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Reinforcing the pulmonary artery autograft in the aortic position with a textile mesh: a histological evaluation

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Abstract

OBJECTIVES: The Ross procedure involves replacing a patient's diseased aortic valve with their own pulmonary valve. The most common failure mode is dilatation of the autograft. Various strategies to reinforce the autograft have been proposed. Personalized external aortic root support has been shown to be effective in stabilizing the aortic root in Marfan patients. In this study, the use of a similar external mesh to support a pulmonary artery autograft was evaluated.

METHODS: The pulmonary artery was translocated as an interposition autograft in the descending thoracic aortas of 10 sheep. The autograft was reinforced with a polyethylene terephthalate mesh ($n = 7$) or left unreinforced ($n = 3$). After 6 months, a computed tomography scan was taken, and the descending aorta was excised and histologically examined using the haematoxylin–eosin and Elastica van Gieson stains.

RESULTS: The autograft/aortic diameter ratio was 1.59 in the unreinforced group but much less in the reinforced group (1.11) ($P < 0.05$). A fibrotic sheet, variable in thickness and containing fibroblasts, neovessels and foreign body giant cells, was incorporated in the mesh. Histological examination of the reinforced autograft and the adjacent aorta revealed thinning of the vessel wall due to atrophy of the smooth muscle cells. Potential spaces between the vessel wall and the mesh were filled with oedema.

CONCLUSIONS: Reinforcing an interposition pulmonary autograft in the descending aorta with a macroporous mesh showed promising results in limiting autograft dilatation in this sheep model. Histological evaluation revealed atrophy of the smooth muscle cell and consequently thinning of the vessel wall within the mesh support.

Keywords: Ross procedure • Reinforcement • Pulmonary autograft • Personalized external aortic root support • Histology • Marfan

INTRODUCTION

In the Ross procedure, the healthy pulmonary artery root is used as an autograft to replace the diseased aortic valve [1, 2]. Compared to replacement with an animal tissue valve, the living valve tissue is less prone to failure, and compared with a mechanical valve, the patient is spared mandatory lifelong anticoagulation [2–4]. Published by Ross in 1967, it was an early innovation in the history of aortic valve replacement [5]. It remains an attractive solution for young patients with aortic valve disease but has only been adopted sporadically because of anxiety about surgical complexity, the compromise of a healthy pulmonary valve and later deterioration of either or both the autograft and the replacement pulmonary valve [3, 5, 6]. Autograft dilatation of the pulmonary artery root in the aortic position is the most important failure mode after Ross surgery, occurring in 17–55% of patients at 5–10-year follow-up. Up to 12% of patients ultimately require autograft replacement due to substantial dilatation [2–4, 7, 8]. Clinical experience is that the autograft increases in diameter on exposure to systemic pressure. This is neither detrimental to autograft valve function nor predictive of later dysfunction. There may be further dilatation during the 1st year and beyond [9, 10]. To tackle the drawback of autograft dilatation, various reinforcement techniques have been developed, but none has been consistently successful [11–15].

It is 14 years since personalized external aortic root support was used for the 1st time to halt aortic root expansion in Marfan patients. Personalized external aortic root support is a procedure in which a soft macroporous mesh sleeve is custom made based on the patient's computed tomography (CT) and/or magnetic

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resonance imaging (MRI) images and surgically placed around the dilated area [16]. Note that personalized external aortic root support has only been used when the aorta has reached a diameter sufficient for adult haemodynamic function because it fixes the aortic shape and size. To date, more than 100 patients with aortic root aneurysms, predominately due to genetically determined aortopathy, have had an operation to place an ExoVasc mesh support [17, 18]. A modification of this technique might be a promising new option for autograft reinforcement during the Ross procedure.

It has been found that the external mesh, closely fitting the aorta, becomes fully incorporated in the adventitia and preserves the vascular architecture, in contrast to wrapping with low porosity and poorly fitting Dacron grafts [17, 18]. A clinical case report confirmed these findings and showed that the supported aneurysm had the histological appearance of a normal aorta as opposed to Marfan-related degeneration [19]. Verbrugghe *et al.* [20] investigated the histological characteristics more thoroughly in sheep. They reported full incorporation of the exostent in the outer layer of the carotid artery and minimal structural changes in the wrapped arterial wall. Recently, the principle has been applied to the Ross pulmonary autograft in 7 patients. No follow-up data on these patients are yet published.

