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PREPARATION OF VASCULARIZED BONE ALLOGRAFTS – EXPERIMENTAL STUDY

321.18 ORTHOPEDICS AND TRAUMATOLOGY

Summary of PhD thesis in medical sciences

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The thesis was developed within the Laboratory of Tissue Engineering and Cell Cultures and Department of Orthopedics and Traumatology of the *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova.

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The public defense of the doctoral thesis will take place on 6th November 2024, at 14:00, at the *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova, 165 Stefan cel Mare si Sfânt Ave, room 205, in the meeting of the Commission for the public defense of the doctoral thesis, approved by the decision of the Scientific Council of the Consortium from 26th June 2024 (minutes of the meeting no. 46*)*.

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CONTENT

INTRODUCTION

Topicality and importance of the subject studied

Bone defects of different origins initiate natural regeneration mechanisms in the human body and most of the time sufficient for the recovery of the small bone defects [1]. The bone remodeling process is qualified as one with the most complex mechanisms. Bone cells, having a complex communication with each other, perfectly orchestrate the processes of bone healing (consolidation), the mechanical adaptation of the skeleton and of course regulate calcium homeostasis [2]. However, the treatment of bone defects becomes a real challenge when we talk about massive bone defects, especially those after severe trauma, massive tumor formations, localized and aggressive infectious processes or congenital bone defects. For such diagnoses, traditional treatment methods are used [3, 4, 5, 6, 7]. However, for bone defects exceeding 4-5 cm in length, the bone grafting procedures currently used may be insufficient [8, 9]. The increased demand for interventions to recover bone defects of different etiology, the improvement of technical possibilities, and of course the large-scale development of tissue engineering (TI) induces the approach of the given problem to another level [1, 10, 11].

The ideal material for bone construction should be a biocompatible, biodegradable, osteoconductive, osteoinductive, and with good mechanical properties. The advantages of using natural extracellular matrix (ECM) include primarily their similarity in morphology and 3D structure to that of native bone tissue [12, 13]. Medical engineering at the moment has a huge impact on organ and tissue transplantation, promising the development of obtaining and widely using ECMs of different origins that will offer the possibility of replacing any non-functional organ or organ segment [11].

The goal of bone TI is to regenerate/repair tissue with using a biocompatible and biodegradable graft (natural or synthetic). Taking into account that bone is a very well vascularized tissue, and all its stages of regeneration (inflamation, maturation, remodeling) depend on a strict regulation of blood supply, it is very important to develop strategies aimed at the rapid and efficient vascularization of the implanted graft [14, 15]. In the Republic of Moldova, the need for bone grafts exceeds 1.4 times the availability. Even more, according to a survey of 161 specialists, including 75 trauma orthopedic surgeons from 16 different medical institutions, there is an increased need for bone grafts processed by a method other than freezing [16, 17].

Purpose of the study

Development of the universal protocol for decellularization of tissues with various degrees of mineralization in order to obtain vascularized composite bone grafts with minimal immunogenic qualities.

Objectives of the study

1. Elaboration of the surgical protocol to procure the vascularized bone graft of the tibial bone in laboratory animals;
2. Elaboration of the protocol for decellularization of bone grafts (tibial bone) with preservation of the vascular pedicle;
3. To test the efficacy of the bone graft decellularization used method by qualitative (H&E, DAPI, SEM) and quantitative (DNA quantification) methods;
4. To test the biocompatibility of vascularized bone extracellular matrices using different cell types for *in vitro* recellularization.

Methodology of scientific research

In order to achieve the purpose of the work, we have started a preclinical experimental study that was based on 2 major elements: the increased demand for bone transplantation *vs* the evolution of the TI field. This experimental study was carried out on biological samples taken from laboratory animals (pigs) and is based on soft tissue and hard tissue samples (blood vessels of

porcine origin of three different diameters, periosteum, avascular bone grafts, vascularized composite bone grafts). The objectives of the study were achieved by performing the following steps: (1) was developed the surgical protocol for vascularized bone grafts procurement; (2) a protocol described for the decellularization of small bone grafts was adjusted and tested on large, medium, and small caliber vascular grafts, plus on large avascular bone grafts (cortical and spongy bone grafts), and on vascularized composite bone grafts; (3) the effectiveness of the decellularization protocol was assessed through qualitative and quantitative tests; (4) the biocompatibility of vascular ECMs, avascular bone ECMs and vascularized bone ECM was tested performing *in vitro* recellularization using different cell types.

Decellularization of soft and hard tissue grafts was possible using a protocol that included: an isotonic solution (0.1 % ethylenediaminetetraacetic acid, EDTA + phosphate saline buffer, PBS), a chelating agent (0.1 % EDTA + tris aminomethane buffer solution, TRis buffer), a detergent (0.5 % sodium dodecyl sulfate, SDS) and an enzyme solution (300 U/ml deoxyribonuclease, DNase). Static decellularization was used for vascular and avascular bone grafts, and the decellularization for vascularized composite bone grafts was performed by infusion of solutions using two infusion routes: (1) perfusion of the solutions through the vascular pedicle and (2) perfusion of the solutions through the bone diaphysis. Also, the efficacy of decellularization at increasing the detergent concentration (SDS) from 0.5% to 1% was tested. In order to assess the efficacy of the decellularization protocol, the qualitative analysis of soft and hard tissue samples (histological staining Hematoxylin and Eosin, H&E and 4', 6-diamidino-2-phenylindole, DAPI) was performed, and the quantitative analysis of the decellularization process was performed based on DNA quantification for avascular and vascularized bone grafts. In order to assess the microscopic changes that occurred at the level of the bone component of vascularized bone ECM, scanning electron microscopy (SEM) of native *vs* decellularized cortical and cancellous bone samples was performed.

The most important moment of the study, the assessment of biocompatibility by *in vitro* recellularization of the obtained ECMs, was performed by using human umbilical venous endothelial cells (HUVEC) for vascular grafts, and the allogeneic mesenchymal stem cells from bone marrow – BM MSC (porcine origin) for avascular bone grafts and vascularized ones.

The analysis of histological samples and cell cultures was performed using the optical microscope, fluorescent microscope and stereo microscope. The data were collected and processed with the help of the Excel program from Microsoft Office version 2017. Using the same program, continuous variables were expressed as mean $\pm$ SD. The statistical processing was carried out with the help of the SPSS program (version 17.0). The comparison and assessment of the statistically significant differences between the mean values of the evaluated parameters was carried out with the help of the independent parametric test t-Test. The p-value was interpreted as statistically significant for $p \leq 0.05$.

The study was approved by the Research Ethics Committee of the *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova, with the issuance of the favorable opinion no. 71 on 21.05.2018. This study was carried out at the Laboratory of Tissue Engineering and Cell Cultures of the *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova and the Leibniz Research Laboratory of Biotechnology and Artificial Organs (LEBAO), Hannover School of Medicine (MHH), Germany, during 2017-2022.

Scientific novelty and originality of the results obtained

The scientific novelty of the work consists in the elaboration of a universal decellularization protocol. A universal protocol for the reasons that it can be applied for the decellularization of hard tissue and soft tissue. The final product to obtain such a protocol was an vascularized bone

ECM, biocompatible *in vitro*, vascularized by its vascular pedicle. The basic element of the study was not only the elimination of the immunogenic components from the bone, vascular and periosteal compartment of the graft, but also the obtaining of a qualitative vascular and bone ECM. The biocompatibility of the obtained ECMs was demonstrated by *in vitro* recellularization using different cell types.

Theoretical importance and main scientific results

(1) The surgical protocol for procurement an allogeneic tibial bone graft in complex with its own vascular pedicle for subsequent decellularization was developed. For the first time, this graft model was used as material for obtaining the natural vascularize bone extracellular matrix; (2) the universal decellularization protocol for obtaining vascularized composite bone ECMs has been developed and tested; (3) microscopic changes (SEM) that occur at the level of cortical and cancellous bone after processing/decellularization were analyzed; (4) it was possible to obtain *in vitro* biocompatible bone allografts, which denotes the functionality of the universal protocol tested; (5) Was achieved *in vitro* recellularization of the ECMs obtained by seeding different cell types (HUVEC for vascular ECMs and BM-MSCs for bone ECMs).

