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ORIGINAL ARTICLE

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TRANSLATIONAL RESEARCH

Dichloroacetate inhibits the degeneration of decellularized cardiovascular implants

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Key question

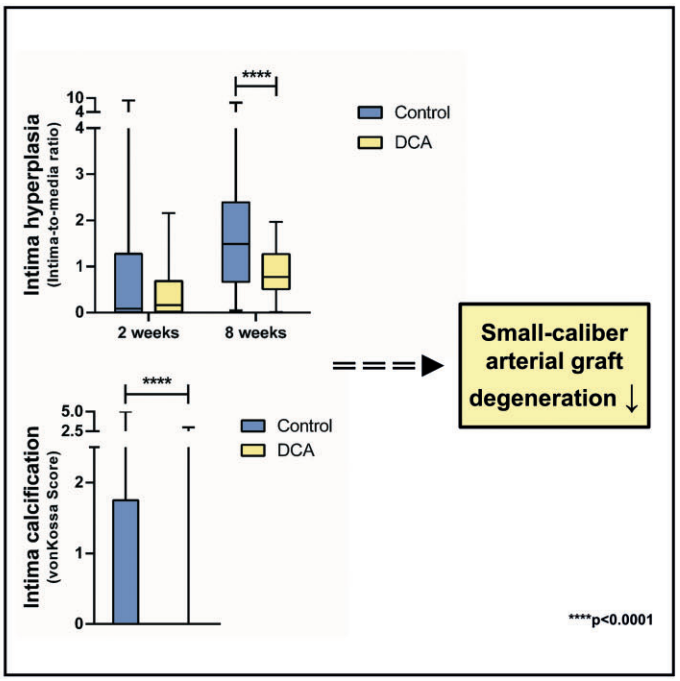
What is the effect of systemic dichloroacetate (DCA) on the degeneration of decellularized small-calibre arterial grafts?

Key finding(s)

Systemic DCA treatment inhibits intima hyperplasia and calcification in decellularized small-calibre arterial grafts.

Take-home message

Systemic DCA treatment is envisioned to improve the durability of acellular biological cardiovascular implants.



Abstract

OBJECTIVES: Intima hyperplasia is a major issue of biological cardiovascular grafts resulting in progressive *in vivo* degeneration that particularly decreases the durability of coronary and peripheral vascular bypasses. Previously, dichloroacetate (DCA) has been reported to prevent the formation of hyperplastic intima in injured arteries. In this study, the effect of DCA on the neointima formation and degeneration of decellularized small-caliber implants was investigated in a rat model.

METHODS: Donor rat aortic grafts ($n = 22$) were decellularized by a detergent-based technique, surface-coated with fibronectin (50 µl ml^{-1}, 24 h incubation) and implanted via anastomoses to the infrarenal aorta of the recipients. Rats in the DCA group ($n = 12$) received DCA via drinking water during the whole follow-up period (0.75 g l^{-1}), while rats without DCA treatment served as controls ($n = 10$). At 2 ($n = 6 + 5$) and 8 ($n = 6 + 5$) weeks, the grafts were explanted and examined by histology and immunofluorescence.

RESULTS: Systemic DCA treatment inhibited neointima hyperplasia, resulting in a significantly reduced intima-to-media ratio (median 0.78 [interquartile range, 0.51–1.27] vs 1.49 [0.67–2.39] without DCA, $P < 0.001$). At 8 weeks, neointima calcification, as assessed by an

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established von Kossa staining-based score, was significantly decreased in the DCA group (0 [0–0.25] vs 0.63 [0.06–1.44] without DCA, $P < 0.001$). At 8 weeks, explanted grafts in both groups were luminally completely covered by an endothelial cell layer. In both groups, inflammatory cell markers (CD3, CD68) proved negative.

CONCLUSIONS: Systemic DCA treatment reduces adverse neointima hyperplasia in decellularized small-caliber arterial grafts, while allowing for rapid re-endothelialization. Furthermore, DCA inhibits calcification of the implants.

Keywords: Tissue engineering • Decellularization • Vascular graft • Intima hyperplasia • Graft degeneration • Dichloroacetate

ABBREVIATIONS

αSMA	Alpha smooth muscle actin
DCA	Dichloroacetate
IQR	Interquartile range
PBS	Phosphate-buffered saline
PDK	Pyruvate dehydrogenase kinases
vWF	von Willebrand factor

INTRODUCTION

Tissue-engineered vascular grafts represent an implant source that promises to overcome the limitations of clinical standard grafts. Such optimized implants may have crucial impact particularly on the surgical treatment of coronary and peripheral arterial disease requiring small-caliber grafts.

Methods to create a more biocompatible vascular graft are focused on the decellularization of allogeneic or xenogenic tubular tissues, the creation of biodegradable scaffolds and biopolymers, as well as on coating and surface modification [1]. Since the 1960s, despite continuous innovation in decellularization strategies, decellularized scaffolds have been reported to promote thrombotic events and antigenicity-related complications, albeit at low rates [2]. To overcome the issue of immune response- or thrombosis-mediated graft failure, recent research has focused on controlled *in vivo* recellularization by means of scaffold surface modification with bioactive agents such as stromal cell-derived factor 1 alpha, fibronectin or heparin [3, 4]. Recent studies have shown that fibronectin coating of decellularized grafts improves their cellular repopulation without causing thrombosis or immune rejection but also stimulates intimal hyperplasia [4].