Currently, there are very limited data concerning the incorporation of the ExoVasc mesh support and its influence on the histological properties of the aorta. Concerns about thinning of the media of the aorta within the ExoVasc mesh support and the potential for aortic dissection within and beyond the support have been raised by critics. The neoaorta no longer relies on the media for its strength, and relative thinning can reasonably be reviewed as an adaptive change, and to date, dissection within or beyond the support has never been seen in 470 patient-years of follow-up [17, 18]. If the technique is to have a place in the clinical use of the Ross procedure, further investigation on the impact of ExoVasc mesh implantation around the pulmonary artery could bring further insights. Our goal was to assess in a large animal model whether the macroporous mesh can be used to protect pulmonary artery tissue in the aortic position from dilatation and to evaluate the impact of that mesh on the histological features of the arterial wall.

MATERIALS AND METHODS

Surgical procedure

The animal experiments were approved by the Animal Ethics Committee of the KU Leuven (P053/2013). In 13 Lovenaar sheep, a pulmonary artery interposition graft was placed in the aortic position. Three of them died during surgery and were excluded from further analysis. Only female sheep were used to avoid inter-gender differences. The sheep were sedated with an intramuscular injection of ketamine (15 mg/kg). Subsequently, anaesthesia was induced and maintained with isoflurane (5% and 2–3%, respectively). Through a left thoracotomy, the pulmonary artery was carefully exposed. During cardiopulmonary bypass, ±15 mm of pulmonary artery was resected and relocated as an interposition graft in the descending aorta. In 7 sheep (age 40.1 ± 7.3 weeks), the pulmonary autograft was reinforced with a polyethylene terephthalate mesh with a pore size of 0.7 mm (Exstent Ltd., Tewkesbury, UK). The amount of overlap of the mesh on the aorta was approximately 1 cm on both sides. In contrast, the autograft was left without reinforcement in 3 control sheep (age 37.2 ± 5.8 weeks). Six to 8 months later, a CT scan was taken, and the sheep were euthanized with euthasol (120 mg/kg). After sacrifice, cylindrical samples of both the pulmonary artery and the descending aorta were excised in all sheep. Additionally, the reinforced aorta and pulmonary artery tissue of the exostent sheep and pulmonary tissue in the aortic position of 1 control sheep were collected. A diagram of the surgical procedures and the tissues collected is shown in Fig. 1.

Aortic diameter

The diameter of the pulmonary artery and the pulmonary autograft was measured using the CT images. In addition, the diameter of the descending thoracic aorta approximately 1.5 cm proximal and distal to the pulmonary autograft was measured.

Histological analysis

The obtained samples were fixed in paraformaldehyde (6%) and dehydrated (Medite TES 99), before being embedded in paraffin. Five-micrometre-thick serial cross-sections were created (Microm HM360) and stained with haematoxylin and eosin and Elastica van Gieson stains using standard laboratory protocols. All specimens were examined with the use of a Zeiss Imager M2 microscope and pictures were taken with an Axiocam MRc5 camera. Measurements of the wall thickness and the smooth muscle cell (SMC) and elastin content were performed with AxioVision software (carl Zeiss AG, Oberkochen, Germany).

Statistical analysis

Data were analysed using Matlab R2016b (MathWorks Inc., Natick, MA, USA) and Microsoft Office Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as mean ± standard deviation. A P-value <0.05 was considered statistically significant. Variables were compared using the unpaired t-test.

RESULTS

Macroscopic evaluation

During the initial surgery, as in clinical experience with the Ross procedure, immediate dilatation of the autograft in both the control and reinforcement groups was visible. After 6–8 months, macroscopic examination showed that the ExoVasc mesh was entirely surrounded by an inhomogeneous fibrotic sheet, extending to either end of the material. The lumen was well preserved and showed no erosions or obstructions. Finally, the aorta proximal and distal to the autograft appeared normal in both groups (Fig. 2).

Aneurysmatic dimensions

The diameter of the thoracic aorta proximal and distal to the pulmonary autograft served as a reference to indicate the amount of dilatation. In the control group, the autograft/aortic diameter ratio was 1.59 ± 0.40 at sacrifice. A significant smaller ratio of 1.11 ± 0.06 was measured in the reinforced group ($P < 0.05$) (Table 1).