Applicative value of research

The study represents a positive argument to the question of whether or not we can use a universal decellularization protocol in order to obtain vascularized composite bone ECMs (ducted tissue + soft tissue). Thus, we can consider the obtained results a strong scientific basis in the further elaboration of the action plan for research projects in vascularized bone allotransplantation based on *in vivo* studies, using MECOv obtained through IT.

Implementation of research results

The results collected in this study were used for another experimental *in vivo* study – ,,Treatment of large bone defects using a decellularized vascularized bone allograft incorporated into the recipient's vascular circuit". Thus, we expect the implementation of a new decellularization protocol for vascularized composite bone allgrafts within the Laboratory of Tissue Engineering and Cell Cultures of the *Nicolae Testemitanu* State University of Medicine and Pharmacy.

Approval af scientific results

The results of the study were presented within the following scientific forums: *MedEspera International Medical Congress for Students and Young Doctors*, Chişinau, Moldova, 3-5 mai, 2018. *Conferinţa ştiinţifică anuală a cadrelor ştiinţifico-didactice, doctoranzi, masteranzilor, rezidenţilor şi studenţilor*, USMF „Nicolae Testemiţanu". Chişinău, Republica Moldova, 16-18 octombrie 2019. *11th BAPRAS Congress, 12th National Congress of RSSH and 13th National Congress of RSRM*, Cluj-Napoca, România, May 9-11, 2019. *MedEspera International Medical Congress for Students and Young Doctors*, Chişinau, Moldova, 24-26 septembrie, 2020. *Conferinţa ştiinţifică anuală a cadrelor ştiinţifico-didactice, doctoranzi, masteranzilor, rezidenţilor şi studenţilor*, USMF „Nicolae Testemiţanu". Chişinău, Republica Moldova, 20-22 octombrie 2021. *International european conferince on interdisciplinary scientific research*. Warsaw, Poland, August 8-9, 2021. *19th National Congress of the Romanian Association of Plastic Surgeons, 14th National Congress of the Romanian Society of Reconstructive Microsurgery, 13th National Congress of the Romanian Society for Surgery of the Hand*. Timişoara, România, April 28-30, 2022. *Conferinţa ştiinţifică naţională a ortopezilor şi traumatologilor din Republica Moldova, ediţia a XVIII-a ,,Leziunile locomotorului – principii minim invasive de tratament"*, Chişinău, Republica Moldova, 25 iunie 2022. *National Scientific Conference with International Participation „Cells and tissues transplantation. Actualities and*

perspectives" dedicated to the 10 anniversary of the founding of the Human Tissue and Cells Bank and to the 15th anniversary of the founding of the Laboratory of Tissue Engineering and Cells Culture of „Nicolae Testemitanu" State University of Medicine and Pharmacy. Chişinau, Republic of Moldova, March 17-18th 2023. *6th International Conference on Nanotechnologies and Biomedical Engineering – ICNBME-2023*. Chişinau, Republic of Moldova, September 20-23, 2023.

Research publications

On the research topic, 18 scientific papers were published, including: articles in international journals with IF – 2, articles in Conference Proceeding indexed in SCOPUS – 3, articles in national journals B+ – 1, materials/theses at international conferences – 3, materials/theses at international conferences organized in the Republic of Moldova – 2, materials/theses at conferences (national conferences) – 7. During the research period, 2 innovator certificates were obtained.

Structure of the thesis

The thesis is presented over 143 pages and includes: table of contents, list of abbreviations, 13 tables, 45 figures, introduction, 4 chapters, general conclusions, and practical recommendations. The work concludes with a reference list composed of 257 titles, 2 appendices, the author's CV, and a declaration of responsibility. **Keywords:** bone allograft, composite allograft, vascularized allograft, decellularization, tissue engineering, extracellular matrix, recellularization, biocompatibility. **Keywords:** bone allograft, composite allograft, vascularized allograft, decellularization, tissue engineering, extracellular matrix, recellularization, biocompatibility.

THESIS CONTENT

1. The chapter **ROLE OF TISSUE ENGINEERING IN OBTAINING ALLOGRAFTS** represents an review of the specialized publications in view of the methods of obtaining vascularized bone ECM. The basics for a qualitative ECM are reviewed, as well as the most acute problems encountered in the *in vivo* transplantation of natural ECM.

TE is a promising branch, with amazing evolution in recent years, which aims at tissue regeneration [15, 18, 19, 20]. Through the use of nanotechnologies, 3D bioprinting techniques, multiple cell types (stem) and of course through the use of ECMs, IT has as its ultimate goal the creation of tissue grafts for the effective use of replacement therapy for non-functional organs or segments, through cell replacement or regeneration [21, 22, 23, 24]. Decellularization of organs, organ segments and tissues ensures the obtaining of three-dimensional acellular scaffolds that can later be used as an material in reconstruction [25, 26].

At the moment, the problem of massive bone defects is one of the basic pillars in causing functional disability [21]. The gold standard autologous graft represents a perfect combination of mineralized ECM, bone marrow and osteogenic cells, thus forming the most osteogenic material available, with osteoinductive and osteoconstructive properties. However, the available amount of such biological material is limited [27, 28, 29]. The naturally occurring ECM is an ideal biological scaffold for the adhesion, growth and differentiation of new cells in the process of remodeling different types of tissues or organs both *ex vivo* and *in vivo* [30, 31, 32]. Currently, several techniques, along with their advantages and disadvantages, are used to achieve bone tissue decellularization [33, 34]. The competence of removing cells from an organ/segment is based on physical, chemical or enzymatic approaches. The decellularization process is most often based on the combination of these 3 methods to obtain the desired final result [35, 36]. The development of protocols for obtaining the correct ECM, with subsequent recellularization, would allow the

evolution of regenerative medicine to another level, which in combination with technical advances would allow for the creation of personalized ECMs for the future.

The effectiveness of decellularization refers not only to the removal of cellular components, but also to the preservation of ECM. ECM biocompatibility is one of the basic characteristics of an applicable matrix [37]. The basic importance of decellularization processes is the removal of immunogenic elements from allografts or xenografts with the ECM components intact, so as to minimize the release of molecules associated with the lesions after transplantation. I can say with certainty that the interaction between antigens and antibodies at the time of transplants of a damaged, insufficiently decellularized, toxic or insufficiently sterilized of the ECM, further activates the immune cascade and creates a vicious circle that ends with transplant failure. Despite encouraging results on the applicability of ECMs, their immunogenicity may be a barrier that needs to be addressed. Recognizing the factors contributing to the immunogenicity of ECM will allow us to find solutions for the manufacture of scaffolds with good biocompatibility *in vivo* that could be considered applicable for transplantation in the near future [38, 39].

2. RESEARCH MATERIALS AND METHODS

This work relates to a preclinical experimental study, carried out on biological samples taken from laboratory animals (pigs). This study was carried out at the Laboratory of Tissue Engineering and Cell Cultures of the *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova and the Leibniz Research Laboratory of Biotechnology and Artificial Organs (LEBAO), Hannover School of Medicine (MHH), Germany, during 2017-2022. The biological material (vacular grafts, avascular bone grafts, vascularized composite bone grafts) was collected from laboratory animals, pigs (females and males) with an age around 3 months, and a body weight of 35-40 kg. The basic objective of this chapter is the detailed description of the material and methods that I've used during the experimental study to obtain vascularized bone ECM by describing: **(**i) the surgical protocol for procurement of the vascular grafts, avascular bone grafts (cortical and spongy) and bone grafts on the vascular pedicle; **(**ii) the decellularization protocol used; **(**iii) the qualitative and quantitative methods used to test the efficacy of the protocol; (iv) the method used to test the *in vitro* biocompatibility of the obtained allografts (vascular and bone).

Surgical procurement

The hind limb of the piglet, in the first hour after euthanasia, was guided by the anatomical landmarks described in the literature [40, 41]. The dissection was performed on 8 hind limbs, from 8 different piglets. The dissection were done in sterile conditions. Following the dissection, I've collected vascular grafts of different diameters, avascular bone grafts and vascularized bone grafts. In order to test the functionality of the vascular pedicle in vascularized bone graft, and to select the exact dimensions of the bone component, I infused the bone graft with a solution containing methylene blue for 24 h using an infuzomate. Thus, in the end I've obtained a tibial bone graft with a length of about 5 cm, vascularized by the vascular pedicle, the nutritive artery - the branch of the popliteal artery

In order to eliminate confusion, since most of the data in the literature use the term ,,small diameter blood vessels" for arterioles, I will use the combination of words ,,large diameter vessel" for the carotid artery, ,,medium diameter vessel" for the femoral artery, and ,,small diameter vessel" for the caudal tibial artery. I consider this classification real because in some sources, the classification of arteries by diameter uses the term ,,small-caliber vessel" for vessels with a

diameter of 0.3 mm to 10 μm. The vascular and bone grafts collected for the study are shown in Figure 1.