Several studies on intima hyperplasia in clinical cases and experimental models showed that particularly late graft failure is associated with the proliferation of vascular smooth muscle cells [5]. Various drugs as well as biological agents such as antibodies or nucleotides have been demonstrated to inhibit vascular smooth muscle cell proliferation [6–8].

In this context, the xenobiotic dichloroacetate (DCA) was shown to decrease pulmonary hypertension by causing smooth muscle cell apoptosis in remodelled pulmonary arteries [8]. Even more interestingly, DCA has been reported to reduce intima hyperplasia in injured vessels by increasing apoptotic activity due to the induction of mitochondrial oxidative stress [9].

The aim of this study was to examine the potential of DCA to influence the intimal hyperplasia and the calcifying degeneration of decellularized aortic grafts, surface-coated with fibronectin, in a standardized rat implantation model.

MATERIALS AND METHODS

Experimental design

Male Wistar rats ($n = 44$, 22 donor rats, 22 recipient rats) weighing 200–250 g were obtained from the local animal care facility of the University Duesseldorf, Germany, and fed *ad libitum* with regular diet. All experiments were performed in agreement with the national animal welfare act and approved by the state animal care committee—reference number 84—02.04.2012.A391.

After explantation from donor animals, the aortic grafts were decellularized by means of a detergent-based protocol and coated with fibronectin. Thereafter, coated aortic grafts were implanted into the infrarenal aortas of the recipients. The recipient animals were distributed to 2 experimental groups: The DCA group ($n = 12$) received engineered aortic grafts coated with fibronectin and systemic DCA therapy during the whole follow-up period (0.75 g l^{-1} via drinking water; ingested water monitored daily), while the control group ($n = 10$) received only engineered aortic grafts coated with fibronectin and no systemic treatment. Animals in each group were observed for 2 and 8 weeks, respectively, resulting in $n = 6$ DCA rats and $n = 5$ control rats at each time point for implant readout. The sample size has been calculated estimating the effect size by Cohen's *d* based on previous data on intima hyperplasia in fibronectin-coated decellularized grafts [4]. A schematic representation of the experiments including technical aspects is presented in Supplementary Material, Fig. S1, and a timeline of the experiments in Supplementary Material, Fig. S2.

All surgical procedures, the graft engineering as well as the readout methods were conducted according to standards previously established in our group [4, 10]. In the following, brief descriptions are provided.

Surgical procedures

Donor operation. Animals were euthanized by an overdose of isoflurane, after which a median sternotomy was performed followed by *en bloc* removal of the heart and the thoracic aorta. The aortic grafts were prepared containing the ascending aorta, aortic arch and descending aorta. After through preparation, the U-shaped aortic grafts were rinsed with heparinized phosphate-buffered saline (PBS).

Graft decellularization. The decellularization started with 4 cycles of 12 h with 0.5% sodium dodecyl sulphate + 0.5% deoxycholate + 0.05% sodium azide and with 2 cycles 2 h with DNase (2.3 mg ml^{-1} in PBS), followed by 4 repetitive 24 h washing cycles with PBS containing 1% penicillin/streptomycin (Sigma-Aldrich, Taufkirchen, Germany; Aldrich and Merck, Darmstadt, Germany). All steps were conducted in 15-ml tubes, filled with 12 ml, containing a maximum of 2 grafts.

Graft coating. All decellularized rat aortic prostheses intended for implantation ($n = 22$) were coated with fibronectin on both surfaces by incubation under the following conditions: fibronectin concentration 50 μg ml^{-1} in PBS (Sigma-Aldrich), incubation time 24 h and incubation temperature 37°C.

Recipient operation. The heterotopic implantation of the engineered aortic grafts was carried out according to a previously standardized approach [11], which is described in brief: Recipient rats were intubated, anaesthetized with 2.0–2.5% isoflurane, a central venous jugular vein catheter was inserted and carprofen was injected intraperitoneally. A midline laparotomy was performed, the intestines were mobilized and the aorta was dissected away from the vena cava at the level of the infrarenal aorta. After systemic administration of 300 IU kg^{-1} (body weight) heparin via the central venous catheter and aortic clamping within this segment, 2 incisions were made to create distal and proximal openings for the anastomoses. The anastomoses were sutured in an end-to-side manner, with a continuous 10–0 suture (Ethicon, Norderstedt, Germany). Following release of blood flow through the graft, the native aorta between the 2 anastomoses was ligated to improve perfusion of the implant. After clinical observation, particularly paying attention to the perfusion of the lower extremities, the abdomen was closed, and after sonographic confirmation of unimpaired graft perfusion with a Philips HDX 11 ultrasonography system equipped with a 15-MHz probe (Philips, Amsterdam, Netherlands), the recipients were allowed to recover from anaesthesia.

