Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

A Large-Diameter Vascular Graft Replacing Animal-Derived Sealants with an Elastomeric Polymer

Manon Van Hecke, MD,[a,*] *Lucas Van Hoof, MD,*[b] *Magdalena Sikole, MD,*[b] *Hozan Mufty, MD,*[c] *Piet Claus, MSc, PhD,*[d] *Peter Verbrugghe, MD, PhD,*[b] *John Ely, MS,*[e] *Geoffrey A. Berg, ChM, FRCS,*[e] *Tania Roskams, MD, PhD,*[a] *and Bart Meuris, MD, PhD*[b]

[a] *Department of Pathology, University Hospitals Leuven, Leuven, Belgium*
[b] *Department of Cardiac Surgery, University Hospitals Leuven, Leuven, Belgium*
[c] *Department of Vascular Surgery, University Hospitals Leuven, Leuven, Belgium*
[d] *KU Leuven, Department of Cardiovascular Sciences, Cardiovascular Imaging and Dynamics, Leuven, Belgium*
[e] *RUA Life Sciences, Irvine, United Kingdom*

ARTICLE INFO

Article history:
Received 19 April 2022
Received in revised form
25 October 2022
Accepted 20 November 2022
Available online xxx

Keywords:
Elastomer
Gelatin
Large animal models
Sealed grafts
Vascular grafts

ABSTRACT

Introduction: To assess the safety and efficacy of an experimental large-diameter vascular graft externally sealed with an elastomeric polymer when used as an interposition graft in the descending aorta of sheep.

Methods: The experimental vascular grafts as well as control gelatin sealed interposition grafts were inserted into the descending aorta of juvenile sheep. The grafts were assessed by time to hemostasis and blood loss during surgery and hematology and biochemistry panels at distinct time points. Magnetic resonance imaging (MRI) was performed at 3 and at 6 mo after surgery, after which the animals were euthanized and necropsies were carried out including macroscopic and microscopic examination of the grafts, anastomoses, and distal organs.

Results: All animals survived the study period. There was no perceivable difference in the surgical handling of the grafts. The median intraoperative blood loss was 27.5 mL (range 10.0-125.0 mL) in the experimental group and 50.0 mL (range 10.0-75.0 mL) in the control group. The median time to hemostasis was 5.0 min (range 2.0-16.0 min) minutes in the experimental group *versus* 6.0 min (range 4.0-6.0 min) in the control group. MRI showed normal flow and graft patency in both groups. Healing and perianastomotic endothelialization was similar in both groups.

Conclusions: The experimental graft has a similar safety and performance profile and largely comparable necropsy results, in comparison to a commonly used prosthetic vascular graft, with the experimental grafts eliciting a nonadherent external fibrous capsule as the major difference compared to the control grafts that were incorporated into the periadventitia. Survival, hemostatic sealing, and hematologic and radiologic results were comparable between the study groups.

Presented at the 35th EACTS Annual Meeting, Barcelona, Spain, October 15, 2021.

* *Corresponding author*. Department of Pathology, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium. Tel.: +32 487112639.
E-mail address: manon.vanhecke@kuleuven.be (M. Van Hecke).

https://doi.org/10.1016/j.jss.2022.11.041

Introduction

Prosthetic vascular grafts are frequently used in the treatment of cardiovascular diseases such as aneurysms or occlusions, congenital malformations, or to provide peripheral vascular access in the context of end-stage renal disease. Although uncommon, complications still occur, with often high morbidity and even high mortality numbers. Hence, the quest for better and readily available synthetic alternatives is still relevant.[1] Of the different available types of prosthetic vascular grafts, Dacron grafts have been used since the 1950's and provide the cardiovascular surgeon with an extremely versatile material with excellent long-term durability. First-generation grafts required preclotting to prevent oozing through the graft interstices during implantation. Sealing the luminal surface of large-bore woven and knitted vascular grafts with collagen, elastin or albumin proteins eliminated this step, reducing blood loss, and enabling the grafts to be used in emergency surgery and in situations of total heparinization.[2,3]

At present, the available sealing products are animal-derived.[4,5] While the graft coating typically resorbs by 1 to 2 wk postoperatively,[6] coated grafts seem to be associated with a postoperative systemic inflammatory response. However, the clinical repercussions are generally limited and outweigh the downsides associated with uncoated grafts.[7] In rare cases, insufficient healing or graft oozing may lead to seroma formation, potentially leading to pleural effusions or intra-abdominal collections.[8] Replacement of the animal-derived sealant by a synthetic and inert polymer has the potential to reduce the inflammatory response to vascular grafts and further improve tissue healing and incorporation.

Moreover, apart from eliminating these by-product risks, alternatives to animal products would have the advantage of improving the control of supply and manufacture, since the currently used compounds are sourced from a small resource of Bovine Spongiform Encephalopathy-free herds. Lastly, it provides patients a choice to avoid implants made from animal products.

The RUA Vascular graft has been designed as an alternative to the currently used prosthetic vascular grafts coated with an animal-derived sealant. The RUA vascular graft is a woven polyester graft, externally sealed with the Elast-Eon copolymer. The external sealing is preferred to provide a porous surface on the luminal side for cell attachment, while still preventing oozing through the exterior graft wall. Elast-Eon polymers are formed from copolymers of hydroxyl-terminated polydimethyl siloxane and methylene diphenyl diisocyanate–based polyurethanes.[9] The resulting compounds have the favorable mechanical strength of polyurethanes, a class of materials suitable for long-term implantable medical devices.[10] The addition of the silicon component provides the Elast-Eon polymer with more flexibility, biostability, and biocompatibility.[11,12] By combining the advantages of both polyurethanes and silicones, the Elast-Eon polymer is unique and therefore widely used for coating of implantable medical devices.

In this noninferiority study, we hypothesize that the application of the Elast-Eon polymer in RUA vascular grafts has a similar safety and performance profile in comparison to a commonly used prosthetic vascular graft, providing an alternative to animal-derived products.

Materials and Methods

Ethical statement

All animals were cared for by a veterinarian in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.[13] The local Ethics Committee approved the study (Ethische Commissie Dierproeven, KU Leuven, Study number P093/2020 on January 14, 2020) independent from the study sponsor.

Study devices

The RUA Vascular graft (RUA Vascular Ltd, Irvine, Ayrshire KA11 5AN, UK) is a straight large-bore (18 mm diameter) polyester woven crimped graft, externally sealed with Elast-Eon, a biostable polymer (Biomerics LLC, Utah, USA), under license from RUA Life Sciences plc (Fig. 1). Control grafts implanted were Gelweave straight grafts of the same size. (Terumo Aortic (UK) Vascutek Ltd, Inchinnan, Renfrewshire PA4 9RR, UK). Both grafts are composed of the same polymer, polyethylene terephthalate.

Animal model

Fifteen female juvenile sheep (Lovenaar breed), between 1 and 2 y old and weighing 60-70 kg, were obtained from TRANS-farm, KU Leuven, and were quarantined at the animal facility of the Medanex Clinic (Webbekom, Belgium) before undergoing surgery. No sample size calculation was performed for this exploratory study. Animals revealing signs of pregnancy, infection or disease, found through clinical examination by the Center's veterinarian, were excluded. Male animals were excluded since the handling of male sheep is less safe, and to avoid possible impregnation of female sheep during the study period. After surgery, the animals were observed and cared for at this facility for 6 mo.