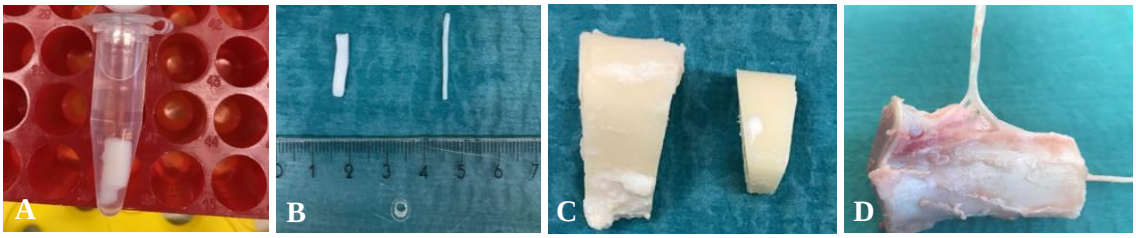

Figure 1. **The grafts used in the study for decellularization.** (A) large caliber vessel, carotid artery, (B) medium and small caliber vascular grafts, superficial femoral artery and caudal tibial artery, (C) avascular bone grafts, (D) vascularized composite bone graft.

Decellularization protocol

The vascular and bone grafts were processed according to the following steps: 1h - isotonic solution, which was obtained from the dilution of 0.5 ml of 0.5 mM (equivalent to 0.1 g of 372.24 g/mol) EDTA (ethylenediaminetetraacetic acid/Ethylenediamine tetraacetic acid) per 100 ml PBS solution (phosphate buffer saline solution/Phosphate Buffered Saline); 16 h - a solution containing a chelating agent, which was obtained by diluting 0.5 ml of 0.5 mM (equivalent to 0.1g of 372.24 g/mol) EDTA per 100 ml TRis buffer 10 mM with ph 7.5; 1 h - PBS solution; 24 h - with a solution containing an anionic detergent obtained by dissolving 0.5 gr SDS (Sodium dodecyl sulfate) in 100 ml Tris buffer 10 mM with pH 7.5; 7 h - PBS, with the solution changed every 1 h; 24 h - a solution containing a non-ionic detergent that was obtained by diluting 1 ml Triton X-100 per 100 ml Tris buffer 10 mM with pH 7.5; overnight - PBS, with changing the solution 7 times in the first hour; 48 h - DNase 300 U/ml, at 37°C, the solution being changed every 24 h, DNase 300 U/ml was obtained by diluting 1 mg DNase with the activity of 5279 U/mg in 17.5 ml PBS and 17.5 μl 1 M MgCl2.

For vascularized bone grafts, in the 8th step of graft processing, where the enzymatic solution was used, we applied measures to rationalize the research costs (decrease the volume of the solution used) and to reduce the number of animals needed for the study (use of technical replicates). Thus, I've collected 3 bone samples from the cancellous segment of the graft and 3 bone samples from the cortical segment (the dimensions of the graft allowed this). The technical replicates were collected with the help of a manual beige and placed in 50 ml tubes, at 37°C, being subjected to enzymatic processing with the help of a digital roller stirrer. Subsequently, the samples were examined qualitatively and quantitatively. The remaining graft was stored at 4°C in PBS+ antibiotic solution for further examination. The solution was changed every 24 hours for grafts that required longer storage.

Static-decellularization using a digital shaker with oscillating movements was used for vascular grafts, and a roller mixer for avascular bone grafts. Perfusion-decellularization was used for the processing of the vascularized composite bone graft, the solutions were perfused through the vascular pedicle *vs* diaphysis using the peristaltic pump with a closed circuit of solutions and at a speed of 15 ml/h. The methods used are presented in Figure 2.

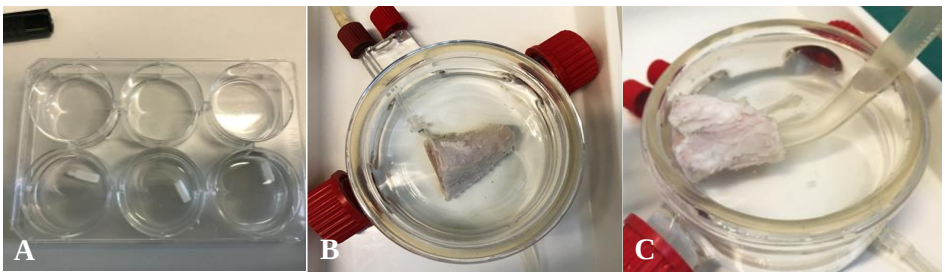

Figure 2. **The decellularization process.** (A) static-decellularization, (B) diamanic decellularization with perfusion of solutions through the popliteal artery, (C) dynamic decellularization with perfusion of solutions through the diaphysis of the tibial bone.

Assessing the effectiveness of decellularization

The effectiveness of the decellularization protocol was tested using qualitative and quantitative histological examination, as well as *in vitro* biocompatibility assay. After the decellularization was completed, the vascular grafts, periosteum and avascular bone grafts were qualitatively examined. For the qualitative histological examination were performed H&E and DAPI staining which allowed the visualization of cell nuclei and DNA. The histology was performed in the same time for decellularized tissue *vs* native tissue. The native tissue was collected from the same graft until the beginning of the processing process and it was used as a control sample. For avascular bone grafts, were additionally performed the DNA quantification test. The vascularized bone grafts were examined quantitatively (DNA quantification) and qualitatively (SEM) separately for the cortical segment of the graft and the cancellous segment (native *vs* decellularized). The *in vitro* biocompatibility test was performed for vascular ECM, avascular bone ECM, and vascularized bone ECM.

Biocompatibility test

Biocompatibility by *in vitro* recellularization has been tested for vascular ECM, avascular bone ECM and vascularized bone ECM. For recellularization, different types of cell cultures were used. The biocompatibility test of vascular ECM was performed using human umbilical endothelial cells (HUVEC). The HUVEC cells used in this study were transposed by lentiviral transduction with green fluorescent protein (GFP) for visualization under a fluorescent microscope, cryopreserved and stored at -80°C. Bone ECM recellularization was performed using allogeneic BM MSC. BM MSC cells were stained with PKH26 for viewing under a fluorescent microscope (Figure 3).

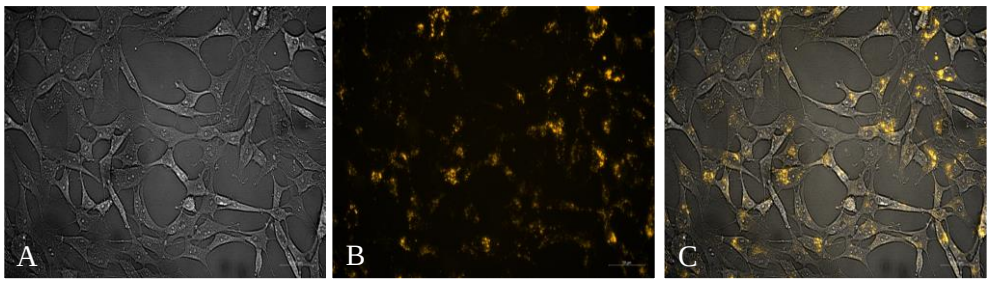

Figure 3. **Primary stem cells (BM MSC). PKH26 staining.** (A) the image of the cells obtained in the light field of the microscope. (B) image of cells obtained in Cy3 field (stained cells provides a gold color). (C) the image of the cells obtained in the overlapping fields (bright + Cy3). Scale. A, B, C: 50 μm

3. RESULTS OF THE STUDY

Vascular permeability test results for vascularized bone graft

This method of testing the functionality of the vascular pedicle was used to evaluate the perfusion of the bone segment specific to the vascular pedicle in an *in vitro* controlled environment, which means that the dimensions of the bone graft were adjusted depending on the degree of blue staining of the graft. The most pronounced colored area has been preserved (Figure 4).

