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RESEARCH ARTICLE

Surgical procedure for acellular vascular xenografts testing in sheep carotid artery

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Objective: The objective of this study is to demonstrate the safety and reproducibility of our surgical technique for implanting decellularized vascular xenografts in the carotid artery of sheep.
Methods: Acellular porcine carotid arteries were implanted as interposition xenografts in seven sheep. An intravascular shunt was used for cerebral protection, and a flowmeter was utilized to assess graft performance.
Results: There were no intraoperative deaths or postoperative neurological complications observed. Acute graft thrombosis occurred in one sheep during surgery, but was successfully managed with thrombectomy to restore blood flow. Post-implantation flowmetry and Doppler ultrasound confirmed graft functionality.
Conclusions: Our study demonstrates the successful application of our surgical method for implanting decellularized vascular xenografts in the carotid artery of sheep. The implanted grafts maintained patency, normal blood flow, and favorable wound healing and neurological outcomes post-surgery.

Keywords: vascular xenograft, carotid artery, ovine model

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Introduction

Vascular diseases are a leading cause of mortality and morbidity worldwide, resulting in numerous deaths [1]. Treatment modalities may encompass lifestyle modifications, pharmacotherapy, and surgical excision of affected vessels with subsequent placement of a vascular graft [2].

The primary objective of tissue-engineered vessels (TEVs) is to serve as an alternative to autologous grafts in cases where a patient's own vessels are not viable [3]. Given that many individuals with vascular conditions lack suitable arteries and veins for autologous grafts, the advancement of TEVs has been a focal point of research for an extended period. The challenges posed by small-diameter vascular grafts present a significant obstacle for vascular bypass alternatives, constituting a substantial public health concern [4]. Current clinical approaches for bypassing small-diameter (<6 mm) blood vessels in the management of cardiovascular disease are frequently hindered by the absence of suitable autologous arteries and veins due to conditions such as atherosclerosis, trauma, or varicose vein disease [5].

Advancements in tissue engineering provide innovative strategies for overcoming the limitations associated with small-diameter prosthetic vascular grafts. These conventional grafts are frequently hindered by complications such as inflammatory responses, thrombogenicity, bleeding secondary to anticoagulant therapy, mismatched compliance, and the potential for long-term aneurysm formation [6,7]. The development of a blood vessel constructed from autologous cells and a biocompatible scaffold, capable of remodeling, repairing, and growing, signifies a significant therapeutic advancement. Novel techniques are emerging that facilitate the cultivation of various tissues both in laboratory settings and within living organisms, often utilizing naturally-derived scaffolds [8].

In our prior published research, we obtained a functional acellular vascular xenograft through a NaOH-based decellularization solution, from porcine carotid arteries [9].

In vivo animal model testing of these grafts is essential on the way to a clinical scenario. All criteria for testing a graft in systemic circulation were met by positioning them in the carotid artery and the xenogeneic character was achieved by implantation on an ovine model.

Our hypothesis was that these grafts would be repopulated with the host organism's cells within a three-month period. Following this timeframe, the grafts will be explanted, and comprehensive macroscopic and histological evaluations will be performed.

The aim of this study is to demonstrate that our surgical method of implanting decellularized vascular xenografts in the carotid artery of sheep is a safe and reproducible procedure.

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Methods

XenoGraft Fabrication

The acellular scaffolds were developed at the Regenerative Medicine Laboratory within the Center of Advanced Medical and Pharmaceutical Research, and the experimental procedures were conducted at the Experimental Station, both facilities of „George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology of Targu Mureș, Romania. Our team [9] previously published the decellularization protocol and the quality control protocols.

Anesthesia management

The study adhered to specialized guidelines including Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes, ARRIVE guidelines, and the AVMA euthanasia guidelines 2020. Ethical approval was granted by the University's Ethics Committee under approval number 1434, dated July 8, 2021.