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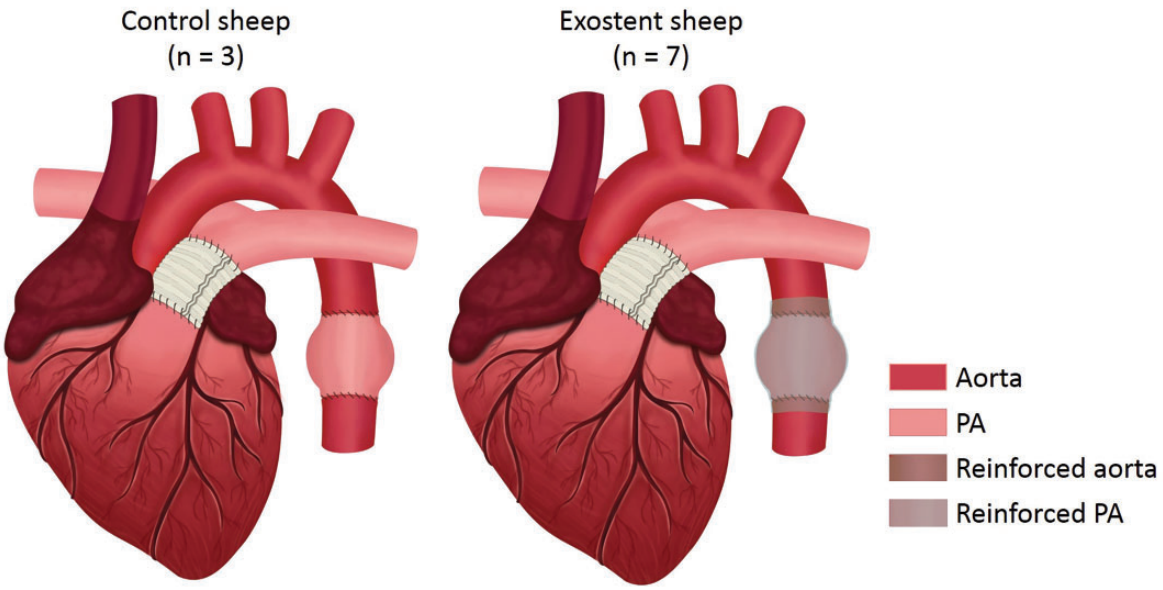

Figure 1: The surgical procedure with a list of the collected tissues. The removed portion of the main trunk of the PA has been replaced with standard low-porosity vascular interposition tube graft (white). The colour key identifies the aorta and PA and where they have been reinforced. For the ease of interpretation, the illustrations are based on human anatomy. PA: pulmonary artery.