The animals were operated under supervision of a fully trained, practicing cardiovascular surgeon with over 15 y of experience in aortic surgery with assistance of several cardiac surgery residents. Procedures were performed under general anesthesia after premedication with 10-20 mg/kg body weight intramuscular ketamine (100 mg/mL, Nimatek, Dechra, Bladel, The Netherlands). Anesthesia was induced and maintained with 1%-5% isoflurane (Isoba, Schering-Plough Animal Health, Middlesex, UK) in oxygen. After endotracheal

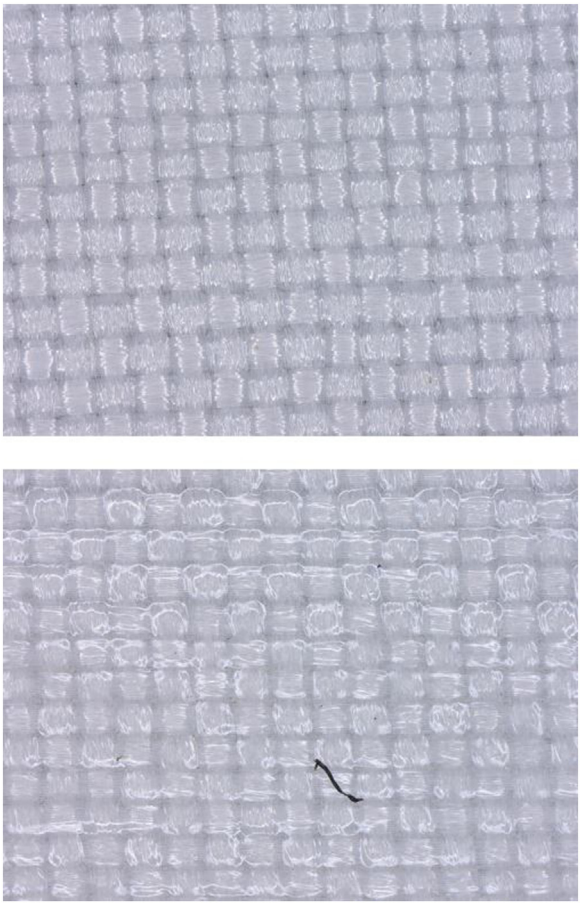

Fig. 1 – Magnified view of the luminal, uncoated (top) and exterior, coated (bottom) surface of the RUA vascular graft.

intubation, mechanical ventilation was started. All ventilation parameters were adjusted to keep the arterial blood gasses and pH within the physiologic range. A large-bore oro-gastric tube was placed to prevent ruminal distention. Electrocardiographic limb leads were connected and monitored. A maintenance intravenous drip of Ringer's solution was started. Gentamicin (6.6 mg/kg, Genta-Kel 10%, Hoogstraten, Belgium) and benzylpenicillinum natrium (40,000 U/kg, penicillin, 1,000,000 units, Kela, Hoogstraten, Belgium) was administered intravenously. The animal was placed on the operating table in the right lateral recumbent position. A left thoracotomy was carried out through the 4th intercostal space, and a cervicotomy was performed to expose the common carotid artery. After administration of heparin (200 IU/kg), a passive shunt was inserted between the left common carotid artery and the distal descending aorta in order to enable distal perfusion during aortic clamping. No other medication was given. Activated clotting time was maintained above 400 s during the procedure. The aorta was clamped proximally and distally, and a small segment was removed to allow for a 5 cm interposition graft to be implanted. The grafts were implanted with running 5/0 polypropylene sutures. Time to intraoperative hemostasis, by which from removal of cross clamps to hemostasis is meant, was recorded along with any measures used to aid hemostasis for example glues or extra sutures. When the hemodynamic parameters were stable, the shunt was removed. The chest was closed in layers with a chest drain in the left pleural space.

Postoperative care

The animal was weaned from the ventilator as soon as there was spontaneous respiration with adequate tidal volumes and stable hemodynamics. Chest drains were removed before extubation. The animals were carefully observed during the immediate postoperative period for up to 7 d. Following this, the animals returned to the controlled animal facility where the general health of the sheep was checked daily and follow-up data were obtained. No postoperative medication was given.

MRI

At 12 wk and 24 wk postoperatively, the animals were sedated for MRI. Animals were premedicated with ketamine (intramuscular 10-20 mg/kg body weight), intubated and anesthesia was maintained with 1%-2% of isoflurane. Animals were scanned in right lateral position during breath-hold on a 3T MRI unit (Prisma-Tim, Siemens, Erlangen, Germany). High resolution cine images were acquired in transverse and longitudinal planes of the descending aorta at the graft position. Contrast enhanced aortograms (3D angio sequences) as well as a 3D Dixon sequence,[14] with water and fat separation images were obtained. A 3D mesh of the graft was obtained from segmenting the 3D MR angio sequences, based on thresholding. Proximal and distal anastomoses were determined and a segment of approximately 1 cm proximal and distal to the graft was also reconstructed. Cross-sections were determined at these proximal and distal aortic sites, as well as at the proximal and distal anastomoses and in the middle of the graft. Mean diameters per cross-section were calculated. The length of the graft was measured as the distance between the centroids of the two anastomoses.

Hematology and biochemistry

Serial blood samples were obtained before surgery, on the day of implantation, at 90.2 (±6) d and at 178 (±4) d. Standardized complete blood counts were obtained for each sheep as well as LDH, free hemoglobin in plasma and haptoglobin to evaluate hemolysis and CK, creatinin, and urea to evaluate kidney function.

Explant procedures

The animals were euthanized at 6 mo. They were prepared and anesthetized as described earlier. After the MRI assessment, the sheep received heparin (3 mg/kg) intravenously and were euthanized with an overdose of a Euthasol solution intravenously. The grafts were explanted along with the two anastomotic sites and rinsed in saline. The grafts were opened longitudinally to allow for internal inspection and the inflow and outflow aspect of the grafts were photographed. Examination of the grafts included examination for delamination of the sealant, thrombosis, endothelialization, intimal

hyperplasia, patency, inflammation or infection and structural integrity. The liver, spleen, and kidneys were removed and biopsies were stored in 4% buffered formalin for further macroscopic and histologic examination.

Histologic data

For histology, 5-μm thick cross-sections were prepared from the anastomotic and mid-graft areas. The sections were embedded in paraffin. All sections were stained with hematoxylin and eosin (HE). Selected sections were stained with commercially available antibodies for CD11b (ab133357 by Abcam), CD3 (ab5690 by Abcam), Vimentin (m0725 by Dako) and vWF (ab6994 by Abcam) after optimizing the staining protocols for ovine tissue in our lab. Using light microscopy, the histologic integrity of the tissue, the presence of an inflammatory response in the tissue, and the extent of the healing process were evaluated by a pathologist.