For the in vivo experiments, we chose 7 "Tigaie" sheep breed females, aged 1-3 years, weighing 45-65 kg, based on our previously experiment on sheep [10, 11].

A few days before the surgical intervention, the sheep underwent sanitation procedures and were moved indoors to acclimatize to the environment and get acquainted with the staff. Given their sociable nature, they were consistently relocated in groups to enhance their comfort. Solid and fluid food intake were restricted 24 hours and 8-12 hours before the surgery, respectively.

To facilitate the handling and transfer of the animal to the operating room, and to alienate the anxiety, the sheep was sedated 30 minutes before the surgery with 0.015 mg/kg Medetomidine Hydrochloride (Produlab Pharma B.V, Netherlands), administered intramuscularly, along with 0.01 mg/kg of Atropine (Accord Healthcare, Poland) subcutaneously. The animal was securely positioned on the operating table using harnesses and straps. To maintain homeostasis (circulation, oxygenation, ventilation, temperature), vital parameters were monitored as follows: heart rate and rhythm through electrocardiography (ECG) with 3 leads, oxygen saturation (SpO2) using a lingual pulse oximeter, and invasive monitoring of arterial pressure by placing an arterial catheter at the level of the left auricular artery.

Anesthetic induction was achieved by intravenous administration through a previously placed peripheral venous catheter in the left ventral limb. A dose of 4.0 mg/kg IV of Propofol (Fresenius Kabi GMBH, Austria), 0.02 mg/kg IV of Atropine, and 0.2 mg/kg IV of Atracurium (Glaxo Smithkline Pharmaceuticals, Poland) was administered.

We employed the standard intubation procedure using a laryngoscope with a straight blade measuring 15 cm and a blade-handle opening of 125 degrees. The endotracheal tube was connected to the ventilation circuit. The experimental animal was ventilated in pressure control ventilation mode, with a tidal volume (Vt) of 5-10 mL/kg body weight (maximum 15 mL/kg) and a respiratory rate of 12-20 breaths per minute. This was done to maintain a pCO2 within the 30-40 mmHg range, a ventilation pressure of up to 30 mmHg, and a positive end-expiratory pressure (PEEP) of 5-10 cm H2O. Anesthesia maintenance was achieved with 1-2.5% Sevoflurane (Rompharm, Romania) in 50% oxygen (MAC 2,3%).

Prophylactic antibiotherapy (Amoxicilin-clavulanic acid – 3 mg/kg IV), analgesics and anti-inflammatory treatment (Ketoprofen 1 mg/kg IV, Dexamethasone 0,2 mg/kg IM) was administered up to three days.

Xenograft implantation into the carotid artery of the sheep

After sterile preparation of the surgical field, a 20 cm left lateral-cervical incision was made. Subcutaneous tissue was dissected and sectioned. The common carotid artery was then dissected in the jugular groove, located posteromedially to the jugular vein, over a 10-15 cm length, with ligature of small arterial branches. Blood flow at this level was measured using a Medistim Flowmeter (MiraQ cardiac, Norway).

Following systemic heparinization (Heparin Galenika a.d, Belgrade, 1.5 mg/kg), the carotid artery was clamped, excluding a 10-12 cm segment of the carotid artery. For cerebral protection, an intravascular shunt was inserted in the native carotid artery and fixed proximally and distally on tourniquets, previously passed through the arterial xenograft. The carotid artery was unclamped to restore blood flow through the shunt. End-to-end anastomoses of the arterial xenograft to the native carotid artery were performed at the proximal and distal ends using continuous sutures of non-absorbable 6.0 polypropylene. The vascular shunt was gradually suppressed after reclamping the carotid artery to evacuate air and prevent gas embolism.

Finally, the arterial xenograft was unclamped, and we assessed the blood flow proximally and distally using the Medistim Flowmeter (Figure 1).

Two parameters, mean graft flow (MGF) and pulsatility index (PI), commonly measured in aorto-coronary bypass procedures, were taken in consideration.