Graft explantation. Two or 8 weeks after implantation, the recipient rats were anaesthetized and Doppler sonography was conducted to control the perfusion of the prostheses. After median laparotomy, the aortic grafts were excised and further processed for histology and immunohistology.

Morphological characterization of explanted grafts

Histology. Cryo-sections (5-μm thick) were generated for histological staining. For characterization of the vascular wall and cell layers, haematoxylin/eosin staining was used. Von Kossa staining was applied to determine the degree of calcification. Movat pentachrome staining was chosen for the detection of matrix components.

Comparative quantification of the luminal neointima formation and graft calcification was performed by standardized scoring as previously published [12] (Supplementary Material).

Immunohistology. Cryo-sections were incubated for 10 min with 4% formalin, for 10 min with 0.25% Triton-X-100 (Sigma-Aldrich) and for 1 h with 5% bovine serum albumin (Sigma-Aldrich) at room temperature.

Smooth muscle cells were visualized with the anti-alpha smooth muscle actin (αSMA, Sigma-Aldrich). Endothelial cells were detected using anti-von Willebrand factor (vWF, Dako, Hamburg, Germany). For inflammatory processes, anti-CD3 (Sigma-Aldrich) for T cells and anti-CD68 (Abcam, Cambridge, UK) for macrophages were evaluated. Thereafter, the sections were washed 3 times in PBS containing 0.1% Tween-20. As secondary antibodies, Alexa 546 and Alexa 488 (Invitrogen, Carlsbad, USA) + 1% bovine serum albumin were applied for 45 min at 37°C. Control sections were incubated with PBS without primary antibodies. After 1 h incubation, sections were washed again 3 times in PBS. Sections were covered with Vectashield antifade mounting medium with 4′,6-diamidino-2-phenylindole (Vector Labs, Peterborough, UK) and image acquisition was performed with a microscope system DM 2000, equipped with a digital Application Suite V3.7 software.

Statistical analysis

Data are presented as median and interquartile range (IQR) for all continuous variables. Following normality assessment (D'Agostino-Pearson test), direct group comparisons were conducted by two-tailed Mann–Whitney *U* tests or two-tailed Student's *t*-tests, respectively. Statistical significance was considered if *P*-values were lower than 0.05. Data analysis was conducted using Graph Pad Prism v6.01 (Graph Pad Software, San Diego, USA).

RESULTS

Operative results

All 22 decellularized aortic grafts, coated with fibronectin, were successfully implanted and remained functional for up to 2 and 8 weeks, after which they were explanted. All animals showed normal clinical function during the follow-up and recovered from surgery without signs of neurological or ischaemic symptoms of the lower limbs.

Cellular graft repopulation

Histology of the explants revealed a progressive luminal re-endothelialization within 8 weeks after transplantation, with significantly increased luminal coverage compared to the data at 2 weeks ($P < 0.001$), however, without a statistically significant inter-group difference regarding treatment (Fig. 1). After 8 weeks,

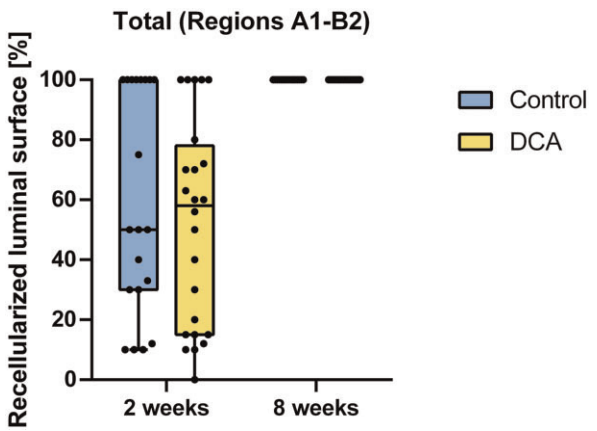

Figure 1: Semiquantitative analysis of the luminal recellularization with neointima in the control and dichloroacetate group after 2 and 8 weeks *in vivo*. In all 4 regions, the aortic grafts were completely covered with neointima after 8 weeks in both groups without significant intergroup differences. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values.