Data management and statistical analysis

All data was tested for normality using normal probability plots or Shapiro–Wilk tests. The hematology and biochemistry data as well as the MRI data showed normal distribution, justifying the use of parametric tests and reporting the values as means and standard deviations. Since the measurements of perioperative blood loss were not normally distributed, nonparametric tests were used and values are represented as medians. Means were compared using a standard *t*-test and medians using a Mann–Whitney-*U*-test.

Results

Twelve animals were allocated to the study group and had 18 mm RUA Vascular grafts implanted and three animals to the control group, which had commercially available 18-mm Gelweave graft implants.

Mortality and morbidity

No animals died as a result of the surgical intervention, anesthesia or during follow-up. The animals remained in a healthy condition as assessed by regular inspections by veterinarians. Consequently, no data were excluded from the study.

Device handling characteristics

Grafts were implanted using standard suturing techniques employing polypropylene 5/0 sutures for all anastomoses. Additional anastomotic sutures were required in eight of the 12 study procedures and two of the three control procedures to secure hemostasis. Surgeons reported similar handling and suturing characteristics with no unusual difficulties noted with the surgical implantation of the experimental graft. There was no significant bleeding from suture holes and no glues or sealants were required for either the study or control grafts. Surgeons reported a difference only in stretch characteristics, with the longitudinal length of the RUA Vascular graft extending less under manual tension compared to the control grafts. This had to be taken into account when estimating the correct implant length. No systematic survey of the handling was performed.

Hematology and biochemistry

Serial blood samples were obtained before surgery, on the day of implantation, at 90.2 (±6) d and at 178 (±4) d. Taking into account the limited number of animals, there were no major differences between the RUA Vascular grafts and the control grafts regarding the complete blood counts, hemolytic parameters or kidney function. The results of the blood tests and statistical analyses are available in the supplementary material.

Perioperative blood loss

The median intraoperative blood loss, measured by the volume gathered in the suction recipient, was 27.5 mL (range 10.0-125.0 mL) for the RUA Vascular graft and 50.0 mL (range 10.0-75.0 mL) for the control grafts ($P = 0.945$). RUA Vascular graft outliers included one animal with 125.0 mL intraoperative blood loss secondary to bleeding from the aortic cannulation site and one with 100.0 mL intraoperative blood loss due to bleeding from an aortic side-branch. The median time to hemostasis, as defined by the time necessary to stop the graft and anastomoses from oozing, was 5.0 min (range 2.0-16.0 min) for the RUA Vascular graft *versus* 6.0 min (range 4.0-6.0 min) for the control grafts ($P = 0.839$). Eight of the 12 RUA Vascular graft group had no drainage from the chest drains with the median postoperative blood loss 0.0 mL (range 0.0-20.0 mL). All three control animals had some chest drainage with a median blood loss of 10.0 mL (range 10.0-15.0 mL, $P = 0.101$).

MRI

At the 3-mo interval, MRI imaging revealed a graft length of 45.0 ± 3.3 mm and a graft diameter of 18.95 ± 1.2 mm for the RUA vascular graft, which was not different from the Gelweave control graft, in which case the graft length was 46.0 ± 2.6 mm and the graft diameter was 17.7 ± 0.6 mm. These dimensions remained stable at the 6-mo interval, at which point the RUA vascular graft revealed a graft length of 45.4 ± 3.3 mm and a graft diameter of 19.0 ± 0.8 mm and for the Gelweave control graft a graft length of 45.3 ± 2.9 mm and a graft diameter of 17.7 ± 0.7 mm. These findings indicate that all implanted grafts maintained their structure, without evidence for dilatation at the 3- or 6-mo' time intervals (Fig. 2). Additionally, preserved graft patency and performance was reported for all RUA vascular grafts with no occlusion or false aneurysm formation and normal flow characteristics in all implants. In four out of 12 animals with a RUA vascular graft, small peri-graft collections were described, and three out of 12 had a large collection. These collections fully resolved in five out of seven animals and decreased in the remaining two at the 6-mo MRI scans. Two out of three control animals showed small intraluminal masses, of which one had an associated

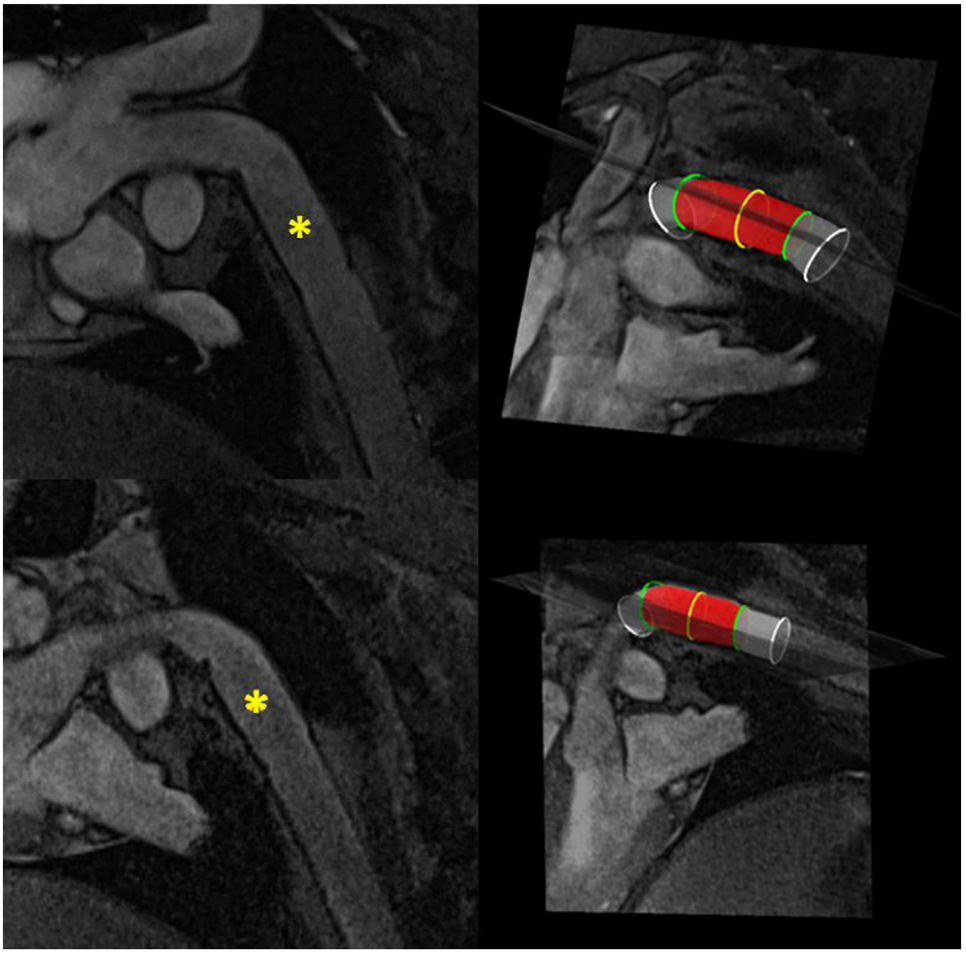

Fig. 2 – MRI with on the right side a 3D reconstruction of RUA Vascular graft (top) and Gelweave control graft (bottom) in descending thoracic aorta at 6 mo indicating preserved structure of both graft types (*).

peri-graft collection at 3 mo with the masses increasing in size at 6 mo.

