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RECONSTRUCTION OF TUBULAR BONE DEFECTS USING ALLOGRAFTS INCLUDED IN THE ADOPTIVE VASCULAR CIRCUIT (experimental study)

321.18 – ORTHOPEDICS AND TRAUMATOLOGY

SCIENTIFIC ABSTRACT
of the PhD Thesis in Medical Sciences

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The thesis was developed within the Department of Orthopedics and Traumatology and the Laboratory of Tissue Engineering and Cell Cultures, at the Nicolae Testemițanu State University of Medicine and Pharmacy, a founding member of the Consortium of the Doctoral School in Medical Sciences.

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INTRODUCTION

The use of bone transplantation represents a successful step in the treatment of a wide range of congenital, degenerative, infectious, tumoral, traumatic, and post-traumatic disorders of the osteoarticular system [1, 14, 17, 19]. Currently, numerous bone grafting techniques and substitutes are available for defect reconstruction, applied either as standalone methods or in combination. These include bone transport (Ilizarov technique), cryopreserved bone allotransplantation, vascularized and non-vascularized autologous bone grafting, endoprosthetic reconstruction, as well as synthetic bioinert or bioactive alternatives [1, 3, 8, 13, 78].

Critical-sized bone defects (CSDs), particularly circular defects located at the diaphyseal level of tubular bones of the pelvic limb, represent a major challenge for contemporary reconstructive orthopedic surgery [8, 15]. Vascularized autologous bone grafting is considered by some authors to be the "gold standard" due to the preservation of the full biological properties of bone tissue [2, 4]. However, other studies indicate that, to date, no ideal solution exists for the reconstruction of such extensive tubular bone defects [5, 8, 14, 16].

Adequate bone regeneration depends on optimal vascularization of the fracture site, provided by intramedullary arteries, periosteal vessels, and metaphyseal circulation. The nutrient artery plays a key role throughout the entire consolidation process, including in the healing of pseudoarthroses [3]. Microvascular anastomosis of the allograft pedicle enables the creation of optimal conditions for revascularization, resulting in improved consolidation, increased strength, and enhanced rigidity of the reconstructed segment [21, 22]. Thus, it has been demonstrated that vascularized bone grafts are clearly superior, particularly for the reconstruction of critical-sized tubular bone defects, as they preserve osteogenic capacity and an intrinsic source of vascularization. However, the availability of vascularized autologous bone grafts is limited, which necessitates the investigation of vascularized bone allografts [8, 9, 10, 16]. The interaction between immunocompetent blood cells and the vascular endothelium of the graft represents the first stage in the cascade that induces ischemia, reperfusion dysregulation, and graft rejection, which is considered the main cause of post-transplant failure [6, 7].

Immunosuppression represents a hallmark of progress in contemporary transplantology [10, 12]. Transplantation of limbs, joints, and bone tissue differs fundamentally from the transplantation of vital parenchymal organs such as the heart or liver. In the case of muscular and osteoarticular tissues, immunosuppressive medication is required at doses two to three times higher than those used in solid organ transplantation and must be administered long-term to prevent systemic complications [9, 12]. Widespread long-term immunosuppression is not justified due to the associated risks of organ toxicity, malignancy, and other systemic complications; therefore, it becomes unjustifiable in these non–life-critical clinical situations [20, 21]. As a compromise, short-term immunosuppression has been proposed [9], or alternatively, the identification of strategies that allow for its complete omission in the post-transplant period of vascularized bone allografts.

Recent in vivo studies conducted in rats [5], New Zealand White rabbits (NZWR) [20], and porcine models [16, 17, 21] have demonstrated the possibility of inducing

therapeutic and surgical neoangiogenesis comparable to that of the host within vascularized bone allografts under short-term immunosuppression (two weeks). These studies reported favorable long-term outcomes in terms of the mechanical and functional characteristics of the reconstructed bone [1, 5, 16, 18, 20, 21].Decellularization involves the removal of cells and associated immunogenic antigens from vascularized bone allografts using physical, chemical, and enzymatic agents while preserving the integrity of the extracellular matrix (ECM), including the architecture of the vascular wall [94]. Another important advantage of this technique is the preservation of vascular channel integrity, making the graft suitable for cellular repopulation (recellularization) and for supporting cellular migration and proliferation during the postoperative period [58, 59, 60]. The omission of immunosuppression, even short-term, through the creation of non-immunogenic vascularized bone allografts would allow the development of a standardized algorithm for the effective reconstruction of large bone defects.

Could graft decellularization represent one of the solutions? Would a decellularized vascular pedicle, processed using methods effective for bone tissue, allow safe microvascular manipulation? Would it withstand blood flow during revascularization and enable internal graft repopulation by host cells, ultimately restoring all biological properties of bone tissue? These questions remain to be investigated.

The thesis is structured according to the traditional simple format. Based on the above considerations, the following goal was formulated:

Goal: investigation of the premises for tubular bone defect reconstruction using decellularized composite allografts integrated into the host vascular system during an *in vivo e*xperimental feasibility study.

Objectives:

1. Anatomical investigation of the vascularization of the hind limb in the laboratory animal (NZWR) and identification of the long tubular bone segment with the optimal vascular pedicle.
2. Development and testing of the procedure for harvesting and preserving the long tubular bone segment with the optimal vascular pedicle for decellularization and inclusion in the host vascular circuit.
3. Evaluation of the feasibility of the anesthesia and surgical method for orthotopic *in vivo* repair of long tubular bone defects using decellularized composite vascularized allografts.
4. Investigation and *in vivo* testing of decellularized vessels using a mixed method (compatible with integral composite bone grafts: bone + vessel) to study mechanical resistance during microsurgical suturing, blood pressure (BP), and pulse wave in the host circuit.
5. Investigation through *in vivo* testing of decellularized bone tissue using a mixed method (compatible with integral composite bone grafts – bone + vessel) revascularized by studying the clinical and paraclinical outcomes of tubular bone defect repair.

Research hypothesis: The proprietary method developed for the preparation and testing of decellularized vascularized bone allografts is feasible for transplantation (repair of tubular bone defects) and exhibits reduced immunoreactivity.

Summary of research methodology and justification of chosen methods:

The study was conducted during 2017–2024 as part of postgraduate doctoral studies within the Doctoral School of Medical Sciences. The research stages corresponded to the objectives listed above.

1. Analysis of bibliographic data through medical databases Google Scholar, PubMed (Medline), and Hinari, including investigations of the most relevant and recent articles and publications, to accumulate theoretical information (2017–2023), with the storage of over 600 articles and abstracts in Paperpile software – Annex 1.

2. Investigation of the anatomy and vascularization of the hind limb in NZWR, as well as the method of harvesting femoral diaphyseal segments while preserving optimal or sufficient vascular sources of the long tubular bone (nutrient artery). This stage was conducted at the Morphology Block and the Department of Anatomy and clinique anatomie, USMF "N. Testemițanu", 2017–2019.

3. Study of the procedure for harvesting the long tubular bone segment with the optimal vascular pedicle for preservation (at -84°C) and decellularization while maintaining the vascular continuity of the pedicle, followed by incorporation into the adoptive vascular circuit.

4. Development of anesthesia and intraoperative protocols for orthotopic osteosynthesis of decellularized vascularized femoral allografts – conducted at the Tissue Engineering and Cell Culture Laboratory, USMF "N. Testemițanu", within the research project "GaN-based Nanoarchitectures and 3D Biological Matrices for Microfluidics and Tissue Engineering Applications" – 20.80009.5007.20, funded by the National Agency for Research and Development of the Government of the Republic of Moldova, 2020–2022.

5. Investigation through in-vivo testing of decellularized bone tissue using a mixed-method (compatible with integral composite bone grafts – bone + vessel), revascularized by studying the clinical and paraclinical manifestations in the host organism in medium-sized laboratory animals – NZWR – with the development of a set of recommendations for the use of decellularized vascularized bone allografts in the preclinical stage of tubular bone defect treatment. This stage was conducted at the Tissue Engineering and Cell Culture Laboratory and the Department of Orthopedics and Traumatology, USMF "N. Testemițanu", 2022–2023.

The study was approved by the Research Ethics Committee of USMF "Nicolae Testemițanu," examined at the session on 21.05.2018, with a favorable opinion no. 75, and by the Research Ethics Committee no. 118/18.10.2021 of UMF "G.T. Popa," Iași, Romania, with registration number 25807, dated 19.10.2021. Project authorization no. 44/04.11.2021 was granted by the Sanitary-Veterinary Directorate and Food Safety, Iași, Romania.

The pilot experimental surgical study provides valuable preliminary information but has limitations related to sample size, biological variability, extrapolation to humans, and statistical power. The results must subsequently be validated in a larger,

randomized, controlled study to obtain robust clinical conclusions for the clinical testing phases of the treatment method.

Innovation and scientific originality of the obtained results: Integration of the method for preservation and decellularization of vascularized bone allografts with advanced microsurgical revascularization techniques, by connecting the composite grafts to the host vascular circuit, allows the creation of a bone allotransplant that fully preserves the biological properties of bone (osteoconductive, osteoinductive, and osteogenic), with inside-out vascularization for efficient bone consolidation and reduced immunoreactivity.

Based on the research conducted within the doctoral thesis in medical sciences, two innovation applications were filed, resulting in the issuance of innovator certificates from the following institutions:

IP USMF "Nicolae Testemițanu," Republic of Moldova

1. Pavlovschi E., Stoian A., Nacu V., Verega Gr., "Decellularized Vascularized Bone Allografts as a Method for Treating Critical Bone Defects". Innovation Certificate No. 6052, May 16, 2023.

2. Stoian A., Pavlovschi E., Verega Gr., Nacu V., "Decellularization Method for Vascularized Composite Bone Grafts". Innovation Certificate No. 6058, May 24, 2023.

