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Dear colleagues,

We present to your attention a special issue of the journal *Genij Ortopedii*. The articles selected for this issue unite the authors from various countries and institutions with a very important topic. They are devoted to various aspects of the development of bioactive implants and to biological process control in orthopedics. This direction is now being actively developed by scientists and researchers from all over the world. Russia does not lag behind, and even surpasses them in some developments. Universities, institutes, medical and research centers are joining their efforts to build a domestic market for high-tech medical products.

The issue contains articles written by authors from Russia, Serbia, France, Switzerland, the USA, Hungary, and India. Several studies were prepared in collaboration with colleagues from the Ilizarov Center.

The articles present both theoretical and experimental developments, as well as original research on the practical application of innovative technologies and literature reviews on the topic. It is noteworthy that out of 13 articles in the issue, seven studies are devoted to the study of bioresorbable materials and implants that are in demand in contemporary medicine. Thus, Pierre Lascombes, Pierre Journeau and Dmitry Popkov present their own experience of using resorbable implants in pediatric orthopaedics and traumatology. Seven children with long bone fractures were treated using resorbable screws (ActivaScrew™). In the immediate postoperative period, no cases of excessive swelling, hyperemia or other pathological reaction from the soft tissues were detected. In all cases, pain disappeared by the seventh postoperative day. Restoration of weight-bearing ability, the possibility of full weight bearing on the operated limb, and normal physical activity was noted within a standard term for such injuries. The colleagues conclude that the main indications for the use of resorbable implants in children remain fractures and osteotomies that need to be fixed with screws, while the development of the production of plates and elastic screws from resorbable materials will expand the indications for their use.

Authors from Hungary (Gergő Jozsa, Tamas Kassai, Marcell Varga) share their results of using resorbable elastic intramedullary nails for fractures of the forearm bones in 4 patients. Bone union without secondary displacement along with anatomical and functional recovery was observed 5-7 months after surgery in each case. It is claimed that the resorbable osteosynthesis material provides reliable stability and similar results as when using metal nails. The obvious advantage of resorbable implants is that there is no need to remove them. Irritation of soft tissues by the protruding end of the nail is also excluded, since according to the technology it is cut off at the level of the bone. Thus, surgical treatment of forearm fractures using resorbable implants is a reasonable alternative to metal intramedullary nails.

The results of tibial lengthening using an intramedullary degradable implant are presented by authors from Kurgan (Popkov AV, Gorbach ES, Mamedov UF, Stepanov RV). For the first time in clinical practice, a case of surgical lengthening of the tibia with the Ilizarov apparatus and an intramedullary degradable implant made of polycaprolactone (PCL) saturated with hydroxyapatite was used in a 10-year-old patient to stimulate reparative regeneration of the tibia. The process of lengthening the tibia was accompanied by a pronounced formation of a bone "coupling" around the implant, which was directly connected to the endosteum of the tibia. It is concluded that the implant used is not inferior in characteristics to titanium wires coated with hydroxyapatite in terms of osteoinduction and does not require a repeated surgical intervention for removal.

The use of bioactive biodegradable implants made of polycaprolactone for the treatment of osteochondral defects was the topic of the study by Popkov AV, Gorbach ES, Gorbach EN , Kononovich NA , Kireeva EA , Popkov DA. Specialists from the Ilizarov Center conducted a comparative study on 76 Wistar rats, divided into 2 groups, in which an osteochondral defect of the medial femoral condyle was modeled. In the experimental group, the defect was treated with a biodegradable bioactive membrane made of polycaprolactone with hydroxyapatite. In the control group, the simulated defect was not managed. Results were assessed over a one-year period using clinical, anatomical, histological, biomechanical and statistical methods. The range of motion in the knee joint in the animals of the experimental group at all stages of the experiment was significantly better than in the control group. The implant ensured the integrity and congruence of the articular surface. On the 180th day, at the site of the defect filled with the implant, a newly formed area of the articular surface of an organotypic structure was observed with the subchondral bone being replenished with bone tissue, and the articular surface with cartilaginous tissue. The authors conclude that a bioresorbable polycaprolactone implant impregnated with hydroxyapatite particles is effective for healing osteochondral defects.

Experimental work on the production of bioresorbable implants and the study of their properties is presented in three studies.

Scientists from Tomsk Polytechnic University and their co-authors from Kurgan propose a method for applying hydroxyapatite to the surface of three-dimensional scaffolds made of ε-polycaprolactone by processing in a "good/bad" solvent mixture. The proposed processing method ensures uniform coverage of the external and internal surfaces of polycaprolactone scaffolds manufactured by 3D printing with a layer of hydroxyapatite particles, while maintaining their porous structure. The presence of a bioactive layer on the surface of bioresorbable polymer scaffolds can expand their use in clinical practice for surgical treatment of bone defects.

Stogov MV and co-authors (Kurgan, Tomsk) presented the results of studying the rate of degradation of a material with a polylactide (PLLA)/hydroxyapatite (HA) composition depending on the crystallinity of the polymer

structure. The study showed that the crystallinity of PLLA influenced the kinetics of HA release from the samples of the studied materials. As crystallinity increases, the rate of HA hydrolysis increases. This observation can be explained by the fact that the polymer in the crystalline phase underwent hydrolysis faster than in the amorphous state. The authors show that changing the HA content and the PLLA crystallinity enables to control the biological characteristics of PLLA/HA composite materials.

An *in vitro* study of the bactericidal activity of implants made of biodegradable material (polycaprolactone) impregnated with hydroxyapatite and an antibiotic is the topic of the study by Popkov DA et al. (Tomsk, Moscow). The authors demonstrated that porous implants made from PCL and impregnated with an antibiotic have significant antimicrobial activity against the most common gram-negative and gram-positive bacteria that cause purulent complications in surgical practice. Nanostructured hydroxyapatite on the surface of the implant does not decrease bactericidal activity. The proposed implants will help stimulate bone regeneration and simultaneously provide an antimicrobial effect.

The experience of using customized implants is of great interest. Thus, Korytkin AA et al. (Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, Novosibirsk) in *in vitro* experiments and clinical studies investigated the biological fixation of customized implants in managing post-traumatic deformities of the acetabulum. The results of the experiment to study the penetration of living fibroblasts into the porous structure of implants with different pore sizes showed that metal structures with a pore size of 400-499 microns can be distinguished from all others, since at a given pore size the penetration of living fibroblasts into the structure of the implant surface is the greatest. Management of bone tissue defects in the acetabulum area using customized implants with a mesh porous structure surface (400-499 microns) showed signs of biological fixation in the bone tissue surrounding the customized implant in the study group after 12 months.

Issues of new treatment methods are discussed in four publications. Leonchuk SS and his co-author from India present a literature review and clinical case of a new surgical approach to the treatment of aneurysmal bone cyst (ABC) of the medial cuneiform bone. A 47-year-old woman with a 10-month history of pain and swelling in her right foot underwent en-bloc resection (complete removal of the medial cuneiform bone remnant), the defect was filled with a fibula graft from the right leg, and an allograft ("Bio-Ost"®) was placed along with the autograft. The postoperative period was uneventful with complete healing of the bone defect without relapse after 12 months of follow-up. The AOFAS score increased significantly from 34 points preoperatively to 92 points at a 1-year follow-up. Based on their work, the authors conclude that the use of a combination of Ilizarov external fixation and bone grafting provided favorable conditions for foot bone defect healing in this ABC case without complications, maintaining the patient's mobility and early axial load.

The use of combined osteosynthesis in the treatment of diaphyseal fractures of the tibia is discussed in the article by Popkov AV et al. (Kurgan), which assessed the effectiveness of a combination of transosseous osteosynthesis with intramedullary reinforcement with elastic titanium nails coated with hydroxyapatite (HA-coated nails) in the treatment of fractures of long bones. It has been shown that the advantages of the combined method contribute to reducing the time of external fixation, reducing the number of wires and half-pins in the external fixation apparatus, stimulating the formation of callus and preventing secondary displacement of bone fragments.

A mini-review of current concepts of mechanical methods of distraction regenerate stimulation is presented by Cherkashin A (Texas Scottish Rite Hospital for Children). It is proposed to define axial dynamization as the ability to provide axial load on the bone regenerate with minimal displacement or bending forces. Axial dynamization can be carried out through direct stimulation of the regenerate by axial cyclic loads and the exclusion of bending and displacement forces. The author concludes that axial dynamization, together with other non-invasive methods of mechanical stimulation of the distraction regenerate, should become a mandatory element in limb lengthening.

Popkov AV and Popkov DA aimed to identify new directions in the study, production and clinical use of bioactive implants for indications similar to autografts. The authors conclude that the main current trends in orthopedic bioengineering are 3D-printed implants that provide deterministic cell migration, proliferation and differentiation and maintain sufficient mechanical strength of their structure for the required time. The combination of biodegradable implants with impregnation with bone morphogenetic protein stimulates the regeneration of the reconstructed bone. Programmed and controlled resorption of implants along with filling the tissue with new bone is the main vector in the development of bone tissue engineering.

We are confident that this thematic issue will be interesting and useful to specialists and will acquaint the expert community not only with the current state of the field, but also outline promising projects for future cooperation.

Have a nice and useful reading!