Necropsy and histology

At necropsy, none of the animals revealed recent thromboembolic damage to the peripheral organs (liver, spleen, kidneys); no macroscopic particularities were observed. No dilatation or infection of the implanted grafts was observed. All anastomotic sites showed normal healing with no hematoma or dissection formation. Perigraft tissue was formed at the anastomoses of all RUA Vascular grafts. However, moving away from the anastomoses toward the central part of the graft, this perigraft tissue was no longer adherent to the graft, resulting in a clear plane of dissection between the graft and the surrounding tissue in the central parts. In five of 12 animals, this perigraft space contained a thrombus (Fig. 3). Other than this occasional thrombus, no other excess perigraft fluid was present in any of the cases and therefore no seroma fluid was analyzed. All control grafts had firm adhesion of the perigraft tissue to the graft, but two out of three control animals had red-colored intraluminal masses (Fig. 4).

Histology confirmed the absence of thromboembolic damage to the peripheral organs in both groups. At the

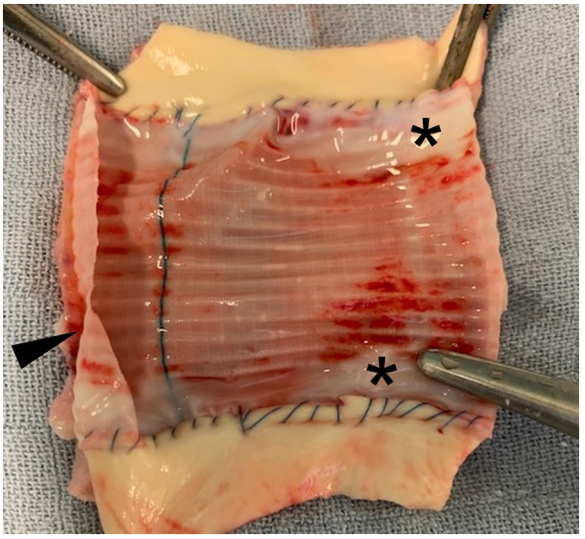

Fig. 3 – RUA Vascular graft showing perianastomotic endothelial coverage (*) and external capsule not adherent to the graft (arrowhead).

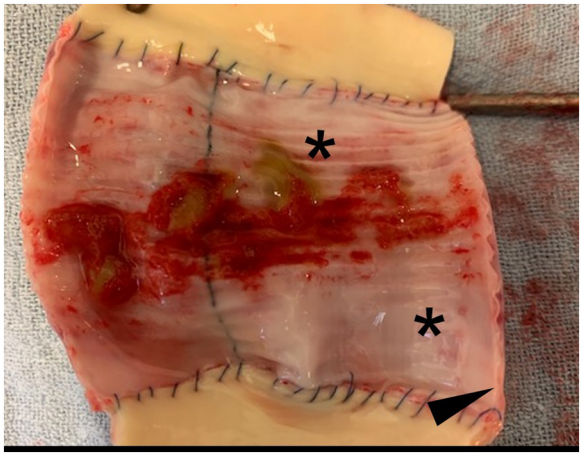

Fig. 4 – Gelweave graft showing extensive endothelial coverage (*) as well as intraluminal mass formation and external capsule (arrowhead) attached to the graft.

anastomoses, the RUA Vascular grafts showed presence of mature and organized connective tissue on the luminal as well as the external side of both the proximal and the distal anastomosis. The luminal sides of the anastomoses were covered by a layer of endothelium. Surrounding the fibers of the graft on the luminal side, there were some multinucleated giant cells as illustrated by the CD11b stain (Fig. 5). On the external side of the graft, there was organized connective tissue with deposition of hemosiderin pigment. In the central section of the graft, the luminal side only showed intermittent covering by fibrin. The reaction on the external side consisted of peri-graft tissue formation composed of interstitial cells and collagen, illustrated by a Vimentin stain (Fig. 6). This perigraft tissue was not adherent to the graft, yet showed the same features as at the anastomoses, with additional presence of fibrin covering its internal surface. There was no observable delamination of the polymer covering. The macroscopically observed peri-graft thrombi consist of fibrin and red blood cells, which were partially organized and incorporated in the connective tissue of the peri-graft (Fig. 7 and 7bis). Apart from the multinucleated giant cells on the luminal side adjacent to the graft fibers, there was no mentionable inflammatory component present, as illustrated by the CD3 stain (Fig. 8).

The control grafts showed similar histologic features, with again presence of mature and organized connective tissue on both sides of the anastomoses. The luminal sides of the anastomoses were covered by a layer of endothelium as illustrated by the vWF stain (Fig. 9). On the external side of the graft there was organized connective tissue with only minor deposition of hemosiderin pigment. Unlike in the RUA grafts, the central section of the control grafts did show adherent peri-graft tissue, with presence of multinucleated giant cells on both the luminal and the external side of the grafts. The macroscopically observed intraluminal masses consist of organizing thrombus material attached to the luminal surface of the graft. This thrombus was variable in its composition, near the anastomoses it was organized into connective tissue while in the center it still consisted of fibrin admixed with white blood cells (Fig. 10).

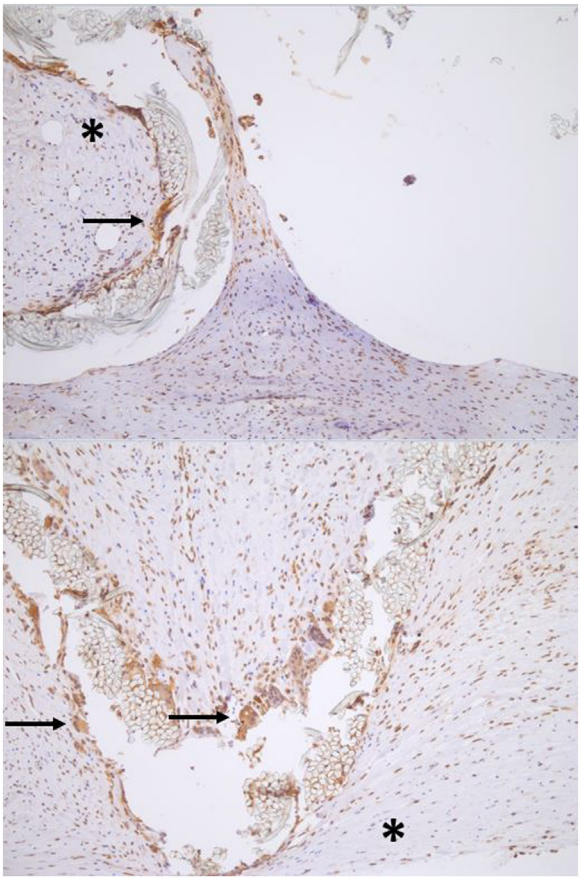

Fig. 5 – Illustration of the macrophages and multinucleated giant cells (arrow) present around both grafts. Note that in the case of the RUA (top) graft, the giant cells are only present on the luminal side (*), suggesting that there was no physical contact between the exterior side of the graft and the perigraft tissue. In the case of the Gelweave (bottom) graft, there are clearly giant cells present on both sides of the graft fibers. The other macrophages are present in a scattered manner, as expected in this remodeling context. Staining was performed with a commercially available anti-CD11b antibody.