Scientific problem solved: The elimination of immunosuppression, even of short duration, through the creation of vascularized bone allografts with reduced immunogenic potential allows for the establishment of a standardized preclinical—and later clinical—algorithm for orthotopic repair of segmental and critical tubular bone defects using decellularized vascularized bone allografts.

Practical value of the work: This thesis proposes a complete algorithm for the harvesting, preservation, and orthotopic repair of decellularized vascularized bone allografts under *in-vivo* conditions. Detailed analysis of the *in-vivo*, clinical, and paraclinical post-transplant experimental results obtained in the pilot study on laboratory animals (Wistar rats and domestic rabbits – NZWR) supports the validation of the proprietary experimental model. This approach opens the perspective for extending the repair of tubular bone defects with decellularized vascularized bone allografts in large animals, with potential translational applications in human clinical practice, without the need for post-transplant immunosuppression or immunomodulation.

Implementation of research results: The practical impact of this study is particularly relevant at the preclinical stage, through the description of an innovative technique for the treatment of long tubular bone defects within the Tissue Engineering and Cell Culture Laboratory, Department of Orthopedics and Traumatology, State University of Medicine and Pharmacy "Nicolae Testemițanu," Chișinău, Republic of Moldova. The obtained results (protocol for harvesting, preservation, and orthotopic bone repair through inclusion of the decellularized, vascularized bone allograft into the host vascular circuit) were presented at meetings of the Tissue Engineering and Cell Culture Laboratory and the Department of Orthopedics and Traumatology. The results were also implemented within the research project: 1."GaN-based Nanoarchitectures and 3D Biological Matrices for

Microfluidics and Tissue Engineering Applications" – 20.80009.5007.20, funded by the National Agency for Research and Development of the Government of the Republic of Moldova; 2. Bilateral Project: Nanostructured bone grafts with predetermined properties (2024–2026) (Grant No. 29ROMD/20.05.2024) – Laboratory of Tissue Engineering and Cell Cultures – "Nicolae Testemițanu" State University of Medicine and Pharmacy (Republic of Moldova) and the Personalized Medicine Center – National University of Science and Technology POLITEHNICA Bucharest, Romania.

Innovations within the thesis topic implemented within the scientific-practical process at IP USMF "Nicolae Testemițanu," Republic of Moldova:

1. "Decellularized Vascularized Bone Allografts as a Method for Treating Critical Bone Defects", implementation act no. 82, May 16, 2023.

2. "Decellularization Method for Vascularized Composite Bone Grafts", implementation act no. 88, May 24, 2023.

The thesis materials were presented and discussed at meetings of the Tissue Engineering and Cell Culture Laboratory and the Department of Orthopedics and Traumatology, State University of Medicine and Pharmacy "Nicolae Testemițanu," Chișinău, Republic of Moldova.

Publications on the research topic: The materials of the thesis were reflected in five scientific publications on the research topic, including: 2 articles in international journals indexed in Web of Science; 1 article in journals listed in the National Register of Specialty Journals; 3 materials/abstracts presented at international conferences; 5 materials/abstracts presented at international conferences organized in the Republic of Moldova; and 4 materials/abstracts presented at national conferences. The results of the thesis were approved during meetings of the Department of Orthopedics and Traumatology and the Tissue Engineering and Cell Culture Laboratory at the State University of Medicine and Pharmacy "Nicolae Testemițanu," Chișinău, Republic of Moldova.

GENERAL RESEARCH METHODOLOGY

1. General characteristics of the study: stages and design. The experimental study was conducted between 2017 and 2024 as part of Cycle III Doctoral University Studies at the Doctoral School of Medical Sciences of the "Nicolae Testemițanu' State University of Medicine and Pharmacy (IP USMF). The research was carried out within the Department of Orthopaedics and Traumatology, specializing in 321.18 Orthopaedics and Traumatology. The study was approved by the Research Ethics Committee of USMF "Nicolae Testemițanu," examined at the session on 21.05.2018, with a favorable opinion No. 75, and by the Research Ethics Committee No. 118/18.10.2021 of UMF "G.T. Popa," Iași, România, registration number 25807, dated 19.10.2021. Project authorization No. 44/04.11.2021 was granted by the Sanitary-Veterinary Directorate and Food Safety, Iași, Romania.

The structure of the thesis follows the Guidelines 2025 Edition, No. 2, for the preparation of doctoral theses and is organized according to the Traditional Simple type. This chapter describes all the materials and methods used during the research stages and pilot experimental studies, corresponding to the pre-established objectives

of the study to achieve the proposed research aim. All *in-vitro* and *in-vivo* experiments were conducted at the Tissue Engineering and Cell Culture Laboratory of USMF "N. Testemițanu," the Department of Anatomy and Clinical Anatomy, Orthopedics and Traumatology of USMF "N. Testemițanu," as well as at CEMEX, UMF "G.T. Popa," Iași, Romania, under the AUF "Eugen Ionescu" doctoral research fellowship, conducted from 15.09.2021 to 15.02.2022.

2. Methods for studying the anatomy and vascularization of the hind limb in NZWR, as well as the method for harvesting femoral diaphyseal segments while preserving sufficient vascular sources of the bone (nutrient artery) for preservation. The study was performed on cadavers of 20 adult NZWR rabbits, obtained from an abattoir with the mandatory sanitary-veterinary euthanasia certificate – "ECO-FER-MER" SRL. The average age of the sacrificed animals was 120 days, and the body weight ranged from 2.6 to 2.8 kg. At the Department of Anatomy and Clinical Anatomy of USMF "N. Testemițanu," the anatomical arterial and venous vascularization of the hind limb was studied in 10 NZWR rabbit cadavers. After identifying the optimal tubular bone segment with a vascular pedicle suitable for a vascularized bone allograft, 20 composite femoral bone grafts (bone + vessel) were harvested from 10 of these 20 sacrificed animals and stored by freezing at -82.5°C.

3. Angiographic study of the vascularization of the hind limb in NZWR laboratory animals

Angiographic investigation was performed by introducing the contrast agent (Urografin® 30%) into the abdominal aorta. The contrast agent was injected under pressure to visualize and study the arterial course of the hind limb in NZWR. A micro-angiographic study of the vascularized femoral bone segments followed. Catheterization of the external iliac artery was performed, and Urografin® 30% solution was introduced under pressure. Standard radiologic examinations were performed at 1, 3, and 5 minutes after the start of contrast agent injection to assess the vascularization area of the femoral bone through the pedicle formed by the external iliac artery – lateral circumflex femoral artery – nutrient artery, for anatomical correlation with the femoral bone.

4. Morphological study of the dorsal aorta, external iliac artery, and vascularized bone segments – femur. This stage of the research was performed on segments of arteries and bone harvested from NZWR laboratory animal cadavers. Histological investigations were conducted at the following levels: dorsal aorta, external iliac artery, and proximal femoral artery segments (nutrient artery). The morphological study and histological examination focused on the extracellular matrix content of the muscular vessel walls at these levels. The aim of this investigation was the microscopic visualization of structure and extracellular content, which is critically important for subsequent mechanical resistance during microsurgical insertion into the host circulation after the complex mixed decellularization process for composite grafts (bone + vessel), considering vascular wall thickness and extracellular matrix composition. The histological appearance of the femoral bone was also investigated to compare it with the results of cortical bone decellularization. Histological sections were analyzed to determine the optimal segment for mixed-method decellularization and to evaluate the quantitative potential of the extracellular matrix.

5. Calculation of graft length to structurally match a critical-sized bone defect.

The concept of a massive bone defect includes a segmental and/or cortical bone defect with a critical-size defect (CSD). Such defects are generally accepted as being ≥1.5–2 times the diaphyseal diameter of the long bone, a circumferential defect ≥50%, or a surface area ≥2 cm², although the values may vary depending on the bone and patient. The femoral bone length in NZWR laboratory animals was measured in 10 subjects at this stage of the study. The data were recorded and statistically processed using IBM SPSS Statistics 26.0.0.1 FP001. Based on these results, the required length of the composite graft (bone + vessel) for the repair of critical bone defects was calculated.

6. Preservation and decellularization of composite grafts (bone + vessel)

Twenty vascularized bone grafts of predetermined size were harvested from both hind limbs of 10 laboratory animals and stored at -82.5°C. Composite grafts (bone + vascular pedicle) were obtained according to the criteria for repairing a critical defect of a long tubular bone. The grafts were stored at -82.5°C for subsequent decellularization using a combined method effective for cortical and spongy bone tissue, as well as vascular soft tissue (artery). Grafts were fixed for decellularization in a sterile, closed-box system connected to a peristaltic pump, which facilitated the washing of the grafts with pre-established solutions according to a nine-step decellularization protocol.

7. Investigation of microsurgical anastomosis feasibility and *in-vivo* testing of decellularized vascular pedicles of composite bone grafts (bone + vessel) using a mixed-method

Decellularized grafts were microsurgically anastomosed end-to-end into the femoral arterial circulation of five Wistar rats at CEMEX, UMF "G.T. Popa," Iași, Romania.

For 60 minutes, the arterial-type vascular grafts, subjected to preventive decellularization and included in the host circulation via end-to-end anastomosis, were monitored in vivo in five subjects (Wistar rats). The following parameters were recorded:

- maintenance of a pulsatile wave at 10, 30, and 50 minutes,
- maintenance of blood pressure (BP) at 10, 30, and 50 minutes,
- maintenance of distal graft blood flow through a milking test at 10, 30, and 50 minutes.

These parameters were used to evaluate the viability and performance of the decellularized vascular graft after microsurgical anastomosis in order to assess its ability to integrate into the circulatory system and supply blood from the inside to the outside of the bone tissue, which is subsequently transplanted orthotopically as a decellularized vascularized bone allograft.