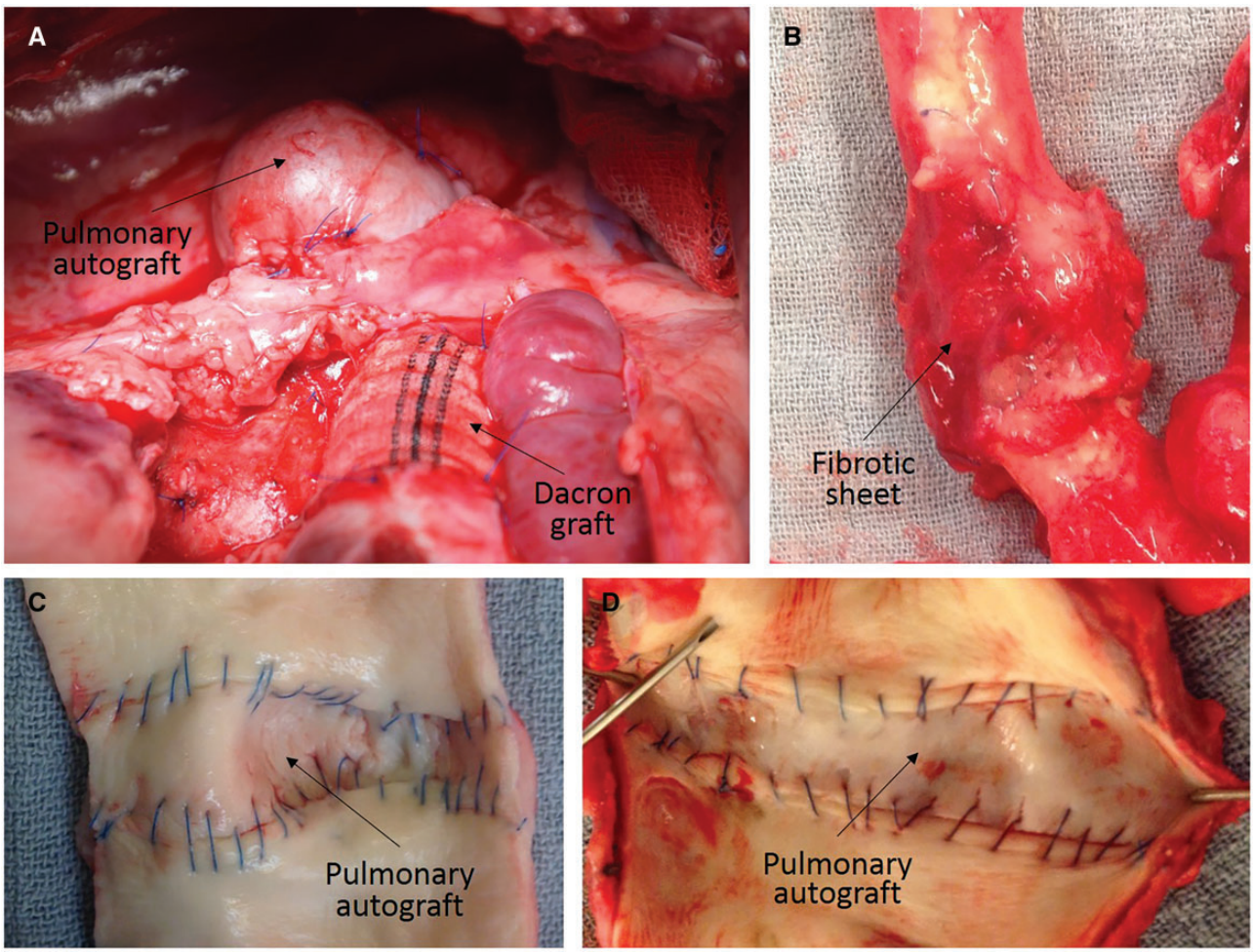

Figure 2: (**A**) Surgical view of the pulmonary artery in the aortic position. An instantaneous dilatation of the autograft is noticed. (**B** and **D**) Macroscopic analysis of the reinforced pulmonary autograft after 6–8 months, revealing a fibrotic sheet covering the mesh and a preserved lumen. (**C**) Macroscopic analysis of the pulmonary autograft of a control sheep after 6–8 months.

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Table 1: Diameter data of the reinforced group and control group at sacrifice (reprinted from Vastmans *et al.* [29], with permission from Elsevier)

Sheep	Diameter of the aorta (mm)	Diameter of the autograft (mm)	Autograft/aortic diameter ratio (mm)
Reinforced group			
0091	19.95	21.13	1.06
0073	21.85	23.02	1.05
0385	19.39	20.86	1.08
0393	17.88	21.66	1.21
0434	Missing	20.99	Missing
0320	19.37	22.51	1.16
0418	19.89	21.29	1.07
Mean ± SD	19.72 ± 1.17	21.64 ± 0.76	1.11 ± 0.06
Control group			
0321	20.00	22.24	1.11
1983	22.21	46.45	2.09
1858	19.88	31.08	1.56
Mean ± SD	20.70 ± 1.07	33.26 ± 10.01	1.59 ± 0.40

The diameter of the aorta is the average of the aortic diameter approximately 1.5 cm proximal and distal to the interposition graft.
SD: standard deviation.

Histological evaluation

The mean native aortic and pulmonary arterial wall thicknesses of the reinforced group were 2.86 ± 0.47 mm and 1.61 ± 0.59 mm, respectively. After reinforcing the pulmonary autograft and the adjacent aorta, the mean wall thicknesses, measured from the tunica intima to the tunica adventitia, significantly decreased to 1.36 ± 0.63 mm (53% decrease) and 0.84 ± 0.22 mm (42% decrease), respectively, 6–8 months after surgery ($P < 0.05$ and $P < 0.05$, respectively). In contrast, if the mesh and fibrotic sheet are included, there will be an increase in the mean wall thicknesses by 3% and 57%, respectively (Table 2). However, there is a large variation in increase, ranging from -27% to 37% for the aorta and from -12% to 132% for the pulmonary artery due to the variable thickness of the fibrotic sheet.

Atrophy of the vascular SMCs was present in all the samples of both the wrapped pulmonary autograft (Fig. 3) and the surrounding wrapped aorta (Fig. 4), causing the uniform thinning. An average decrease of 34% ± 21% and 36% ± 27% in SMC concentration was measured in the wrapped pulmonary autograft and wrapped aorta, respectively. Overall, the elastin fibres appeared intact, although in some areas, fragmented elastin fibres were seen. As a consequence of vessel wall thinning, the density of the elastin fibres increased by 28% ± 36% for the pulmonary autograft and 25% ± 21% for the aorta. The SMC/elastin ratio in the pulmonary artery and aorta decreased from 3.00 ± 0.62 to 1.12 ± 0.54 and from 0.81 ± 0.40 to 0.39 ± 0.19, respectively, again illustrating the atrophy of the SMC after wrapping. The evolution in SMC and elastin fibre content per sheep is given in Table 3.

In this experiment, the macroporous mesh was not custom made to fit as it has been in clinical use. After 6–8 months, the gap between the vessel wall and the mesh was mainly filled with fluid and a limited amount of fibroblasts. Additionally, oedema between the elastin fibres in the media of the vessel wall was sometimes present (Fig. 4B). The mesh itself was entirely covered by a fibrotic sheet, consisting of collagen fibres, fibroblasts, neovessels and foreign body giant cells.

In 1 control sheep, samples of the aorta, pulmonary artery and pulmonary artery in the aortic position were collected (Fig. 5). The initial thicknesses of the aortic and pulmonary arterial wall were 1.90 mm ± 0.11 mm and 1.07 mm ± 0.05 mm, respectively. Overall, after placing the pulmonary artery in the aortic position, the wall thickness remained the same. However, more variability in wall thickness was observed (1.06 mm ± 0.18 mm). Concerning the SMC and elastin amount, no conclusion can be drawn since samples of only 1 sheep were available, and these samples show a large variability.