Discussion

Commonly used large-bore vascular grafts are sealed with animal-derived sealants to prevent intraoperative bleeding through the graft. Since animal-derived products implicate difficulties concerning biocompatibility, availability, cost, etc. there is interest in development of a synthetic alternative for this sealant. The RUA Vascular graft is externally sealed with the Elast-Eon copolymer as a possible synthetic alternative to provide impermeability. This unique polymer has undergone extensive *ex vivo* performance testing[11,12] and has shown improved long-term biostability in applications in previous animal research as a drug-bearing surface, a mechanical heart valve or as an insulation material for pacemaker leads.[15-20] RUA has performed and passed ISO10993 biocompatibility testing on Elast-Eon applied in the context of vascular grafts before this study as well. Applications using the Elast-Eon

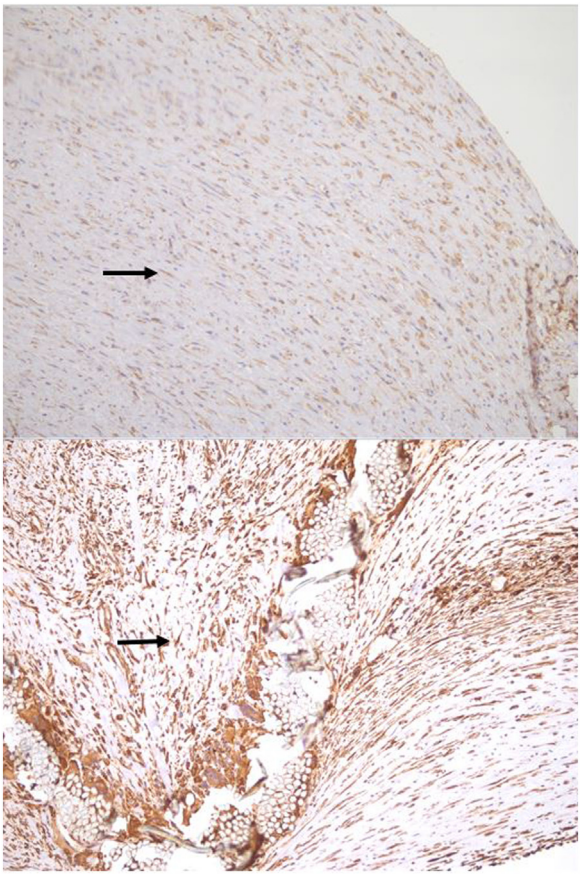

Fig. 6 – Illustration of the interstitial cells in the fibrous tissue formed around both the RUA (top) and Gelweave (bottom) grafts, stained by a commercially available anti-Vimentin antibody. Note that Vimentin stains more cells than the spindled interstitial cells (arrow) alone, as it is not a specific marker.

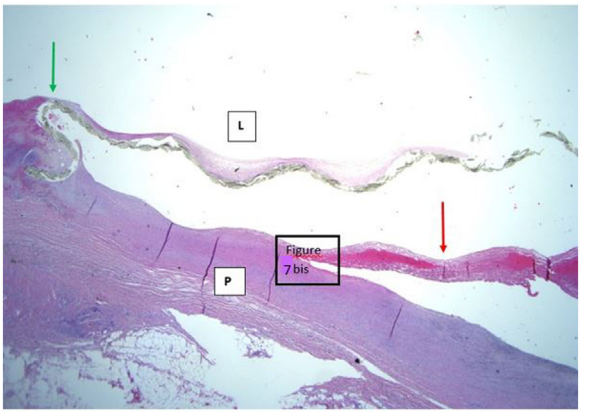

Fig. 7 – Microscopic image (x1.25) of HE section of the distal anastomosis (green arrow) toward the central part of the graft of sheep 0736, showing covering of the luminal side (L) by organized connective tissue originating from an organizing fibrin layer. There is endothelialization of the luminal surface starting from the anastomosis. On the external side there is presence of a perigraft hematoma (red arrow) originating from the perigraft tissue (P) with partial organization into connective tissue, as illustrated by the x10 image below (Fig. 7bis). Note the lack of attachment of the perigraft tissue to the graft.
Fig. 7bis Detailed image (x10) of Fig. 4 The thrombus (A) consists of fibrin and red blood cells, and gradually gets incorporated in the connective tissue of the peri-graft (B).

polymer have been implanted in man for over 15 y, mainly as lead insulation of implanted devices.[21] Although widely applied in various human applications, there is only limited evidence of the compounds' clinical performance,[22-25] highlighting the importance for more research such as this study.

Polymer coating of vascular grafts has been suggested previously,[26] but although Elast-Eon is associated with good biostability, mechanical performance and low thrombogenicity, it has relatively poor cell attachment and increased fibrosis when compared to some coblended elastomers.[27] However, technological advances now allow the coating of the external surface only, thereby preserving the exposure of intra-luminal blood to the porous polyester weave. Porosity was found to be important to vascular grafts early on in the development of grafts as noted in 1986 by Rahlf and colleagues.[28] Particularly, lack of porosity can lead to detachment of the inner capsule resulting in embolization. This phenomenon was also reported by Wang *et al.* where grafts with an inner wrap exhibited this complication.[29] Moreover, Wang *et al.* established that an outer barrier does not necessarily affect tissue ingrowth from the anastomoses or from deposits of pluripotent cells from the circulation as both externally wrapped and fully porous grafts have essentially identical luminal surfaces.

The sheep model was chosen for this study as it is an established model for evaluating the preclinical safety and efficacy of such grafts. The sheep is affordable and relatively easy to care for long-term. The rate of somatic growth of the sheep is less than that of the calf which reduces potential size mismatch. One important difference with human tissue reactions is the expected higher rate of endothelialization, since the vascular graft is tested in healthy, juvenile sheep but will eventually have its main purpose in diseased vessels of older humans. Relative hypercoagulability of sheep in comparison to humans is another difference to be taken into account,

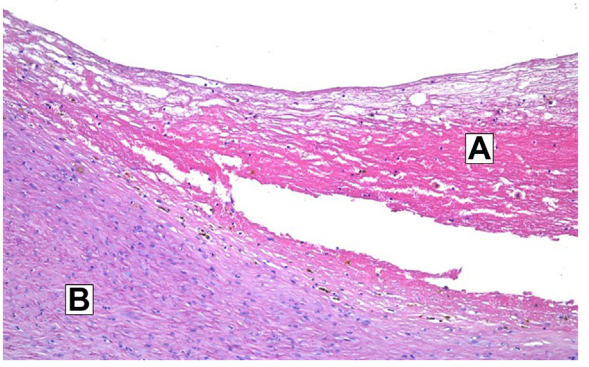

Fig. 7 – *(continued)*.