8. Assessment of feasibility through the establishment of anesthesia and surgical protocols for the repair of tubular bone defects via osteosynthesis and inclusion of decellularized vascularized bone allografts into the adoptive vascular circuit in NZWR

In-vivo testing of the anesthesia and surgical protocols for the repair of tubular bone defects via osteosynthesis and subsequent inclusion into the adoptive vascular circuit of decellularized vascularized bone allografts in NZWR was approved by the Research Ethics Committee No. 118/18.10.2021 of UMF "G.T. Popa," Iași, Romania, registration number 25807, dated 19.10.2021. Project authorization No. 44/04.11.2021 was granted by the Sanitary-Veterinary Directorate and Food Safety, Iași, Romania.

At this stage, in-vivo research was conducted on six NZWR laboratory animals to:

I. Development of the anesthesia protocol for NZWR laboratory animals, enabling long-duration procedures with two surgical stages: microsurgical anastomosis and orthotopic osteosynthesis of a long tubular bone – femur (including sedation, induction, and maintenance).

II. Development of the intraoperative protocol for in-vivo repair of tubular bone defects using decellularized vascularized allografts reintroduced into the adoptive vascular circuit.

During this stage, the following approaches were studied:

A. Lateral intermuscular approach – for osteotomy and orthotopic osteosynthesis of the decellularized bone allograft.

B. Medial approach – for latero-terminal anastomosis of the decellularized pedicle of the bone allograft with the femoral artery of the host circulation.

9. Study of postoperative local and systemic manifestations following implantation of vascularized bone allografts in the host organism.

A pilot surgical study helps test and optimize a new surgical technique before wider application. This type of experiment allows for early identification of methodological issues, complications, or variability in results. It reduces risks for animals and optimizes the design of the main study.

The surgical technique described in Stage IV enabled evaluation of feasibility, safety, and the impact of this novel surgical method. Pilot studies frequently use 4–6 animals per group to estimate variability and guide larger subsequent studies. Reduction in animal numbers is ethically justified according to the 3R principle (Reduce, Refine, Replace). Statistical power can be optimized through repeated measurements on the same animal (e.g., histological and functional evaluations at multiple time points). If promising trends are observed, a larger study with 8–12 animals per group is recommended for confirmation.

Following the anesthesia and surgical protocols established in the previous stage (lateral intermuscular approach for osteotomy and osteosynthesis, medial approach for latero-terminal anastomosis of the pedicle with the femoral artery), a comparative study was conducted on three groups of four NZWR laboratory animals each:

- Group 1 – repair of extensive bone defects using autologous vascularized bone grafts
- Group 2 – repair using native vascularized bone allografts
- Group 3 – repair using decellularized vascularized bone allografts

Between 06–12.2022, 12 NZWR laboratory animals from the doctoral school-funded cohort were moved to the vivarium of IP USMF "N. Testemițanu". Postoperative assessment for each subject included:

A. Local postoperative manifestations: Wound appearance: swelling, heat,

redness, exudate, and healing – evaluated on postoperative days 1, 5, 10, and 15. Operated limb functionality: weight-bearing, gait, and jumping – assessed on postoperative days 1, 5, 10, and 15.

B. Paraclinical postoperative manifestations: Complete blood count (CBC) with leukocyte formula – blood collected from the posterior auricular vein of NZWR and stored in tubes for analysis of the post-graft inflammatory response. Biochemical blood analysis with bone indices: calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) – to examine the degree of post-transplant osteolysis in the study groups.

Standard digital radiography of the operated hind limb on postoperative days 14 and 30 in one of two projections (anteroposterior or lateral-oblique) using a system for evaluating fracture healing of bone defects. Four criteria were selected for imaging evaluation of vascularized graft repair of tubular bone defects: formation of bone tissue, total bone consolidation, resorption of graft bone tissue, and the presence of a bony junction between the graft and host bone.

Histological examination of grafts: segments were collected pre- or intraoperatively and post-sacrifice at 30 days postoperatively, and stained with hematoxylin-eosin to examine bone and vascular components. The femoral bone of the operated limb was resected, soft tissues not of interest were removed, and the grafted segment was sectioned. For histology, anatomical specimens were fixed in 10% neutralized formalin. Fragments from the defect site were decalcified in 15% nitric acid and embedded in paraffin.

10. Mathematical methods for synthesis of experimental research results

To analyze statistical data from this study, quantitative descriptive statistics were applied using IBM SPSS Statistics 26.0.0.1 FP001. The results of each study stage, corresponding to its objectives, were statistically analyzed according to the type of collected data and the variables used. Three databases were developed in SPSS Statistics for this purpose. All statistical tests were compared with P-values <0.05 to determine statistical significance.

RESEARCH PREMISES FOR THE REPAIR OF TUBULAR BONE DEFECTS USING DECELLULARIZED VASCULARIZED BONE ALLOGRAFTS

1. Arterial vascularization of the hind limb in medium-sized laboratory animals

Macroscopic vascular analysis of the hind limbs in 10 New Zealand White Rabbits (NZWR) revealed that the common iliac artery bifurcates into the internal iliac artery, which supplies the pelvic viscera, and the external iliac artery, which continues as the femoral artery. The external iliac artery gives rise to the lateral and medial circumflex femoral branches, contributing to the trochanteric anastomosis, and to the anterior cervical arteries, which descend toward the femoral neck. Crucially, the primary nutrient artery of the femur was found to originate from the lateral circumflex femoral artery, entering the nutrient foramen on the medial diaphysis distal to the lesser trochanter. Contrast-enhanced flow measurements demonstrated that this vessel provides 70–80% of total femoral perfusion, emphasizing its dominant role in epiphyseal, metaphyseal, and diaphyseal blood supply.

2. Calculation of allograft length to match a critical-sized bone defect

Morphometric analysis of 10 NZWR subjects established a mean femoral length of 96.53 ± 3.61 mm (range: 89–107 mm), indicating a homogeneous sample distribution. The mode (89 mm) and median (97.75 mm) confirmed a slight negative skewness while maintaining adequate structural stability for the study. Validation of the critical-sized bone defect model was achieved by comparing the mean femoral length to the 42 mm allograft length. A one-sample t-test revealed a highly statistically significant difference ($p < 0.001$, $df = 9$). The 42 mm graft represents approximately 43.5% of the total femoral length, fulfilling the criteria for a critical tubular bone defect (exceeding the 2 cm^2 threshold; at a width of 1 cm, the defect area is 4.2 cm^2). The regenerative capacity required for this defect exceeds the natural physiological self-repair threshold in rabbits.

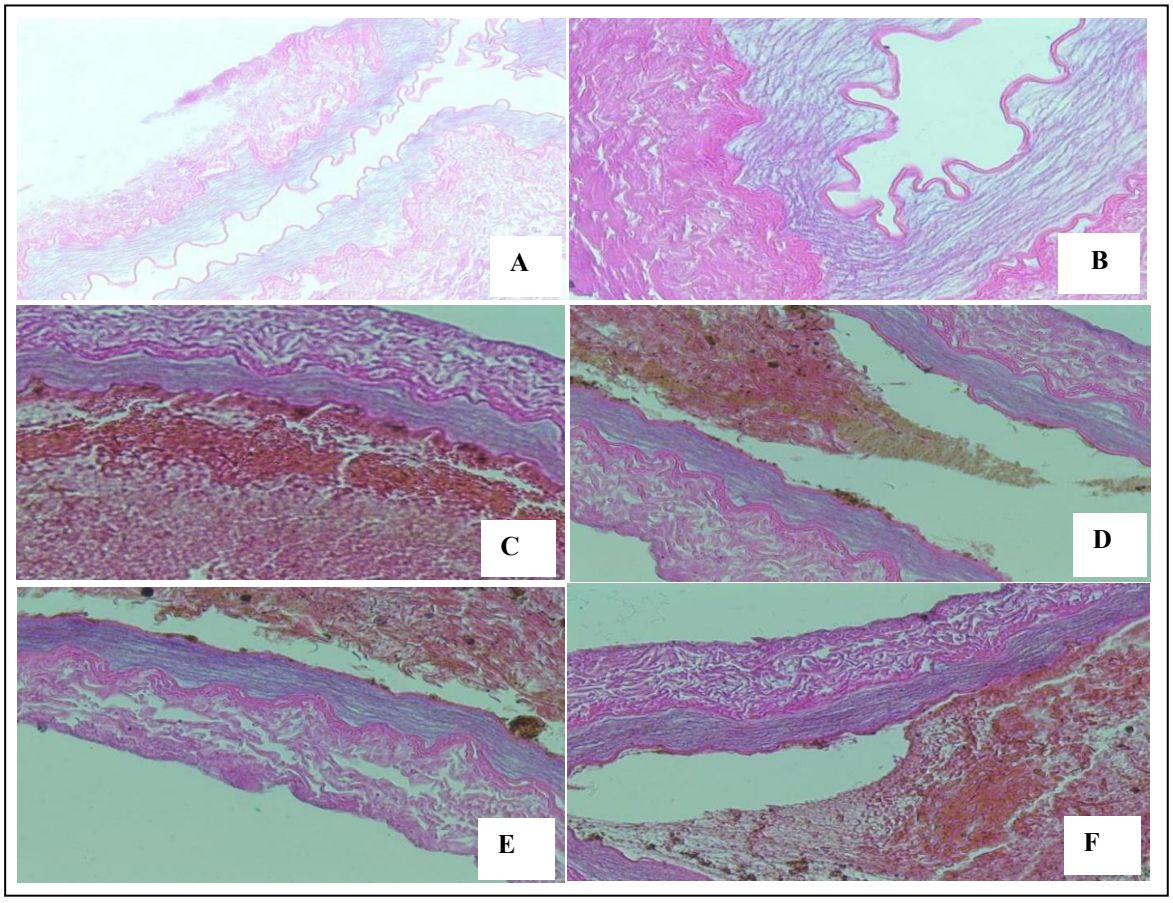


Figure 1. Histological examination of decellularized arterial segments microsurgically incorporated into the host vascular circuit, HE staining.