D.A. Popkov
Professor of the Russian Academy of Sciences,
corresponding member of French Academy of Medical Sciences

S.I. Tverdokhlebov
Candidate of Physical and Mathematical Sciences

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Solvent/non-solvent treatment as a method for surface coating of poly(ε-caprolactone) 3D-printed scaffolds with hydroxyapatite

V.S. Bocharov[1], G.E. Dubinenko[1], D.A. Popkov[2], A.V. Popkov[2], S.I. Tverdokhlebov[1]✉

[1] Tomsk Polytechnic University, Tomsk, Russian Federation
[2] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Sergey I. Tverdokhlebov, tverd@tpu.ru

Abstract

Introduction Over the last decades numerous new materials and techniques for bone tissue engineering have been developed. The use of bioresorbable polymeric scaffolds is one of the most promising techniques for surgical management of bone defects. However, the lack of bioactive properties of biodegradable polymers restricts the area of their application for bone tissue engineering. **The aim of study** was to apply solvent/non-solvent treatment to coat the surface of 3D-printed bioresorbable poly(ε-caprolactone) scaffolds with bioactive hydroxyapatite particles and report on the physicochemical properties of the resulting materials. **Material and Methods** In the present study, biomimetic poly(ε-caprolactone) scaffolds were 3D-printed via fused deposition modeling technology and their surface was treated with the solvent/non-solvent method for coating with bioactive particles of hydroxyapatite. **Results** It has been found that treatment in the mixture of toluene and ethanol is suitable for the coating of poly(ε-caprolactone) scaffolds with hydroxyapatite. The scaffolds maintain porous structure after treatment while hydroxyapatite particles form homogeneous coating. The amount of hydroxyapatite on the treated scaffolds was 5.7 ± 0.8 wt. %. **Discussion** The proposed method ensures a homogeneous coating of outer and inner surfaces of the poly(ε-caprolactone) scaffolds with hydroxyapatite without a significant impact on the structure of a scaffold. Fourier-transform infrared spectroscopy confirmed that the solvent/non-solvent treatment has no effect on the chemical structure of PCL scaffolds. **Conclusion** Coating of biomimetic 3D-printed PCL scaffolds with bioactive hydroxyapatite by the solvent/non-solvent treatment has been successfully carried out. Upon coating, scaffolds retained their shape and interconnected porous structure and adsorbed hydroxyapatite particles that were uniformly distributed on the surface of the scaffold.

Keywords: bone tissue engineering, scaffolds, polycaprolactone, hydroxyapatite

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INTRODUCTION

The development of new functional materials for the fabrication of biodegradable tissue engineering scaffolds is an important task in medical materials science [1-3]. Two-dimensional and three-dimensional scaffolds made from natural and synthetic polymers have found applications in the regeneration of biological tissues and the restoration of tissue defects. One of the widely studied biodegradable materials used for tissue defect replacement is poly(ε-caprolactone) (PCL) [4]. However, PCL, like most biodegradable polyesters, lacks functional properties, making its use without bioactive additives not effective [5-8]. Hydroxyapatite (HAP), a mineral that supports the proliferation and differentiation of mesenchymal stem cells in the osteogenic direction and stimulates the mineralization of bone regenerate, is frequently used for bone tissue regeneration in medicine [9-12]. The main methods for combining biodegradable polymers and bioactive hydroxyapatite are the fabrication of polymer composites and the deposition of coatings on the surface of polymer scaffolds [13-16]. Composites have demonstrated their effectiveness for bone defect management in a number of studies [17-19]. However, an important drawback of composites is the lack of bioavailable hydroxyapatite on the surface of the fabricated composite scaffold. Hydroxyapatite in the subsurface layer of the composite scaffold is covered by a thin layer of polymer, which hinders the contact of the hydroxyapatite particles with the surrounding tissues during the first weeks after implantation.

Currently, there are two main methods for forming a layer of bioavailable hydroxyapatite on the surface of biodegradable polymer scaffolds: etching composite polymer/hydroxyapatite scaffolds in alkaline solutions to exposure bioactive particles on the surface, and in situ precipitation of hydroxyapatite on the surface of polymer scaffold [11, 16, 20]. The disadvantage of the first method is the initiation of the hydrolysis

process of polymer chains in the surface layers of the scaffold, which often leads to the loss of the mechanical properties of the scaffold and changes in its degradation profile. On the other hand, in situ mineralization results in the formation of a relatively thick continuous layer of hydroxyapatite on the surface of the scaffold, which isolates the polymer from the surrounding environment and hinders the degradation process of the polymer matrix.

A promising approach for modifying the surface of PCL involves treatment of the polymer with a mixture of organic solvents to partially swell its surface [21]. The swelled surface layer of the polymer can adsorb biologically active molecules and particles from the contacting medium. However, this technique has not yet been applied to the relevant task of fabrication bioactive coatings on the surface of 3D-printed porous scaffolds. In this study, we report on the application of a solvent/non-solvent treatment to coat the surface of 3D-printed PCL scaffolds with bioactive hydroxyapatite particles and on the physicochemical properties of the resulting materials.

The main objective of this study was to investigate the application of solvent/non-solvent treatment as a method for surface coating of PCL 3D-printed scaffolds with HAP. The study proposes a method for applying dispersed HAP particles onto the surface of PCL scaffolds using a mixture of a solvent toluene, and a non-solvent ethanol. Based on the Design of Experiments (DOE), optimal coating parameters and the optimal ratio of solvent" and non-solvent were determined.

MATERIALS AND METHODS

Poly(ε-caprolactone) (PCL; Mn 80000 $g \cdot mol^{-1}$) was purchased from Sigma-Aldrich (Sigma-Aldrich, Gillingham, United Kingdom), hydroxyapatite (HAP; nanoXIM•HAP203, average particle size 10.0 ± 5.0 μm) was purchased from Fluidinova (Fluidinova S.A., Maia, Portugal), toluene (anhydrous, 99.8%) was purchased from EKOS-1 (EKOS-1, Moscow, Russia), ethanol (≥ 99.5 %, water ≤ 0.20 %) was purchased from Merck (Merck KGaA, Darmstadt, Germany).

3D printing of polycaprolactone scaffolds

PCL pellets were melted and extruded with the use of Filabot EX2 (Filabot HQ, Barre, Vermont, USA) single screw extruder to fabricate filament of 2.8 ± 0.15 mm in diameter. The temperature of extrusion was 80 ± 3 °C and the rate of extrusion was 2 $m \cdot min^{-1}$. Extruded filament was used for the 3D printing of scaffolds with a commercial FDM 3D printer Ultimaker S5 (Ultimaker B.V., Utrecht, Netherlands). The temperature of the glass substrate was 35 °C and 200 °C for the printer nozzle. The printing was performed at a printing rate of 6 $mm \cdot s^{-1}$. Scaffolds had a shape of porous cylinders with the diameter of 10 mm and the height of 3 mm. Internal porous structure of scaffolds was printed with the gyroid infill with the infill struts distance of 1 mm.

Hydroxyapatite coating

Solvent/non-solvent treatment of scaffolds was performed in the mixture of toluene and ethanol at 3:7 v/v ratio. HAP was mixed with the toluene/ethanol mixture at 10% w/w and stirred with the use of magnetic stirrer for 30 minutes to obtain suspension. Scaffolds were dipped into the suspension for 2 minutes at room temperature under continuous stirring. Coated scaffolds were washed with ethanol and dried for 24 hours under vacuum (1 mbar) at room temperature.

Scaffolds characterization

Investigations of the surface of the scaffolds and dispersion of HAP coating on the scaffolds were performed by the scanning electron microscopy (SEM) on a JEOL JCM-6000 (JEOL Ltd., Tokyo, Japan). All SEM imaging processes were performed in low vacuum at 15 kV accelerating voltage. The scaffolds were sputter-coated with gold on a JEOL Smart Coater (JEOL Ltd., Tokyo, Japan) prior to SEM examinations.

The chemical composition of the scaffolds was investigated by attenuated total reflectance (ATR) Fourier-transform infrared spectroscopy (FTIR) on Tensor 27 (Bruker Optik GmbH, Ettlingen, Germany) with a Miracle™ single reflection ATR attachment (PIKE Technologies, Madison, Wisconsin, USA). The measurements were performed with a ZnSe crystal at an incident angle of 45°. All FTIR spectra were recorded in the spectral range of 530-4000 cm^{-1} with a resolution of 4 cm^{-1}.

Thermal stability of the scaffolds and solid inorganic residue from HAP were studied by the thermogravimetric analysis (TG) in an inert atmosphere on a simultaneous thermal analyzer SDTQ 600 (Artisan TG, Champaign, Illinois, USA) in the range of 40-800 °C with 10 $°C \cdot min^{-1}$ heating rate. For the TG analysis 20 mg samples were cut from the middle porous part of the coated scaffolds.

RESULTS

The 3D-printed scaffolds had the appearance of porous cylinders with a smooth glossy surface. After surface treatment, the translucent glossy scaffolds changed their appearance to the matte white color of HAP. Surface analysis of the scaffolds by scanning electron microscopy demonstrated a change in the morphology

of the struts. After treatment of the scaffolds in a mixture of solvents, the struts became thicker and had a smoother and more rounded morphology (Fig. 1, top row). Despite the visual decrease in the size of the pores, the scaffolds retained its internal interconnected pore structure and full permeability. The surface of the scaffold treated in the solvent mixture was uniformly coated with segregate particles of HAP (white particles in Figure 1, middle row). It should be noted that there were no agglomerates or HAP particles (Fig. 1, middle and bottom rows). The coating was observed both on the top layers of the scaffold and in its depth within the pores.

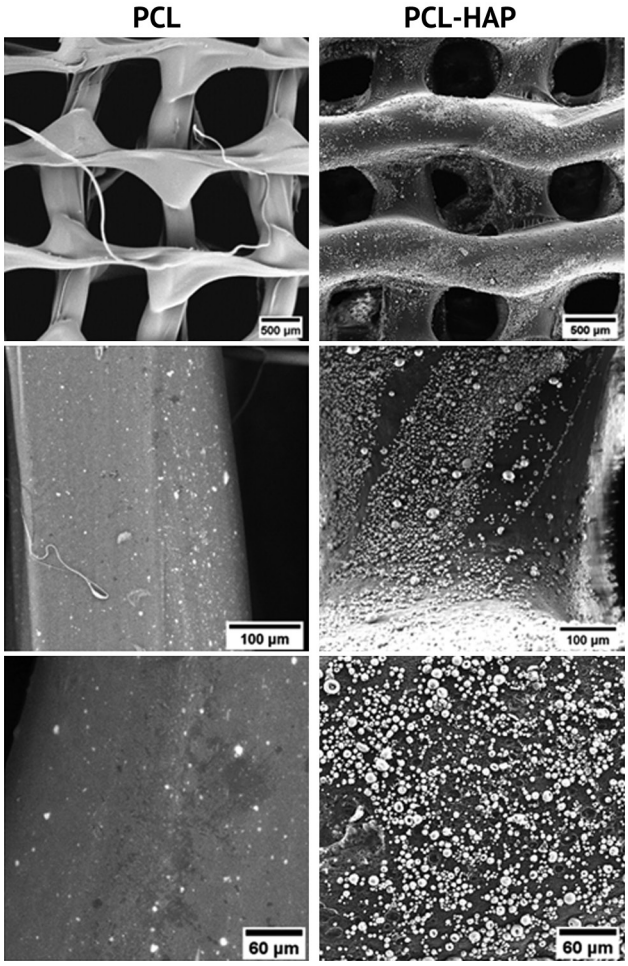

Fig. 1 Microscopic appearance of the PCL scaffold surface at different magnifications: 3D-printed gyroid PCL scaffold on the left; 3D-printed PCL gyroid scaffold with HAP coating on the right

