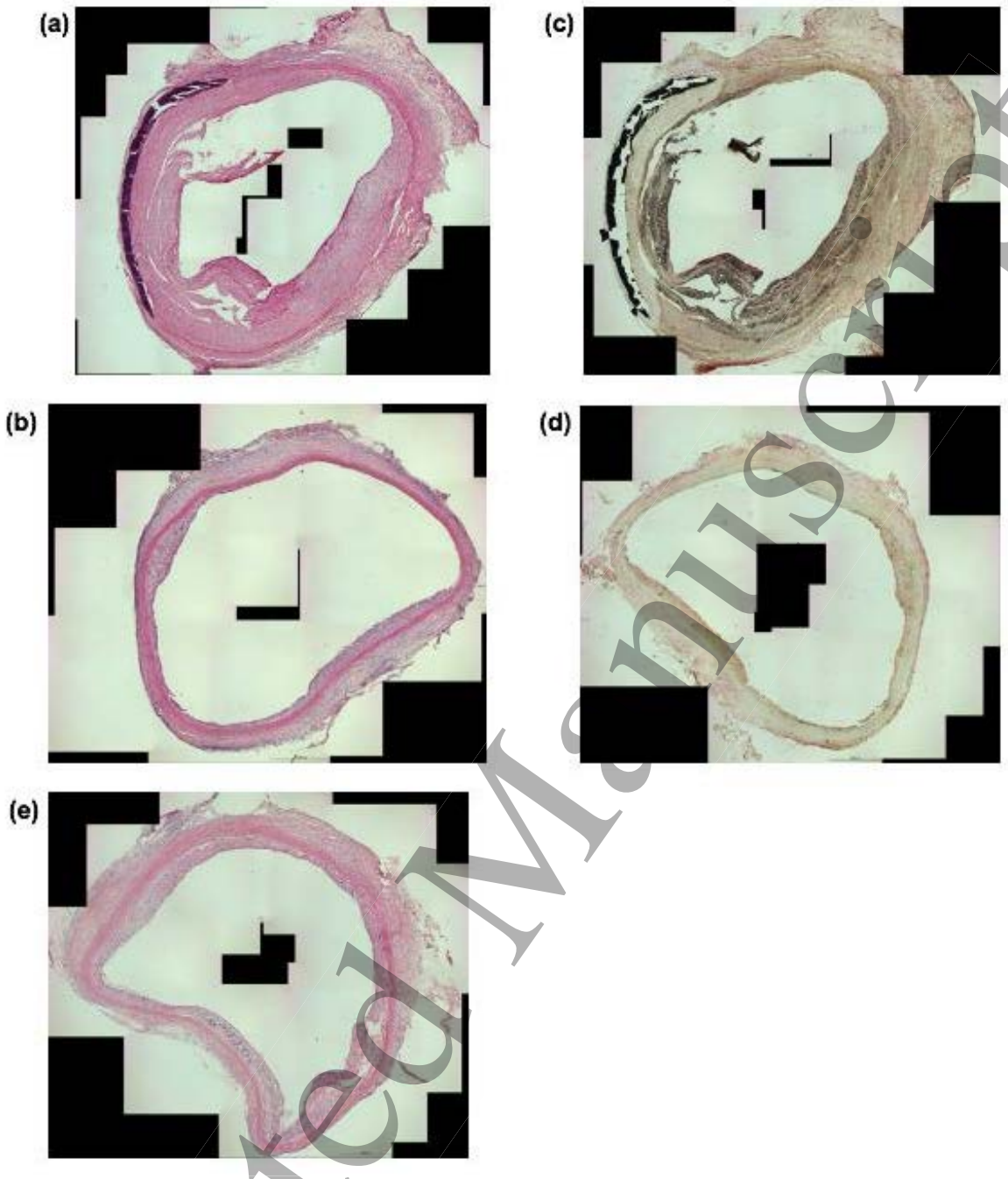

Suppl. 2 Representative low magnification images of decellularized AGs after 8 weeks. *hematoxylin/eosin (a, b, e), and von Kossa (c, d). Cross sections of tissue from the B1 region explanted after 8 weeks are depicted. in the SDF1α group for region A1 (a), A2 (b), B1 (c) and B2 (d). (a), (c), (e): Control group; (b), (d): SDF1α group. SDF1α, stromal cell-derived factor 1α. Scale bars = 400 μm.*

