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ORIGINAL RESEARCH

Bioengineered Small-Diameter Vascular Xenografts as an Alternative to Autologous Vascular Grafting for Emergency Revascularization – a Preliminary Study

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ABSTRACT

Background: Autologous vascular arterial or venous graft are not available in 10-40% of patients, due to vascular pathologies, and the utility of decellularized biological scaffolds would be a solution for those cases. The purpose of this research was to obtain a functional acellular xenograft, prior to in-vivo testing as a vascular graft in an experimental animal. **Materials and method:** Two batches of carotid vasculo-nervous bundles were collected from porcine models from a local slaughterhouse. The arterial grafts were dissected and isolated, obtaining carotid arteries with a caliber of 5–6 mm and a length of approximately 10–12 cm. Two decellularization protocols were used, immersion (n = 10) and perfusion (n = 9). The resulting grafts underwent histological examination, DNA analysis, electrophoresis and spectrophotometry. **Results:** Due to severe tissue damage and friability, the batch that was decellularized using perfusion was not examined. The histological examination of grafts stained with hematoxylin-eosin and DAPI highlighted the absence of nuclei. Spectrophotometry revealed a 90% decellularization, and electrophoresis of revealed the migration band of the material extracted from the fresh tissue, as well as the absence of migration bands in the case of the material extracted from decellularized tissues. **Conclusion:** We successfully used the immersion protocol

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to obtain a functional acellular vascular graft, in contrast to perfusion decellularization, where intraluminal high pressures damage the extracellular matrix.

Keywords: vascular grafting, emergency revascularization, bioengineering

INTRODUCTION

Autologous vascular arterial or venous grafts are not available in 10–40% of patients, due to vascular pathologies (e.g., atherosclerosis, varicose veins disease), acute trauma, or a previous surgical procedure.[1] Emergency surgical revascularization in acute coronary syndromes or acute/traumatic limb ischemia has a challenging approach, especially in patients in whom qualitative vascular harvesting is not available.

Simultaneously with the advance and improvement of tissue engineering in the last decades, biological scaffolds have drawn significant interest in this area, due to their mechanical performances, biocompatibility, and bioactivity.[2] Biologic scaffolds composed of extracellular matrix (ECM) are commonly used in reconstructive surgery and gaining practice in regenerative medicine as tissue and organ substitutes.[3] When donor vessels are not available or are unsuitable, tissue-engineered vascular grafts are achieving clinical success and are becoming a promising choice for grafting.[4,5]

Non-thrombogenicity, non-immunogenicity, biocompatibility, hemocompatibility, and appropriate pore size are the main proprieties that are required for an ideal scaffold, in order to reflect the biomechanical properties of blood vessels.[6] The application of decellularized biological scaffolds could be a solution for these cases, but the reconstruction of small-diameter vascular grafts remains a challenge, decellularization processes leading to a high risk of obstruction, due to thrombogenicity of those vessels.[7,8]

The purpose of this research was to obtain a functional acellular xenograft, prior to in vivo testing as a vascular graft in an experimental animal.

MATERIALS AND METHOD

We used two different decellularization methods – perfusion and immersion. Sierad et al. demonstrated that tissue exposure to intraluminal high pressure increases the penetration of decellularization agents through the aortic media layer.[9] Based on this hypothesis, we aimed to compare the two techniques in order to obtain a biological vascular graft.

Two batches of carotid vasculo-nervous bundles were collected from porcine models from a local slaughterhouse (Figure 1), in order to be processed in our laboratory – TERMLab (Tissue Engineering and Regenerative Medicine Laboratory) of the "George Emil Palade" University



FIGURE 1. Carotid vasculo-nervous bundles from porcine models



FIGURE 2. Carotid arteries from porcine models after dissection



FIGURE 3. Carotid arteries ready for the decellularization process



FIGURE 4. Immersion decellularization process

of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Romania. The arterial grafts were dissected and isolated, obtaining carotid arteries with a caliber of 5–6 mm and a length of approximately 10–12 cm, sectioning any collaterals (Figure 2). The arteries were fixed on a plastic support, in order to maintain them straight (Figure 3).