FTIR spectra of the scaffolds are shown in Figure 2. The PCL scaffold spectrum is characterized by the following main bands: 2945 cm^{-1} ($\nu_{as}CH_2$), 2868 cm^{-1} ($\nu_{s}CH_2$), 1724 cm^{-1} (νC = O), 1294 cm^{-1} (ν_{s}C-O, ν_{s}C-C), 1240 cm^{-1} (ν_{as}C-O-C) and 1168 cm^{-1} (ν_{s}C-O-C) [22]. In the coated scaffold spectrum, there are also HAP-related bands at 1042 cm^{-1} (νPO), 958 cm^{-1} (δPO), 730 cm^{-1} (δPO), 706 cm^{-1} (δPO) present. There are no significant differences in the shape, width and wavenumber position of PCL bands both in spectrum of the 3D-printed and coated scaffolds. It is important to note that the presence of hydrophilic HAP on the surface of the scaffold increases the oxidative reaction of PCL and introduces hydroxyl groups into the polymer backbone [23]. However, there is no evidence for active oxidative degradation of the PCL scaffolds, which is usually indicated by a broadening of the carbonyl peak of the ester groups of polyesters at 1724 cm^{-1}.

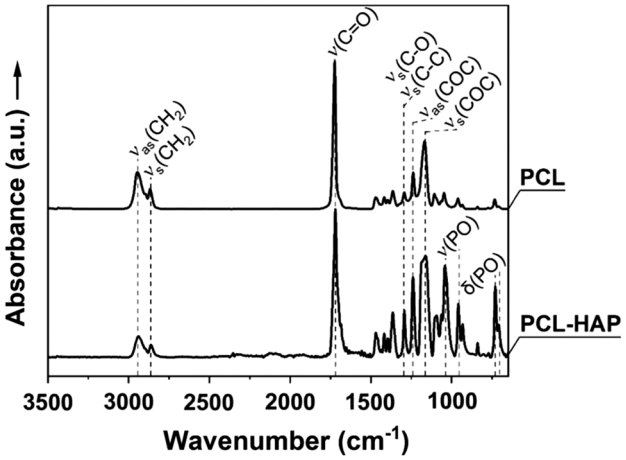

Fig. 2 Chemical characterization of the scaffolds by FTIR

Results of the thermogravimetric analysis (TG) are shown in Figure 3. HAP coating decreased the temperature of the beginning of PCL decomposition from 265 ± 11 °C for PCL scaffold to 190 ± 19 °C for PCL-HAP coated scaffold. After the decomposition and loss of the organic components of the scaffolds, the amount of nonorganic residual from HAP was 5.7 ± 0.8 wt. %. Considering that the samples for TG analysis were cut from the middle porous part of the scaffolds, the amount of HAP confirms successful fabrication of the coating on the inner surfaces.

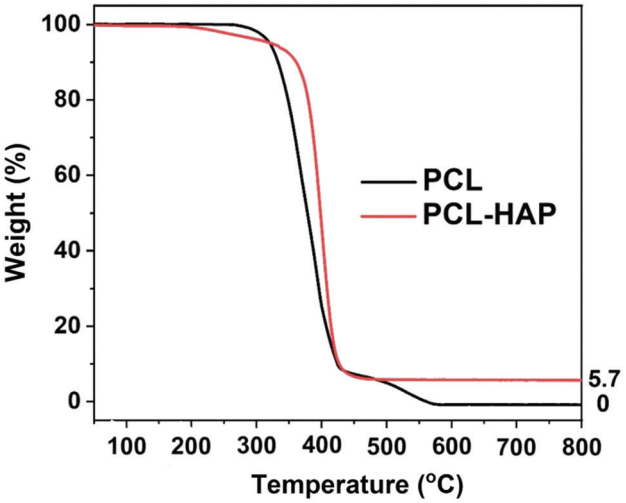

Fig. 3 Thermogravimetric analysis of the scaffolds with indicated solid inorganic residue from HAP

DISCUSSION

Over the last decades, biomedical scaffolds made from bioresorbable polymers for the application in orthopaedics have been gaining interest due to the disadvantages of traditional implants made of metal alloys: the need for their surgical removal from the body, exemption to use in children and adolescents, difficulty to examine bone regeneration in radiography and magnetic resonance imaging due to overlapping, possible mechanical stress at the bone and metal interface. Bioresorbable polymers change their macromolecular structure and physicochemical properties upon contact with the biological environment but do not produce a harmful effect during their resorption [24]. Such polymers contain hydrolytically unstable functional groups which degrade as a result of hydrolysis, and their by-products are removed through normal cellular metabolism [24-26]. Moreover, biodegradable polymers are known for they X-ray transparency, mechanical properties in the range of biological tissues properties. However, the bsence of metal implant shortcoming does not make the use of bioresorbable polymer scaffolds justified. To increase efficiency of treatment with biodegradable polymeric scaffolds, new requirements are imposed on them: osteoinductivity, osteoconductivity, biocompatibility and biodegradation.

Various substances have the ability to induce early bone formation. Thus, calcium phosphates have excellent osteoinductivity and osteoconductivity for maintaining the proliferation and differentiation of osteoblasts, and also prevent encapsulation of the implant by fibrous tissues [27, 28]. The application of such materials to the surface of bioresorbable scaffolds can significantly improve their biological properties as the scaffolds' surface interacts with body fluids and tissues and, therefore, plays a key role in osseointegration. Several methods to expose HAP on the surface of bioresorbable scaffolds are available: biomimetic method which imitates the natural process of bone growth [29, 30], sol-gel method which consists of treatment the surface with colloidal suspension and condensation of calcium phosphate precursors [31], surface etching of the composite scaffold made of bioresorbable polymer and HAP [32]. Despite the fact that the described methods are suitable for scaffold coating with HAP, such techniques could damage porous scaffold structure due to aggressive long-term treatment. The present study proposes a technically simple and inexpensive method for depositing hydroxyapatite particles onto polycaprolactone scaffolds.

During the pilot study, a series of experiments were conducted according to design of experiment (DOE) approach. The influence of the solvents ratio, temperature, immersion time, and HAP concentration in the suspension on the quality of the formed coating was evaluated. The optimal parameters were selected to obtain a uniform coating of the scaffold surface with HAP particles while maintaining the original scaffold structure. When the immersion time, temperature, and content of the "good" solvent in the mixture were increased, the scaffold lost its original structure due to partial dissolution. On the contrary, when these factors were decreased, a coating was not formed on the scaffold surface. An important consideration for a coated scaffold is that it should maintain initial structure of the polymer matrix and improve its' functional properties. HAP coated scaffolds in this study show high level of conformity to the pristine PCL scaffold. Coating with HAP only slightly decreased sizes of the pores of gyroid infill and ensured corresponding physico-chemical properties of the scaffolds. Thereby, the optimized parameters of coating ensured homogeneous absorbance of HAP particles on the PCL scaffold surface without significant damage to the scaffold structure.

CONCLUSION

The development of new functional materials for the fabrication of bioresorbable tissue engineering scaffolds is an urgent task of biomedical materials science. Scaffolds made of natural and synthetic polymers find their application in the research on regeneration of biological tissues and restoration of tissue defects. However, they should possess specific bioactive properties to have an advantage over traditional implants. In the present study, the method of coating biomimetic 3D-printed PCL scaffolds with bioactive hydroxyapatite by the solvent/non-solvent treatment has been successfully used. It could show that the proposed method ensures a homogeneous coating of outer and inner surfaces of the PCL scaffolds with HAP without a significant impact on the scaffold structure a according to the findings of scanning electron microscopy. Fourier-transform infrared spectroscopy confirmed that the solvent/non-solvent treatment did not affect the chemical structure of PCL scaffolds and the thermogravimetric analysis revealed 5.7 ± 0.8 wt. % of HAP related to the whole mass of coated sample. The uniformly distributed HAP that is potentially beneficial for osteogenic differentiation of osteoblasts adhered to the surface of coated scaffolds. However, further studies are required to investigate calcium and phosphorous ions release from the coating under the hydrolytic degradation conditions together with an *in vitro* investigation of osteogenic differentiation of osteoblasts on the coated scaffolds to confirm their bioactive properties.

Conflict of Interest *The authors declare no conflict of interest.*

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Information about the authors:

1. Vadim S. Bocharov – post-graduate student, vsb27@tpu.ru;
2. Gleb E. Dubinenko – junior research fellow, dubinenko@tpu.ru, https://0000-0001-9466-469X;
3. Dmitry A. Popkov – Doctor of Medical Sciences, Professor of the Russian Academy of Sciences, Corresponding Member of the French Academy of Medical Sciences, Head of the Clinic, dpopkov@mail.ru, https://orcid.org/0000-0002-8996-867X;
4. Arnold V. Popkov – Doctor of Medical Sciences, Professor, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989;
5. Sergey I. Tverdokhlebov – Candidate of Physical and Mathematical Sciences, Associate Professor, tverd@tpu.ru, https://orcid.org/0000-0002-2242-6358;

Contribution of the authors:

Bocharov V.S. – Investigation, Validation, Writing – original draft.
Dubinenko G.E. – Conceptualization, Investigation, Writing – original draft, Writing - Review & Editing, Supervision.
Popkov D.A. – Conceptualization, Supervision.
Popkov A.V. – Conceptualization, Supervision.
Tverdokhlebov S.I. – Conceptualization, Supervision, Project administration, Funding acquisition.