Figure 4. **Arterial perfusion test results.** (A) the first hour after perfusion of the methylene blue solution. (B) the parameters of the infuzomate. (C) adjustment of the size of the bone part of the graft 24 h after perfusion.

Histological examination (H&E and DAPI)

H&E and DAPI histological staining demonstrated the efficacy of the decellularization protocol by effectively removing cell nuclei in vascular grafts (large, medium and small diameter) and in cortical and cancellous avascular bone grafts. DAPI staining also showed a lack of DNA material in all processed grafts. All these under the conditions of preserving the integrity of the vascular and bone ECMs (Figure 5, Figure 6, Figure 7).

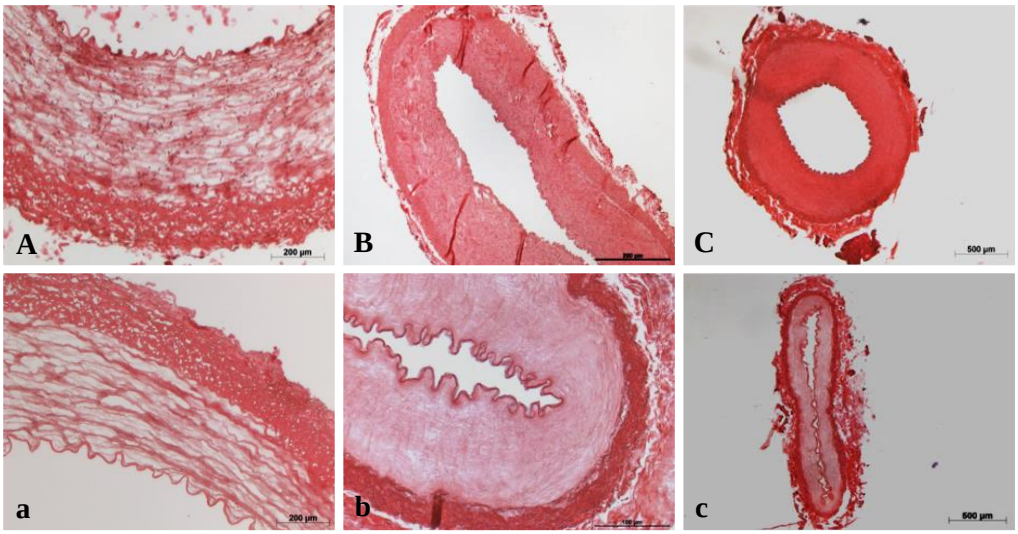

Figure 5. **Native *vs* decellularized vascular graft. H&E staining.** (A, B, C) native vascular grafts. (a, b, c) decellularized vascular grafts. (A, a) carotid artery. (B, b) superficial femoral artery. (C, c) caudal tibial artery. Scale. A, B, a: 200 μm; b: 100 μm; C, C: 500 μm

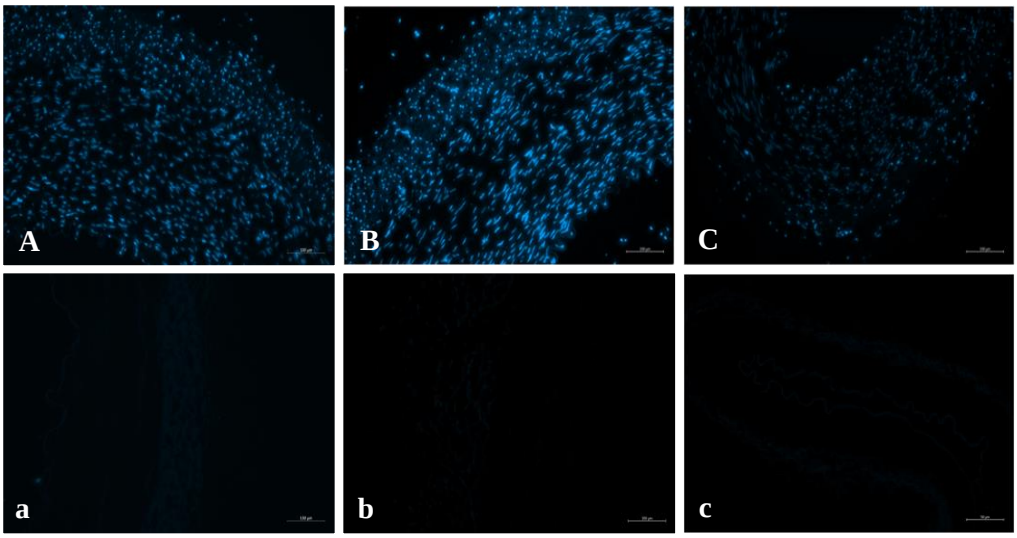

Figure 6. **Native *vs* decellularized vascular graft. DAPI staining.** (A, B, C) native vascular grafts. (a, b, c) decellularized vascular grafts. (A, a) carotid artery. (B, b) superficial femoral artery. (C, c) caudal tibial artery. Scale. A, B, C, a, b, c: 100 µm

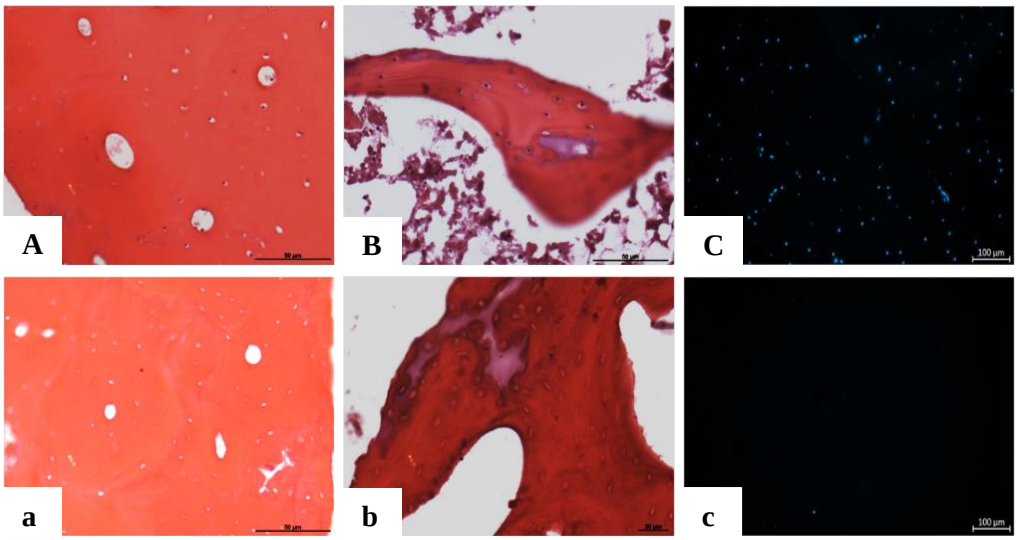

Figure 7. **Native *vs* decellularized bone graft. H&E and DAPI staining.** (A, B, C) native grafts. (a, b, c) decellularized grafts. (A, a) H&E staining for cortical graft. (B, b) H&E staining for cancellous graft. (C, c) DAPI staining cortico-cancellous graft. Scale. A, B, a: 50 µm; b: 20 µm; C, c:100 µm

Scanning electron microscopy

The analysis of the images obtained at SEM, shows that the decellularization process did not significantly modify the cortical bone ECM structure, confirming the efficacy and preservation of the structural integrity of the bone during the decellularization treatment. As for the cancellous bone samples, they were found to be more sensitive to decellularization processing. This sensitivity was manifested by the presentation of rougher surfaces in the processed samples compared to the native cancellous bone samples. In addition, the bone structure lost its contour uniformity over the entire examined surface, indicating a change in the microstructural appearance of the cancellous bone tissue following the decellularization process. The images obtained at SEM for cortical and cancellous native samplies *vs* decellularized samplies are presented in Figure 8.