3.6. Media repopulation

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The numbers of autologous cells migrating into the media did not differ between the groups at 8 weeks (2,326 ± 658 /nm^2 vs. 2,923 ± 274 /nm^2, p=0.43; SDF1α vs. Control). Furthermore, there was no inter-group difference in any graft region (in A1: 6,109 ± 832 /nm^2 vs. 6,143 ± 787 /nm^2, p=0.98; in A2: 46.0 ± 13.5 /nm^2 vs. 36.4 ± 10.7 /nm^2, p=0.59; in B1: 80.3 ± 30.9 /nm^2 vs. 192 ± 111 /nm^2, p=0.36; in B2: 5,457 ± 630 /nm^2 vs. 5,203 ± 846 /nm^2, p=0.81; SDF1α vs. 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 2 or 8 weeks (2 weeks: 5.60± 1.66 vs. 5.40 ± 1.69, p=0.93; 8 weeks: 9.60 ± 1.75 vs. 20.8 ± 6.22, p=0.18; SDF1α vs. Control). However, in the anastomotic regions, no intima calcification was observed in group SDF1α at 8 weeks, while the control group exhibited calcified areas in these regions.

3.7.2. Media calcification

After 2 weeks, the semi-quantitative von Kossa scoring system revealed a significant difference between the two groups in the non-anastomotic regions (1.40 ± 0.87 vs. 7.80 ± 0.86, p<0.001 for A2; 2.00 ± 0.84 vs. 8.00 ± 2.17, p<0.05 for B1; SDF1α vs. Control) **(Fig. 8a, b)**. Similarly, significant differences were shown after 8 weeks (2.95 ± 0.87 vs. 9.05 ± 1.60, p<0.01 for all regions; 3.80 ± 1.43 vs. 9.40 ± 0.98, p<0.05 for A2; 8.00 ± 1.14 vs. 16.0 ± 2.67, p<0.05 for B1; SDF1α vs. Control) **(Fig. 9a-e)**. Interestingly, no media calcification was found in the anastomotic regions in SDF1α-

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coated grafts at 8 weeks **(Fig. 9f, g)**. Furthermore, the area of calcification in the media was significantly decreased in the SDF1α group as compared to controls ($p<0.01$ for 8 weeks) **(Fig. 10)**.

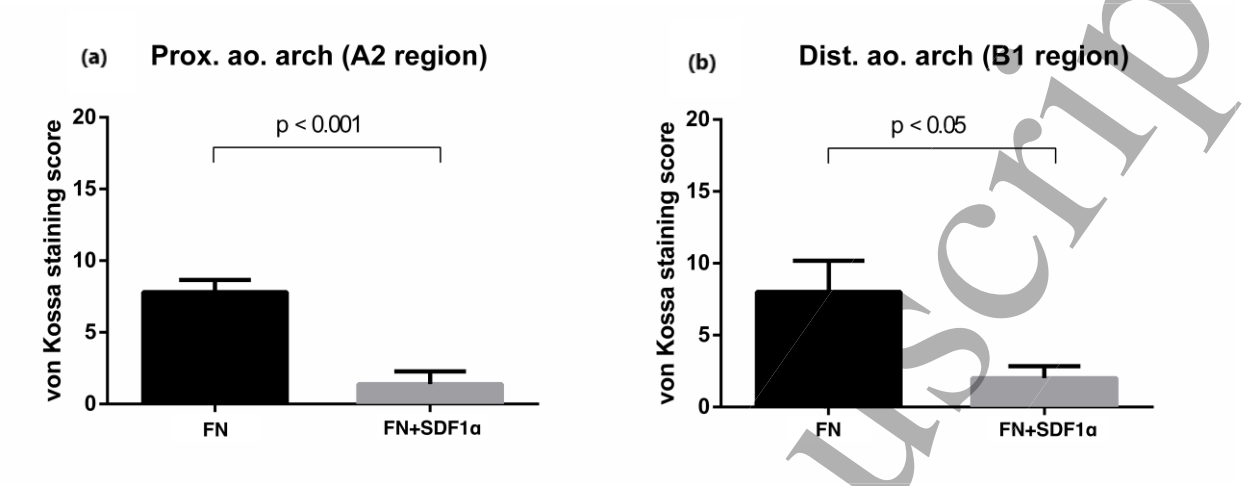

Fig. 8 Media calcification in decellularized AGs at 2 weeks. *SDF1α significantly inhibited media calcification in the regions A2 (a) und B1 (b) of implanted grafts, as evident by von Kossa staining and semiquantitative scoring analysis. ao, aort(a/ic); Dist, distal; FN, fibronectin; Prox, proximal; SDF1α, stromal cell-derived factor 1α.*