DISCUSSION

Effect of external wrapping on autograft dilatation

In theory, the Ross procedure is an attractive alternative to the standard aortic valve replacement for young patients allowing the potential of many years of free from anticoagulation and reoperation. This has been achieved for many patients, but it has not been widely adopted due to major concerns about technical difficulty, trading 'single-valve disease for the double-valve disease' and the long-term failure due to autograft dilatation and consequent aortic regurgitation [6]. To avoid the deterioration of the autograft, several reinforcement techniques and materials have been developed [11–13, 15]. In our study, a macroporous ExoVasc mesh was used to successfully limit dilatation of the pulmonary interposition graft. Nappi *et al.* [21] used a similar approach to reinforce the pulmonary interposition graft in growing sheep. Their semiresorbable macroporous mesh prevented autograft dilatation while allowing the natural process of growth [21–23]. Overall, studies investigating pulmonary autograft dilatation after wrapping with different materials provided the same conclusion, namely reduction or complete prevention of dilatation [11–15]. However, the experiences of using a low porosity Dacron and a Gore-Tex graft were unsatisfactory [2].

Effect of external wrapping on histological features

One of the most frequently voiced concerns associated with historical 'wrapping' of the aorta is thinning of the arterial wall.

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Table 2: Wall thickness data of the reinforced group

Sheep	Native		After reinforcement			
	Wall thickness of the aorta (mm)	Wall thickness of the PA (mm)	Wall thickness of the aorta (mm)	Wall thickness of the PA (mm)	Total wall thickness of the aorta (mm)	Total wall thickness of the PA (mm)
0091	3.14	1.58	1.89	1.18	2.95	2.95
0073	2.53	1.11	1.04	0.74	3.11	2.58
0385	2.48	1.32	1.95	0.65	3.41	1.50
0393	2.05	1.71	0.49	0.60	1.61	1.45
0434	3.02	2.83	1.42	1.13	3.28	3.12
0320	3.30	1.10	1.47	0.90	2.41	2.42
0418	3.49	Missing	1.25	0.67	3.72	2.72
Mean ± SD	2.86 ± 0.48	1.61 ± 0.59	1.36 ± 0.47	0.84 ± 0.22	2.93 ± 0.66	2.39 ± 0.62

The wall thickness includes the tunica intima, tunica media and tunica adventitia. The total wall thickness includes the 3 layers of the vascular wall as well as the mesh and the fibrotic sheet.

PA: pulmonary artery; SD: standard deviation.