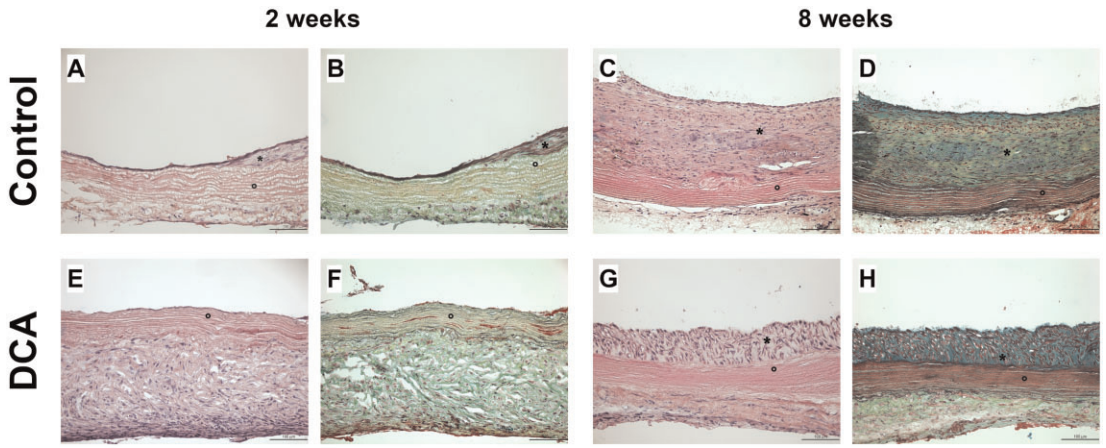

Figure 2: Representative images of explanted aortic grafts in the control and dichloroacetate group after 2 and 8 weeks *in vivo*. Early hyperplasia (asterisks in **A**, **B**) and multi-layered hyperplastic neointima (asterisks in **C**, **D**, **G**, **H**) were enhanced in the control group. Circles display the graft media. Haematoxylin/eosin (**A**, **C**, **E**, **G**) and Movat's pentachrome staining (**B**, **D**, **F**, **H**). Scale bars = 100 µm.

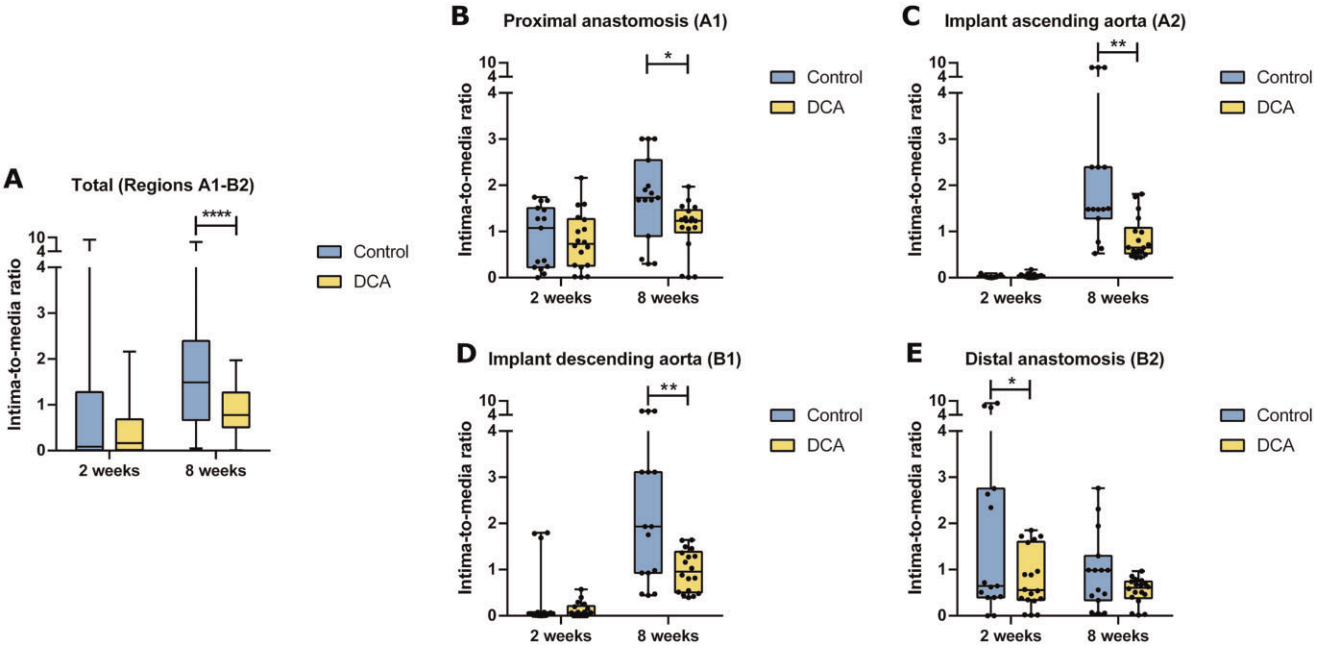

Figure 3: Semiquantitative analysis of intima hyperplasia measured by means of the intima-to-media ratio in all regions (**A**) and separate regions (**B**–**E**) of the aortic graft explants in the control and dichloroacetate group after 2 and 8 weeks *in vivo*. The intima hyperplasia was significantly lower in the dichloroacetate group. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values. **P* < 0.05; ***P* < 0.01; *****P* < 0.0001.

neointima coverage was complete throughout the length of the grafts in both groups (Supplementary Material, Fig. S3).

After 2 weeks, the neointima in the control group presented with hyperplastic areas, while the DCA group showed predominantly single-layer endothelium. By week 8, intima hyperplasia had progressed in both groups, however, to a larger extent in group control. By Movat's pentachrome staining, hyperplastic intima in the control group showed higher collagen content and generally more intercellular substance (Fig. 2).