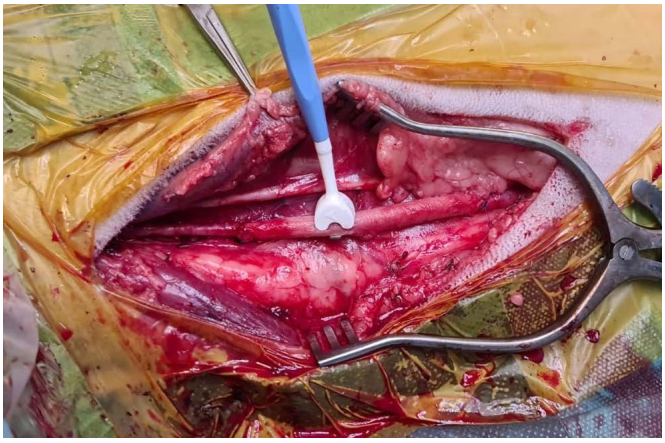

Fig. 1. Intraoperative flow measurement of the vascular graft

The surgical wound was closed in 3 layers: platysma, subcutaneous tissue and skin.

In the day after the surgery, Doppler ultrasound was performed to check the neo-artery permeability.

Statistical analysis

Mann Whitney U Test was performed for statistical analysis, using SPSS for Mac OS, version 29.0.1.1 (SPSS, Inc., Chicago, IL, USA).

Results

The surgical procedures were performed without any deaths occurring during the operation. In the sheep no. 5, the flowmeter initially indicated no blood flow at the xenograft site, suggesting acute thrombosis, which was later confirmed upon the removal of thrombotic material.

Flow was subsequently restored, and post-implantation results showed optimal blood flow.

Comparative flowmeter measurements: MGF (Figure 2) and PI (Figure 3), did not show significant differences between the native carotid flow, with the first measurement at the time of implantation (T0).

The neurological examination showed positive results, indicating favorable outcomes in all sheep, as they maintained consciousness, engaged in rumination, and exhibited vocalization.

One day after the surgery, on ultrasound assessment, all animals showed complete patency, with laminar flow and no signs of stenosis at the anastomosis sites. The surgical wounds progressed favorably, without infections or other complications in healing.

Discussions

Diseases such as atherosclerosis, which affect small-caliber muscular arteries (< 6 mm), can result in vessel occlusion, leading to decreased blood flow and contributing to conditions like myocardial infarction, peripheral arterial disease, or stroke due to occlusion of carotid or cerebral arteries [12].

Arterial replacement has become a common approach for treating many patients with vascular disease [13].

Autologous vessels like saphenous veins are considered the standard for bypass surgery. However, approximately one-third of patients lack suitable veins for grafting due to peripheral vascular disease, previous vein harvesting, or vein stripping from prior procedures, vascular trauma or limb amputation [14, 15].

The advancement of tissue-engineered vascular grafts for small-caliber arteries has the potential to significantly enhance the management of vascular diseases, thereby improving patients' quality of life [16].

The aim of this report was to establish a dependable and replicable method for implanting tissue-engineered vessels into the carotid artery of sheep. Our model facilitated graft implantation under the cerebral protection of an intravascular shunt, without any observed neurological deficits.

Interposition of vascular grafts without intravascular shunt was also described [17], with good results, the contralateral carotid flow would increase 50%-100% to maintain blood flow to the brain. The decrease in arterial pressure values recorded by the invasive arterial catheter in the ipsilateral auricular artery at the time of carotid clamping, made us believe that the placement of the intravascular shunt represents a necessary measure of brain protection.

The placement of a decellularized graft in the carotid position often results in several notable complications. One of the primary concerns is thrombosis, which can occur within the first 24 hours after implantation and sometimes during surgery [18-20].

The surgical technique described here has been successfully implemented with graft patency and favorable outcomes. However, in this report one sheep experienced acute thrombosis during surgery.