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The influence of polylactide/hydroxyapatite composite implant crystallinity on the polymer structure degradation

M.V. Stogov[1]✉, E.A. Kireeva[1], G.E. Dubinenko[2], S.I. Tverdokhlebov[2]

[1] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] National Research Tomsk Polytechnic University, Tomsk, Russian Federation

Corresponding author: Maksim V. Stogov, stogo_off@list.ru

Abstract

Introduction Assessment of biological characteristics of polylactide/hydroxyapatite (PLLA/HA) biodegradable materials is requiered to specify indications for the use of PLLA/HA composite implants in clinical practice. **The present study** was aimed to measure the kinetics of calcium and phosphate release from PLLA and its dependence on polymer structure crystallinity. **Material and methods** Four types of biodegradable materials were studied *in vitro*. Samples of type 1 and type 3 made of crystalline PLLA after annealing contained 25 % and 50 % of HA mass fraction, respectively. Samples of type 2 and type 4 made of amorphous PLLA (without annealing) contained 25 % and 50 % of HA mass fraction, respectively. In every group, 6 samples were tested. The samples were incubated in an aqueous medium at 37 °C for 52 weeks. The rate of PLLA degradation was assessed by the accumulation of lactate monomer in the hydrolysate. The concentrations of calcium ions and phosphate ions were determined for assessment the HA hydrolysis rate. The degree of crystallinity of the polymer matrix was evaluated by scanning calorimetry. **Results** The hydrolysis of PLLA and HA in the samples was not simultaneous. The PLLA was hydrolyzed first followed by HA hydrolysis. By the moment of complete hydrolysis of PLLA, there was only 15 % of hydrolyzed HA. The release of calcium ions occurred from the sixth week of incubation for all tested samples, that of phosphate ions from the third week. The total amount of the released calcium ions and phosphate ions decreased in the line: material 3 > material 4 > material 1 > material 2. Calcium ions in the hydrolysates were detected up to 42 weeks of incubation, phosphate ions up to the 52nd week. **Conclusion** Higher crystallinity of PLLA achieved by annealing results in increased rate of hydrolysis of HA from PLLA matrix. Biological activity of PLLA/HA implants can be determined by degree of polymer crystallinity and saturation with HA.

Keywords: biodegradable implant, polylactide (PLLA), hydroxyapatite (HA), crystallinity, hydrolytic degradation

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INTRODUCTION

Recently developed biomaterials are designed to stimulate regeneration of host tissues providing the surgeon with new options for restoring shape and function. Medical devices made of polymeric biodegradable materials based on polylactide (PLLA) with inclusions of hydroxyapatite (HA) are promising for clinical practice [1-4]. The hydroxyapatite in the polymer content provides osteoinductive and osteoconductive characteristics of the products based on PLLA [5]. It was demonstrated that osteoinductive properties of the material amplify along by increasing the saturation of HA in PLLA due to greater elimination of calcium and phosphate from its composition [6]. However, an increase in the content of HA in the polymer decreases its biomechanical properties. Therefore, to date, along with an increase in the osteogenic properties of PLLA-based materials, their porosity and mechanical resistance are important and required characteristics [7, 8]. Numerous studies have demonstrated the role of crystallinity of PLLA for mechanical stability and resistance of PLLA to hydrolysis [9-11]. It can provide even greater potential for the use of structured PLLA for bioengineering purposes, such as artificial bones and tissue scaffolds [12]. Thereby, the assessment of the relationship between the rate of hydrolysis of PLLA/HA composite materials and their crystallinity presents a problem of relevance.

Our study evaluates the kinetics of calcium and phosphate release from PLLA and its dependence on crystallinity of the polymer structure.

MATERIALS AND METHODS

Product samples were extruded from PLLA-based composite filled with 25 % HA and 50 % mass fractions (wt. %). To increase the crystallinity of PLLA, one part of samples was annealed at the temperature of 110 °C. The samples had cylindrical shape and were 1 cm long and 2 mm in diameter.

Four types of materials were studied. Samples of materials 1 and 3 made of crystallized PLLAc (by annealing) contained 25 % and 50 % of mass fractions of HA, respectively. Samples of materials 2 and 4 based on amorphous PLLAa (without annealing) contained 25 % and 50 % of mass fractions of HA,

respectively. In each material group, 6 samples were examined.

The differential scanning calorimetry (NETZSCH DSC 204 F1 Phoenix apparatus, Germany) was applied to measure the crystallinity of PLLA polymer matrix in the groups of samples (Table 1).

Table 1

Crystallinity of the tested samples

Material	Degree of crystallinity, %
Material 1 – PLLAc/HA25 %	46.1 ± 3.0
Material 2 – PLLAa/HA25 %	34.3 ± 2.7
Material 3 – PLLAc/HA50 %	17.6 ± 3.2
Material 4 – PLLAa/HA50 %	12.4 ± 1.8

Each sample was placed in a separate measuring cell filled with distilled water, the volume of which was determined at the rate of 4 ml per 1 cm^2 of the sample surface. Next, the samples were incubated in a thermostat at the temperature of 37 °C. After a week of incubation, the medium was changed. A new solution was poured in. The hydrolysate was subjected to chemical analysis for lactate, calcium ions and inorganic phosphate. The rate of PLLA degradation was assessed by the accumulation of its monomer in the hydrolysate. The concentration of calcium ions and phosphate ions reflected the progress of HA hydrolysis. The duration of incubation of the samples of all the materials was 52 weeks. The reagents of BioSystems (Spain) on Hitachi 902 biochemical analyzer (Hitachi Ltd., Japan) were used for analysis of lactate, calcium, and phosphate.

The arithmetic mean (M) and standard deviation (SD) were determined for quantitative parameters. The reliability of intergroup differences was assessed using the Kruskal-Wallis H-test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using AtteStat 13.1 for Excel.

RESULTS

The hydrolysis of PLLA in material 1 (crystalline phase, HA content of 25 wt. %) increased during the first and the second week of incubation. In contrast to that, the samples of group 3 (PLLAc/HA 50 % sample) had an increased hydrolysis only the first week (Table 2). The hydrolysis of amorphous PLLA samples (HA content of 25 wt. %, material 2 and PLLAa/HA 50 %, material 4) was significant throughout the first three weeks.

The dynamics of the release of calcium ions and phosphate ions is shown in Figure 1.

Table 2

PLLA hydrolysis progression; % of total lactate formed as a result of hydrolysis

Week(s) of incubation	Material 1 PLLAc/HA 25 %	Material 2 PLLAa/HA 25 %	Material 3 PLLAc/HA 50 %	Material 4 PLLAa/HA 50 %
1	47.9 ± 5,5	47.4 ± 6.0	90.2 ± 8.2	67.0 ± 4.7
2	20.9 ± 3.2	15.0 ± 3.3	3.3 ± 1.1	10.8 ± 3.6
3	7.5 ± 1.1	19.7 ± 4.9	3.0 ± 0.7	16.2 ± 2.0
4	9.5 ± 2.0	10.9 ± 3.0	3.5 ± 1.8	3.4 ± 0.9
5	7.2 ± 1.4	7.9 ± 2.0	0	1.3 ± 0.5
6	4.2 ± 0.9	4.7 ± 1.8	0	1.2 ± 0.3
7	2.7 ± 0.8	3.9 ± 0.6	0	0
8	0	2.2 ± 0.7	0	0
10	0	5.9 ± 1.0	0	0
11	0	0	0	0
Total duration of hydrolysis (weeks)	7	10	4	6

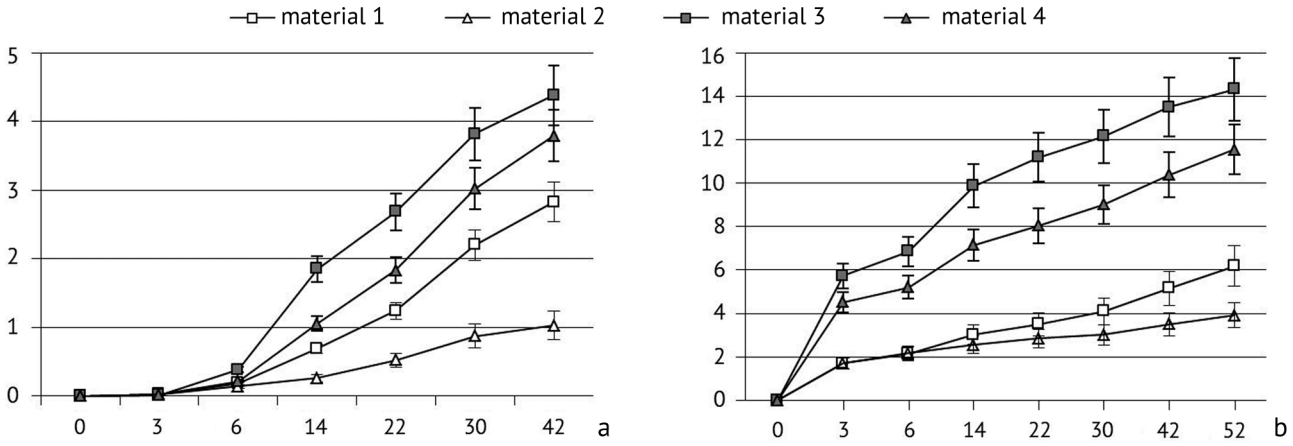

Fig. 1 Diagram of accumulation: a – calcium ions released from the samples during incubation; b – released inorganic phosphate ions from the samples during incubation. OX axis – weeks of incubation; OY axis – the number of ions (μmol/cm^2)

We stated that significant release of calcium ions began from the sixth week of incubation for all the samples while that of phosphate ions occurred from the third week. There was at the same time, the total number of released calcium ions and phosphate ions decreased in the line: material 3 > material 4 > material 1 > material 2.

Calcium ions in the hydrolysates of the samples of all materials were detected up to 42 weeks of incubation, phosphate ions – up to the 52nd week. The total amount of the released calcium and phosphate is presented in the Table 3. We noticed higher release of ions from the samples with high content of HA (samples 3 and 4) ($p < 0.05$). The accessibility of these ions from the crystalline polymers was higher relative to the samples of amorphous PLLA phase.