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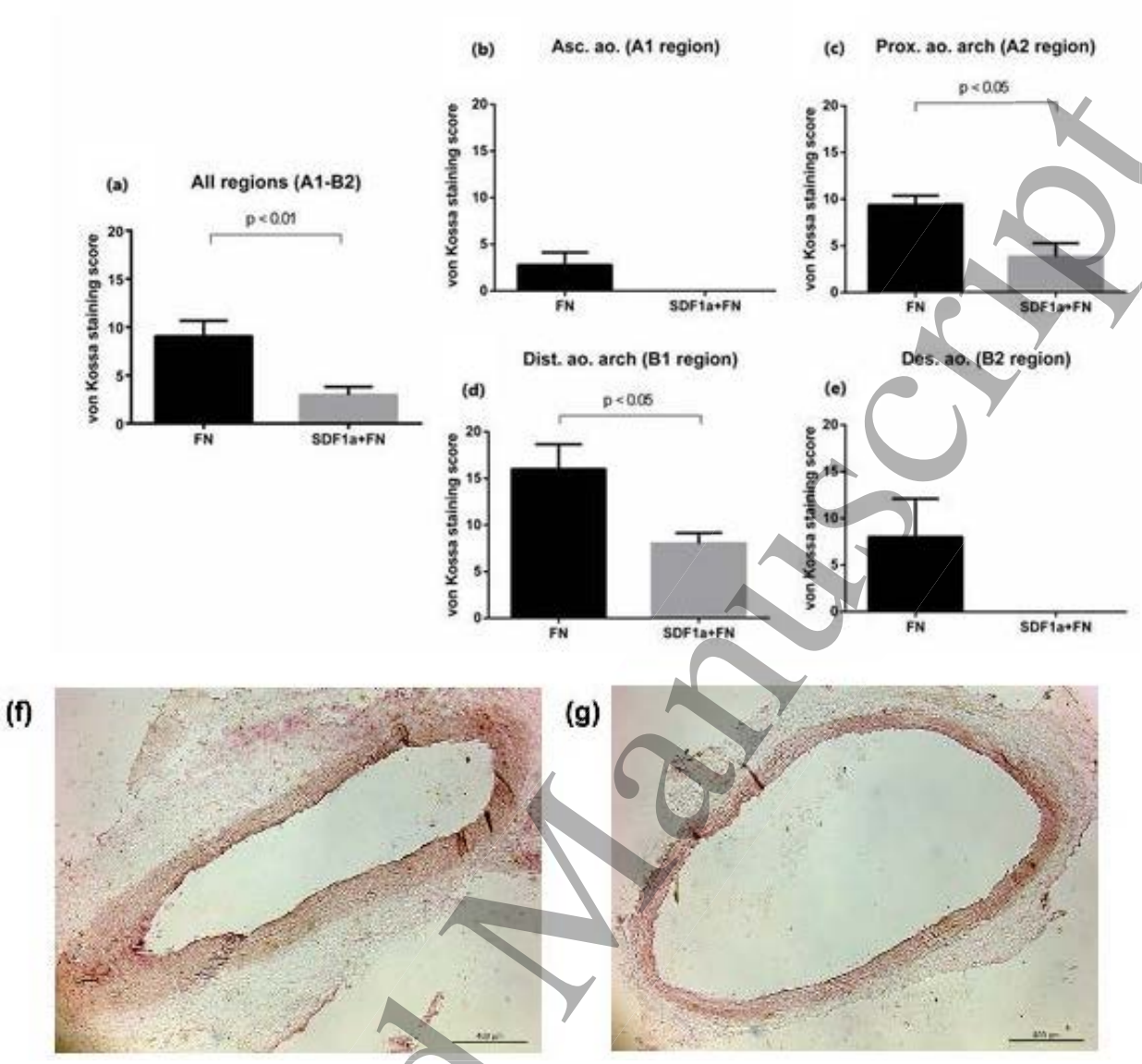