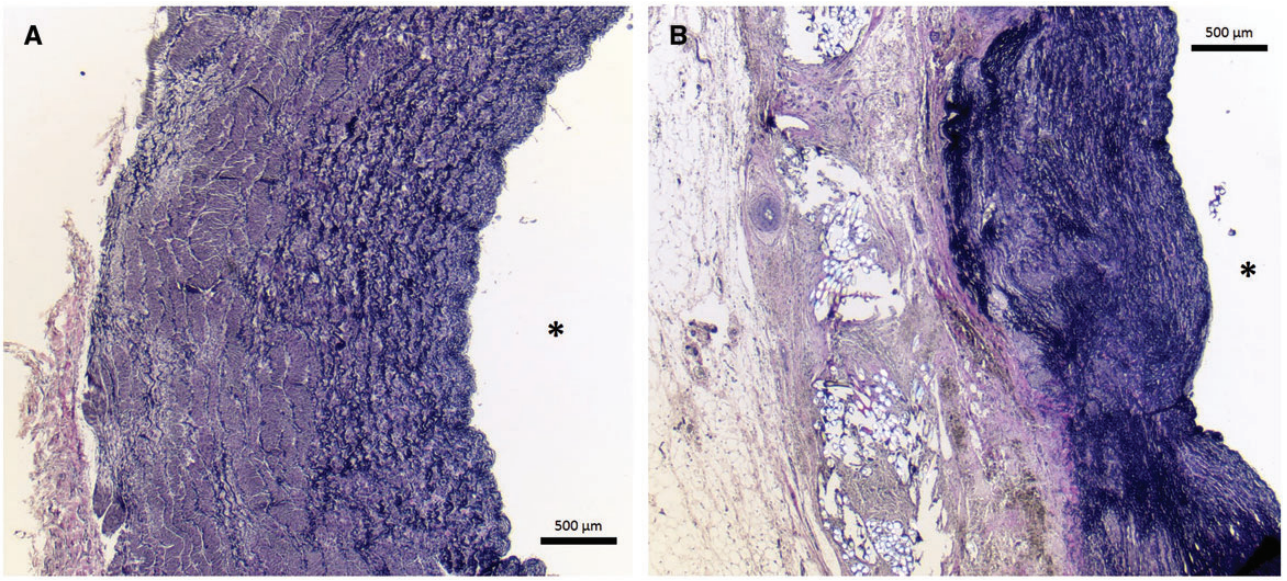

Figure 3: Transverse microscopic sections of the native pulmonary artery and wrapped pulmonary autograft of sheep 0091. Elastica van Gieson stain, magnification ×25. The lumen is marked with an asterisk. (**A**) The native pulmonary artery. (**B**) The wrapped pulmonary autograft with increased density of the elastin fibres due to atrophy of the smooth muscle cells.

This concern arose mainly from 2 case reports describing an extremely thin aortic wall several years after Dacron graft-supported aortoplasty [24]. Robicsek *et al.* [25] coined the term under-the-wrap atrophy. These observations may be inherent to the use of a low porosity vascular graft material, which was not designed for this purpose but to be a prosthetic replacement for the aorta. In a previous experiment of our research group, a microporous Dacron mesh and a macroporous Dacron mesh were implanted around the abdominal aorta of the same 3 sheep for 12 months. Atrophy of the vascular SMC in the tunica media was present with a Dacron wrap, whereas changes were much less pronounced in the aortic wall sleeved with the macroporous mesh [26]. In this study, depletion of the SMC in the mesh supported pulmonary arterial wall and aortic wall, and the corresponding thinning of those vessel walls was also seen. An overall increase in wall thicknesses was seen due to the fibrotic sheet covering the mesh.

In contrast to our results, Nappi *et al.* [22, 23] reported thinning of the media in their control group and an intact media in the reinforced group. Also, Verbrugghe *et al.* [20] reported minimal structural changes in the tunica media of carotid arteries in growing sheep after implantation of a macroporous mesh for 4–6 months. Similar observations were mentioned in 2 follow-up studies of patients with aortic wall reinforcement with a highly porous mesh. The aortic wall architecture was well preserved after wrapping, and no erosion of the mesh through the aortic wall was observed [27, 28]. A more recent patient report confirmed these findings, additionally mentioning that the supported aortic root had the histological appearance of a normal aorta. Also, the fact that the unsupported aortic arch showed medial degeneration raises the possibility of microstructural recovery of the damaged aorta after wrapping [19].

As stated above, our results are in line with the previously mentioned concern of thinning. However, in this context, thinning of the media does not necessarily result in loss of strength or an increased propensity for dissection [30].

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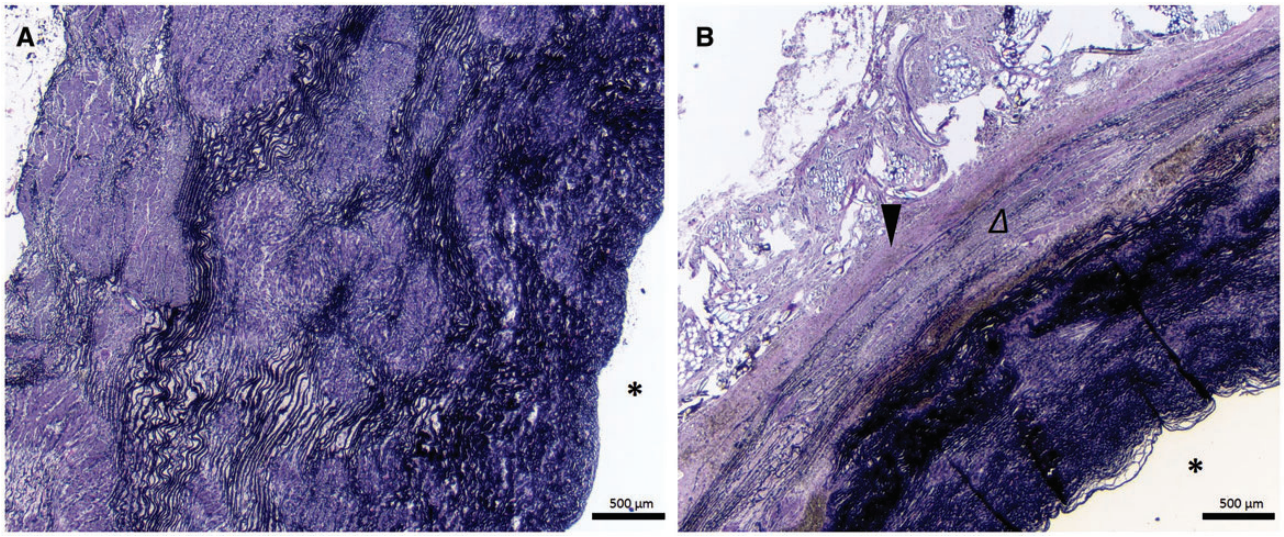

Figure 4: Transverse microscopic sections of the native and wrapped aorta of sheep 0091. Elastica van Gieson stain, magnification ×25. The lumen is marked with an asterisk. (**A**) The native aorta. (**B**) The wrapped aorta with uniform thinning of the media. Fluid accumulation between the vessel wall and the mesh (arrowhead) and peripheral within the media of the vessel wall (Δ) is clearly visible.