The comparison of kinetics in hydrolysis of PLLA and HA are presented in Table 4. During the period of PLLA of degradation (the duration of hydrolysis is shown in Table 1), no more than 15 % out of the total released calcium and from 40.9 % to 61.1 % of phosphate were released

Table 3

Total amount of released calcium and phosphate from the studied materials

Ion	Material 1	Material 2	Material 3	Material 4
Ca, μmol/cm^2	$2.83 \pm 0.34^{2,3,4}$	$1.03 \pm 0.21^{1,3,4}$	$4.38 \pm 0.82^{1,2}$	$3.79 \pm 0.46^{1,2}$
P, μmol/cm^2	$6.18 \pm 0.68^{2,3,4}$	$3.92 \pm 0.23^{1,3,4}$	$14.31 \pm 0.49^{1,2,4}$	$11.55 \pm 1.06^{1,2,3}$

Notes: superscript – the material relative to which there were significant differences at $p < 0.05$.

Table 4

Percentage of released calcium and phosphate for the period of complete decay of PLLA

Ion	Material 1	Material 2	Material 3	Material 4
Ca, %	$11.03 \pm 1.52^{3,4}$	$15.12 \pm 1.81^{3,4}$	$5.46 \pm 0.62^{1,2}$	$5.75 \pm 0.69^{1,2}$
P, %	40.9 ± 2.8^{2}	$61.1 \pm 4.1^{1,3,4}$	41.6 ± 3.0^{2}	48.2 ± 2.5^{2}

Notes: superscript – the material relative to which there were significant differences at $p < 0.05$.

DISCUSSION

Our studies revealed that the degree of PLLA crystallinity had an impact on the kinetics of HA release from the samples. In particular, the progression of HA hydrolysis correlates with the crystallinity of PLLA. This finding can be explained by the fact that the polymer in the crystalline phase underwent hydrolysis faster than in the amorphous one.

A typical feature of the hydrolysis kinetics found in the study is that the hydrolysis of PLLA and HA occurred stepwise. The polymer hydrolyzed earlier than HA. This is confirmed by the fact is that at the time of complete PLLA hydrolysis, the hydrolyzed HA was no more than 15 %. In general, such a sequence of the breakdown process of PLLA and filler (HA) degradation represents an important feature of the material for practical application. This fact implies a delayed release of calcium ions into host tissue environment after implantation.

We found that the saturation of crystalline PLLA with hydroxyapatite might be a promising option for production of materials designed for orthopedics. Although the issue of optimal amount of HA in PLLA remains open. On the one hand, the biological efficiency of materials containing both a small amount of HA (up to 10 wt. %) and its significant portion (up to 80 wt. % HA) was demonstrated [13, 14]. On the other hand, the osteogenic activity of the materials based on PLLA improves with an increase HA amount in the polymer composition [6].

Findings of our study are consistent with the study of study of Zhang et al. [15]: the crystallinity of the polymer saturated with HA determines its degradability and consequently the biological characteristics of the PLLA/HA material. That is why the PLLA crystallinity and its saturation with HA are the main characteristics of the PLLA/HA material, which determine its effectiveness and indications for use.

Based on the results of the analysis performed, it is not correct to speak about the exceptional superiority of any of the materials we studied. We believe that all the studied materials and products based on them could be used in clinical practice. It all depends on the indications for their use. The clinical experience in the use of products made from the PLLA/HA material shows that the choice of a biodegradable product should be made after considering the nature of degradation of its polymer [16].

In this regard, we believe that the indications for the use of PLLA products saturated with HA by more than 50 wt. %, might be cases of large defect management as the HA in the polymer actually becomes a calcium reserve for local bone tissue formation [17]. An additional advantage of PLLA/HA implants saturated with HA is their ability to reduce development of biofilms formed by S. aureus and P. aeruginosa on the surface of PLLA materials [18]. Less HA-saturated materials (< 25 wt. %) might address small bone defects [19].

Obviously, varying the content of HA and the Obviously, varying the content of HA and the degree of PLLA crystallinity may ensure options for using PLLA/HA materials for creating customized implants [14, 20, 21]. The material might be also promising for creation of scaffolds providing controllable degradation rate to compliment cell/tissue in-growth and maturation [22, 23].

Nevertheless, we should mention potential risks of the materials with high HA saturation for clinical use. The accelerated PLLA/Ha degradation results in the risks of early mechanical instability of implanted devices. The risk of heterotopic ossification should not be negligible either.

CONCLUSION

Thus, the performed study showed that increased crystallinity of PLLA treated by annealing increases the rate of hydrolysis of HA included in the PLLA matrix. Changes in the HA content and in PLLA crystallinity allow control over the biological characteristics (mechanical stability, calcium release, osteogenic properties) of the PLLA/HA composite materials. This also expands the indications for their possible clinical use.

Conflict of interest *Authors declare no conflict of interest.*

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Information about the authors:

1. Maksim V. Stogov – Doctor of Biological Sciences, Associate Professor, Head of Department, stogo_off@list.ru; https://orcid.org/0000-0001-8516-8571;
2. Elena A. Kireeva – Candidate of Biological Sciences, Senior Researcher, ea_tkachuk@mail.ru; https://orcid.org/0000-0002-1006-5217;
3. Gleb E. Dubinenko – junior researcher, dubinenko.gleb@gmail.com; https://orcid.org/0000-0001-9466-469X;
4. Sergey I. Tverdokhlebov – Candidate of Physical and Mathematical Sciences, Associate Professor, tverd@tpu.ru; https://orcid.org/0000-0002-2242-6358.

Contribution of the authors:

Stogov M.V. – formulation or evolution of the overarching goals and objectives of the study; development and design of methodology; creation of models; preparation and writing of the initial draft (draft) of the work.
Kireeva E.A. – application of statistical, mathematical, computational or other formal methods for analysis or synthesis of research data; carrying out the research process.
Dubinenko G.E. – development or design of methodology; application of statistical, mathematical, computational or other formal methods to analyze or synthesize research data; conducting the research process; making adjustments to the original version, preparing the work for publication.
Tverdokhlebov S.I. – development or design of methodology; making adjustments to the original version, preparing the work for publication.

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Original article

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Induction of bactericidal activity by degradable implants

A.V. Popkov[1], N.V. Kononovich[1], D,A, Popkov[1]✉, N.V. Godovykh[1], S.I. Tverdokhlebov[2], E.N. Bolbasov[2], M.V. Stogov[1], E.A. Kireeva[1], E.N. Gorbach[1], Yu.Yu. Litvinov[3]

[1] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] School of Nuclear Science & Engineering, Tomsk Polytechnic University, Tomsk, Russian Federation
[3] All-Russian Research Institute of medicinal and aromatic plants, Moscow, Russian Federation

Corresponding author: Dmitry A. Popkov, dpopkov@mail.ru

Abstract

Introduction The problem of implant-associated infections is far from being solved in arthroplasty, osteosynthesis of fractures, and spinal pathology. The development of biodegradable implants with bioactive properties is a promising direction. **The purpose** of this study was to evaluate the *in vitro* bactericidal activity of implants made from a degradable material polycaprolactone (PCL) impregnated with hydroxyapatite and an antibiotic. **Material and methods** To study antibiotic availability, antibiotic-impregnated PCL cylindrical samples (n = 6) were incubated in distilled water at 37 °C. To evaluate the antibacterial properties, samples in the form of porous disks were used: control samples from PCL; 1) PCL samples coated with antibiotic and hydroxyapatite; 2) PCL samples coated only with antibiotic; 3) PCL samples coated only with hydroxyapatite; (n = 6 for each type of tested samples). The disk diffusion method was used to determine the sensitivity of microorganisms to antibiotics. The microbial strains used were *S. aureus* ATCC 25923, P. aeruginosa ATCC 27853 and E. coli ATCC 25922. Test microorganisms were cultivated on beef peptone agar (MPA) at 37 °C for 24 hours. Quantitative data were subjected to statistical processing. **Results** It was determined that 82.6 % of the antibiotic was released during the first day of incubation and 8.2 % on the second day. Control samples did not show a bactericidal effect. Samples 3 showed an antibacterial effect against E. coli culture. Samples 1 and 2 equally demonstrated significant inhibition of the growth of *S. aureus*, *P. aeruginosa*, and *E. coli*. **Discussion** Most of the antibiotic is released into the hydrolyzate during the first two days of incubation. Porous implants made of PCL and impregnated with an antibiotic have pronounced antimicrobial activity against the most common gram-negative and gram-positive bacteria that cause purulent complications in surgical practice. Nanostructured hydroxyapatite on the surface of the implant does not reduce bactericidal activity. **Conclusions** Porous polycaprolactone implants filled with hydroxyapatite and antibiotics are targeted to stimulate bone regeneration and simultaneously ensure antimicrobial activity. Nanostructured hydroxyapatite on the implant surface does not decrease bactericidal activity.

Keywords: bioactive implant, polycaprolactone, hydroxyapatite, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, antimicrobial activity, hydrolytic degradation

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INTRODUCTION

Over the last ten years remarkable progress has been made in the development of surgical techniques for bone reconstruction using bioresorbable implants having osteoinductive activity. The first fixation devices fabricated from biodegradable materials have become available since the early 1980s [1, 2]. They are still used in traumatology as pins and screws and do not need surgery for their removal [3-7]. Such pins are mostly made of polylactic acid and have no osteogenic activity; therefore fracture healing occurs in the usual terms [4, 8].

The risks of septic complications following internal osteosynthesis should not be negligible. The studies aimed at enhancing the bioactivity of polymer implants filled with antibiotics (Biomatrix, Allomatrix-implant, Osteomatrix, CollaPan G, CollaPan L) [9, 10] demonstrated efficiency of such approach. However, the matrix of such implants is shaped as a fine-grained material or a thin fibrous film. Thus, they do not enable stable osteosynthesis. Fused deposition modeling [11] for printing of implants with 3D-structure currently uses a filament made of linear bioresorbable polyesters, such as polylactic acid (PLLA), polycaprolactone (PCL), polyglycolic acid (PGA) and their copolymers. Saturation of these implants with antibiotics could provide antimicrobial activity associated with structural integrity and controlled resorption of an implant.

The aim of this study was to study the *in vitro* accessibility of antibiotics during hydrolytic degradation of polycaprolactone (PCL) products and bactericidal activity.