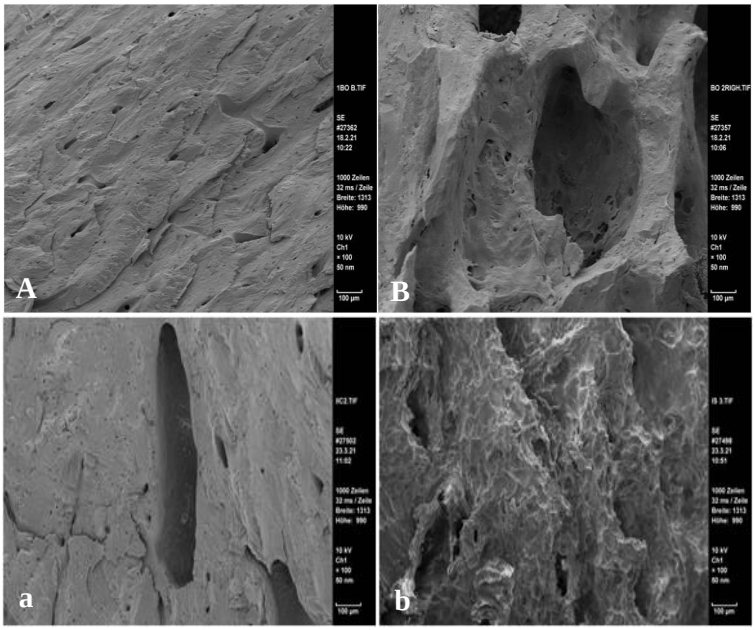

Figure 8. **Cortical and cancellous bone graft, native *vs* decellularized. SEM.** (A) native cortical bone, (a) decellulized cortical bone. (B) native cancellous bone, (b) decellularized cancellous bone. Scala. A, B, C, D: 100 μm

DNA quantification

The statistical analysis of the obtained results demonstrated a statistically insignificant decrease after decellularization of DNA in all grafts for the cortical component ($p>0.05$) and a statistically significant decrease in DNA for the cancellous component ($p<0.05$). All data obtained from the DNA measurement are presented in Table 1.

Other preliminary conclusions after analyzing the results suggest that compared to native cancellous bone, native cortical bone contains a much lower amount of DNA, even more, in some cases the amount of DNA in native cortical bone was less than in decellularized cancellous bone. Thus, I can say that the removal of DNA from the cortical component can be considered less important than the removal of DNA from the spongy component.

Since the literature does not provide precise data on the relationship between the amount of DNA present in the transplanted graft and the provoked immune response, we assume that the amount of DNA remaining in the decellularized vascularized composite graft may be insufficient to trigger an immune response and, implicitly, an acute or chronic rejection of the vascularized composite bone implant. However, definitive confirmation of this can only be obtained from *in vivo* studies.

As for the results indicating an insignificant increase in the amount of DNA in the cortical component of the composite graft when infusing decellularization solutions through the popliteal artery, I assume that these results are not an error. For 3 different grafts, taken from 3 different animals, decellularized with perfusion of solutions through the popliteal artery, we determined the insignificant growth of DNA in the cortical area in 2 grafts. This phenomenon can be explained by the washing of cells/cellular components along the bone structure, which led to the migration of DNA from the spongy region during graft processing.

Considering the above considerations, I assume that the way of perfusion of decellularization solutions through the popliteal artery, provides a better result when removing DNA from the vascularized bone graft after decellularization.

Tabelul 1. **DNA quantification of the bone grafts.**

Tissue type		**DNA concentration (µg/mg), average of technical triplicates**		
		Sample I	**Sample II**	**Sample III**
DNA quantification of avascular cortico-cancellous graft				
Native tissue		3.964	4.547	4.868
Decellularized tissue		3.156	3.676	4.01
DNA quantification of vascularized bone ECM processed by perfusion through the vascular pedicle				
NATIVE	Cortical	1.015	2.647	3.835
	Cancellous	9.978	6.654	8.51
DECELLULARIZED	Cortical	1.949	3.706	4.122
	cancellous	0.862	0.488	0.969
DNA quantification of vascularized bone ECM processed by perfusion through the diaphysis				
NATIVE	Cortical	0.912	1.622	1.564
	Cancellous	4.705	3.931	3.653
DECELLULARIZED	Cortical	0.706	1.086	1.097
	Cancellous	0.949	1.069	1.074
DNA quantification of vascularized bone ECM processed by perfusion through the vascular pedicle using 1% SDS				
NATIVE	Cortical	2.097	2.638	3.729
	Cancellous	20.132	19.383	20.404
DECELLULARIZED	Cortical	0.970	3.899	4.067
	Cancellous	3.462	4.645	5.695
DNA quantification of vascularized bone ECM processed by perfusion through the vascular pedicle and used for *in vitro* recellularization				
NATIVE	Cortical	4.950	3.962	4.508
	Cancellous	9.991	8.386	9.722
DECELLULARIZED	Cortical	5.166	4.520	3.211
	Cancellous	2.134	2.928	2.049

Biocompatibility test (*in vitro* recellularization)

Vascular ECM biocompatibility was tested by seeding of HUVEC-GFP cells on the vascular lumen. After 72 hours of preconditionong of the vascular ECM in specific cellular media at 37°C Thus, cell seeding and monitoring demonstrated that the vascular graft decellularized by the protocol used allows the attachment and multiplication of seeded cells. A progressive increase in the number of cells on the vascular ECM was observed for 8 days, the period for which cell growth was performed (Figure 5).

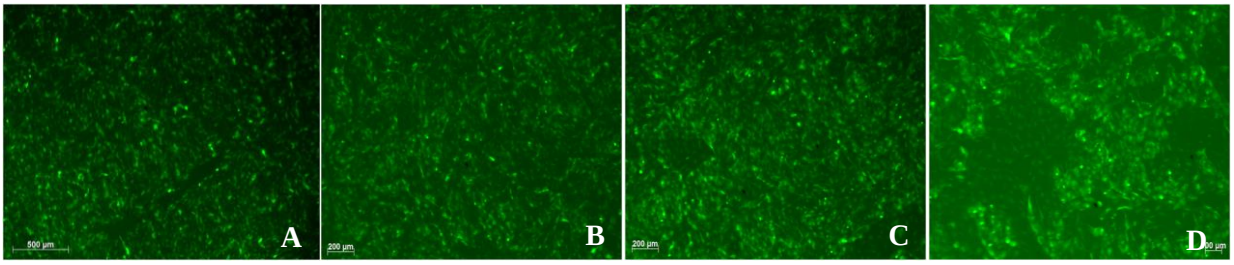

Figure 9. **Recellularization of vascular ECM. Preconditioning – 72h. HUVEC-GFP cells on the vascular ECM lumen.** (A) HUVEC-GFP cells on day 2. (B) HUVEC-GFP cells on day 4. (C) HUVEC-GFP cells on day 6. (D) HUVEC-GFP cells on day 8. A: 500 μm; B, C: 200 μm; D: 100 μm

The *in vitro* biocompatibility of avascular bone ECM was tested by seeding PKH26-labeled BM MSC. Thus, a progressive increase in the number of cells on the bone extracellular scaffold was observed for 8 days, during which time cell growth was performed (Figure 10).

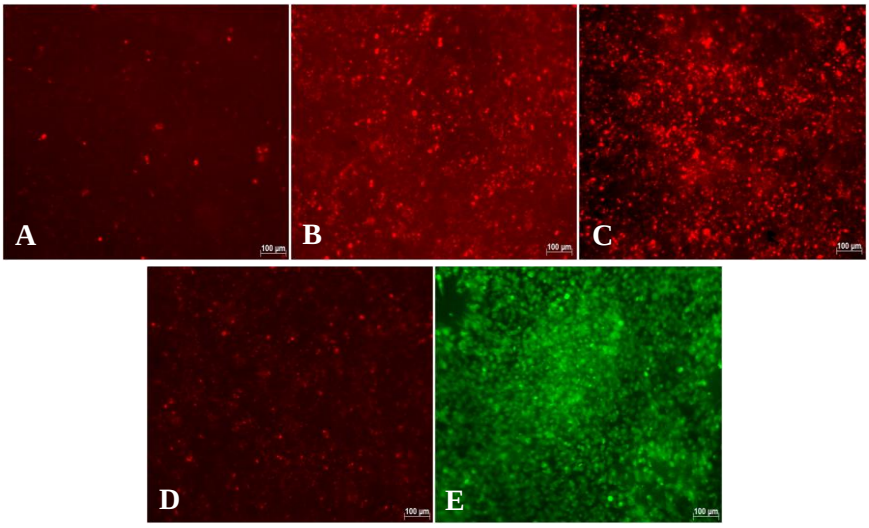

Figure 10. **Recellularization of avascular bone ECM. BM MSC, PKH26 and Calcein staining.** (A) MB MSC cells on day 2, PKH26 staining. (B) MB MSC cells on day 4, PKH26 staining. (C) MB MSC cells at day 6, PKH26 staining. (D) MB MSC cells at day 8, PKH26 staining. (E) MB MSC cells on day 8, Calcein staining. Scale. A, B, C, D, E: 100 μm;

The biocompatibility of vascularized bone ECM was tested by seeding BM MSC labeled PKH26. The cells were seeded on the external surface of the bone graft, at the level of the periosteum and inside the vascular pedicle. On day 2, a satisfactorily number of cells was determined. Towards day 4, a slight decrease in the cell number on the external surface of vascularized bone ECM was determined, however, the presence of viable cells inside the graft was determined, even if the cells were not seeded inside. Likewise, on the last day of testing (day 5), we determined viable cells at the level of the periosteum and on the external surface of the vascular pedicle (Figure 11).