The decellularization process aimed to remove all resident cells by exposing the tissue to a series of physical, chemical, and mechanical agents, isolating the ECM that would represent the new arterial graft. Two decellularization protocols were used, perfusion and immersion, differing by the decellularization method. We used the same decellularization agents for both protocols, as described below.

The first batch was decellularized using the using the immersion process (n = 10), and the second batch (n = 9) was decellularized using the perfusion process. We aimed to compare the decellularized tissue (batch 1 and 2) with a fresh carotid artery (control). The immersion decellularization process consisted of placing the tissue in the decellularization agents, while being subjected to agitation (Figure 4). Perfusion decellularization process is based on continuous infusion of the decellularization agents inside the arterial graft lumen (Figure 5), using a peristaltic pump. We used penta-galloyl glucose (PGG) to treat acellular scaffolds, for tissue stabilization, preventing early scaffold degradation and to minimize calcification.

We used the immersion protocol of Simionescu *et al.*[10], as follows: treatment with NaOH 0.1 M for 3 h, which was stirred for immersion in water at 37 °C for 3 h. The scaffolds were extensively rinsed, repeated with double-dis-



FIGURE 5. Perfusion decellularization process

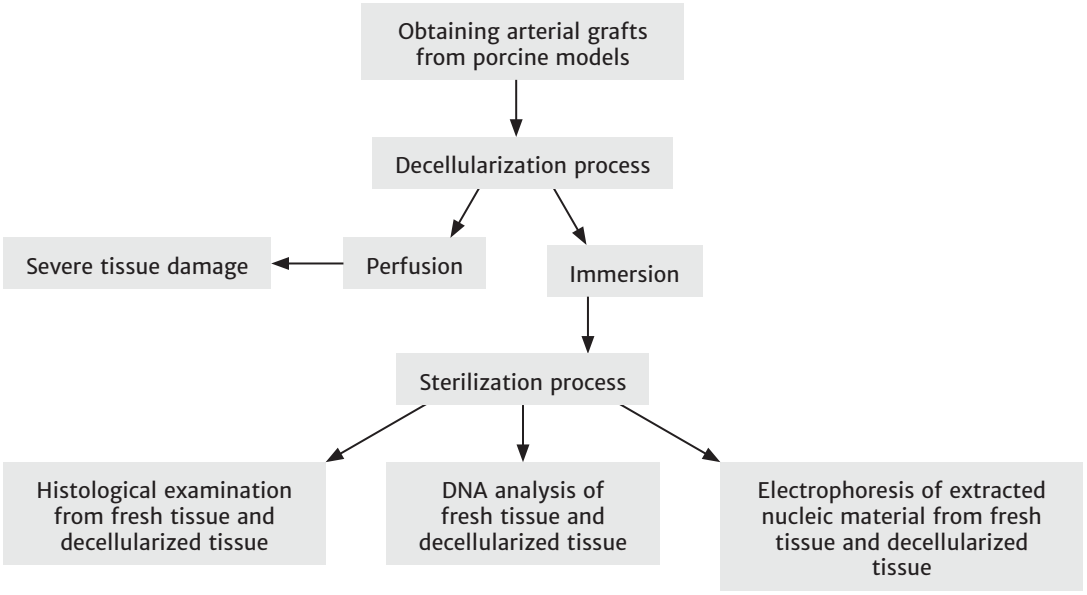


FIGURE 6. Protocol of the study

tilled water by immersion for 24 h. The carotid arteries were sterilized with 0.1% peracetic acid for one 1 h, washed with sterile PBS, and then fixed with 0.3% pentagalloyl glucose (PGG) for 24 h. After 24 h, the carotid arteries were transferred to sterile PBS at 4 °C. The same protocol was used for perfusion, infusing the decellularization agents through the arterial grafts instead of stirring. The sterility of the arteries was tested by collecting a small fragment that was microbiologically tested. All samples were found to be sterile. Later, tissue fragments were taken for quality control through histological staining, in order to observe the integrity of the ECM and the absence of nuclei. The study protocol is illustrated in Figure 6.