To quantify the extent of hyperplastic neointima formation, the intima-to-media ratio was calculated (Fig. 3). After 2 weeks, the intima-to-media ratio in group DCA was significantly decreased at the level of the distal anastomosis (region B2) (0.55 [IQR, 0.34–1.60] vs 0.65 [IQR, 0.40–2.76] in group control; $P = 0.043$). After 8 weeks, overall hyperplastic intima formation was significantly decreased in the DCA group as compared to the control group (0.78 [IQR, 0.51–1.27] vs 1.49 [IQR, 0.67–2.39], $P < 0.001$). In 3 of the 4 regions of the implants, there was a significantly lower intima-to-media ratio at 8 weeks (region A1: 1.23 [IQR, 0.97–1.47] vs 1.73 [IQR, 0.90–2.54], $P = 0.0225$; region A2: 0.65 [IQR, 0.52–1.08] vs 1.48 [IQR, 1.28–2.39], $P = 0.001$; region B1: 0.96 [IQR, 0.51–1.39] vs 1.93 [IQR, 0.93–3.11], $P = 0.024$; region B2: 0.61 [IQR, 0.38–0.74] vs 0.99 [IQR, 0.33–1.30], $P = 0.057$).

Immunofluorescence analysis of the cell populations participating in the development of neointima of the vessel grafts revealed a vWF-positive monolayer of endothelial cells at the luminal surfaces of the grafts, and multi-layered intima regions stained positive for αSMA (Fig. 4). Inflammatory cell markers were not relevantly positive in any group at any time point (Supplementary Material, Fig. S4).

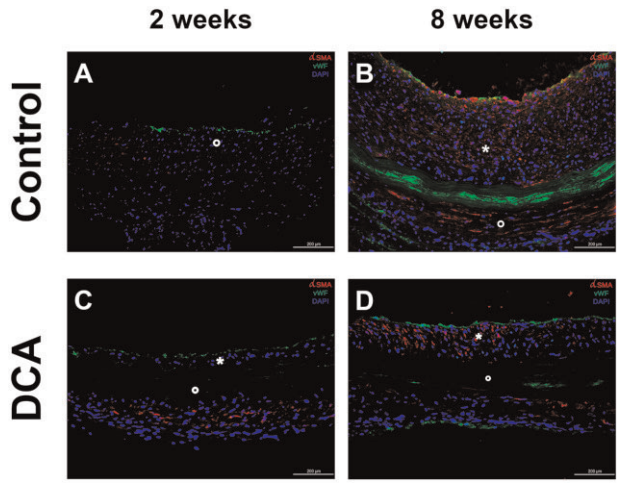

Figure 4: Representative images of immunohistochemically stained explanted aortic grafts in the control (**A**, **B**) and dichloroacetate (**C**, **D**) group after 2 and 8 weeks *in vivo* (asterisks, neointima; circles, media). Single-cell layers on the luminal surface stained positive for von Willebrand factor (green) (**A**–**D**), and multi-layered hyperplastic regions stained positive for a-smooth muscle actin (red) (**B**, **D**). 4′,6-Diamidino-2-phenylindole, blue. Scale bars = 200 μm.

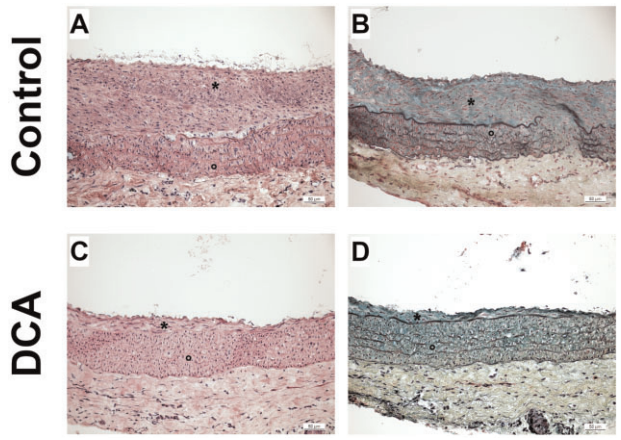

Figure 5: Representative images of the repopulation of the aortic graft media in the control (**A**, **B**) and dichloroacetate (**C**, **D**) group after 8 weeks *in vivo* (asterisks, neointima; circles, media), displaying substantial media repopulation by autologous cells in both groups. Haematoxylin/eosin (**A**, **C**) and Movat's pentachrome (**B**, **D**) staining. Scale bars = 50 μm.

Autologous cell migration into the media was observed after 2 and 8 weeks in both groups (Fig. 5). Media repopulation in anastomotic regions was presented earlier and to a substantially larger extent than in regions remote to the anastomoses (Fig. 6), and originated predominantly from the adventitial side of the graft wall (Fig. 2F). The cells that invaded the media were positive for αSMA. Inflammatory cell markers were not relevantly positive in any group at any time point. In all grafts, the extracellular matrix structure was well maintained as visualized by Movat pentachrome staining with the typical yellow staining indicating collagen, blue/green staining indicating glycosaminoglycans, and black staining for elastic fibres.