A, B – Histological examination by H&E of decellularized arterial segments from NZWR laboratory animals shows the absence of cellular nuclei and preservation of the extracellular matrix across all three layers of the arterial wall (endothelium, tunica media, adventitia).

C, D, E, F – The vascular wall of arteries microsurgically incorporated into the host vascular circuit for 60 minutes demonstrates preservation of the extracellular matrix structure, presence of intraluminal thrombus not adherent to the decellularized endothelium, and absence of host cellular material in the decellularized vessel matrix.

providing the necessary conditions to evaluate the orthotopic integration of decellularized composite allografts into tubular defects.

3. *In-vivo* testing of the decellularized vascular pedicle for composite bone allografts.

The viability of decellularized arterial grafts was monitored for 60 minutes in a cohort of five Wistar rats following end-to-end microsurgical anastomosis with the host artery. Functional and hemodynamic outcomes assessed at 10, 30, and 50-minute intervals during the in vivo experiment indicated 100% immediate patency. The maintenance of the pulsatile wave and arterial pressure (AP) across all time points demonstrated the matrix's structural resistance to the host's systemic pressure.

Dynamic patency testing (milking test) showed constant distal blood flow until the 50-minute mark, at which point a decrease in flow rate was observed (without complete obstruction). This finding suggests the initiation of a hemodynamic thrombotic process within the decellularized graft lumen. Throughout the observation period, macroscopic integrity was confirmed; the grafts maintained an intact tissue architecture with no evidence of ruptures or trans-mural extravasation. Suture-line bleeding was minimal and self-limited within 1–2 minutes.

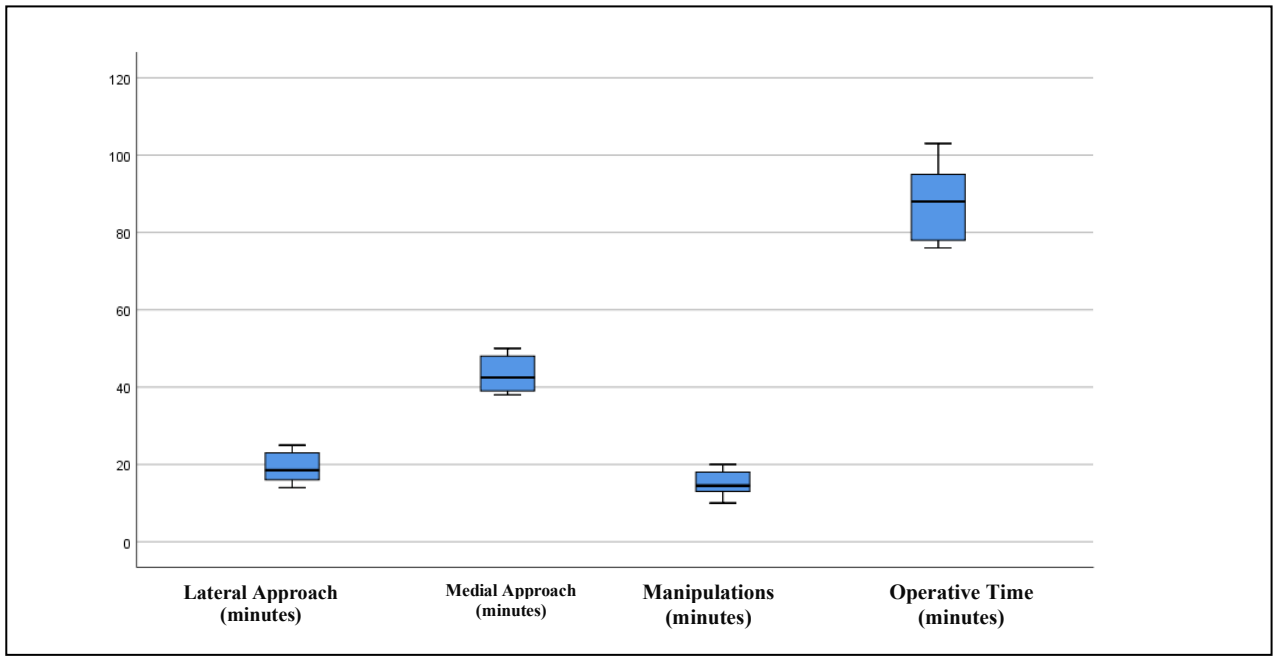


Figure 2. Boxplot representation of the variable: Surgical Intervention Duration (minutes).

Post-implantation histological analysis using light microscopy with Hematoxylin and Eosin (H&E) staining confirmed the preservation of the extracellular matrix (ECM) structure and the absence of acute inflammatory infiltrate (macrophages, lymphocytes), validating the efficiency of the decellularization process. Although a distal intraluminal thrombus was identified, it was non-adherent to the internal surface of the graft. This indicates that the decellularized surface is
not intrinsically thrombogenic; rather, the thrombosis represents a hemodynamic reaction rather than an immune or surface-driven response - figure 1.

The decellularized vascular graft is capable of providing the necessary perfusion for the future composite bone allograft, demonstrating structural stability and immediate biological compatibility for the treatment of critical-sized bone defects.

4. Feasibility of tubular bone defect reconstruction using vascularized bone allografts: an NZWR laboratory study.

The feasibility of this pilot surgical study focuses on assessing the technical viability and the experimental reproducibility of the protocol. We examined the practical implementation of the in vivo surgical intervention to validate the proposed methodology.

The specific elements defining this feasibility include: anatomical accessibility, host cellular integration within the decellularized vessel matrix, and the mechanical stability of the bone allograft to prevent traction or torsion of the vascular pedicle. Central tendency indicators for the pre-established variables were calculated using quantitative descriptive statistics:

- Lateral intermuscular approach: Mean duration of 19.17 min (range: 14–25 min).
- Medial approach for anastomosis: Mean duration of 43.33 min (range: 38–50 min).
- Other manipulations: Mean duration of 15 min (range: 10–20 min).
- Total operative time: Mean duration of 88 min, with a minimum of 76 min and a maximum of 103 min.

The mean total duration of the procedure was 85 minutes, with the primary stage consisting of the microsurgical anastomosis (mean: 43.33 min). Statistical analysis using one-way ANOVA demonstrated the absence of statistically significant differences between the studied groups across all surgical stages ($p = 0.212$ for total duration, $p = 0.437$ for the lateral approach, and $p = 0.203$ for the medial approach). These results confirm that the use of decellularized grafts and the applied anesthesia protocols does not negatively influence operative time. It was observed that time variations were exclusively determined by technical complexity and anesthetic management, rather than the subjects' body weight (Figure 2).

In conclusion, the proposed experimental surgical model, utilizing both medial and lateral surgical approaches to the thigh, is technically feasible. It ensures the mechanical stability of the allograft and efficient blood flow from the interior to the exterior of the bone graft—an essential indicator for the success of vascularized bone reconstruction.

5. In-vivo Testing of Decellularized Bone Tissue via Transplantation with Vascularized Bone Grafts in the Host Organism in Animal Study Groups. According to the established anesthesia and surgical protocol (lateral intermuscular approach for osteotomy and osteosynthesis, medial approach for lateral-terminal anastomosis of the pedicle with the femoral artery), in the third stage of the study, a comparative investigation was conducted on three study groups, each consisting of four NZWR laboratory animals:

- Group 1: Critical bone defect repair with vascularized autologous bone graft.
- Group 2: Critical bone defect repair with vascularized allogeneic bone graft (native).
- Group 3: Critical bone defect repair with vascularized decellularized allogeneic bone graft.

Postoperative research for each subject in the study groups was strictly monitored.

A. Local Postoperative Manifestations (Days 1, 5, 10, and 15):

Wound appearance: *Tumor, calor, rubor*, wound discharge, and wound healing. For comparative analysis of differences between conditions, variables were coded as binary, where "1" indicates success (e.g., presence of an effect or event of interest – presence of Tumor), and "2" indicates the absence of the event. The only continuous variable was the mean subject weight, which was 3597.5 g, ranging from 3350 to 3810 g. All binary variables were measured on a sample of 12 subjects with no missing data. Most variables (except weight in grams) were measured on a

Postoperative monitoring of the 12 subjects (mean weight: 3597.5 g) over a 15-day period was conducted. Statistical analysis of binary variables (scale: 1.00–2.00) demonstrated a clear transition from the presence of inflammatory signs on Day 1 (means near 1.00; e.g., tumor = 1.08) to their absence by Days 10 and 15 (means approaching 2.00; e.g., calor = 1.91) (Table1).

A progressive decrease in wound secretions and discharge was also noted, with mean values improving from 1.08 to 1.91. The results indicate a steady remission of inflammatory parameters (*calor, rubor, tumor*) across all three observation groups, providing an objective numerical overview of local therapeutic success during the critical postoperative period.

B. General Postoperative Manifestations:

Postoperative functional dynamics of the operated limb—evaluated as binary variables (1 = success/presence; 2 = absence)—highlighted a progressive recovery of motor capacities over the 15-day monitoring period. While total locomotor incapacity (absence of walking and jumping) was recorded on Day 1, weight-bearing began to resume by Day 5 (mean: 1.16), followed by significant improvement in walking by Day 10. By Day 15, walking was almost fully recovered across the entire sample, whereas jumping—the most complex motor function—reached its peak study value (mean: 1.41), yet remained the slowest parameter to recover.

Comparative group analysis demonstrated major differences in recovery quality:

Group I (Autograft): exhibited optimal performance, with the fastest inflammatory remission and the earliest resumption of jumping.