Decellularization quality control aimed to observe the integrity of the ECM and the absence of nuclei at the level of the processed tissue and compare it with the control batch. It was performed by histological examination and DNA extraction. Due to severe tissue damage and important friability not suitable for surgical suturing, the second batch that was decellularized using the perfusion process was not examined, so we are only mentioning the batch that was decellularized using the immersion protocol.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

For histological examination, samples from fresh tissue and decellularized tissue were fixed in 10% formalin, then imbibed in paraffin and sectioned at dimensions of 5 µm. In the first histological control method, they were stained with hematoxylin-eosin (HE) and examined under an optical microscope to visualize the general structure. The second method involved DAPI (4',6-diamidino-2-phenylindole) fluorescent nuclear staining, which allows examination of the presence of nuclei by fluorescence microscopy, through strong binding to DNA domains.

DNA ANALYSIS

For DNA analysis, approximately 20–29 mg of tissue fragment were extracted and purified, from both decellularized and fresh native tissue, with a special extraction kit, DN-easy Blood & Tissue (Qiagen). Obtained data from Nano-Drop spectroscopy were adjusted according to the weight of the sample. As a final step, we performed the electrophoresis of extracted nucleic material using agarose gel and ethidium bromide.

RESULTS

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

The histological examination of staining with HE (Figure 7A–F) and DAPI (Figure 7G–I) highlighted the absence of nuclei at this level for the decellularized tissue compared with the control artery.

DNA ANALYSIS

The results obtained using spectrophotometry for the immersion batch revealed a 90% decellularization (Table 1).