Graft degeneration

To visualize graft calcification processes, von Kossa staining was performed (Supplementary Material, Fig. S6). The amount of hydroxyapatite deposition in the neointima after 8 weeks was significantly lower in the DCA group than in the control group (region A1: 0 [IQR, 0–0] vs 0 [IQR, 0–1], $P = 0.030$; region A2: 0 [IQR, 0–0] vs 0 [IQR, 0–2], $P = 0.015$; region B1: 0 [IQR, 0–0.75] vs 1 [IQR, 0–2], $P = 0.008$; region B2: 0 [IQR, 0–0] vs 1 [IQR, 0–1], $P = 0.003$) (Fig. 7A). The difference in calcium burden in the media showed statistical significance in the perianastomotic regions at 8 weeks, with decreased calcification in the DCA group (region A1: 0 [IQR, 0–0] vs 0 [IQR, 0–1], $P = 0.008$; region B2: 0 [IQR, 0–0] vs 1 [IQR, 0–1], $P = 0.047$) (Fig. 7B).

DISCUSSION

Graft decellularization techniques aim at effective cell removal while preserving the extracellular matrix structure. The most commonly used decellularization techniques include methods of chemical, enzymatic and mechanical treatment [1]. The resulting decellularized scaffolds are prone to cause thrombosis, intima hyperplasia or aneurysms due to the absence of a closed endothelial layer [13]. Bioactive surface coating prior to graft transplantation can be used to accelerate the autologous *in vivo* recellularization, thereby counteracting the above mentioned adverse effects.

In the current study, we have used fibronectin coating since we had previously shown that fibronectin induces accelerated medial graft repopulation in the absence of an inflammatory reaction [4]. Unfortunately, fibronectin in our previous study had not only accelerated neointima formation, but also aggravated intima hyperplasia, so that a different strategy was necessary to overcome this issue. To inhibit neointimal hyperplasia, systemic DCA treatment was administered, and the effect was analysed 2 and 8 weeks after graft implantation.

DCA is a small molecule, a structural analogue of pyruvate, that is commonly used to inhibit pyruvate dehydrogenase kinases (PDK), reduce cellular proliferation and induce apoptosis [14]. DCA activates the pyruvate dehydrogenase complex by inhibition of the PDK isoform 2. Pharmacologic PDK2 blockade with DCA prevents hyperpolarization of the mitochondrial membrane potential, thereby inducing apoptosis and thus reducing hyperplastic neointima formation in injured vessels [15].

In numerous studies, DCA has shown protective effects on myocardial ischaemia [16], cancer [14], pulmonary hypertension [8], platelet aggregation and arterial thrombosis without altering hemostasis [17]. It was also reported that neointima formation in native human arteries is driven by hyperpolarization of the mitochondrial membrane potentials in vascular smooth muscle cells, which can be counteracted by DCA treatment [9].

While synthetic vascular prostheses offer good long-term patency for the replacement of large arteries with high blood flow, such as the aorta, synthetic grafts with small diameters (<6 mm), such as in coronary or peripheral vascular surgery, show poor patency rates [18, 19]. Similarly, small-caliber allogenic and xenogenic grafts, independently of their usage in a fresh or a cryopreserved state, have proven not to be adequate substitutes for available autologous bypass vessels, such as saphenous veins or internal thoracic arteries or radial arteries [18, 20]. For synthetic as well as biological small-caliber grafts, thrombogenicity due to the lack of autologous endothelium and progressive intima hyperplasia resulting in calcifying degeneration are the driving forces of graft failure. In this context, we envision that the inhibition of intima hyperplasia and subsequent calcification in tissue-engineered