Table 3: Data of the impact of mesh implantation on the vascular SMC and elastin amount

Sheep	Tissue	SMC/elastin ratio		Elastin increase (%)	SMC decrease (%)
		Native	After reinforcement		
0091	PA	4.18	0.75	73.99	-28.86
	Aorta	0.84	0.17	27.56	-70.12
0073	PA	2.47	1.81	-12.39	-27.55
	Aorta	0.77	0.33	32.24	-33.94
0385	PA	2.74	1.41	-2.46	-40.61
	Aorta	0.60	0.52	22.04	10.00
0393	PA	2.45	1.58	38.26	0.57
	Aorta	0.75	0.69	-11.76	-1.24
0434	PA	3.44	0.19	74.03	-70.10
	Aorta	0.65	0.17	40.04	-34.65
0320	PA	2.71	1.41	-0.82	-38.94
	Aorta	1.03	0.60	7.27	-41.03
0418	PA	Missing	0.76	Missing	Missing
	Aorta	1.86	0.27	58.26	-64.08
Mean ± SD	PA	3.00 ± 0.62	1.12 ± 0.54	28.34 ± 35.99	-34.25 ± 20.95
	Aorta	0.81 ± 0.40	0.39 ± 0.19	25.09 ± 20.93	33.58 ± 27.43

PA: pulmonary artery; SD: standard deviation; SMC: smooth muscle cell.

Mechanical analysis

Mechanical testing of similar samples is reported by Vastmans *et al.* [29]. The difference in behaviour of aortic and pulmonary arterial tissue was clearly visible. The stress–strain curves indicated that the pulmonary artery behaves stiffer than the aorta. After mesh support, the difference in stiffness was less evident. In addition, when exposed to aortic pressure, no difference between the arterial tissues with or without mesh was visible, because at low pressures, the macroporous mesh nicely fits around the artery and does not contribute significantly to the mechanical stiffness. Only at higher pressures, the textile fibres of the mesh are put under tension and start to contribute mechanically. These results indicate the importance of a personalized mesh. The mesh should have no influence at physiological stresses and only restrict motion at higher pressures, which is only possible if the mesh encloses the vessel precisely. Moreover, it is of great importance that no prestretch is created during surgery to allow unrestricted dilatation during the entire cardiac cycle.

Experimental sheep model

Sheep are widely used for testing cardiovascular surgical devices because of the cardiovascular similarities between sheep and humans [30]. Therefore, we developed an experimental model of a pulmonary artery interposition graft in sheep. Performing an actual Ross procedure from our perspective is not feasible in sheep due to anatomical differences [21, 30]. First, the ascending aorta is too short and immobile. Second, reimplantation of the coronary ostia on the pulmonary autograft is challenging as they are positioned very low. Third, and most important, the failure