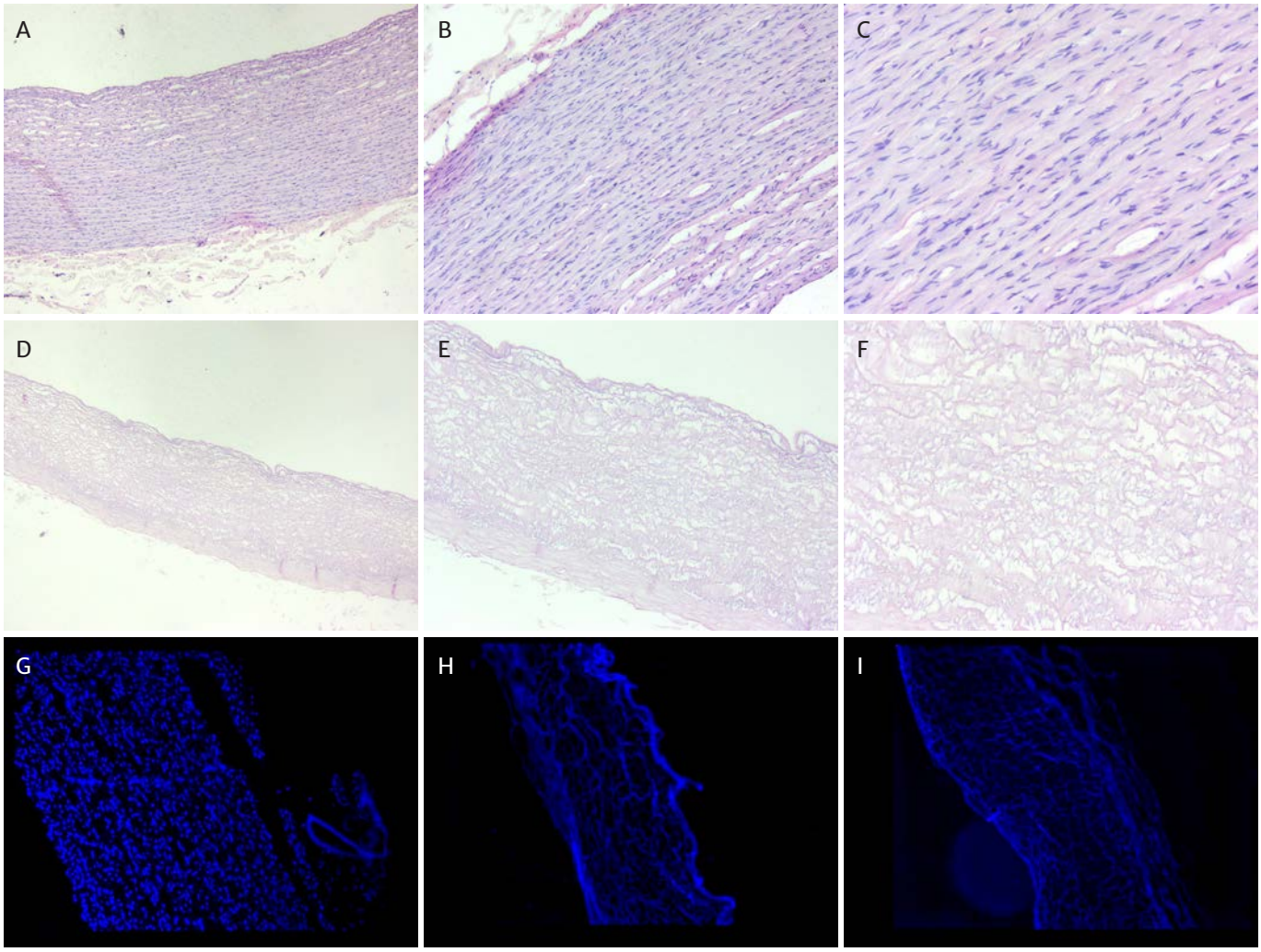


FIGURE 7. **A–C** – Histological appearance of the control carotid artery, HE coloration, 5x (**A**), 10x (**B**), 20x (**C**); **D–F** – Histological appearance of the carotid arteries after decellularization, HE coloration, 5x (**D**), 10x (**E**), 20x (**F**); **G–I** – Histological aspect of the carotid arteries, DAPI fluorescence: control carotid artery (**G**), decellularized carotid artery at exposure 150, gain 1.5 (**H**) and exposure 200, gain 2 (**I**)

Electrophoresis of extracted nucleic material in ethidium bromide revealed the migration band of the material extracted from the fresh tissue, as well as the absence of migration bands in the case of the material extracted from decellularized tissues (Figure 8).

DISCUSSION

The absence of proper autologous grafts and the unfeasibility of synthetic prostheses for small-diameter vascular reconstruction made mandatory the development of an alternative vascular graft.[11,12] The currently available synthetic grafts used in practice are made from expanded polytetrafluoroethylene (ePTFE) and polyethylene terephthalate (Dacron).[13] They are used in large-diameter bypass grafting, preventing mechanical deformation in high-pressure and high-flow-rate arteries like the aorta.[14] Numerous synthetic vascular graft types have been developed but are not suitable for small-diameter artery reconstruction due to host inflammatory and thrombogenic responses.[15]

The main factors associated with vascular graft failure are intimal hyperplasia, atherosclerosis, thrombosis, or infection. Given the limitations of the currently available vascular grafts, tissue engineering has become one of the fastest-growing areas of research.[16,17] Rosenberg et al. were the first to develop a decellularized vascular prosthesis using bovine carotid in the 1960s. Since then, numerous decellularization processes have been developed.[18] Several studies have found slightly improved patency outcomes comparing bovine carotid artery grafts with ePTFE grafts.[19,20]

Three classes of materials are mainly used in tissue engineering grafting: synthetic polymers, natural polymers,

TABLE 1. DNA extraction – immersion batch

		Tissue weight (mg)	DNA concentration (µg/mL)	DNA purity
1	Control	22.9	182.3	1.79
2	Carotid artery 1	22.2	19.7	1.82
3	Carotid artery 2	21.8	16.5	1.9
4	Carotid artery 3	20.6	14.2	1.18
5	Carotid artery 4	23.1	16.8	1.89
6	Carotid artery 5	23.5	13.1	1.88
7	Carotid artery 6	20.1	18.9	1.87
8	Carotid artery 7	23.1	14.5	1.92
9	Carotid artery 8	20.4	13.5	1.97
10	Carotid artery 9	21.3	17.9	1.94
11	Carotid artery 10	28.5	14.8	1.83

and bio-derived materials.[21] The goal of the design of a vascular graft is to resemble the native vessel, both in structure and function. Therefore, a functional scaffold should have appropriate architectural and mechanical properties, along with biocompatibility, bioactivity, and antithrombogenicity.[22,23] The materials used for vascular graft engineering, including synthetic polymers and collagen matrices, can cause profound inflammatory and thrombogenic responses when placed in contact with blood.[24] Components of the ECM must be preserved after the decellularization process in order to meet the requirements of a functional scaffold.[25] Advances in specific techniques are made, enabling the removal of cellular components using a combination of physical, chemical and enzymatic methods.[26]