MATERIAL AND METHODS

Two types of implant samples were studied *in vitro*. Type 1 was shaped as a nail used in orthopedic surgery (cylindrical PCL samples, 10.0 mm long and 2.4 mm wide). The accessibility of antibiotics was assessed. To study bactericidal activity, disks made from polycaprolactone (PCL) using 3D printing technology, 10 mm in diameter and 1 mm thick, were used. The disks had cells with a diameter of 1-1.5 mm, limited by crossbars of 1 mm (like implants designed to treat bone defects). The surface of all implants was impregnated with cefotaxime, a broad-spectrum antibiotic.

The implants were designed and manufactured at Tomsk Polytechnic University. The components for the preparation of composite materials were ε-polycaprolactone (Sigma-Aldrich, United States; Mn 80000) and hydroxyapatite (Fluidinova, Portugal; 10 ± 5 μm). For preparation of the composite, PCL was dissolved in high purity acetone (EKOS-1, Russia) with a concentration of 15 wt %. Hydroxyapatite (HA) was pre-ground in a ball mill in a ceramic chamber with ceramic grinding media with the addition of acetone in a mass ratio of 1.5: 1 at a rotation speed of 72 rpm for 12 hours. After HA grinding, the PCL solution was added to the chamber and mixed in the ball mill. After drying, the obtained composite was crushed in a low-speed polymer crusher (Shini SG-1621N, Taiwan). The ground composites were extruded using a Filabot EX2 single screw extruder (Filabot, USA) to obtain 4-mm filaments. Additionally, HA particles were applied to the implant surface by dipping into a suspension of HA powder and antibiotic cefotaxime in a solvent, and then dried to remove residual solvent.

To study hydrolytic degradation, each cylindrical PCL sample (n = 6), impregnated with an antibiotic, was placed in a separate measuring cell filled with distilled water, the volume of which was determined at 4 ml per 1 cm^2 of the sample surface. Next, the samples were incubated in a thermostat at a temperature of 37 °C. The incubation medium was changed daily. The hydrolysate was subjected to a chemical analysis for the content of the antibiotic, which was determined on a spectrophotometer by the absorption intensity at a wavelength of 243 nm, relative to the standard calibration curve. The duration of incubation was 7 days.

The disk-diffusion method for determining the antibiotic sensitivity of microorganisms was applied to reveal the bactericidal activity [12].

The following strains of microbes were used to evaluate the antibacterial properties: *Staphylococcus aureus* ATCC 25923 (gram-positive bacteria), *Pseudomonas aeruginosa* ATCC 27853 (gram-negative bacteria) and *Escherichia coli*, *E. Coli* ATCC 25922 (intestinal bacteria).

Conditions for cultivating test microorganisms. Test microorganisms were cultivated on beef-peptone agar (MPA) at 37°C for 24 hours. A working suspension of test cultures was prepared from a culture of this test strain grown on dense nutrient medium (MPA) at 37 °C for 24 hours. Nutrient medium for evaluating the bactericidal properties of products was Muller – Hinton agar. The method of direct suspension in a sterile isotonic solution of colonies of a pure 18-24-hour culture of bacteria grown on a dense non-selective nutrient medium (MPA) was used for the preparation of the inoculum. The density of the suspension is 0.5 McFarland turbidity standard.

The discs of the test products were applied on a day-old fresh medium of microbial test culture. The time between the preparation of the microbial culture lawn and the application of disks on it was no more than 15 minutes.

Four types of products were studied for antibacterial activity. Discs without calcium phosphate coating and without antibiotics served as control. The other types were discs coated with hydroxyapatite and antibiotic (1), discs coated only with antibiotic without hydroxyapatite (2), discs coated only with hydroxyapatite (3).

Incubation after application of the discs was performed at 35 ± 1 °C, and lasted for 18 hours. A total of 36 studied tests were conducted (n = 6 for each type of tested samples).

The bactericidal activity of the implant was assessed by the zone of growth inhibition of the tested microorganisms around the disks. The checking was carried out in reflected light. For measuring the zone of growth inhibition, we were guided by the zone of complete suppression of visible growth. The bactericidal activity of the products was considered significant if the zone of growth inhibition around the disks was more than 1 mm.

Statistical analysis was performed using AtteStat 13.1 program (Russia): median values (Me), standard deviation (SD) and the lower and upper quartiles (Q1-Q3). The evaluation of the normal distribution of samples was performed using the Shapiro-Wilk test.

RESULTS

The study showed that 82.6 % of the antibiotic was released on the first day of incubation (Table 1). During that period, the mass of the samples increased slightly what can be explained by the absorption of water by the polymer. On the remaining days of the observation period, there was no significant change in the sample weight. The integrity of all samples was maintained throughout the entire incubation period.

Table 1

Average values of cefotaxime in hydrolysate and average weight of samples

Days of incubation	CANT, mg/cm^2, M ± SD	Release of antibiotic*; %	Weight, mg	% of weight change from initial level
0	0	0	66.2 ± 2.9	0
1	0.534 ± 0.074	82.6	66.5 ± 3.0	100.5
2	0.044 ± 0.009	8.2	66.4 ± 2.8	99.8
3	0.019 ± 0.012	3.6	66.3 ± 2.9	99.8
4	0.022 ± 0.008	4.0	66.0 ± 3.0	99.6
5	0.004 ± 0.001	0.8	66.0 ± 3.0	99.6
6	0.002 ± 0.001	0.4	65.9 ± 3.0	99.6
7	0.002 ± 0.001	0.4	65.5 ± 2.9	99.3
Total for 7 days	0.627 ± 0.050	100	–	–

Notes: CANT – is the concentration of the antibiotic in the hydrolysate; * – % release of antibiotic relative to the final value.

Control discs applied to lawns against all types of bacteria did not show a bactericidal effect. A continuous growth of microbial cultures was observed around the disks (Fig. 1). In all samples, the zone of growth inhibition around the discs was not determined (Table 2). This series confirmed that the implant matrix of pure polycaprolactone does not have a bactericidal effect.

Disks of product 3, coated only with hydroxyapatite (without antibiotic), showed a bactericidal effect only against the *Escherichia coli* culture (Fig. 2 c). The average zone of growth retardation in these samples exceeded 4 mm (Table 2).

Discs with antibiotic or antibiotic combined with hydroxyapatite demonstrated significant inhibition of bacterial growth (Fig. 3).

This experimental study revealed high activity of the products with the antibiotic against *P. aeruginosa*, *S. aureus* and *E. coli* cultures. The zone of complete inhibition of bacterial growth was 15.5-23.0 mm in sample 2 and 15.8-25.7 mm in sample 1 (Table 2). It should be emphasized that the application of a nanostructured hydroxyapatite on the surface (product 2) does not decrease the bactericidal effect of the antibiotic.

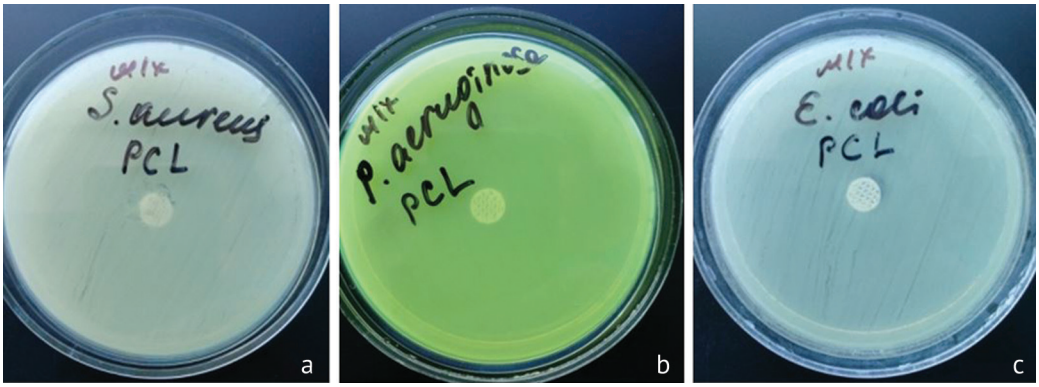

Fig. 1 Modified disk-diffusion method for determining the antibiotic sensitivity of microorganisms, absence of a zone of inhibition of the growth: a – *S. aureus*; b – *P. Aeruginosa*; c – *E. coli*

Table 2

Bactericidal properties of products in relation to microbial test-cultures (Me (Q1-Q3))

Microbes	Zone of growth inhibition, mm			
	Product 1	Product 2	Product 3	Product 4
	Pure PCL	Antibiotic	HA + antibiotic	HA
Staphylococcus aureus	0	21.25 (20.54-21.56)	23.11 (22.77-23.41)	0
Pseudomonas aeruginosa	0	15.85 (15.33-16.0)	17.23 (15.88-17.47)	0
Escherichia colli	0	22.37 (21.12-23.08)	23.80 (23.10-25.77)	4.27 (2.89-4.94)

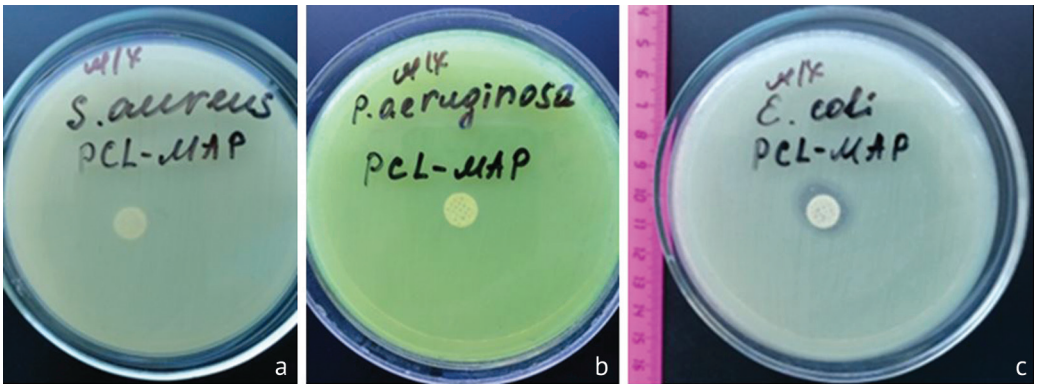

Fig. 2 Modified disk-diffusion method for determining the antibiotic sensitivity of microorganisms: a – no growth inhibition of *S. aureus* culture; b – no growth inhibition of the *P. aeruginosa* culture; c – zone of significant growth inhibition of *E. coli* is 4.27 (2.89 – 4.94) mm