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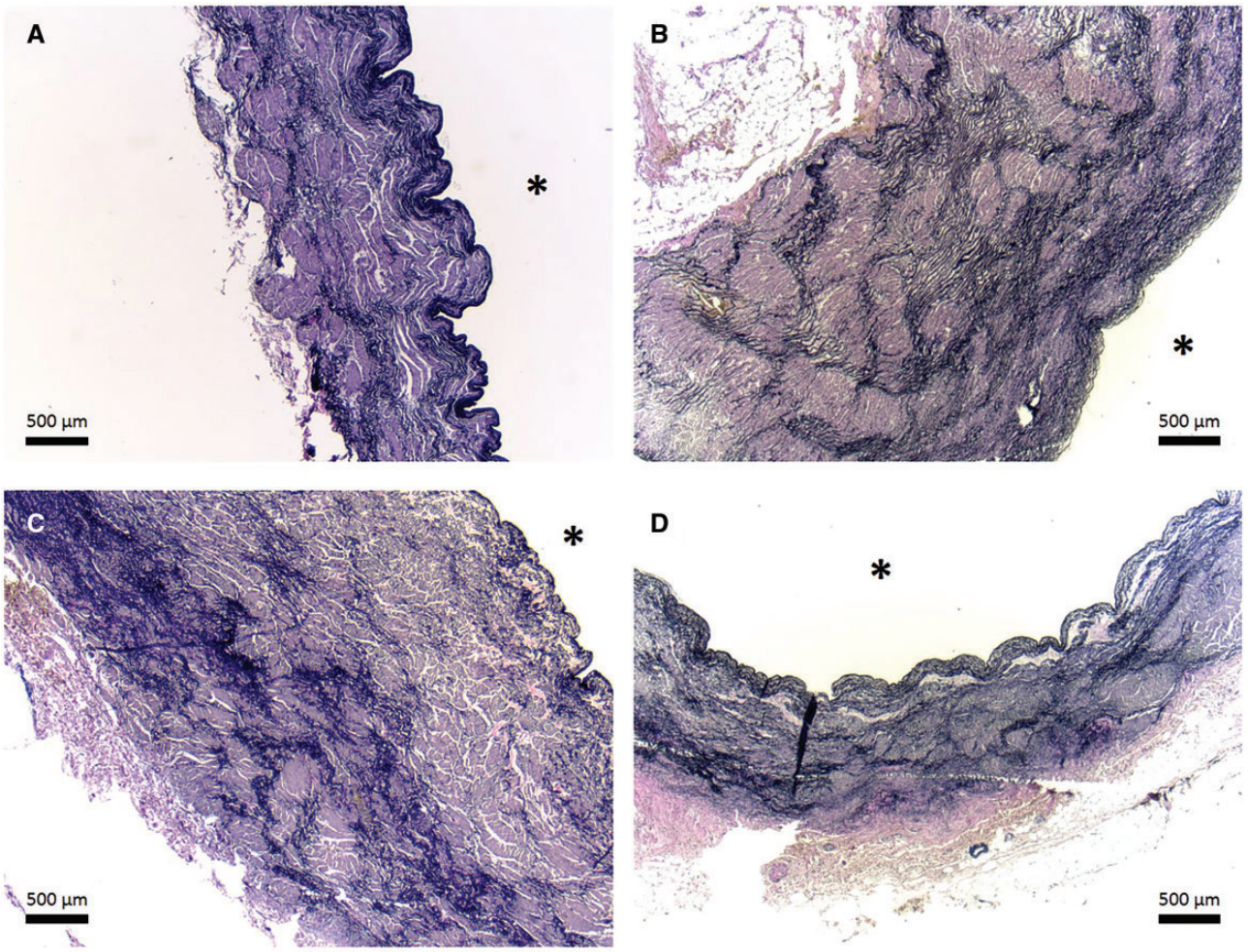

Figure 5: Transverse microscopic sections of the aorta, pulmonary artery and pulmonary artery in the aortic position of control sheep 0321. Elastica van Gieson stain, magnification ×25. The lumen is marked with an asterisk. (**A**) The pulmonary artery. (**B**) The aorta. (**C** and **D**) The pulmonary artery in the aortic position. Both pictures are taken from the same transverse microscopic section, showing the large variability in wall thickness and composition (reprinted from Vastmans *et al.* [29], with permission from Elsevier).

mode of the human Ross operation takes place over decades. This is not evaluable in animal experiments. In our model, the behaviour of the pulmonary artery under systemic pressure was examined, avoiding the complexities of the valve leaflets, coronary ostia and the sinuses of Valsalva. The 1 cm overlap of the mesh onto the aorta protects the anastomosis. In an actual Ross procedure, this would not be possible at the proximal end. Despite these limitations, we consider reimplanting the pulmonary artery in the descending aorta to be a clinically relevant model. This experimental approach is of low risk for the survival of the animal, reproducible and allowed us to assess the histological and structural effects of mesh reinforcement on the pulmonary artery under systemic haemodynamic conditions.

Limitations

We acknowledge the fact that only 1 CT scan per sheep makes it difficult to evaluate autograft dilatation. The baseline diameter of the pulmonary interposition graft was not measured using CT, although the 6 months/postoperative pulmonary autograft diameter ratio describes the differential effect. In addition, no knowledge on the cardiac phase during which the CT scan was taken is available. As a final remark, the lack of sufficient control sheep is one of the limitations of this study, leaving uncertainty as to the reproducibility of the changes in wall thicknesses and composition. In any further studies, more imaging and more control sheep can be considered.

CONCLUSION

To evaluate the effect of exostent reinforcement on dilatation of the pulmonary artery interposition graft and on the histological features of the arterial wall, we developed a reproducible and clinically relevant sheep model. Reinforcing the pulmonary autograft with a macroporous mesh, currently used to halt aortic root expansion in Marfan patients, successfully limited autograft dilatation. Thinning of the media, due to atrophy of the vascular SMC, was present in all the samples. However, the mesh that supported pulmonary arterial wall was stronger when tested mechanically. We propose for discussion that a macroporous mesh is likely to be applicable to circumvent the major drawback of the Ross procedure. This is being considered for clinical use, and the 1st clinical use will be reported soon.

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