Fig. 9 Media calcification in decellularized AGs at 8 weeks. *SDF1α significantly inhibited media calcification in all regions of implanted grafts, as evident by von Kossa staining and semiquantitative scoring analysis over all regions (a). In sub-group analyses, a statistical significance was observed for regions distant from the anastomotic sites (regions A2 (c) and B1 (d), respectively), whereas complete absence of calcification in the regions A1 (b,f) and B2 (e,g) of SDF1α grafts did not allow for statistical testing, however, a remarkable difference was also present in these regions. ao, aort(a/ic); Asc, ascending; Dist, distal; Des, descending; FN, fibronectin; Prox, proximal; SDF1α, stromal cell-derived factor 1α. Scale bars = 400 μm.*

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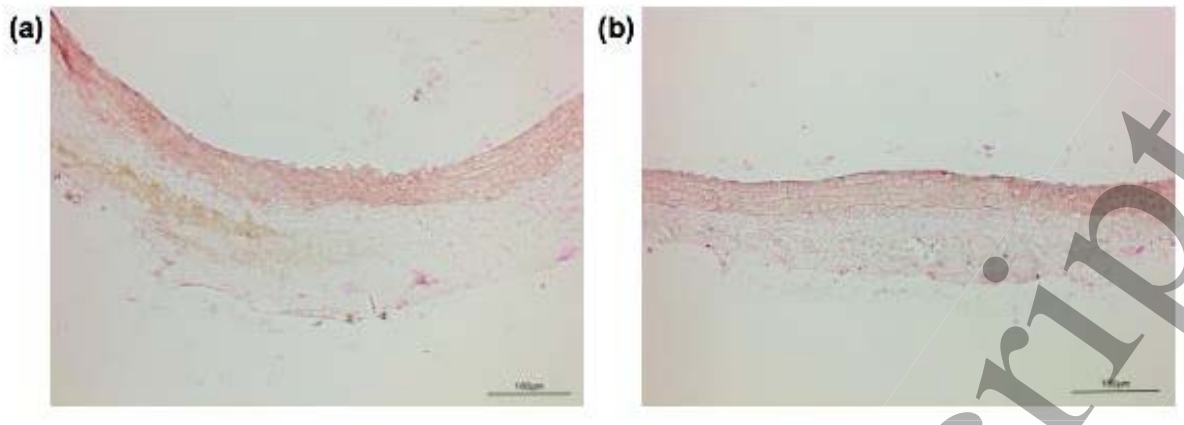

Suppl. 3 Representative high magnification images of von Kossa staining of decellularized AGs after 8 weeks in the SDF1α group. *Cross sections of tissue from the A1 region (a) and B2 region (b) explanted after 8 weeks are depicted. SDF1α, stromal cell-derived factor 1α. Scale bars = 100 µm.*