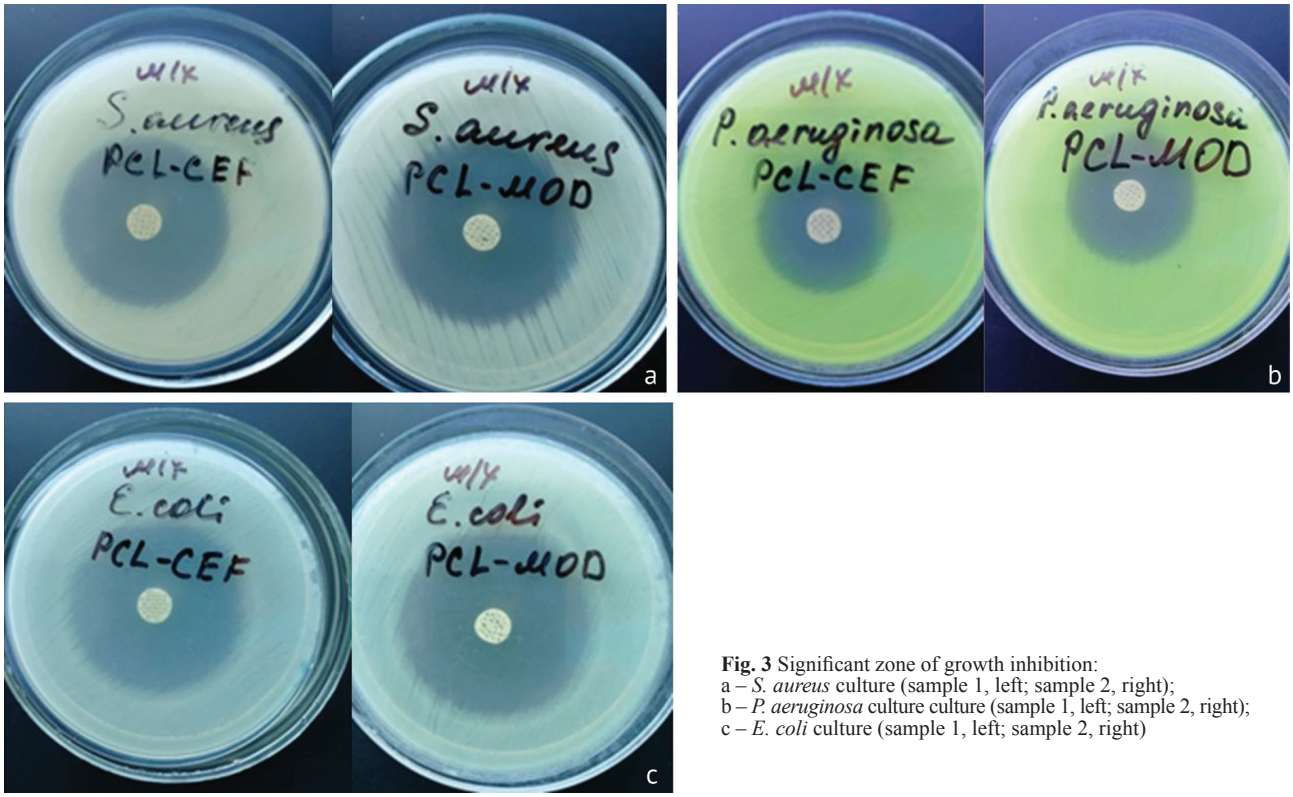

Fig. 3 Significant zone of growth inhibition:
a – *S. aureus* culture (sample 1, left; sample 2, right);
b – *P. aeruginosa* culture culture (sample 1, left; sample 2, right);
c – *E. coli* culture (sample 1, left; sample 2, right)

DISCUSSION

The most promising synthetic polymers for medical use are aliphatic polyesters based on hydroxyalkanocarboxylic acids: polylactide, polyglycolide, polyhydroxybutyrate. Their degradability under the influence of biological factors can be used in new devices and implants. Bioinert implants should be distinguished from bioresorbable polymers used in reconstructive surgery. Resorbable polymers ensure required function and structure over the period of host tissue regeneration. They must be able to decompose under the influence of body fluids with the formation of non-toxic products. The rate of decomposition of a solid bioresorbable implant polymers into liquid products should be controllable and not exceed the rate of tissue regeneration (in bone regeneration process, it is a period of months) [13, 14].

Currently, products made from polylactic acid and polycaprolactone have been frequently used in medicine. Introduction of inorganic substances into the composition of the polymer matrix enables the control over the physicochemical and mechanical properties of polylactide [15-17]. However, their application for bone fragments fixation has not been widely used as they do not meet the requirements of the AO/ASIF principles [13, 18].

New research trends are aimed to develop materials and implants enabling osseointegration of an implant followed by controlled matrix resorption [19, 20]. However, new generation of implants does not exclude risks of septic complications related to surgery [21, 22].

The relationship of bacterial agent with polymers or with the ceramic surface of implants remains unclear.

Nevertheless, a large clinical material on the use of intraosseous implants shaped as wires with a bioactive hydroxyapatite surface revealed complete absence of inflammatory complications [23]. The technology of intramedullary reinforcement with HA-coated wires is applied for treatment of fractures and pseurarthrosis [24, 25].

We chose polycaprolactone (PCL) as the matrix for the degradable implant due to its chemical properties providing control of its 3D mechanical structure (resorption lasts 1.5-4 years) [26, 27]. The HA-filled PCL maintains mechanical strength and demonstrates an osteoinduction effect on the bone regeneration [28, 29]. We hypothesized that the impregnation of an PCL implant with an antibiotic would have an antibacterial effect, what is especially required in early postoperative period.

The antibacterial activity of ceramic nanoparticles (ZnO, TiO_2) applied as a coating on metal implants was demonstrated in the study of Colon et al. [30]. This coating significantly decreased adhesion of *Staphylococcus epidermidis* on the implant surface. In contrast to that, an increased adhesion of osteoblasts was stated. This finding allowed drawing a conclusion that the large surface areas of the nanophase compared to the microphase and high surface energy present in the surface layers of the nanoparticles could lead to increased dissolution rate of the ceramic surface, which disrupts the functions of bacteria. That study suggests that the technology of bioactive coating of an implant with hydroxyapatite allows its additional saturation with a desirable broad-spectrum antibiotic or in accordance with its antimicrobial activity against infected patient tissues. Our findings are consistent with this hypothesis demonstrating the bactericidal effect of nanostructured hydroxyapatite coating of PCL against *Escherichia coli*.

We propose to fill the matrix of bioresorbable implants with antibiotics. Combined with HA-nanostructured coating, degradable implants will ensure stability of osteosynthesis (related to controllable resorption), stimulation of host bone regeneration and prevention of septic complications. Our experimental study demonstrated antibacterial activity of the implant bioactive surface against the most common gram-negative and gram-positive bacteria causing septic complications in surgical departments: *P. aeruginosa*, *S. aureus*, *E. coli*. Most of the impregnated antibiotic released from porous PCL products into the hydrolysate within the first two days of incubation. We established that nanostructured hydroxyapatite on the surface of a biodegradable implant in its pure composition has a pronounced bactericidal activity only against *Escherichia coli*.

CONCLUSION

Porous polycaprolactone implants filled with hydroxyapatite and antibiotics are targeted to stimulate bone regeneration and simultaneously ensure antimicrobial activity. Nanostructured hydroxyapatite on the implant surface does not decrease bactericidal activity.

Conflict of interest *All authors declare no conflict of interest.*

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Information about the authors:

1. Arnold V. Popkov – Doctor of Medical Sciences, Professor, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989;
2. Natalia A. Kononovich – Candidate of Veterinary Sciences, Lead Researcher, n.a.kononovich@mail.ru, https://orcid.org/0000-0002-5990-8908;
3. Dmitry A. Popkov – Doctor of Medical Sciences, Professor of the Russian Academy of Sciences, Corresponding Member of the French Academy of Medical Sciences, Head of the Clinic, dpopkov@mail.ru, https://orcid.org/0000-0002-8996-867X;
4. Natalia V. Godovykh – junior researcher, natalia_nvn@mail.ru, https://orcid.org/0000-0001-8512-4165;
5. Sergei I. Tverdokhlebov – Candidate of Physical and Mathematical Sciences, Associate Professor, tverd@tpu.ru, https://orcid.org/0000-0002-2242-6358;
6. Evgeny N. Bolbasov – P Candidate of Technical Sciences, Researcher, ebolbasov@gmail.com, https://orcid.org/0000-0002-9789-2185;
7. Maxim V. Stogov – Doctor of Biological Sciences, Associate Professor, Head of Department, stogo_off@list.ru, https://orcid.org/0000-0001-8516-8571;
8. Elena A. Kireeva – Candidate of Biological Sciences, Senior Researcher, ea_tkachuk@mail.ru, 0000-0002-1006-5217;
9. Elena N. Gorbach – Candidate of Biological Sciences, Leading Researcher, gorbach.e@mail.ru, https://orcid.org/0000-0002-9516-7481;
10. Yury Yu. Litvinov – Candidate of Biological Sciences, Leading Researcher, https://orcid.org/0000-0003-2718-2038.