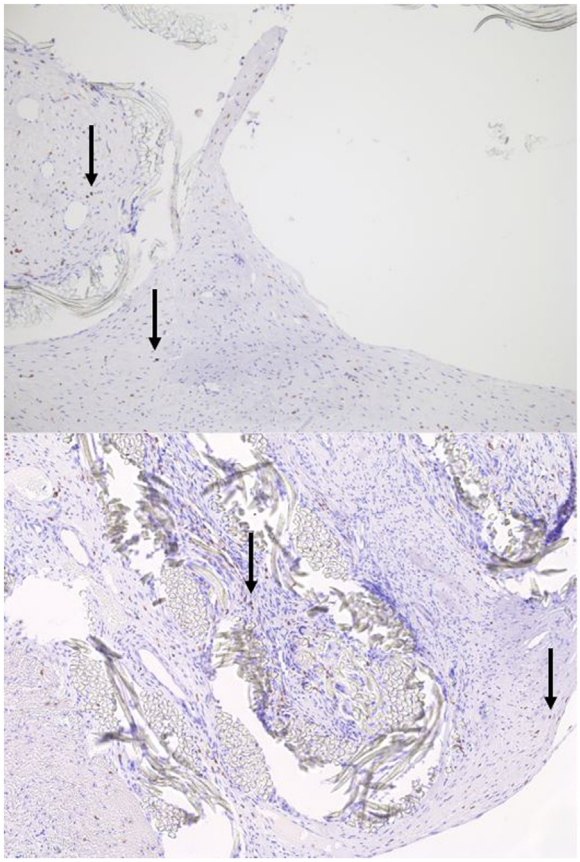

Fig. 8 – Illustration of the T-lymphocytes (arrows) present on each side of both the RUA (top) and the Gelweave (bottom) grafts. T-lymphocytes are involved in reactive processes such as remodeling around a graft like in this experiment. They are present in a scattered manner, as expected, and stain at their membrane with a commercially available anti-CD3 antibody.

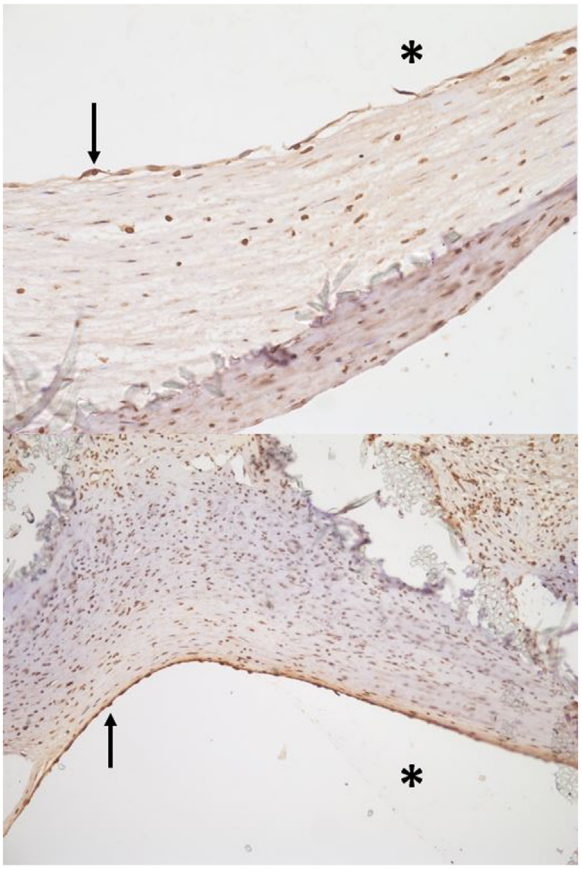

Fig. 9 – Illustration of the endothelial overgrowth of both the RUA (top) and the Gelweave (bottom) grafts. The endothelial cells are flat cells of one layer thick, covering the luminal graft surface (*), and stain intensely with a commercially available anti-vWF antibody (arrow).

although the coagulation system of sheep is closer to that of humans than dogs or pigs.[30] In this study, only female sheep were used for practical reasons. Considering the sex-based differences regarding hemostasis and vascular healing properties in sheep, there is only limited data available. One study showed significantly higher activated partial thromboplastin clotting time (aPTT) values in male sheep compared to female sheep, while there was no significant difference in prothrombin time (PT) values between both sexes.[31] To the best of our knowledge, no other research has been conducted in this field.

Histologic evaluation of the RUA Vascular grafts showed similar features compared to the Gelweave control grafts. The tissue reaction was as expected and is consistent with the general healing process for all implants as described in detail by Anderson *et al.*[32] In both cases, the anastomoses were typically in a mature phase of the healing process with trans-anastomotic endothelialization. The central portions of Gelweave control grafts were luminally covered by thrombus material, consisting mainly of fibrin, that gradually differentiated into connective tissue. Since the gelatin sealant is specifically designed for its prothrombogenic effect,[33] it was

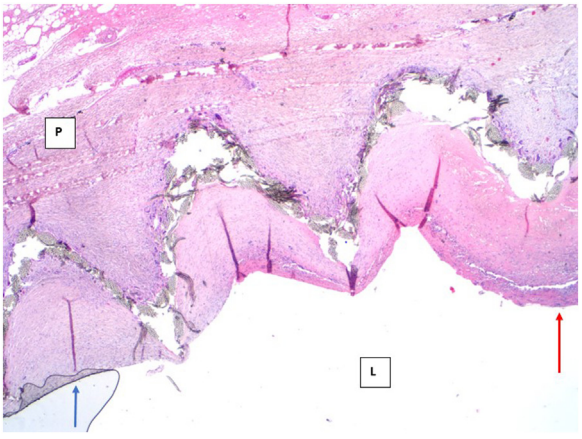

Fig. 10 – Central section of the Gelweave control graft with on the luminal side (L) covering by organized connective tissue (blue arrow), originating from an organizing thrombus (red arrow). The perigraft tissue (P) is firmly attached to the fibers of the graft.

to be expected that this reaction would be seen to a lesser extent in the RUA Vascular grafts. The fibrin reaction and extent of endothelialization is exactly as described for humans by Zilla and colleagues. Large bore polyester grafts have been implanted in humans for over 50 y and their long-term clinical performance is excellent despite the luminal surface remaining largely devoid of endothelium.[34] The major difference between the two grafts was found at the level of the peri-graft tissue (Fig. 11). In the case of the Gelweave control graft, the perigraft tissue was adherent to the external graft surface, with presence of multinucleated giant cells on both sides of the graft fibers, emphasizing the adhesion. The RUA graft however was not attached to the peri-graft tissue. Since there were no multinucleated giant cells seen on the internal surface of this peri-graft tissue, there was no adhesion between the peri-graft tissue and the Elast-Eon polymer on the external side of the graft, a phenomenon that was to be expected, as mentioned before. To a certain degree, the internal surface of the peri-graft tissue was in all cases covered with fibrin, and in some cases even with a thrombus that was already partially incorporated in the connective tissue of the peri-graft. Moreover, the peri-graft tissue showed foci of hemorrhage and hemosiderin-laden macrophages. This might suggest friction between the two components as a possible cause of the fibrin or thrombus covering the internal surface of the peri-graft, a finding consistent with previous histologic research in pacemaker leads.[35]