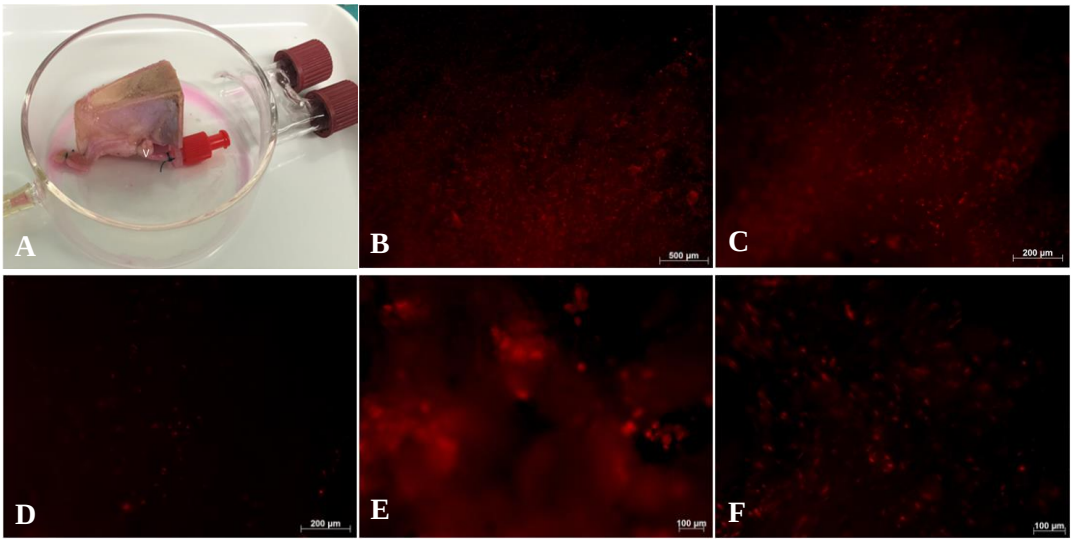

Figure 11. **Vascularized bone ECM *in vitro* recellularization. MB MSC-PKH26 cells.** (A) The vascularized bone allograft. (B) BM MSC cells on the outer surface of the graft, day 2. (C) BM MSC cells on the outer surface of the graft, day 4. (D) BM MSC cells on the outer surface of the graft, day 5. (E) BM MSC cells on the inside part of the graft, day 5. Scale. B: 500 µm; C, D: 200 µm; E, F: 100 µm

GENERAL CONCLUSIONS

1. The proximal third of the tibial bone is a promising source for the development of vascularized composite bone allografts. These grafts can serve as a platform for studying the processes of obtaining the vascularized and decellularized bone extracellular matrix.
2. The optimal method of decellularization of the bone graft while preserving the vascularization source can be a protocol based on SDS detergent with a concentration of 0.5% and an enzymatic solution (DNase). This protocol allows the obtaining of vascularized bone extracellular matrices, preserving the integrity of the vascular pedicle.
3. The use of qualitative methods (H&E, DAPI, SEM) and quantitative methods (DNA quantification) is mandatory for testing the efficacy of the decellularization method of vascularized composite bone grafts. Only the overall interpretation of the results obtained can predict the *in vivo* biocompatibility of the allograft.
4. The use of HUVEC cells and bone marrow stem cells is a valuable source of cells for *in vitro* testing of the recellularization potential of the vascular and bone extracellular matrix. Achieving *in vitro* recellularization of vascularized bone extracellular matrix may suggest a low immunogenic potential and a high degree of allograft biocompatibility.

PRACTICAL RECOMMENDATIONS

Recommendations that may be useful for future preclinical studies are:

1. Using the proximal third of the tibia as a safe material for *in vitro* testing of methods of obtaining vascularized bone extracellular matrices decellularization;
2. To use the enzyme solution in the decellularization protocol as a mendatory step for the efficient removal of DNA;
3. To use preconditioning methods of the decellularized extracellular matrix to emphasize its basic properties and support cell viability, adhesion and multiplication;

4. To test *in vivo* the biocompatibility and degree of immunogenicity of vascularized bone allograft obtained by decellularization;
5. To assess the degree of integration *in vivo* by evaluating the remodeling process of the decellularized vascularized bone allograft and testing its mechanical strength after transplantation using functional and imaging tests.

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LIST OF PUBLICATIONS AND SCIENTIFIC EVENTS

at which the results of the research for the doctoral thesis in Medicine Science with the topic **„Preparation of vascularized bone allograft – experimental study"** were presented

I. Articles in scientific journals

- **Articles in international scientific journals:**

1. **Alina Stoian**, Aisha Adil, Felor Biniazan, Siba Haykal. Two Decades of Advances and Limitations in Organ Recellularization. In: *Current Issues in Molecular Biology* 2024, 46(8), 9179-9214; DOI: 10.3390/cimb46080543 (IF: 2.8).
2. Felor Biniazan*, **Alina Stoian***, and Siba Haykal. Adipose-Derived Stem Cells: Angiogenetic Potential and Utility in Tissue Engineering. In: *International Journal of Molecular Sciences.* 2024 Feb; 25(4): 2356. Published online 2024 Feb 16. PMCID: PMC10889096. PMID: 38397032. DOI: 10.3390/ijms25042356 (IF: 5.6).

- **Articles in accredited national scientific journals:**
- ✓ **Articles in scientific journals category B+**

3. Pavlovschi E., **Stoian A.**, Verega Gr., Nacu V., In vivo experimental study of the arterial supply of the rabbit posterior limb. In: *Moldovan Medical Journal*. 2021; 64(6): pp. 26-32. ISSN 2537-6373. DOI: 10.52418/moldovan-med-j.64-6.21.05.

- **Articles in the papers of scientific conferences:**
- ✓ **Articles in conference papers included in the Web of Science and SCOPUS database**

4. Malcova T., Globa L., Vascan A., Ţugui E., **Stoian A.**, Nacu V. Evolution of the efficacy of decellularization treatment in preparing decellularized umbilical cord artery. In: *Tiginyanu I., Sontea V., Railean S. (eds). Springer, Cham, 4th International Conference on Nanotechnologies and Biomedical Engineering.* ICNBME 2019. IFMBE Proceedings, 2020; vol 77: pp. 589-593. ISBN: 1680-0737 / ISBN: 1433-9277 (electronic). ISBN 978-3-030-31865-9 ISBN 978-3-030-31866-6 (eBook). DOI: 10.1007/978-3-030-31866-6 (SJR: 0.155, SCOPUS).
5. **Stoian A.**, Pavlovschi E., Capros N., Verega Gr., Nacu V. Effectiveness of tissue engineering in obraining of the vascularized composite bone extracellular matrix. Experimental study. In: *Tiginyanu I., Sontea V., Railean S. (eds). Springer, Cham, 6th International Conference on Nanotechnologies and Biomedical Engineering. ICNBME 2023; IFMBE Proceedings*. 2023; vol 91: pp. 357-365. ISBN 978-3-031-42774-9 / ISBN: 978-3-031-42775-6 (online). DOI: 10.1007/978-3-031-42775-6_39 (SJR: 0.155, SCOPUS).
6. Pavlovschi E., **Stoian A.**, Verega Gr., Nacu V. The Critical Size Bone Defects - In-Vivo Experimental Method of the Treatment with the Decellularized Vascularized Bone Allografts. *In: Tiginyanu I., Sontea V., Railean S. (eds). Springer, Cham, 6th International Conference on Nanotechnologies and Biomedical Engineering. ICNBME 2023; IFMBE Proceedings.* 2023; vol 91: pp. 332-347. ISBN 978-3-031-42774-9 / ISBN: 978-3-031-42775-6 (online). DOI: 10.1007/978-3-031-42775-6_37 (SJR: 0.155, SCOPUS).