The initial step of the decellularization approach is cell destruction through the solubilization of the cytoplasmic membrane and DNA fragmentation. Then, the cellular and nuclear debris must be completely removed from the ECM. The final step of the decellularization procedure is the sterilization of the produced scaffold.[3,27,28] Decellularized ECM scaffolds obtained from natural ECM after acellular treatment stand out compared to other biomimetic materials because of their inherent natural components and microenvironment.[29,30] The biomechanical proprieties of an ECM can be affected after being exposed to physical and chemical stress during the decellularization process; on the other hand, the process removes the immunogenic genetic material, and by preserving the native structure of ECM, it provides cell replication and differentiation when stem cells are activated.[14,31] Decellularized tissues have been found to be very similar in structure to native blood vessels, and the mechanical strength is comparable to native vasculature.[4,32,33]

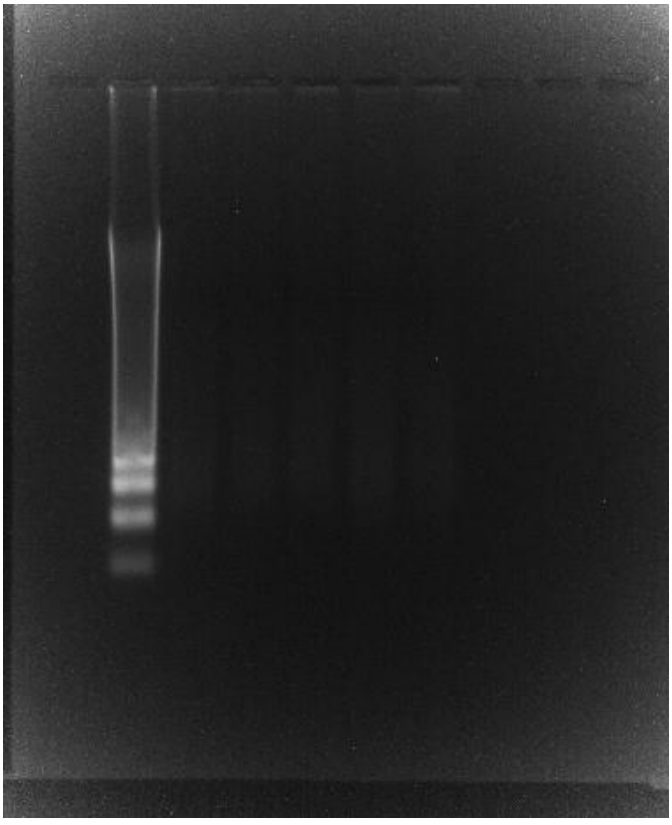


FIGURE 8. Electrophoresis of extracted nucleic material in ethidium bromide

This study aimed to compare two decellularization methods, perfusion and immersion. Due to severe tissue damage during the process, the carotid arteries that were decellularized using perfusion have not been assessed using histological examination and DNA analysis. The immersion protocol showed promising results, the efficiency of this decellularization process being ensured by the absence of remaining cellular DNA, achieved by both direct staining and after DNA extraction.

A large number of studies have been published using different materials and methods for developing tissue-engineered vascular grafts, but graft thrombosis and intimal hyperplasia remains a challenge.[12,34–36]

CONCLUSION

Vascular tissue engineering offers enormous potential to meet the growing needs of patients in all fields of cardiovascular surgery, but the main challenges for vascular grafts are still related to materials, cells, and the surface that comes in contact in blood. In this study, we successfully used the immersion protocol to obtain a functional acellular vascular graft, in contrast to perfusion decellularization, in which intraluminal high pressures damage

the ECM. Future studies will demonstrate whether this xenograft can be used as a feasible vascular alternative in an ovine experimental model.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Romania (no. 1434/July 8, 2021)..

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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