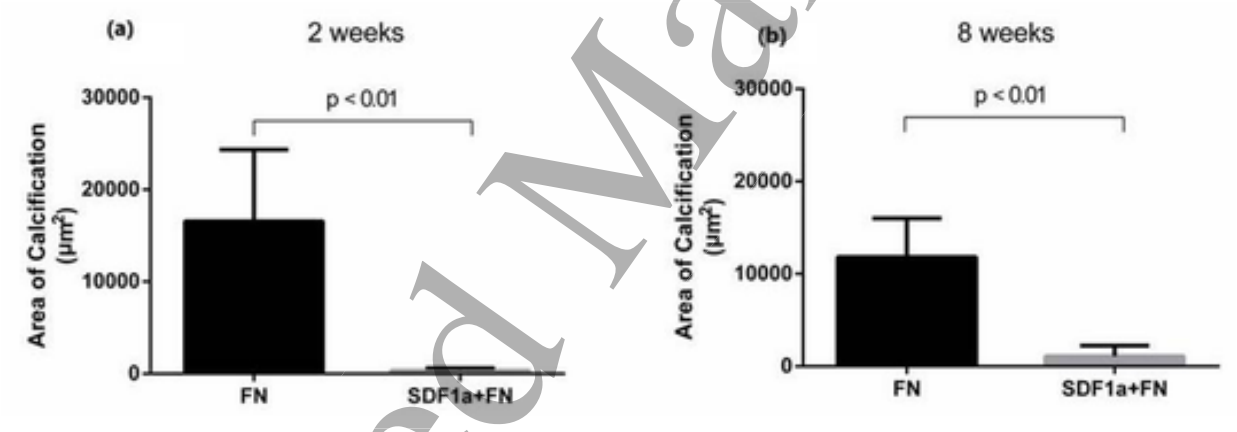

Fig. 10 Area of media calcification in decellularized AGs at 8 weeks. *The area of media calcification was decreased in group SDF1α for the region A2 at 8 weeks, as determined by a software-based quantification of the total cross-sectional area affected by calcification according to von Kossa staining. ao, aortic; FN, fibronectin; Prox, proximal; SDF1α, stromal cell-derived factor 1α.*

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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 **(Fig. 11a, b)**.

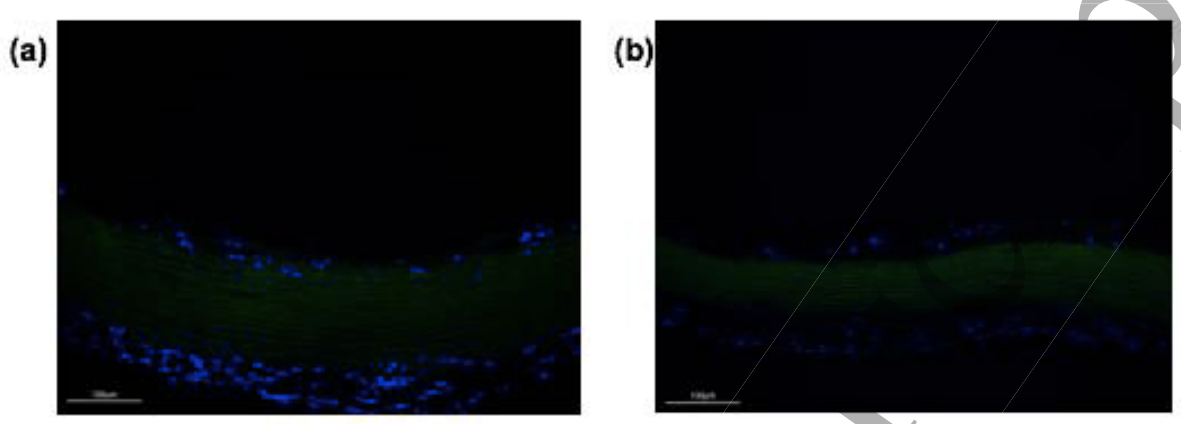

Fig. 11 Immunohistological staining of CD3+/CD68+ cells in decellularized AGs at 2 weeks. *No inflammatory reactions were detected for the region A2 in the control group **(a)**, or in the SDF1α group **(b)**. FN, fibronectin; SDF1α, stromal cell-derived factor 1α. Blue, 4',6-diamidino-2-phenylindole (DAPI); green, CD3; red, CD68. Scale bars = 100 μm.*

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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 decellurized AGs. After coating, it was confirmed that luminal site of AGs was covered with SDF1α. However, no SDF1α was observed on AGs at 2 weeks. Generally, the half time of injected SDF1α is very short in the blood stream [23]. Furthermore, it has been *in vitro* confirmed that 60% of membrane loaded SDF1α is burst released in the first 4 hours and then gradually released to approximately 80% until 35 days [24]. Thus, some strategies in order to sustain SDF1α 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 decellurized 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 for early implant population. In these areas, the significance of cellular recruitment from the blood stream is generally higher.

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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 should 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 supports our assumption of this study results about the impact of the luminal coating of SDF1α on the early endothelialization.