Group III (Decellularized Allograft): achieved intermediate results, nearly equaling Group I in jumping recovery (3/4 subjects), despite slightly slower local wound healing.

Group II (Native Vascularized Allograft): recorded the poorest outcomes, characterized by persistent inflammation and a total failure to recover jumping function by Day 15. In conclusion, the data confirm that the decellularized vascularized allograft (Group III) represents an effective therapeutic solution, ensuring functional recovery significantly superior to conventional allografts and comparable to the 'gold standard' represented by the autograft."

Table 2. Evaluation of Operated Limb Functional Recovery by Study Groups.

Parameter	Initial Status	Recovery Status	Observations by Group
Support	Very low	Complete/almost complete recovery	All groups recover well
Walking	Absent	Complete recovery	All groups recover well
Jumping	Absent	Lot I: 3/4; Lot II: 0/4; Lot III: 3/4	Major difference – Lot II does not recover this advanced function

Table 2. Mean values and standard deviations for Leukocyte, Granulocyte, Lymphocyte, and Monocyte parameters on Days 14 and 28 by study group.

Parameter	Day	Group I	Group II	Group III
Leukocytes	14	11.68 ± 4.20	14.31 ± 4.02	9.91 ± 0.89
	28	8.74 ± 1.37	11.77 ± 4.76	7.56 ± 3.20
Granulocytes	14	6.83 ± 2.76	7.10 ± 2.89	6.21 ± 1.83
	28	6.46 ± 1.98	11.62 ± 4.39	6.17 ± 3.11
Lymphocytes	14	6.38 ± 2.52	8.53 ± 3.54	4.48 ± 1.37
	28	6.64 ± 2.36	10.86 ± 3.97	7.00 ± 3.46
Monocytes	14	0.54 ± 0.50	0.35 ± 0.49	0.69 ± 0.35
	28	0.47 ± 0.44	0.75 ± 0.37	0.49 ± 0.36

C. Paraclinical Postoperative Manifestations:

1. Complete Blood Count (CBC) – Assessment of systemic inflammatory response: Comparative analysis revealed distinct immunological profiles depending on the type of graft used. Statistical analysis demonstrated no significant differences between the study groups. Although mean White Blood Cell (WBC) counts showed a slight normalization trend by day 28 (decreasing from 12.00 to 11.76 $\times 10^3/\mu L$), the right-skewed distribution observed in the final stage indicates a prolonged inflammatory response in a subset of subjects (Table 2).

Group II (Native Vascularized Allograft): Exhibited the most pronounced inflammatory response, consistent with a sustained immune reaction to allogeneic antigens.

Group I (Autograft) and Group III (Decellularized Allograft): Manifested similar hematological profiles, characterized by moderate values and early immunological stabilization. Statistical validation using the Kruskal–Wallis test with post-hoc analysis (Bonferroni correction) confirmed these observations. Statistically significant differences were identified between Group III and Group II (adjusted $p = 0.032$), as well as between Group I and Group II (adjusted $p = 0.007$). Conversely, the comparison between Group III (decellularized) and Group I (autograft) showed no statistically significant differences (adjusted $p = 0.607$), indicating that the

decellularized allograft induces a systemic inflammatory response comparable to the autograft (Group I).

2. Serum Biochemical Analysis

Evaluation of the biochemical profile at 14 and 28 days postoperatively revealed intense dynamics in phospho-calcic metabolism, reflecting bone remineralization and consolidation processes. Across the entire study sample, a significant increase in mean values for all monitored indicators was recorded between the two time intervals: calcium (from 3.46 to 4.68 mg/dL), phosphorus (from 2.40 to 3.18 mg/dL), and alkaline phosphatase (ALP) (from 202.21 to 245.32 U/L).

Table 3. Results of central tendency and distribution values for blood biochemical analysis variables as indicators of the osteolytic process across study groups.

Parameter	Day 14	Day 28
Serum Calcium (Ca)	Mean: 3.69 ± 0.27 mg/dL Median: 3.58 mg/dL Range: 2.35 – 5.33 mg/dL	Mean: 4.68 ± 0.53 mg/dL Median: 4.12 mg/dL Range: 2.21 – 7.98 mg/dL
Serum Phosphorus (P)	Mean: 2.41 ± 0.35 mg/dL Median: 1.86 mg/dL Range: 1.31 – 4.86 mg/dL	Mean: 3.18 ± 0.39 mg/dL Median: 3.14 mg/dL Range: 1.12 – 5.01 mg/dL
ALP (Alkaline Phosphatase)	Mean: 202.21 ± 21.61 U/L Median: 186.35 U/L Range: 123.3 – 363.2 U/L	Mean: 245.33 ± 35.85 U/L Median: 202.11 U/L Range: 114.3 – 441.4 U/L

Comparative group analysis highlighted major differences in osteoblastic activity intensity and bone turnover:

Group II (Native Vascularized Allograft): Presented the highest levels for all parameters at both study time points. By day 28, this group reached peak values for calcium (median ≈ 7.0 mg/dL) and ALP (median ≈ 400 U/L), significantly exceeding the other groups. This biochemical hyperactivity, correlated with high data dispersion, suggests a process of accelerated osteolysis and remodeling, typical of the reaction to native allogeneic tissue.

Groups I (Autograft) and III (Decellularized Allograft): Manifested similar and more balanced biochemical profiles. On day 14, both groups showed significantly lower ALP and phosphorus levels compared with Group II, reflecting more stable metabolic activity and smoother biological integration. Although values increased by day 28, confirming active osteogenesis, they remained within tighter physiological limits than in the native allograft (Table 3).

The progressive increase in bone markers, particularly alkaline phosphatase, confirms graft viability and the initiation of de novo bone formation in all groups. However, the biochemical profile of Group III (decellularized) more closely resembles that of the autograft (Group I). This indicates that the removal of cellular components reduces metabolic stress and reactive osteolysis, favoring a more physiological bone consolidation compared to classical vascularized allografts. 2.

Biochemical Blood Analysis (Ca, P, Alkaline Phosphatase) – Bone Indicators for Evaluating Post-Transplant Osteolysis in Study Groups.

C. Radiography of the operated hind limb at 14- and 28-day intervals highlighted significant differences among the three experimental groups, confirming the structural and biological superiority of decellularized grafts.

- Bone tissue formation and consolidation: Group III (decellularized allograft) recorded the most accelerated progression of osteogenesis, with scores increasing from a mean of 1.75 on day 14 to 3.50 on day 28, surpassing even Group I (autograft, mean 3.25). Regarding bone consolidation, Group III reached the maximum score (3.0), indicating optimal maturation of the newly formed bone, while Group II (native allograft) remained stagnant at 1.0, demonstrating osteointegration failure.

- Resorption Dynamics and Bone Junction: An optimal balance between resorption and neoformation was observed in Group III (maintained constant at 1.75), in contrast to Group II, where excessive and accelerated resorption (2.50) compromised graft integrity before effective replacement with new bone occurred. The bone junction between the graft and the host bone developed most dynamically in Groups I and III, both reaching the maximum score of 3.0 at 28 days- tabel 4.

Statistical Validation: The Kruskal-Wallis test confirmed significant differences in favor of Group III, suggesting that "inside-out" vascularization and the absence of cellular antigens promote balanced remodeling (Table 4).

Table 4. Central tendency and distribution results for the renal study variables across the experimental groups.

Parameter		Day 14 Bone Forma-tion	Day 14 Bone Consoli-dation	Day 14 Graft Tissue Resorp-tion	Day 14 Bone Junction Presence	Day 28 Bone Forma-tion	Day 28 Bone Consoli dation	Day 28 Bone Junction Presence	Day 28 Graft Tissue Resorption
Lot I	**Media**	1.75	1.50	1.25	2.0	3.25	2.5	3.0	1.0
	Dev.Std.	.957	.577	.50	.81	.50	.57	.00	.00
Lot II	**Media**	1.00	1.00	2.25	1.75	1.75	1.0	2.0	2.5
	Dev.Std.	.00	.000	.50	.50	.50	.00	.00	.57
Lot III	**Media**	1.750	1.5	1.75	1.50	3.50	3.00	3.00	1.75
	Dev. Std.	.500	.577	.50	.57	.57	.00	.00	.50

The radiographic results demonstrate that the decellularization process (Group III) transforms the graft into a high-performance osteoconductive matrix. This not only matches the "gold standard" (autograft) but also exhibits superior consolidation dynamics, avoiding the pathological resorption characteristic of native vascularized allografts (Group II).

E. Histological Examination of Bone Segments: Microscopic evaluation of the grafted femoral segments revealed major qualitative differences between the three groups, confirming the impact of the graft preparation method on the host's biological response and bone tissue biointegration.

- Group I (Vascularized Autograft): Demonstrated maximum biocompatibility, showing advanced signs of Haversian system remodeling. The formation of immature bone tissue, rich in osteocytes and collagen fibers, evolving toward mature osteonic systems with classical lamellar architecture, was observed. Lymphocyte presence was sporadic, indicating a physiological adaptive response rather than a pathogenic one.

- Group II (Native Vascularized Allograft): Presented deficient dynamics, characterized by the predominance of osteoclastic resorption over new bone apposition.

Group III (Decellularized Vascularized Allograft): Cellular repopulation of the matrix with osteoblasts and osteocytes was identified, demonstrating the absence of cytotoxicity. Haversian remodeling was present, progressing slightly more slowly than in Group I, but without chronic inflammation or giant cell conglomerates. These results confirmed the success of the decellularization method- figure 3, 4, 5.