The exact cause of this remains uncertain, although it may have been related to due to the lack of epithelium, which provides a thrombogenic characteristic [21, 22]. Other potential complication may include stenosis of

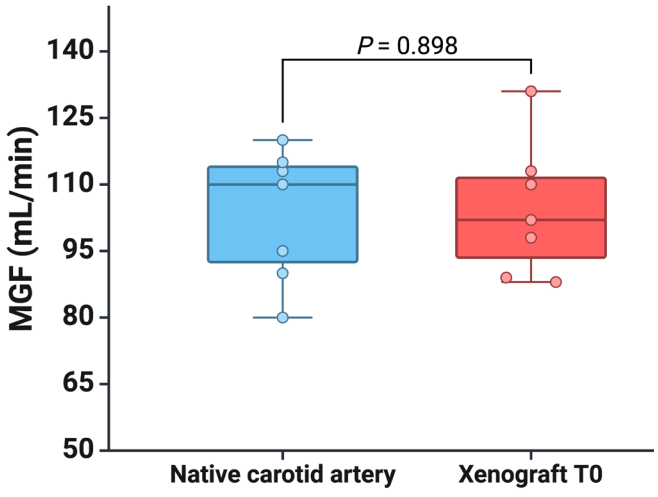

Fig. 2. Comparison of the mean graft flow measurements – native carotid artery versus xenograft

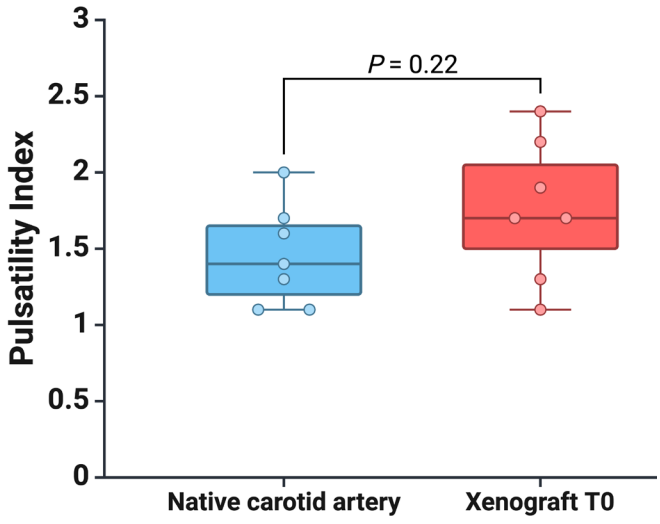

Fig. 3. Comparison of the pulsatility index measurements – native carotid artery versus xenograft

grafts, infection, rupture and bleeding and aneurysm formation. These conditions can be assessed using methods such as physical examination and ultrasound imaging [23, 24], in the follow up period.

Conclusion

Our research demonstrated the successful utilization of our surgical technique. With this method, the implanted grafts maintained patency and normal blood flow post-surgery, along with favorable wound healing and neurological outcomes. However, thrombosis was observed in one sheep. After 3 months of in vivo functionality, the xenografts macroscopic and histologic analysis will demonstrate if our acellular vascular grafts remained patent, biocompatible and repopulated with the host cells.

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Authors' contribution

HMM (Conceptualization, Funding acquisition, Methodology; Project administration, Writing – review & editing), PAI (Investigation; Methodology; Writing – original draft), AED (Data curation: Investigation, Methodology), SDT (Conceptualization; Supervision; Vizualization), TRI (Investigation, Writing – original graft, Data curation), AHH (Investigation; Methodology; Vizualization), CI (Data curation; Investigation; Resources), GC (Project administration, Resources, Investigation), BCM (Formal Analysis, Software, Investigation), CT (Data curation, Investigation, Methodology), HS (Methodology; Supervision; Writing – review & editing), AHH (Investigation; Validation, Visualization)

Conflict of interest

None to declare.

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