4.2.2. Impact of SDF1α on intima hyperplasia

For the biofunctionality of the decellularized AGs, to control 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 8 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 been also demonstrated that the coating with heparin and SDF1α increased endothelialization and reduced myofibroblast-associated intima hyperplasia *in vivo* [27].*Visscher et al.* applied FN and

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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. to promote the early endothelialization and to inhibit the neo-intimal hyperplasia formation, seems to be consecutive 2 steps in terms of 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 8 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 to inhibition of the calcification by luminal SDF1α coating in each graft region at 8 weeks, and proved that the calcification in the media of AGs decreased significantly in group SDF1α as compared to group control at both 2 and 8 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]. Addition of FN to SDF1α coating may support the anti-

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degenerative function of SDF1α by inducing polarization including redistribution of the SDF1α receptor [30].

In 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 2 weeks. In the non-anastomotic regions, the media calcification significantly decreased in the SDF1α group where early intima cellularization was enhanced. Further, no media calcification was observed in the anastomotic regions of the group SDF1α at 8 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

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calcification [13]. Our observation on no inflammatory cells at 2 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 hemotactic protein-1 adhered significantly lower in electrospun scaffolds with SDF1α peptides compared with control scaffolds under pulsatile flow conditions using a mesofluidic device for 14 hours [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. Limitation

There are several limitations of this study. First, the follow-up period was rather short to examine the long-term degenerative effects in the grafts. This may be a reason why we could not demonstrate a significant difference of 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 8 weeks, so that longer observation periods may be indicated. On the other hand, investigation of time points earlier than 2 weeks may reveal early immune-modulating properties of SDF1α that underly 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

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model. As a luminal coating material, SDF1α exhibits good potential to improve the biofunctionality and hemocompatibility of decellularized vascular grafts.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

Y.S., A.C., K.O., S.N., M.T., S.M., M.B., A.K.A., A.L., A.A. and P.A. 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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3. Diskussion und Schlussfolgerung

Das dezellularisierte Allotransplantat, im Allgemeinen die nicht-autologe extrazelluläre Matrix, induziert nach der Implantation die Migration der Wirtszellen *in situ*, was auch zu einer nachteiligen Umgestaltung des Gewebes führt. In früheren Berichten wurde die Effizienz der *in vitro* und *in vivo* EC-Aussaat als *Tissue-Engineering*-Technik zur Optimierung von ECM Gerüsten nachgewiesen [4]. Die EC-Aussaat-Methode ist jedoch für Notfallpatienten nicht möglich, da sie nicht in kurzer Zeit hergestellt werden kann. Andererseits untersuchten einige Forscher auch biofunktionelle Proteine/Peptide, um herauszufinden, wie sich eine frühzeitige funktionelle autologe Endothelialisation und Rezellularisierung in vivo erzielen lässt und wie sich die Immun-Entzündungsreaktion und die Transplantat-Degeneration modulieren lassen [16]. Das Konzept der *in vivo*-Repopulation autologer Zellen in einem zellfreien Transplantat wurde als Geweberegeneration („*guided tissue regeneration*“) bezeichnet und fand insbesondere im kardiovaskulären Bereich große Beachtung [17].

In unserer Studie untersuchten wir die Effizienz von luminalem SDF1α als bioaktive Beschichtung für dezellularisierte AoGs unter Verwendung des translationalen Rattenimplantatsmodel. Wir zeigten, dass die luminale SDF1α-Beschichtung die autologe Endothelialisation *in vivo* frühzeitig beschleunigte und zugleich die IH abschwächte. Andererseits haben wir bewiesen, dass die Verkalkung der AGs in der Tunica Media durch SDF1α signifikant reduziert werden konnte.

Basierend auf den Ergebnissen dieser Studie wurde der Mechanismus der Wirkung von luminal applizierter SDF1a Beschichtung wie folgend gedeutet.

SDF1α ist ein Chemokinproteine, das seinen selektiven Rezeptor CXCR4 hauptsächlich im Knochenmark bindet und eine Mobilisation von EPCs aus dem Knochenmark induziert, woraufhin Reparaturvorgänge an geschädigtem Gewebe anstößt, indem es Helfer-T-Zellen in einen entzündungshemmenden Subtyp verwandelt [16].

EPCs haben die Funktion, im zirkulierenden Blut dort anzuhaften, wo Blutgefäße zu regenerieren sind, und ferner die Revaskularisation für die Geweberegeneration mit folgenden Mechanismen zu steuern [18].

(a) EPCs wirken direkt auf das Endothel, um eine Gewebeverletzung zu reparieren [19].