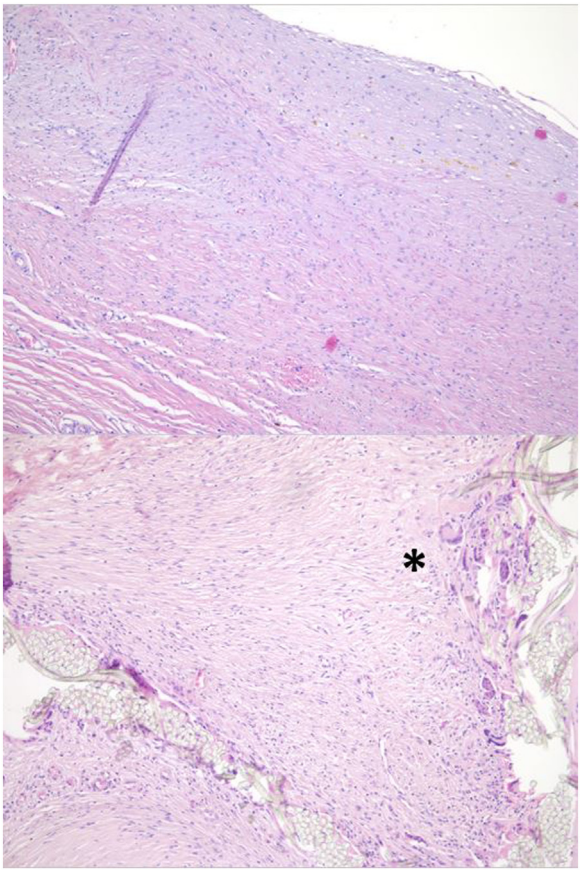

Fig. 11 – Magnified view of the fibrous tissue formed around both the RUA (top) and Gelweave (bottom) grafts. This perigraft tissue consists in both cases of connective tissue with spindled interstitial cells, admixed with remodeling macrophages and lymphocytes as illustrated in the previous figures. The major difference between the experimental groups is that in the case of the RUA grafts, the fibrous perigraft is not attached to the graft material and there are no multinucleated giant cells. In the case of the Gelweave grafts, the perigraft tissue (*) is attached to the graft material and contains multinucleated giant cells, illustrating that tissue incorporation and foreign body response only happens in the case of the Gelweave control grafts.

Postoperative collections surrounding prostheses may occur with all types of grafts. While those that occur early often resolve without treatment, a perigraft seroma may persist in some cases and indicate either graft leakage, excessive inflammation or poor graft incorporation.[8,36] It is reassuring that the early collections seen with RUA Vascular grafts had either resolved or were resolving at the 6-mo sacrifice. No evidence of a growing fluid collection was seen, indicating an effective barrier to leakage. As excessive inflammation was not observed histologically, it cannot be excluded that there is an association between the perigraft collections seen in our experiment and the observation of nonadherence to the external fibrous capsule.

Conclusion

The RUA Vascular graft was well tolerated in all animals with good hemostatic sealing of the grafts by Elast-Eon. Surgical handling characteristics, hematologic and radiologic findings were similar to the Gelweave control grafts. Necropsy results were mainly comparable, with RUA vascular grafts eliciting a nonadherent external fibrous capsule as a major difference compared to the Gelweave grafts that were well incorporated into the periadventitia.

Study limitations

This study has three main limitations. Firstly, our data are based on a large number of experimental animals ($n = 12$) but only limited control animals ($n = 3$). Nonetheless, our findings in the control group are consistent with findings described in literature, therefore we consider the comparison valid. Secondly, vascular grafts are often required to function *in vivo* for decades, while our experiment evaluated results at 6 mo. However, we believe that, considering the histologic findings, this study provides crucial information on the morphology of the early healing reaction. Lastly, we used a sheep model to investigate the safety and performance of the experimental graft. The choice to work with sheep was made with caution and based on literature. Animal models are essential for preclinical, *in vivo*-testing of novelties in the surgical field such as this experimental graft; however, they remain only a model. This should be taken into account when translating our results to the clinical situation.

Article highlights

Type of Research: Single-center prospective pre-clinical animal study.

Key Findings: The implantation of RUA Vascular woven polyester large-bore grafts externally sealed with Elast-Eon polymer in the descending aorta of 12 sheep showed no significant differences in survival, surgical handling, hematologic or radiologic findings compared to 3 control animals implanted with gelatin sealed Gelweave grafts. Necropsy and histology showed no adhesion between the RUA graft and its external fibrous capsule.

Take home Message: the RUA Vascular graft behaves well in surgical handling and overall outcome, but external histological reaction was different compared to standardly used Gelweave grafts.

Table of Contents Summary: Single-center preclinical animal study showing no significant differences in survival, surgical handling, hematologic or radiologic findings between the RUA vascular graft and Gelweave graft. Necropsy results are similar, except for no adhesion of the RUA vascular graft to its external fibrous capsule.

Supplementary Materials

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jss.2022.11.041.

Author contributions

BM, GB, and JE designed the study. GB acquired funding. BM acquired permission from the ethical committee. BM performed the operations with assistance from LVH, MS, HM, and PV. PC conducted and analyzed the MRI's. BM, LVH, MVH, and PV performed the postmortem evaluations. MVH, TR, and LVH performed the histopathological evaluation of tissue samples. Data analysis was performed by BM, PC, GB, and JE with statistical analysis performed by BM. GB and BM wrote the first draft. MVH, JE, and LVH reviewed and further edited the manuscript. All authors approved the final submitted manuscript.

Disclosure

None of the authors report conflict of interest, despite funding of the experiment by RUA. John Ely and Geoffrey Berg are Directors of RUA Life Sciences plc, the owners of RUA Vascular Ltd.

Funding

This study was sponsored by RUA Vascular Ltd, Irvine, UK.

Data Availability Statement

Data underlying this article will be shared upon reasonable request to the corresponding author.