II. **Abstracts / theses at scientific conferences/congresses**

- **International conferences abroad:**

7. Malcova T., Globa T., Vascan A., Ţugui E., **Stoian A.**, Nacu V. Evaluation of the efficacy of decellularization treatment in preparing decellularized umbilical cord artery. In: *Abstract Book. International molecular medicine symposium*. Istanbul, Turcia: 2019: p. 76.
8. **Stoian A.**, Pavlovschi E., Verega Gr., Nacu V., Andree B., Hilfiker A., Experimental study in non/imunogen vascularized bone allograft. In: *Abstract book of 3in1 joint congress in plastic and reconstructive surgery*. Timişoara, România, 2022: p. 36. ISBN: 978-606-786-274-4.
9. Pavlovschi E., **Stoian A.**, Verega Gr., Nacu V., The vascularized bone allotransplantation in vivo experiment. Preliminary report. In: *Abstract book of 4th international european conferince on interdisciplinary scientific researcher*. Warsaw, Poland, 2021: p. 264. ISBN: 978-1-955094-13-9.

- **International conferences organized in Republic of Moldova:**

10. Pavlovschi E., **Stoian A.**, Mihaluta V. The vascularized allotransplant– successful alternative for massive bone defects. In: *Abstract book of Med Espera International Medical Congress for Students and Young Doctors*. Chişinău. Republica Moldova, 2018: pp. 143-144. DOI: ibn.idsi.md/vizualizare_articol/114348.
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- **National conferences:**

12. **Stoian A.**, Pavlovschi E., Verega Gr., Birgit A., Hilfiker A., Nacu V. Experimental study in obtaining of a vascularised composite bone extracellular matrix. In: *Abstract Book. Conferinţa Ştiinţifică Anuală*. Cells and tissues transplantation. Actualities and Perspectives. Chişinau: 2023, pp. 40. CZU: 617.7. DOI: repository.usmf.md/handle/20.500.12710/24272.
13. Pavlovschi E., **Stoian A.**, Gardikiotis I., Verega Gr., Nacu V., Cernei G. The vascularized bone allotransplantation after decellularization process, in vivo testing. In: *Abstract Book. Conferinţa Ştiinţifică Anuală*. *Cells and tissues transplantation. Actualities and Perspectives*. Chişinau: 2023, pp. 37. CZU: 616-089.57.085. DOI: 20.500.12710/24267.
14. Pavlovschi E., **Stoian A.**, Malcova T., Iordăchescu R., Verega Gr. Decelularizarea combinată a allografei osoase vascularizate. Etapă de studiu experimental in vivo. In: *Abstract Book. Congresul Consacrat aniversării a 75-a de la fondarea USMF „Nicolae Testemiţanu”*, Chişinău: CEP „Medicina”, 2020, p. 519. DOI: ibn.idsi.md/vizualizare_articol/126485.
15. **Stoian A.**, Nacu V., Pavlovschi E, Macagonova O., Malcova T., Mihaluta V. Perspectiva de viitor a alotransplantului osos vascularizat. In: *Abstract Book. Congresul Consacrat aniversării a 75-a de la fondarea USMF „Nicolae Testemiţanu”*. Chişinău: CEP „Medicina”, 2020, p. 525. DOI: ibn.idsi.md/vizualizare_articol/126554.
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18. **Stoian A.**, Pavlovschi E., Nacu V., Sladic S., Andree B., Hilfiker A., Principles of decellularization for composite vascularized bone graft. In: *Abstract book. Conferinţa Ştiinţifică Anuală. Cercetarea în biomedicină și sănătate: calitate, excelență și performanță. Chişinău: CEP „Medicina”*, 2021, p. 337. ISBN 978-9975-82-223-7 (PDF). DOI: ibn.idsi.md/vizualizare_articol/144677.

III. **Patents for inventions, patents, registration certificates, materials at invention salons**

19. **Stoian A.**, Pavlovschi E., Verega Gr., Nacu V. *Metodă de decelularizare pentru grefele osoase compozite vascularizate.* Certificat de inovaţie nr. 6058 din 24 mai 2023.
20. Pavlovschi E., **Stoian A.**, Nacu V., Verega Gr. *Alografele osoase vascularizate decelularizate ca metodă de tratament a defectelor osoase critice.* Certificat de inovaţie nr. 6052 din 16 mai 2023.

IV. Participation with communications in scientific forums

- **International:**

21. **Stoian A.**, Pavlovschi E., Mihaluta V., Iordachescu R., Verega Gr., Nacu V. Initiation of the experimental study in obtaining of vascularized bone allograft. In: *11th BAPRAS Congress*,

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V. **Participation with posters in scientific forums / exhibitions**

- **International:**

33. Malcova T., Globa L., Vascan A., Ţugui E., **Stoian A.**, Nacu V. Evolution of the efficacy of decellularization treatment in preparing decellularized umbilical cord artery. In: *International Molecular Medicine Symposium by the Bosphorus.* Istanbul, Turcia, 16-18 mai 2019.

- **National:**

34. **Stoian A.**, Nacu V., Pavlovschi E., Macagonova O., Malcova T., Mihaluţa V. Perspectiva de viitor a alotransplantului osos vascularizat. În: *Congresul consacrat aniversării a 75-a de la fonsarea USMF „Nicolae Testemiţanu".* Ediţie online, Chişinau, 21-23 octombrie 2020.
35. Pavlovschi E., **Stoian A.**, Malcova T., Iordăchescu R., verega G., Nacu V. Deceluarizarea combinată a alografei osoase vascularizate. Etapă de studiu experimental in vivo. În: *Congresul Consacrat aniversării a 75-a de la fonsarea USMF „Nicolae Testemiţanu*". Ediţie online, Chişinau, 21-23 octombrie 2020.

VI. Educational courses and scholarship programs

36. State project No. 20.80009.5007.20, *Nanoarhitecturi în bază de GaN şi matrici tridimensionale din materiale biologice pentru aplicaţii în microfluidă şi inginerie tisulară*. GA 810652. Chişinău, January 2020 - June 2023.
37. European Union project No. 810652 *Horizon 2020, NanoMed TWIN*. Hannover, Germania, 01 Octombrie 2020 - 31 Martie 2021.
38. Training Course on Intellectual Property Protection and Technology Transfer in the framework of the Horizon2020 project „NanoMedTwin". Online. Chisinau, 01 October-19 December 2020.
39. *Eugen Ionescu* research fellowship, 2021-2022. Scientific research, volunteering. *Grigore T. Popa* UMPh from Iaşi, România, Decembrie 2021 - Februarie 2022.

ANNOTATION

Stoian Alina „**Preparation of vascularized bone allografts – experimental study**". The thesis for the degree of PhD in medical sciences, Chișinău, 2024.

Thesis structure: the thesis is presented over 143 pages and includes: table of contents, list of abbreviations, 13 tables, 45 figures, introduction, 4 chapters, general conclusions, and practical recommendations. The work concludes with a reference list composed of 257 titles, 2 appendices, the author's CV, and a declaration of responsibility. The results of the study have been published in 18 scientific papers.

Keywords: bone allograft, composite allograft, vascularized allograft, decellularization, tissue engineering, extracellular matrix, recellularization, biocompatibility.

Study Objective: to develop a universal decellularization protocol for tissues with varying degrees of mineralization for the purpose of obtaining vascularized composite bone grafts with minimal immunogenic qualities.

Study objectives: (1) To develop a surgical technique for obtaining vascularized bone grafts from tibial bone in laboratory animals; (2) To establish a decellularization protocol for bone grafts (tibial bone) while preserving the vascularization pedicle; (3) To test the effectiveness of the bone graft decellularization method using qualitative (H&E, DAPI, SEM) and quantitative (DNA quantification) techniques; (4) To test the biocompatibility of vascularized extracellular bone matrix through *in vitro* recellularization using different cell types.