(b) EPCs transportieren angiogene Wachstumsfaktoren wie vaskulären endothelialen Wachstumsfaktoren (VEGF), insulinähnlichen Wachstumsfaktor (IGF) und Granulozyten-/Makrophagenkolonie-stimulierende Faktoren zum verletzten Endothel und drängen auf eine Endothelreparatur (parakrine Signalisierung) [20].

(c) EPCs setzen die MMP-2 und MMP-9 frei und verstärken die Schädigung eines Teils des Endothels und die Reparatur durch Migration neuer EC- und vaskulärer Umbauten [21].

Das Vorhandensein von mehr EPCs, die durch SDF1α auf der luminalen Seite von Transplantaten stimuliert werden, lässt daher eine Beschleunigung der Endotheliasation vermuten und verhindert eine IH.

Diese Hypothese wird auch aus den folgenden früheren Berichten bestätigt.

Zhou et al. beschichteten Heparin und SDF1α auf SDS-basiert dezellularisierten AoK und implantierte diese in Ratten [22]. Vier Wochen nach der Implantation war die Endothelialisation stärker und es gab signifikant mehr EPCs (CD34-positive Zellen) in der beschichteten Gruppe im Vergleich zur Kontrollgruppe. Ferner reduzierte diese Beschichtung signifikant die Anzahl der Blutplättchen, die an Klappen von AoK anhafteten.

Visscher et al. bewiesen, dass FN- und SDF1α-Beschichtungen EPCs zu Transplantaten anziehen [23]. In einer anderen Studie hat dieselbe Gruppe FN und SDF1α zur Beschichtung synthetischer Polyesterimplantateverwendet, die zur Carotis-Interposition bei Schafen verwendet wurden [24]. Das Ergebnis war, dass mehr CD34-positive Zellen nachgewiesen, die Endothelialisation stimuliert und die IH verhindert wurden.

Darüber hinaus haben *Flameng et al.* FN und SDF1α zur Beschichtung von dezellularisierten allogenen Spenderprothesen verwendet und diese in den rechtsventrikulären Abflusstrakt implantiert [15]. Diese Beschichtung verringerte die Pannusbildung, Verkalkung, die Entzündungsreaktion des Immunsystems und stimulierte ferner die Endothelialisation.

Auf dem Gebiet der interventionellen Kardiologie wurde das Potenzial von SDF1α zur Hemmung der In-Stent-Restenose und der Stent-Thrombose nach medikamentenfreisetzender Stentimplantation beschrieben [25].

Bei diesen letzten Berichten handelt es sich jedoch ausschließlich um SDF1α-Vollschichtenbeschichtungen. In Anbetracht der Wirkung von SDF1α auf Endothelzellen sollte gezeigt werden, ob nur eine luminale SDF1α-Beschichtung erforderlich ist. In diesem Sinne glauben wir, dass unsere Studie eine sehr große Bedeutung und Originalität hat.

Zusammenfassend implizierte die vorliegende Studie eine hervorragende Hämokompatibilität der luminalen SDF1α-Beschichtung, um eine frühzeitige funktionelle autologe Endothelialisation mit gleichzeitiger Hemmung der IH und ebenso einer signifikanten Reduktion der Verkalkung zu ermöglichen.

4. Perspektive

Gute klinische Ergebnisse wurden bereits für dezellularisierte AoK gezeigt [26].

In einer prospektiven multizentrischen Studie mit dem Namen ARISE und gefördert durch die Europäische Union wurde während des Beobachtungszeitraums (im Mittel 2,0 ± 1,8 Jahre, Maximum 7,6 Jahre) kein Versagen des eingesetzten dezellularisierten AOKs beobachtet, was auf eine gute Biofunktionalität hinweist

In dieser Studie entwickelte das eingesetzte schmale dezellularisierte AoK (Durchmesser; 10 mm) jedoch 4,5 Jahre nach dem Einsetzen eine subvalvuläre Stenose, die einen Reflux verursachte und eine Reoperation erforderte.

Die Forschung zur Verlängerung der Haltbarkeit kleinkalibriger Blutgefäße und zur Entwicklung von *in vivo* Versuchstiermodellen ist noch im Gange. In Zukunft wird es wichtig sein, eine Verbindung zur klinischen Forschung herzustellen.

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