Vascular Component Analysis (Group III): The decellularized vessels maintained the architecture of all three tunics (intima, media, and adventitia), despite exhibiting some structural disorganization of the extracellular matrix. The lumen remained patent for blood flow, with non-obstructive thrombocytic masses observed adhering to the endothelium. The presence of blood cells within the vascular wall at 30 days indicates progressive biological integration of the pedicle into the host circuit.

Conclusion: Decellularization (Group III) successfully eliminates the immune rejection reaction specific to native allografts (Group II). It transforms the graft into a safe osteoconductive matrix that facilitates bone remodeling similar to that of autografts, while simultaneously providing the essential vascular support for 'inside-out' nutrition.

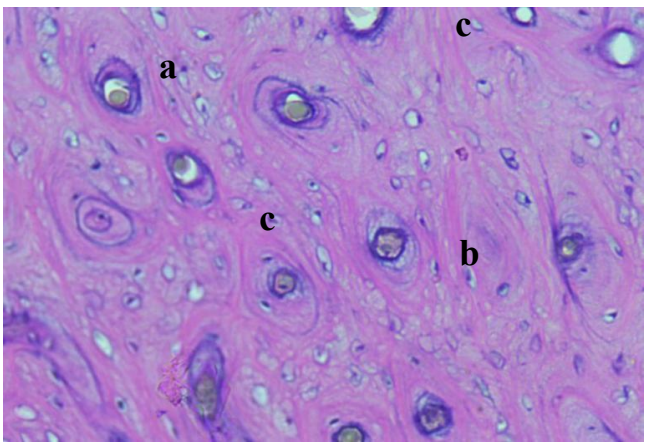
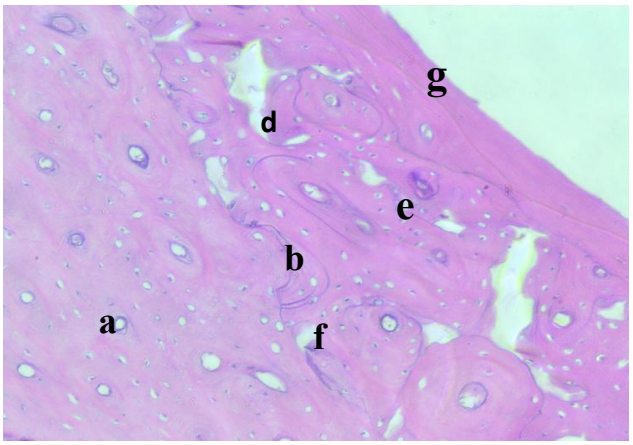
Pre-operative	30 days Post-operative
	

Figure 3. Lot I – Vascularized autologous bone graft.

a. Haversian bone tissue and Haversian vessel, b. Presence of osteocytes in bone lacunae, c. Fundamental bone substance in normal volume, d. Sparse but unmodified fundamental bone substance with fragmentation of bone trabeculae, e. Newly formed Haversian bone tissue. f. Sharp's line, g. Immature bone tissue with numerous osteocytes and disorganized collagen fibers. Magnification: H-EX90

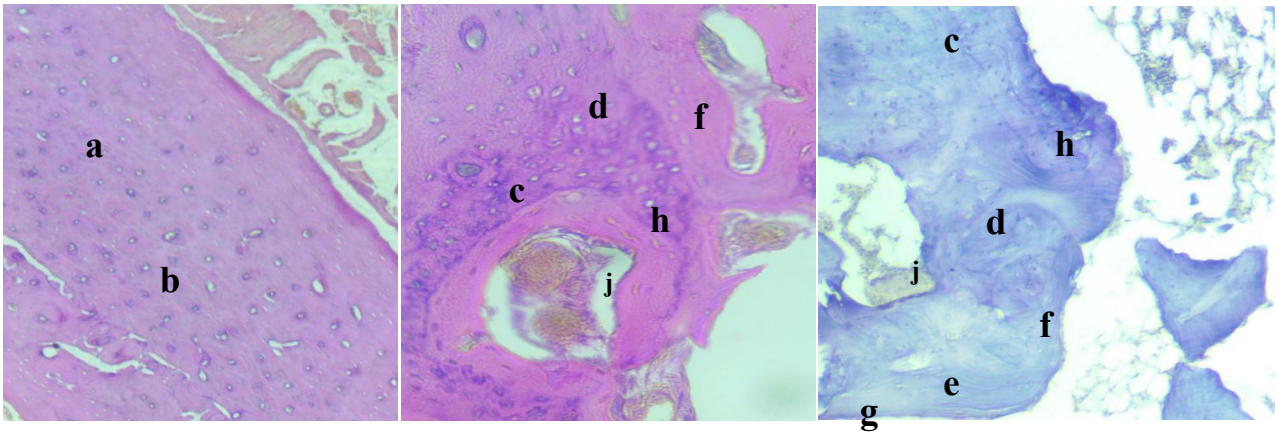


Figure 4. Lot II – Vascularized allogeneic bone graft.

a. Depletion of fundamental bone substance, b. Depletion of osteocytes from bone lacunae, c. Cluster of osteocytes, d. Area of new bone trabeculae with incomplete, fragmented Haversian canals, Necrotic zone with bone resorption, f. Depletion of bone substance, g. Absence of fundamental bone substance, ischemic process, h. Optically empty spaces at the level of the augmented cortex, j. Chron granulomatous giant-cell inflammatory reaction. Magnifications: H-EX90, H-EX40

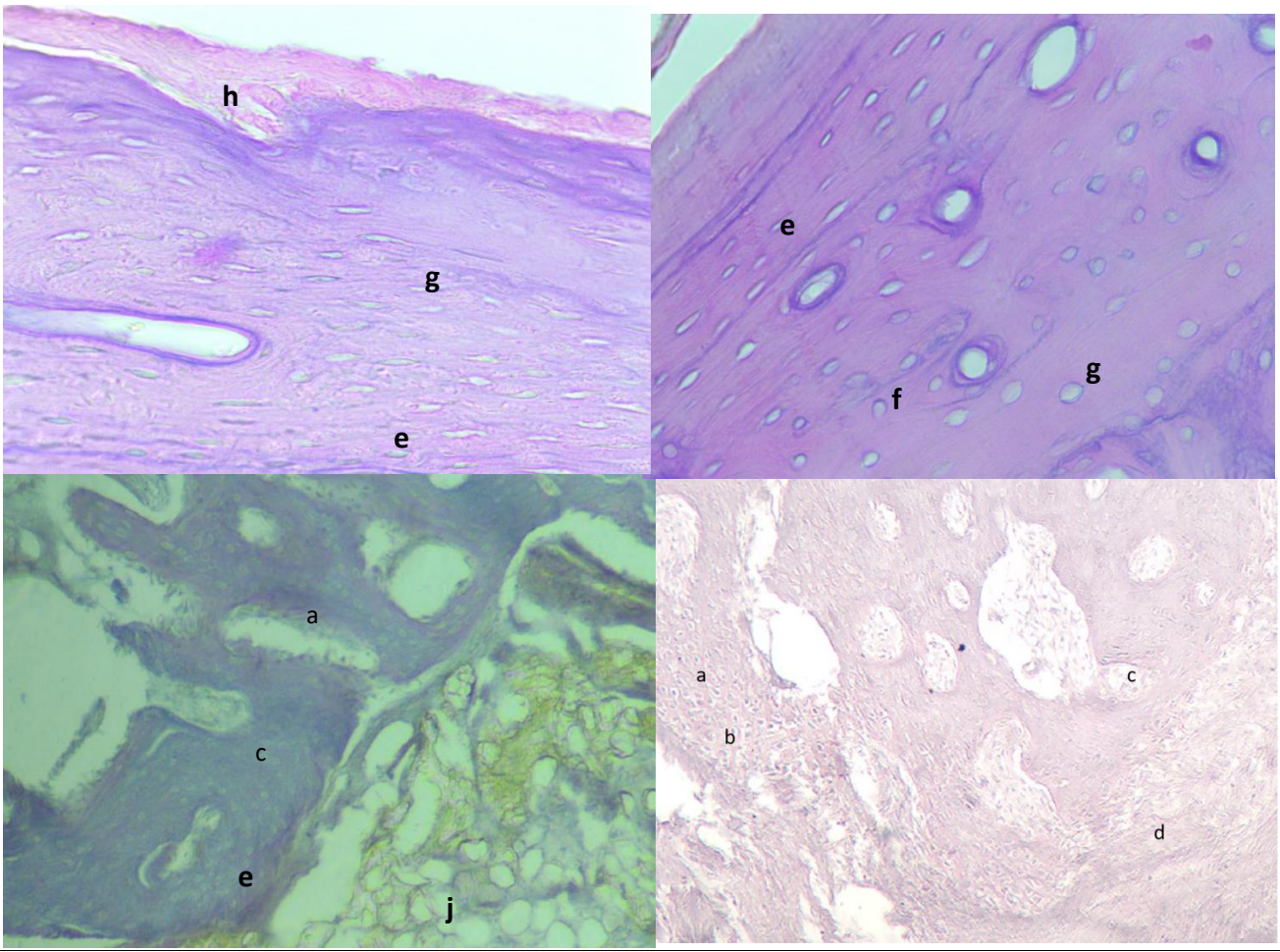


Figure 5. Lot III – Decellularized vascularized allogeneic bone graft.

a. Cluster of proliferating osteoblasts, indicating bone remodeling segments and cellular repopulation of the graft: b. Formation of osteoid; c. Bone resorption; d. Fibrosis, e. Acellular Haversian bone tissue; f. Acellular bone lacunae; g. Depletion of fundamental bone substance; h. Decellularized fibro–connective tissue; j. Medullary bone tissue. Magnifications: H-EX90, H-EX40

CONCLUSIONS

1. The original method of utilizing decellularized composite grafts for the reconstruction of tubular bone defects is characterized by its innovative, interdisciplinary, and pioneering nature, contributing to the advancement of tissue engineering and reconstructive microsurgery of the locomotor system.