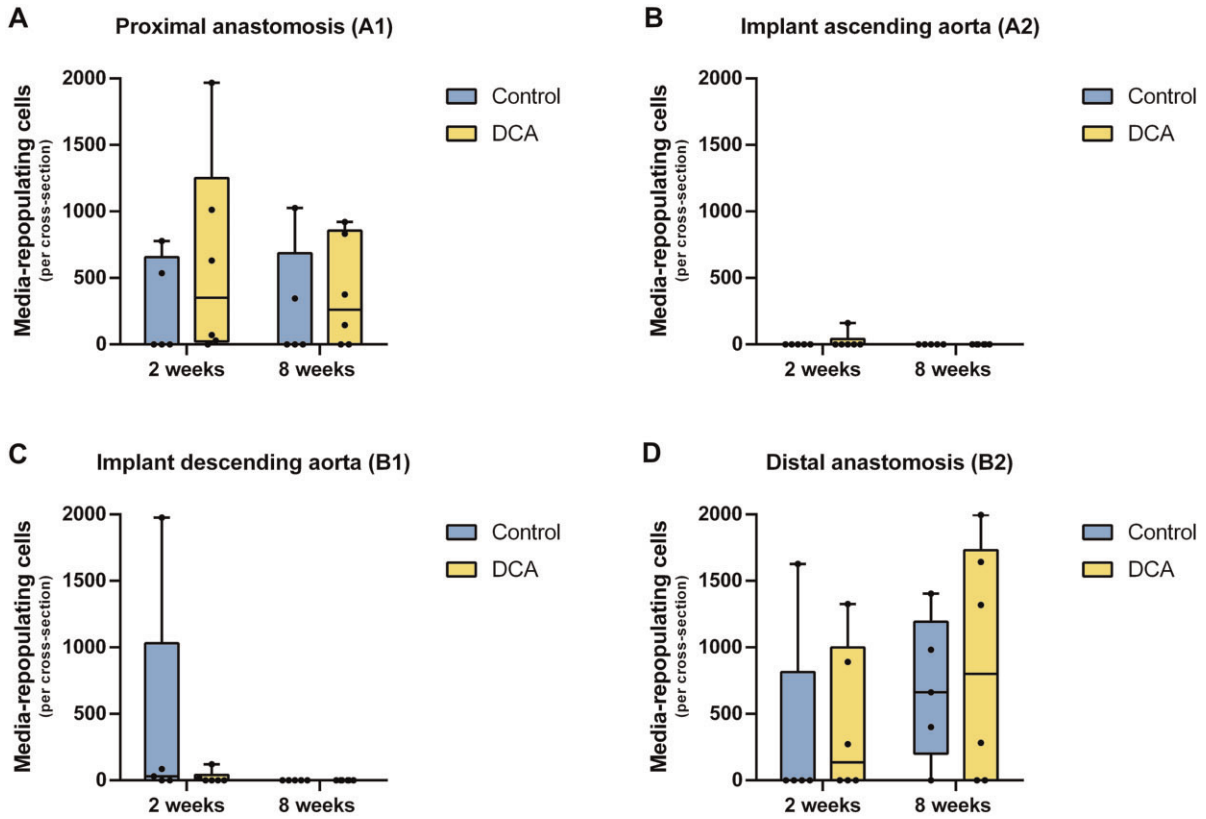

Figure 6: Semiquantitative analysis of the autologous media recellularization in different regions (**A**–**D**) of the graft explants in the control and dichloroacetate group after 2 and 8 weeks *in vivo*, revealing cellular repopulation originating from the perianastomotic graft regions A1 and B2 in both groups. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values.

small-caliber arterial grafts by DCA treatment, while allowing for rapid autologous endothelium formation *in vivo*, can contribute to an improvement in long-term patency of biological implants for arterial replacement or bypass surgery, respectively.

The results of our study show that short-term intake of DCA via drinking water significantly inhibits intima hyperplasia in decellularized small-caliber arterial grafts and does not impair implant re-endothelialization, resulting in complete neoendothelium coverage within 8 weeks *in vivo*. The perianastomotic regions of the grafts were re-endothelialized earlier than the aortic arches, supporting the hypothesis of graft repopulation predominantly via cellular ingrowth from the anastomotic regions, which is in line with previous results from studies on the *in vivo* fate of decellularized aortic grafts [11, 21]. Not only the rapid luminal endothelialization with vWF-positive cells, but also the migration of αSMA-positive interstitial cells from the adventitial side into the graft media confirms previous data from our small animal implant model [4]. Here, we had shown that the migration of adventitial αSMA-positive cells into the media is mediated by fibronectin coating. Furthermore, the observation that media recellularization is emphasized in the perianastomotic regions suggests that ingrowth from the host tissue around the anastomoses, presumably by αSMA-positive activated fibroblasts, plays a role also for the media.

Regarding the pattern of intima hyperplasia in the grafts, the distal preanastomotic region (B2) was less affected after 8 weeks. This finding may be attributed to haemodynamic forces in the implants, particularly to wall shear stress. Low local shear stress and oscillatory shear stress have been reported to favour intima hyperplasia and accelerate atherosclerotic plaque growth [22, 23]. Due to the end-to-side manner of the anastomoses and the mismatch in diameters of the graft and the native aorta, the direction of blood flow changes substantially when entering the graft, and flow disturbances clearly occur in the proximal parts of the implant. This presumably results in heterogeneous wall shear stress patterns with crucial local differences, favouring intima hyperplasia. In the distal preanastomotic part of the graft, which is located at the end of the long graft arch, the blood flow may have restructured to a laminar flow, potentially explaining the lower degree of intima hyperplasia in this area. In order to test this hypothesis, blood flow analyses by computational fluid dynamics should be conducted.

That DCA therapy in animals with implanted decellularized arterial grafts decreases neointima hyperplasia in our study, is in agreement with a previous study on DCA treatment that counteracted intima hyperplasia in injured native vessels [9]. Beyond inhibition of hyperplasia, the present study revealed a significant inhibitory effect of DCA on the degenerative calcification in the neointima within only 8 weeks after graft implantation, and inhibition of media calcification particularly in the perianastomotic regions. As we have previously shown for grafts that are recellularized *in vivo*, calcification of the neointima in the present experiments occurred predominantly in areas of hyperplastic intima formation [10, 12] Therefore, the observed hyperplasia reduction by DCA seems to be causal for the inhibition of implant calcification. According to previous studies on vascular and valvular grafts decellularized with our detergent-based protocol, implantation of the prostheses in the present study did not result in detectable inflammatory response, indicating effective donor cell removal during the decellularization process [4, 11]. This is all