Scientific novelty and originality: a universal decellularization protocol for composite bone grafts (hard tissue + soft tissue) was developed and tested. *In vitro* recellularization of vascular extracellular matrix, avascular bone extracellular matrix, and vascularized extracellular bone matrix was achieved using various cell types.

Scientific problem addressed in the thesis: the most important aspects of decellularization of soft and hard tissues were determined and characterized based on the same decellularization protocol. Subsequent *in vitro* recellularization of the obtained extracellular matrices was successfully achieved.

Theoretical significance of the thesis: drawing up a universal decellularization protocol for composite bone grafts, and the results collected following this study can be a useful scientific basis for subsequent *in vivo* studies.

Applicative value of the thesis: this study represents an important step in supporting future preclinical *in vivo* studies aimed at developing a new surgical method for treating massive bone defects of various origins in the locomotor system, through the use of vascularized decellularized bone allografts obtained via tissue engineering.

Implementation of scientific results: the results obtained have been used in another experimental *in vivo* study titled „Treatment of large bone defects using a decellularized vascularized bone allograft incorporated into the recipient's vascular circuit". Thus, we anticipate implementing a new decellularization protocol for vascularized composite bone grafts within the Tissue Engineering and Cell Cultures Laboratory at SUMF „Nicolae Testemitanu".

ADNOTARE

Stoian Alina **„Prepararea alografelor osoase pe pedicul vascular – studiu experimental".** Teza pentru obţinerea titlului de doctor în ştiinţe medicale, Chişinău, 2024.

Structura tezei: lucrarea este expusă pe 143 pagini şi include: cuprins, lista abrevierilor, 13 tabele, 45 de figuri, introducere, 4 capitole, concluzii generale şi recomandări practice. Lucrarea se finisează cu lista de referinţe alcătuită din 257 titluri, 2 anexe, CV-ul autorului și declaraţia autorului privind asumarea răspunderii. Rezultatele studiului au fost publicate în 18 lucrări ştiinţifice.

Cuvintele-cheie: alografă osoasă, alografă compozită, alografă vascularizată, decelularizare, inginerie tisulară, matrice extracelulară, recelularizare, biocompatibilitate.

Scopul studiului: Elaborarea protocolului universal de decelularizare a ţesuturilor cu divers grad de mineralizare în scopul obţinerii grefelor osoase compozite vascularizate cu calităţi imunogene minime.

Obiectivele studiului: (1) Elaborarea eseului chirurgical pentru obținerea grefei osoase vascularizate a osului tibial la animale de laborator; (2) Elaborarea protocolului de decelularizare a grefelor osoase (os tibial) cu păstrarea sursei de vascularizare; (3) Testarea eficacității metodei de decelularizare a grefelor osoase prin metode calitative (H&E, DAPI, SEM) şi cantitative (cuantificarea ADN-ului); (4) Determinarea biocompatibilităţii matricelor extracelulare osoase vascularizate prin recelularizarea *in vitro* utilizând diferite tipuri de celule.

Noutatea şi originalitatea ştiinţifică: am elaborat şi testat un protocol universal de decelularizare pentru grefele osoase compozite (ţesut dur + ţesut moale). S-a obţinut recelularizare *in vitro* a MEC vasculare, MEC osoase avasculare și a MEC osoase vascularizate utilizând diferite tipuri de celule.

Problema ştiinţifică rezolvată în teză: am determinat și caracterizat cele mai importante aspecte ale decelularizării țesuturilor moi și dure pe baza aceluiași protocol de decelularizare. Am reuşint ulteriora recelularizarea *in vitro* a matricelor extracelulare obţinute.

Semnificaţia teoretică: am elaborat un protocol universal de decelularizare pentru grefele osoase compozite (țesut moale + țesut dur), iar rezultatele colectate în urma acetui studiu pot servi o bază ştiinţifică utilă pentru ulterioarele studii *in vivo*.

Valoarea aplicativă a lucrării: studiul dat reprezintă un pas important în susţinerea viitoarelor studii preclinice *in vivo* pentru elaborarea unei noi metode chirurgicale în tratamentul defectelor osoase masive de diferită geneză la nivelul aparatului locomotor, prin utilizarea alogrefelor osoase decelularizate vascularizate obținute prin inginerie tisulară.

Implementarea rezultatelor ştiinţifice: rezultatele obţinute au fost utilizate în cadrul altui studiu experimental *in vivo* – „Plastia defectelor osoase vaste prin allografaj osos vascularizat decelularizat inclus în circuitul vascular adoptiv". Astfel, preconizăm implementarea în cadrul Laboratorului de Inginerie tisulară și culturi celulare a USMF „Nicolae Testemiţanu", a unui nou protocol de decelularizare pentru grefele osoase compozite vascularizate.

АННОТАЦИЯ

Стоян Алина „**Приготовление костных аллотрансплантатов на сосудистом педикуле – экспериментальное исследование**". Диссертация на соискание степени кандидата медицинских наук, Кишинев, 2024.

Структура диссертации: диссертация представлена на 143 страницах и включает: содержание, список сокращений, 13 таблиц, 45 рисунков, введение, 4 главы, общие выводы и практические рекомендации. Диссертация завершается списком литературы, состоящим из 257 наименований, 2 приложениями, резюме автора и заявлением автора о принятии ответственности. Результаты исследования были опубликованы в 18 научных работах.

Ключевые слова: костный аллотрансплантат, композитный аллотрансплантат, васкуляризованный аллотрансплантат, деклеткация, тканевая инженерия, внеклеточный матрикс, реклеткация, биосовместимость.

Цель исследования: разработка универсального протокола децеллюляризации тканей с различной степенью минерализации с целью получения васкуляризованных композитных костных трансплантатов с минимальными иммуногенными качествами.

Задачи исследования: (1) Разработка хирургической техники для получения васкуляризованных костных трансплантатов из тибиальной кости у лабораторных животных; (2) Установление протокола децеллюляризации костных трансплантатов (тибиальная кость) с сохранением источника васкуляризации; (3) Проверка эффективности метода децеллюляризации костных трансплантатов с использованием качественных и количественных методов; (4) Определение биосовместимости внеклеточных матриц костей с васкуляризацией через реклеткацию *in vitro* с использованием различных типов клеток.

Научная новизна и оригинальность: разработан и протестирован универсальный протокол децеллюляризации для композитных костных трансплантатов (твердая ткань + мягкая ткань). Достигнута рецеллюляризация *in vitro* сосудистого внеклеточного матрикса, avascular костных матриц и васкуляризованных костных матриц с использованием различных типов клеток.

Научная проблема, решенная в диссертации: определены и охарактеризованы наиболее важные аспекты децеллюляризации мягких и твердых тканей на основе одного и того же протокола децеллюляризации. Успешно осуществлена последующая рецеллюляризация *in vitro* полученных внеклеточных матриц.

Теоретическая значимость: разработан универсальный протокол децеллюляризации для композитных костных аллотрансплантатов (мягкая ткань + твердая ткань), а собранные результаты данного исследования могут служить полезной научной основой для будущих *in vivo* исследований.

Прикладная ценность работы: данное исследование представляет собой важный шаг в поддержке будущих доклинических исследований *in vivo* для разработки нового

хирургического метода лечения массивных костных дефектов различного происхождения в опорно-двигательном аппарате с использованием васкуляризованных децеллюляризации костных аллотрансплантатов, полученных с помощью тканевой инженерии.

Внедрение научных результатов: полученные результаты уже использованы в другом *in vivo* исследовании – „Пластика обширных костных дефектов децеллюляризированным васкуляризированным костным аллотрансплантатом, включенным в адоптивный кровоток". Таким образом, планируица внедрение нового протокола децеллюляризации васкуляризированных композитных костных аллотрансплантатов в рамках Лаборатории тканевой инженерии и клеточных культур Государственного Медицинского и Фармацевтического Университета им. Николае Тестемицану.

STOIAN ALINA

PREPARATION OF VASCULARIZED BONE ALLOGRAFTS – EXPERIMENTAL STUDY

321.18 ORTHOPEDICS AND TRAUMATOLOGY

Summary of PhD thesis in medical sciences

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