The extracellular matrix vascular structure of the femoral bone graft in the NZWR rabbit model ensures functional integration and orthotopic plasty of long tubular bone defects in vivo, using a predetermined length of 40–45 mm with an optimal vascular pedicle extending to the external iliac artery.

2. The duration of the microsurgical vascular anastomosis using a decellularized vessel does not significantly differ from the time required for standard orthotopic osteosynthesis of a bone defect, thereby demonstrating the technical feasibility of the developed protocol.

3. The established mixed decellularization method ensures the architectural integrity of the extracellular matrix (ECM) of muscular-type vessels within the composite graft. Decellularized vascular grafts mechanically maintain vascular patency during in vivo microsurgical anastomosis with a native artery, ensuring stable blood flow throughout the 60-minute monitoring interval.

4. Histologically, the wall of the decellularized vessel shows no infiltration of macrophages or lymphocytes, confirming that the decellularization process effectively eliminated immunogenic potential; furthermore, the endothelial matrix is non-thrombogenic.

5. Decellularized vascularized bone allografts (Group III) used for the reconstruction of tubular defects are technically feasible, demonstrating a clinical evolution comparable to the gold standard autograft (Group I). The success of biological integration is supported by the upward dynamics of alkaline phosphatase (from 202.21 U/L to 245.32 U/L) and the maintenance of a metabolic balance between resorption and formation (stable index of 1.75), in contrast to the osteolytic imbalance observed in native allografts (Group II). These biomaterials provide a viable functional and structural alternative, ensuring efficient "inside-out" in vivo recellularization and organized Haversian remodeling.

6. The original reconstruction method using decellularized composite allografts for tubular bone defects is technically feasible, with promising applications in reconstructive surgery and potential for translational research into preclinical, and subsequently clinical, studies. This method offers the major advantage of eliminating the need for immunosuppressive therapy, opening new perspectives in high-complexity bone reconstructive surgery.

RECOMMENDATIONS

Based on the experimental findings, statistical analysis, and achieved objectives, the following comprehensive recommendations are proposed to advance the field of bone bioengineering and clinical transplantation:

1. Strategic Animal Model Selection

A hierarchical approach to animal model selection is essential for validating vascularized bone reconstructions. Initial exploratory phases should utilize small

rodents (rats) for proof-of-concept. Medium-sized animals (New Zealand White rabbits) are recommended for detailed functional and histological assessments. Finally, large animals (pigs) must be employed to simulate human clinical conditions, specifically focusing on subjects with immunologic profiles similar to human HLA to test composite grafts under realistic clinical scenarios (recommended groups of 8–12 subjects). Preliminary vascular mapping is mandatory to identify optimal segments for graft harvesting.

2. Advanced Processing and Cryopreservation

Composite bone-vascular allografts demonstrate structural stability during long-term storage at −84.5°C. It is recommended to perform decellularization on the intact bone-vascular unit without separating the components. This preserves the continuity and mechanical integrity of the nutrient vascular pedicle, ensuring it remains resistant to arterial pressure and pulsatile flow during microsurgical reintegration into the host's systemic circulation.

3. Optimization of Surgical and Infrastructure Protocols

For orthotopic femoral reconstructions, a dual surgical approach (medial and lateral) is recommended to ensure full exposure and precise handling of both vascular and bone structures. To support such complex interventions, the development of specialized preclinical surgical suites is necessary. These facilities must be equipped with advanced microsurgical technology, veterinary anesthesia, and comprehensive perioperative monitoring and intensive care capabilities.

4. Multimodal Postoperative Monitoring and Validation

A systematic, multi-tiered monitoring protocol should be implemented to evaluate graft tolerance and integration:

Clinical & Paraclinical: Monitoring wound healing, limb function, and systemic inflammatory markers.

Microangiographic Evaluation: Serial microangiography should be performed at fixed intervals (e.g., 7, 14, and 28 days) to validate the functional patency and revascularization of the decellularized pedicle.

Histological & Immunohistochemical Analysis: The use of IHC is essential to assess tissue remodeling, revascularization processes, and potential immune rejection patterns.

Implementing these standardized protocols will consolidate the transition from experimental bioengineering to the clinical application of decellularized vascularized composite allografts for the treatment of critical-sized bone defects.

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LIST OF PUBLICATIONS AND PARTICIPATIONS IN SCIENTIFIC FORUMS

Thesis Title:"RECONSTRUCTION OF TUBULAR BONE DEFECTS USING ALLOGRAFTS INCLUDED IN THE ADOPTIVE VASCULAR CIRCUIT" (experimental study)"

Specialty: 321.18 Orthopedics and Traumatology.

I. SCIENTIFIC ARTICLES

1. Articles in International Peer-Reviewed Proceedings (Indexed in SCOPUS/SJR):

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). 6th International Conference on Nanotechnologies and Biomedical Engineering (ICNBME 2023); IFMBE Proceedings. Springer, Cham, 2023; vol. 91: pp. 332-347. ISBN 978-3-031-42774-9. DOI: 10.1007/978-3-031-42775-6_37 (SJR: 0.155, SCOPUS).

Stoian A., **Pavlovschi E.**, Capros N., Verega Gr., Nacu V. Effectiveness of tissue engineering in obtaining the vascularized composite bone extracellular matrix. Experimental study. In: Tiginyanu I., Sontea V., Railean S. (eds). 6th International Conference on Nanotechnologies and Biomedical Engineering (ICNBME 2023); IFMBE Proceedings. Springer, Cham, 2023; vol. 91: pp. 357-365. ISBN 978-3-031-42774-9. DOI: 10.1007/978-3-031-42775-6_39 (SJR: 0.155, SCOPUS).

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). 4th International Conference on Nanotechnologies and Biomedical Engineering (ICNBME 2019). IFMBE Proceedings. Springer, Cham, 2020; vol. 77: pp. 589-593. DOI: 10.1007/978-3-030-31866-6 (SJR: 0.155, SCOPUS, IF: 0.38).

2. Articles in Accredited National Scientific Journals (Category B+):

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.

II. SCIENTIFIC FORUM PARTICIPATIONS

1. National Accredited Journals (Category B+):

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.

2. National Accredited Journals (Category C+):

Pavlovschi E., Verega Gr., Nacu V., Capros N., Cociug A. Decellularized bone allografts – premises for bone defect repair in dentistry. In: Stomatological Medicine. 2024; 69(4): pp. 41-49. ISSN 1857-1328. DOI: 10.53530/1857-1328.24.4.05.

II. CONFERENCE PROCEEDINGS AND ABSTRACTS

1. International Conferences (Held Abroad):

Stoian A., **Pavlovschi E**., Verega Gr., Nacu V., Andree B., Hilfiker A. Experimental study in non-immunogenic vascularized bone allograft. In: Abstract Book of the 3-in-1 Joint Congress in Plastic and Reconstructive Surgery. Timișoara, Romania, 2022: p. 36. ISBN: 978-606-786-274-4.

Pavlovschi E., Stoian A., Verega Gr., Nacu V. The vascularized bone allotransplantation in vivo experiment: Preliminary report. In: Abstract Book of the 4th International European Conference on Interdisciplinary Scientific Research. Warsaw, Poland, 2021: p. 264. ISBN: 978-1-955094-13-9.

Pavlovschi E., Stoian A., Cobzac V., Verega Gr., Nacu V. Vascularized bone allotransplantation – can it be performed without immunosuppression? In vivo experimental study. In: Program Book of the National Congress of the "Romtransplant" Association. Romania, 2020: p. 11 (Online Edition).

Pavlovschi E., Stoian A., Verega Gr. Vascularized allotransplant: a successful alternative for massive bone defects. In: Abstract Book of the National Congress of Orthopedics. Sibiu, Romania, Oct 3–5, 2018: p. 83.

2. International Conferences (Held in the Republic of Moldova):

Pavlovschi E., Stoian A., Mihaluta V. The vascularized allotransplant – a successful alternative for massive bone defects. In: Abstract Book of MedEspera 2018: International Medical Congress for Students and Young Doctors. Chisinau, 2018: pp. 143-144.

Pavlovschi E., Stoian A. Vascularized bone allotransplantation in a rabbit model: preliminary report. In: Abstract Book of MedEspera 2020: 8th International Medical Congress for Students and Young Doctors. Chisinau, 2020: pp. 115-116. ISBN 978-9975-151-11-5.

Mihaluta V., Stoian A., **Pavlovschi E.**, Verega Gr. Reconstruction of scalp defects with the trapezius muscle flap (Clinical Case). In: Abstract Book of MedEspera 2018. Chisinau, 2018: pp. 32-33.

3. National Conferences with International Participation:

Pavlovschi E., Stoian A., Gardikiotis I., et al. In vivo testing of vascularized bone allotransplantation after decellularization. In: Abstract Book of the Annual Scientific Conference: Cells and Tissues Transplantation. Chisinau, 2023: p. 37.

Stoian A., **Pavlovschi E.,** Verega Gr., et al. Experimental study in obtaining a vascularized composite bone extracellular matrix. In: Abstract Book of the Annual Scientific Conference: Cells and Tissues Transplantation. Chisinau, 2023: p. 40.

4. National Conferences (Republic of Moldova):

Pavlovschi E., Stoian A., Malcova T., et al. Combined decellularization of vascularized bone allografts: an in vivo experimental study stage. In: Congress of the 75th Anniversary of "Nicolae Testemițanu" SUMP. Chisinau, 2020: p. 519.

Stoian A., Nacu V., **Pavlovschi E**., et al. Future Perspectives of vascularized bone allotransplantation. In: Congress of the 75th Anniversary of "Nicolae Testemițanu" SUMP. Chisinau, 2020: p. 525.