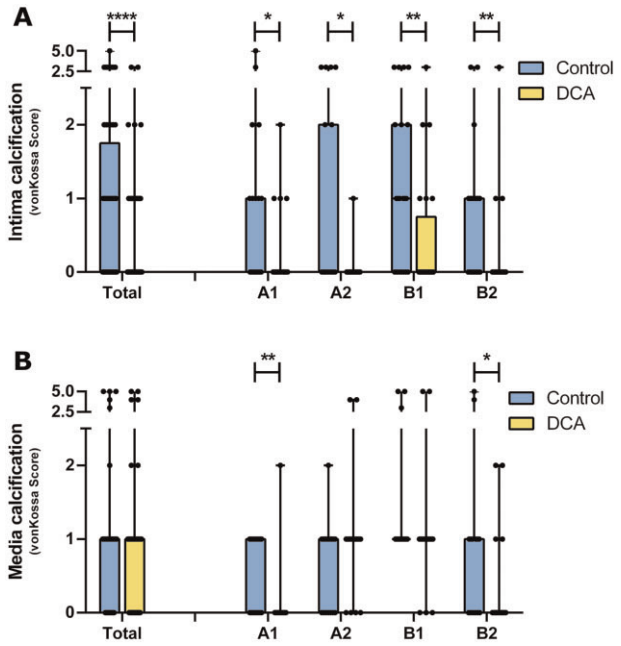

Figure 7: Semiquantitative analysis of hydroxyapatite deposition, measured by a von Kossa staining-based score, in different regions of the intima (**A**) and the media (**B**) of the aortic graft explants. Dichloroacetate treatment significantly inhibited the intima calcification in all graft regions and the media calcification in the perianastomotic regions A1 and B2 within 8 weeks *in vivo*. The upper and lower borders of the boxes represent the upper and lower quartiles. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

the more noteworthy, as incomplete decellularization is considered to be one of the triggers of calcifying degeneration [24]. In this context, it is of great interest that, in opposite to large-caliber human or large animal aorta, small-caliber aortic grafts from rats can be effectively freed from cellular remnants. Another potential source of mineralization are the elastin fibres of the aortic media, the fragmentation of which during decellularization evokes calcium deposition to the scaffolds [25]. That the current decellularization protocol preserves the macro-structure of elastin fibres has been previously demonstrated [4].

Application of DCA in humans has been researched on for many decades, targeting metabolic, cardiovascular and cerebrovascular disease as well as cancer [26]. Therefore, robust data on pharmacokinetics, metabolism, toxicology and dosing in humans already exist [27, 28], which may facilitate future translation of our findings.

The significance of the current findings with regard to human application is limited in so far that a rat model cannot entirely mimic all physiological and pathophysiological events in the human organism. Moreover, considering the short follow-up period of 8 weeks and the small group numbers in our study, in spite of the significant benefit in the DCA group, further research is indicated before clinical translation, particularly to reveal whether DCA predominantly delays or even prevents intima hyperplasia and calcifying degeneration of the grafts. In this context, preclinical long-term experiments evaluating the DCA therapy-associated freedom from graft failure in large animals are required. Additionally, the effect of DCA on graft degeneration may be evaluated also in the presence of typical cardiovascular comorbidities, such as arterial calcification, diabetes, chronic kidney disease or enhanced oxidative stress, all of which have been implemented in our standardized rat model [29].

Another limitation in our data is that a single concentration of administered DCA has been researched on. Although 0.75 g l^{-1} drinking water is an established concentration in rat models, and the weight-normalized calculation (69.9 [IQR, 68.5–74.2] mg kg^{-1} (body weight) day^{-1}) is in the range that has been administered in human trials [8], it may be interesting to examine whether substantially lower DCA concentrations cause similar anti-degenerative effects. In addition, it has to be tested if a short-term intake of DCA over a few weeks after implantation suffices, or if long-term therapy is required to avoid graft degeneration. In this context, addressing controversially discussed potential toxicity of DCA, evaluation of halogen-substituted DCA analogues that have been reported to exhibit lower toxicity in the presence of high binding affinity would be another future research project [30].

Finally, it may sound controversial to treat acellular implants with a substance that affects mitochondria. However, implant deterioration is predominantly driven by invading autologous cells that induce pro-degenerative cascades, and therefore, preventive measures during the repopulation process can effectively inhibit graft degeneration, such as DCA in the present study.

CONCLUSION

The present study demonstrates that systemic DCA therapy decreases adverse neointima hyperplasia in decellularized small-caliber arterial grafts, while allowing for rapid re-endothelialization of the implants in a standardized rat model. Furthermore, to the best of our knowledge, we revealed for the first time that DCA may have a role in anti-calcification of tissue-engineered biological prostheses.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

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Conflicts of interest: none declared.

Author contributions

Agunda Chekhoeva: Data curation; Writing—original draft. **Sentaro Nakanishi:** Data curation; Formal analysis. **Yukiharu Sugimura:** Data curation; Formal analysis; Methodology. **Mahfuza Toshmatova:** Data curation; Formal analysis. **Anna Kathrin Assmann:** Data curation; Formal analysis. **Artur Lichtenberg:** Funding acquisition; Resources; Supervision; Writing—review & editing. **Payam Akhyari:** Conceptualization; Project administration; Resources; Supervision; Writing—review & editing. **Alexander Assmann:** Conceptualization; Project administration; Supervision; Writing—review & editing.

Reviewer information

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