REFERENCES

1. Sohn SH, Kim TH, Kim TS, et al. Evaluation of 3D templated synthetic vascular graft compared with standard graft in a rat model: potential use as an artificial vascular graft in cardiovascular disease. *Materials*. 2021;14:1239.
2. Thompson DF, Letassy NA, Thompson GD. Fibrin glue: a review of its preparation, efficacy, and adverse effects as a topical hemostat. *Drug Intelligence Clin Pharm*. 1988;22:946–952.
3. Gloviczki P, Hollier LH, Hoffman EA, Plate G, Trastek VF, Kaye MP. The effect of preclotting on surface thrombogenicity and thromboembolic complications of Dacron grafts in the canine thoracic aorta. *J Thorac Cardiovasc Surg*. 1984;88:253–258.
4. Terumo heart instructions for use [Internet]. Available at: http://terumoheart.de/downloads/Combined_ROW_Sealed_301-093_7.pdf. Accessed October 12, 2021.
5. Collagen Solutions. BSE-Free Status [Internet]. Available at: https://www.collagensolutions.com/company/bse-free-status/. Accessed October 12, 2021.
6. Drury JK, Ashton TR, Cunningham JD, Maini R, Pollock JG. Experimental and clinical experience with a gelatin-impregnated Dacron prosthesis. *Ann Vasc Surg*. 1987;1:542–547.
7. Suehiro K, Hata T, Yoshitaka H, et al. Impact of collagen-coated and gelatine-impregnated woven Dacron branched grafts on the early postoperative period. *Jpn J Thorac Cardiovasc Surg*. 2003;51:641–645.
8. Kadakol AK, Nypaver TJ, Lin JC, et al. Frequency, risk factors, and management of perigraft seroma after open abdominal aortic aneurysm repair. *J Vasc Surg*. 2011;54:637–643.
9. Wilkoff BL, Rickard J, Tkatchouk E, Padsalgikar AD, Gallagher G, Runt J. The biostability of cardiac lead insulation materials as assessed from long-term human implants. *J Biomed Mater Res B Appl Biomater*. 2015;104:411–421.
10. Rusu LC, Ardelean LC, Jitariu AA, Miu CA, Streian CG. An insight into the structural diversity and clinical applicability of polyurethanes in biomedicine. *Polymers*. 2020;12:1197.
11. Martin DJ, Poole Warren LA, Gunatillake PA, McCarthy SJ, Meijs GF, Schindhelm K. Polydimethylsiloxane/polyether-mixed macrodiol-based polyurethane elastomers: biostability. *Biomaterials*. 2000;21:1021–1029.
12. Pinchuk L, Martin Jr JB, Esquivel MC, MacGregor DC. The use of silicone/polyurethane graft polymers as a means of eliminating surface cracking of polyurethane prostheses. *J Biomater Appl*. 1988;3:260–296.
13. Institute of Laboratory Animal Resources (U.S.) Guide for the Care and Use of Laboratory Animals. NIH Publication, No 85-23. Washington, DC: The National Academies Press.
14. Ma J. Dixon techniques for water and fat imaging. *J Magn Reson Imaging*. 2008;28:543–558.
15. Simmons A, Hyvarinen J, Odell RA, et al. Long-term in vivo biostability of poly(dimethylsiloxane)/poly(hexamethylene oxide) mixed macrodiol-based polyurethane elastomers. *J Biomaterials*. 2004;25:4887–4900.
16. Jenney C, Tan J, Karicherla A, Burke J, Helland J. A new insulation material for cardiac leads with potential for improved performance. *Heart Rhythm*. 2005;2:S318–S319.

17. Jenney C, Millson P, Grainger DW, et al. Assessment of a siloxane poly(urethane-urea) elastomer designed for implantable heart valve leaflets. *Adv. NanoBiomed Res.* 2021;1:2000032.
18. Simmons A, Padsalgikar AD, Ferris LM, Poole-Warren LA. Biostability and biological performance of a PDMS-based polyurethane for controlled drug release. *Biomaterials.* 2008;29:2987–2995.
19. Shams E, Yeganeh H, Naderi-Manesh H, Gharibi R, Hassan ZM. Polyurethane/siloxane membranes containing graphene oxide nanoplatelets as antimicrobial wound dressings: in vitro and in vivo evaluations. *J Mater Sci Mater Med.* 2017;28:75.
20. Brisbois EJ, Davis RP, Jones AM, et al. Reduction in thrombosis and bacterial adhesion with 7 day implantation of S-Nitroso-N-acetylpenicillamine (SNAP)-Doped elast-eon E2As catheters in sheep. *J Mater Chem B.* 2015;3:1639–1645.
21. Abbott product performance report 2021 first edition [Internet]. Available at: https://www.cardiovascular.abbott/content/dam/bss/divisionalsites/cv/hcp/products/product-performance-reports/documents/Abbott-Product-Performance-Report-2021-First-edition.pdf. Accessed October 12, 2021.
22. El-Chami MF, Rao B, Shah AD, et al. Long-term performance of a pacing lead family: a single-center experience. *Heart Rhythm.* 2019;16:572–578.
23. Cairns J, Healey J, Epstein A, Themeles E, Balasubramanian K, Connolly S. Prospective long-term follow-up of silicone-polyurethane–insulated implantable cardioverter-defibrillator leads. *Heart Rhythm O2.* 2022;3:57–64.
24. Hauser RG, Abdelhadi RH, McGriff DM, Kallinen Retel L. Failure of a novel silicone-polyurethane copolymer (Optim™) to prevent implantable cardioverter-defibrillator lead insulation abrasions. *Europace.* 2013;15:278–283.
25. Kleeman T, Nonnenmacher F, Strauss M, et al. Long-term performance and lead failure analysis of the Durata defibrillation lead compared to its previous model, the recalled Riata defibrillation lead. *J Cardiovasc Electrophysiol.* 2019;30:2012–2019.
26. Jean-Baptiste E, Blanchemain N, Martel B, Neut C, Hildebrand HF, Haulon S. Safety, healing, and efficacy of vascular prostheses coated with hydroxypropyl-β-cyclodextrin polymer: experimental in vitro and animal studies. *Eur J Vasc Endovasc Surg.* 2012;43:188–197.
27. Wise S, Liu H, Yeo G, et al. Blended polyurethane and tropoelastin as a novel class of biologically interactive elastomer. *Tissue Eng A.* 2016;22:524–533.
28. Rahlf G, Urban P, Bohle RM. Morphology of healing in vascular prostheses. *Thorac Cardiovasc Surgeon.* 1986;34:43–48.
29. Wang Z, Liu S, Guidoin R, Kodama M. Polyurethane vascular grafts with thorough porosity: does an internal or an external membrane wrapping improve their in vivo blood compatibility and biofunctionality? *Artif Cells Blood Substit Immobil Biotechnol.* 2004;32:463–484.
30. Byrom MJ, Bannon PG, White GH, Ng MKC. Animal models for the assessment of novel vascular conduits. *J Vasc Surg.* 2010:176–195.
31. Carlos MML, Melo MM, Soto-Blanco B. Influence of sex on activated partial thromboplastin time (aPTT) and prothrombin time (PT) in sheep. *Vet Ital.* 2017;53:255–258.
32. Anderson JM. Biological responses to materials. *Annu Rev Mater Sci.* 2001;31:81–110.
33. Vohra R, Drury JK, Shapiro D, Shenkin A, Pollock JG. Sealed versus unsealed knitted Dacron prostheses: a comparison of the acute phase protein response. *Ann Vasc Surg.* 1987;1:548–551.
34. Zilla P, Bezuidenhout D, Human P. Prosthetic vascular grafts: wrong models, wrong questions and no healing. *Biomaterials.* 2007;28:5009–5027.
35. Keiler J, Schulze M, Dreger R, Springer A, Öner A, Wree A. Quantitative and qualitative assessment of adhesive thrombo-fibrotic lead encapsulations (TFLE) of pacemaker and ICD leads in arrhythmia patients-A post mortem study. *Front Cardiovasc Med.* 2020;7:602179.
36. Possible causes and treatment of fluid ultrafiltration and perigraft seroma formation [Internet]. Available at: https://www.goremedical.com/resource/21118151-EN. Accessed October 12, 2021.