Pavlovschi E., Verega Gr., Stoian A., Nacu V. Surgical Protocol for vascularized bone allotransplant: the Next Stage of in vivo experimental study. In: Biomedical and Health Research Conference. Chisinau, 2021: p. 333. ISBN 978-9975-82-223-7.

Stoian A., **Pavlovschi E.**, Nacu V., et al. Principles of Decellularization for composite vascularized bone graft. In: Biomedical and Health Research Conference. Chisinau, 2021: p. 337.

III. INTELLECTUAL PROPERTY & INNOVATION

Innovation Certificate No. 6052 (May 16, 2023): "Decellularized vascularized bone allografts as a Treatment Method for critical bone defects." **Pavlovschi E**., Stoian A., Nacu V., Verega Gr.

Innovation Certificate No. 6058 (May 24, 2023): "Decellularization Method for vascularized composite bone grafts." Stoian A., **Pavlovschi E.**, Verega Gr., Nacu V.

EUROINVENT 2025 – Gold Medal: "Nanostructured Bone Grafts with predetermined properties." Joint Bilateral Project (Moldova-Romania). Team: Ficai A., Nacu V., Pavlovschi E., et al.

IV. EDUCATIONAL COURSES & FELLOWSHIPS

„Eugen Ionescu" Doctoral Research Fellowship (AUF): Sept 2021 – Feb 2022. Microsurgery Laboratory, CEMEX, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania.

State Research Project (No. 20.80009.5007.20): "GaN-based nanoarchitectures and 3D biological matrices for microfluidics and tissue engineering." Chisinau, 2020–2023.

Bilateral Research Project (2024–2026): "Nanostructured bone grafts with predetermined properties." Laboratory of Tissue Engineering and Cell Cultures (SUMP "Nicolae Testemițanu") in collaboration with the Center for Personalized Medicine (POLITEHNICA University of Bucharest, Romania)

V. POSTER PRESENTATIONS AT SCIENTIFIC FORUMS / EXHIBITIONS

International Forums:

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, Turkey, May 16–18, 2019.

National Forums:

Stoian A., Nacu V., **Pavlovschi E**., Macagonova O., Malcova T., Mihaluţa V. Future perspectives of vascularized bone allotransplantation. In: Congress dedicated to the 75th Anniversary of "Nicolae Testemițanu" SUMP. Online Edition. Chisinau, October 21–23, 2020.

Pavlovschi E., Stoian A., Malcova T., Iordăchescu R. Verega Gr., Nacu V. Combined decellularization of vascularized bone allografts: An in vivo experimental study stage. In: Congress dedicated to the 75th Anniversary of "Nicolae Testemițanu" SUMP. Online Edition. Chisinau, October 21–23, 2020.

ADNOTARE

Pavlovschi Elena

„Plastia defectelor oaselor tubulare cu allografă inclusă în circuitul vascular adoptiv” (studiu experimental)

Teză de doctor în științe medicale, Chișinău, 2026.

Lucrarea este expusă pe 136 pagini de text electronic și include: Introducere, III capitole, concluzii generale și recomandări practice. Indicele bibliografic citează 142 surse literare. Materialul ilustrativ este reprezentat în 17 tabele și 32 figuri. Rezultatele obținute sunt publicate în 5 lucrări științifice.

Cuvinte-cheie: defect osos critic, alografă osoasă vascularizată, experiment in *vivo*

Scopul: cercetarea premiselor pentru plastia defectelor osoase tubulare prin metoda experimentală in *vivo* cu alografe osoase vascularizate decelularizate incluse în circuitul vascular, pentru aprecierea fezabilității

Obiective:

1. Cercetarea anatomică a vascularizării membrului posterior la animal de laborator (NZWR) și stabilirea segmentului osos tubular lung cu pedicul vascular optim.
2. Elaborarea procedurii de prelevare și conservare a segmentului osos tubular lung cu pedicul vascular optim pentru conservare, decelularizare
3. Elaborarea protocolului de anestezie și intraoperator pentru plastia ortotopică *in vivo* a defectului osos tubular lung cu alografă vascularizată compozită decelularizată
4. Cercetarea și testarea in *vivo* a vaselor decelularizate prin metodă mixtă (compatibilă pentru grefele osoase integrale compozite - os +vas), pentru studierea rezistenței mecanice la sutura microchirurgicală, a TA, undă de puls in circuitul gazdă la animal de laborator, șobolani Wistar.
5. Cercetarea prin testarea in *vivo* a țesutului osos decelularizat prin metodă mixtă (compatibilă pentru grefele osoase integrale compozite - os +vas) revascularizat prin studierea manifestărilor clinice și paraclinice ale plastiei defectelor osoase tubulare.

Noutatea și originalitatea cercetării: combinarea în premieră a decelularizării alografelor vascularizate osoase cu tehnicile microchirurgicale, pentru includerea lor în circuitul vascular gazdă sau adoptiv, ar permite obținerea unei grefe ortotopice care nu ar necesita imunosupresie, ar păstra integral vascularizarea, proprietățile biologice ale osului pentru consolidare și o funcție reparatorie eficientă.

Semnificația teoretică: cercetarea literaturii de specialitate a permis trasarea vectorului studiului spre tacticile de tratament experimental ale defectelor osoase tubulare prin alografe osoase vascularizate ortotopice, care nu ar necesita imunosupresie postgrefare, testate in *vivo* în premieră după procesul de decelularizare.

Valoarea aplicativă a lucrării: Elaborarea unui algoritm de prelevare și plastie a defectelor osoase tubulare *in vivo* cu alografe osoase vascularizate decelularizate reincluse microchirurgical în circuitul adoptiv. **Implementarea rezultatelor științifice:** rezultatele studiului experimental obținute au fost implementate în cadrul Laboratorului de Inginerie Tisulară și Culturi Celulare, Catedra Ortopedie și Traumatologie a USMF N. Testemițanu. Rezultatele obținute au fost implementate în cadrul proiectelor de cercetare: 1.„Nanoarhitecturi în bază de GaN și matrici tridimensionale din materiale biologice pentru aplicații în microfluidică și inginerie tisulară” - 20.80009.5007.20 oferit de Agenția Națională de Cercetare Dezvoltare a Guvernului Republica Moldova; 2. Proiect bilateral: Grefoni osoși nanostructurați cu proprietăți predeterminate (2024-2026) (nr. 29ROMD din 20.05.2024) – Laboratorul de inginerie tisulară și culturi celulare – Universitatea de Stat de Medicină și Farmacie „Nicolae Testemițanu” (Republica Moldova) și Centrul de Medicină Personalizată – Universitatea Națională de Știință și Tehnologie POLITEHNICA București, România.

SUMMARY

Pavlovschi Elena

„Plasticity of tubular bone defects with allografts included in the adoptive vascular circuit” - experimental study, PhD Thesis of Medical Sciences, Chișinău, 2026

The work is presented on 136 pages of electronic text and includes an Introduction, three chapters, general conclusions, and practical recommendations. The bibliographic index cites 142 literary sources. Illustrative material comprises 17 tables and 32 figures. The results obtained have been published in 5 scientific works.

Key words: critical bone defect, vascularized bone allograft, in vivo experiment.

Main aim of the work: An experimental *in vivo* study on the feasibility of critical-sized tubular bone defects using decellularized vascularized composite allografts integrated into the host circulatory system.

Research objectives:

1. Review of specialized literature to evaluate contemporary methodologies for the treatment of critical bone defects;

2. Anatomical research on hind limb vascularization in laboratory animals (NZWR) to establish the bone segment with the optimal vascular pedicle;

3. Development of a procedure for harvesting bone segments with a vascular pedicle for preservation, decellularization, and subsequent integration into the recipient vascular circuit;

4. In vivo testing of vessels decellularized via a mixed method within composite bone grafts (bone+vessel), to study mechanical resistance to microsurgical suturing, blood pressure (BP), and pulse waves;

5. Investigation of local and systemic postoperative manifestations following placement of the vascularized bone allograft in the recipient organism compared with control groups;

Novelty and originality of the research: Combining the decellularization of vascularized bone allografts with microsurgical techniques by integrating them into the host vascular circuit. This approach allows for obtaining a graft that does not require immunosuppression while fully preserving vascularization, biological properties for bone consolidation, and effective reparative function.

Theoretical significance: The need for orthotopic vascularized bone allografting that does not require post-grafting immunosuppression is a highly relevant and current topic in reconstructive surgery.

Applicative value of the work: Development of an algorithm for sampling and reconstruction (plasty) of critical in vivo bone defects using decellularized vascularized bone allografts.

Implementation of scientific results: The results of this experimental study were implemented within the Laboratory of Tissue Engineering and Cell Cultures, Department of Orthopedics and Traumatology of USMF “Nicolae Testemițanu”. Furthermore, the results were integrated into the research projects: 1. "GaN-based nanoarchitectures and three-dimensional matrices of biological materials for applications in microfluidics and tissue engineering" (20.80009.5007.20), funded by the National Agency for Research and Development of the Government of the Republic of Moldova; 2.Bilateral Project: Nanostructured bone grafts with predetermined properties (2024–2026) (Grant No. 29ROMD/20.05.2024) – Laboratory of Tissue Engineering and Cell Cultures – “Nicolae Testemițanu” State University of Medicine and Pharmacy (Republic of Moldova) and the Personalized Medicine Center – National University of Science and Technology POLITEHNICA Bucharest, Romania.

PAVLOVSCHI Elena

RECONSTRUCTION OF TUBULAR BONE DEFECTS USING ALLOGRAFTS INCLUDED IN THE ADOPTIVE VASCULAR CIRCUIT (experimental study)

321.18 – ORTHOPEDICS AND TRAUMATOLOGY

SCIENTIFIC ABSTRACT
of the PhD Thesis in Medical Sciences

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