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Original article

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Bone defect management with tissue-engineered constructs based on deproteinized cancellous bone: an experimental study

E.A. Anastasieva[1]✉, L.A. Cherdantseva[1], T.G. Tolstikova[2], I.A. Kirilova[1]

[1] Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, Novosibirsk, Russian Federation
[2] Vorozhtsov Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russian Federation

Corresponding author: Evgeniya A. Anastasieva, evgeniya.anastasieva@gmail.com

Abstract

Background Management of bone defects with autologous bone grafting has always been the "gold standard" but it is not always possible to use it for a number of reasons. Preprocessed materials of biological and non-biological origin were developed as an alternative. A new branch of these materials is tissue-engineered constructs that fully imitate autologous bone in required volume. **Aim** is to study *in vivo* the possibility of using deproteinized human cancellous bone tissue as a matrix for creating tissue-engineered constructs. **Methods** The study was carried out on 24 NZW line rabbits, since this line has a fully characterized stromal-vascular fraction formula (SVF). The study design included 3 groups. Fikst group (control) had surgical modeling of bone defects in the diaphysis of the contralateral femur without reconstruction; Group 2 had bone defect reconstruction using fragments of a deproteinized cancellous bone graft; group 3 underwent bone defect reconstruction using fragments of deproteinized cancellous bone matrix along with the autologous adipose tissue SVF (obtained according to ACP SVF technology). Animals were sacrificed with ether anesthesia at 2, 4 and 6 weeks after the operation and subsequent histological study followed. **Result** During all periods of the study, the newly formed bone tissue volume density in the 3rd group (reconstruction with deproteinized human cancellous bone + stromal-vascular fraction) was 1.78 times higher ($p < 0.001$) than in the first group (bone defect without reconstruction), 1.21 times higher ($p < 0.001$) than in the 2nd group (reconstruction with deproteinized cancellous bone alone). The dynamics of changes in the mature bone tissue volume density was similar to those of the newly formed bone tissue. **Discussion** The comparative analysis of reparative processes using a tissue engeneered construst based on deproteinized cancellous human bone with adipose tissue stromal vascular fraction revealed that the use of these bone substitute materials contributes not only to the early activation of reparative regeneration of the main structural elements of bone tissue at the site of bone defect, but also their timely differentiation. **Conclusion** The use of deproteinized cancellous bone matrix combined with stromal-vascular fraction to create a tissue-engineered construct could unleash several regeneration mechanisms and accelerate the process of bone defect site repair, compared with 1st and 2nd group of study.

Keywords: Bone defect; bone matrices; deproteinized cancellous bone; bone defect reconstruction; adipose tissue stromal-vascular fraction

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INTRODUCTION

Bone defect repair remains to be a difficult prom in the field of reconstructive surgery. Despite several trends under study, autogenous bone grafting still is a "gold standard" [1, 2, 3]. However, for a number of reasons, it is not always possible to completely fill in a bone defect with the graft [4, 5, 6]. As an alternative to autogenous bone graft, bone substitute materials have been used. They can have different origin: biological or non-biological one. One of the ways of bone reconstructive technologies development is the use of combined tissue engineered constructs together with the patient's own cell material. Such construct design is able to fully imitate autogenous bone tissue in the required volume [7]. Currently, according to literature data, the most suitable in terms of its properties as the basis (or matrix) for such constructs is bone allograft [3, 8, 9]. Nevertheless, allogeneic bone cannot unleash the stimulation of the osteogenesis processes [3]. That is why autologous non-immunogenic cell material is strongly needed [7].

Aim is to study in vivo the possibility of using deproteinized human cancellous bone tissue as a matrix for creating tissue-engineered constructs.

MATERIAL AND METHODS

The study was carried out on 24 NZW line rabbits, since this line has a fully characterized stromal-vascular fraction formula (SVF) [10, 11, 12, 13, 14, 15]. The study complies with international standards and ethical principles for laboratory research ISO 10993-2, ISO 10993-6-2021. The design of the study included 3 groups. The first group (control) had surgical modeling of bone defects in the areas of the diaphysis of the contralateral femur without reconstruction, similar to the study groups; 2nd group had surgical modelling

of the femoral diaphysis defect with its reconstruction using fragments of a deproteinized cancellous human bone graft (matrix); 3rd group underwent surgical modeling of femoral diaphysis defect with its reconstruction using fragments of deproteinized cancellous human bone matrix together with the autologous adipose tissue stromal-vascular fraction. Stromal-vasular fraction was obtained according to ACP SVF technology (Patent US10512659B2). Animals were sacrificed with ether anesthesia at 2, 4 and 6 weeks after the operation. Under the standard conditions, the material was harvested for subsequent histological assessment to evaluate the bone substitute materials local effect on living tissues and the implementation of reparative osteogenesis in the bone defect reconstruction area.

Adipose tissue material was taken through the dorsal paravertebral approach during the main surgical procedure. It is for this localization in adult rabbits that the largest amount of beige adipose tissue is typical [11, 16]. After obtaining adipose tissue, fragments of cancellous deproteinized bone matrices, 5 × 5 mm in size, were installed paravertebral and subcutaneously to determine their impact on living tissues.

To assess the effectiveness of reparative osteogenesis in the bone reconstruction site, fragments of deproteinized cancellous bone were implanted into simulated bone defects according to the study design. To confirm the absence of variability in the morphological manifestations of bone tissue reparative regeneration in the conditions of each individual animal, an additional defect was formed in the femur diaphyseal part on each limb.

After the harvesting, study samples were fixed in 10 % neutral buffered formalin solution for 72 hours, followed by decalcification in the Richmann-Gelfand-Hill solution for 10 days at a temperature of 20 °C. After standard histological processing in a series of alcohols and xylene increasing concentration, bone tissue samples were embedded in paraffin blocks, followed by making serial sections 4-5 μm thick and staining them with hematoxylin and eosin. For differentiated quantitative assessment of "mature" and emerging connective tissue in the study samples, histological sections were stained according to Van Gieson and impregnated with silver. Light microscopy with obtaining overview micrographs was carried out on an OLYMPUS CX 43 laboratory microscope with an OLYMPUS UC 90 camera (Olympus Medical Systems Corp., Japan). Morphometric study of histological samples of matrices heterotopic and orthotopic implantation sites was performed using the ImageJ software (version 1.53o, 2022, Wayne Rasband and contributors National Institutes of Health, USA) at 200 magnification. The numerical density of the vessels (Nai), the percentage of implantation zone full-blooded vessels (%), the volume density of mature collagen fibers (Vv%), the volume density of argyrophilic connective tissue fibers (Vv%), the volume density of mature and newly formed bone tissue (Vv%) were evaluated in histological sections.

The obtained morphometric data were statistically processed using the RStudio program (version 2022.02.1 Build 461 – © 2009-2022 RStudio, Inc., USA) in the R language (version 4.1.3 (2022-03-10), Vienna, Austria). Comparison of continuous scores between the groups was performed by a non-parametric unpaired Mann – Whitney U-test. The distribution bias was calculated with the 95 % confidence interval. Categorical scores were compared by Fisher's exact two-sided test. Correction for multiple testing error when comparing categories was carried out using the Benjamini – Hochberg method, the difference was considered statistically significant if $p < 0.001$.

RESULTS

From the 2nd week of the study, light microscopy of the heterotopic implant fixation area histological skin samples revealed the formation of a thick-walled connective tissue capsule with weak infiltration of the walls by macrophages and mononuclear leukocytes between the dermis and the muscle. Between the fibers of the connective tissue, a large number of small thin-walled blood vessels without signs of hemocirculatory disorders were revealed. Fragments of mplanted deproteinized cancellous bone matrices were represented by mature bone tissue. In the tissues, perifocal to the area of implantation of bone matrices, the formation of a cellular inflammatory infiltrate was not detected.

Visual examination of the experimental bone defects modeling tissues areas with orthotopic reconstruction using bone-substituting materials showed no signs of a local inflammatory reaction in all animals.

The assessment of reparative osteogenesis according to hystological sections is presented at the Table 1.

From the 2nd week, a significant prevalence of indicators of the vessels numerical density of groups 2 and 3 relative the control group 1 ($p < 0.001$) was determined. There were no significant differences between group 2 and 3 at this stage ($p = 0.699$). A significant prevalence of the group 3 indicators over those in group 2 was noted from the 4th week of the study ($p < 0.001$) and persists by the 6th week ($p < 0.001$) (Fig. 1).

By 6 weeks, the percentage of full-blooded vessels progressively increases in all groups. Only a small decrease in this parameter in group 2 compared to the control group 1 was determined as statistically significant ($p < 0.001$) (Fig. 1 and 2).

Table 1

Histological study results of reparative osteogenesis in orthotopical reconstruction of bone defects with different types of bone substitute material (M ± m)

Study parameters	Study groups								
	1st group (control)			2nd group (deproteinized cancellous bone graft)			3rd group (deproteinized cancellous bone matrix with SVF)		
	Timeline (week of study)								
	2	4	6	2	4	6	2	4	6
Vessels numerical density, Nai	5.61 ± 1.5	8.25 ± 1.5	16.66 ± 5.7	10.43* ± ± 3.5	18.73* ± ± 2.2	23.70* ± ± 6.8	10.40* ± ± 3.41	20.36*.** ± ± 4.5	26.31*.** ± ± 7.9
Full-blooded vessels percentage, %	54.44 ± 0.2	78.82 ± 0.2	96.58 ± 0.1	38.3* ± 0.2	75.49* ± ± 0.2	92.20* ± ± 0.1	81.19*.** ± ± 0.2	64.45* ± 0.2	97.37 ± 0.3
Mature collagen fibers volume density, Vv%	5.15 ± 0.6	7.08 ± 1.1	12.68 ± 2.5	6.35* ± 3.8	8.83* ± ± 2.53	9.26* ± 1.6	6.88* ± 1.5	9.30* ± 1.1	10.68*.** ± ± 1.6
Argyrophilic connective tissue fibers volume density, Vv%	7.33 ± 0.7	11.83 ± 1.1	14.19 ± 2.4	7.25 ± 1.8	9.95* ± 1.6	10.03* ± ± 2.2	9.66*.** ± ± 1.3	10.23* ± 1.9	10.65* ± 2.1
Mature bone tissue volume density, Vv%	2.88 ± 0.7	6.43 ± 0.8	8.98 ± 1.6	5.98* ± 2.8	8.81* ± 1.5	12.83* ± ± 1.5	8.63*.** ± ± 2.2	11.51*.** ± ± 2.5	14.58*.** ± ± 2.2
Newly formed bone tissue volume density, Vv%	3.81 ± 0.7	6.43 ± 0.8	9.26 ± 2.1	6.11* ± 3.2	9.81* ± 1.6	13.53* ± ± 2.7	9.51*.** ± ± 2.2	12.95*.** ± ± 2.71	16.43*.** ± ± 2.1

* – statistically significant differences from indicators in the control group, $p < 0.001$; ** – statistically significant differences from indicators in the 2nd group, $p < 0.001$

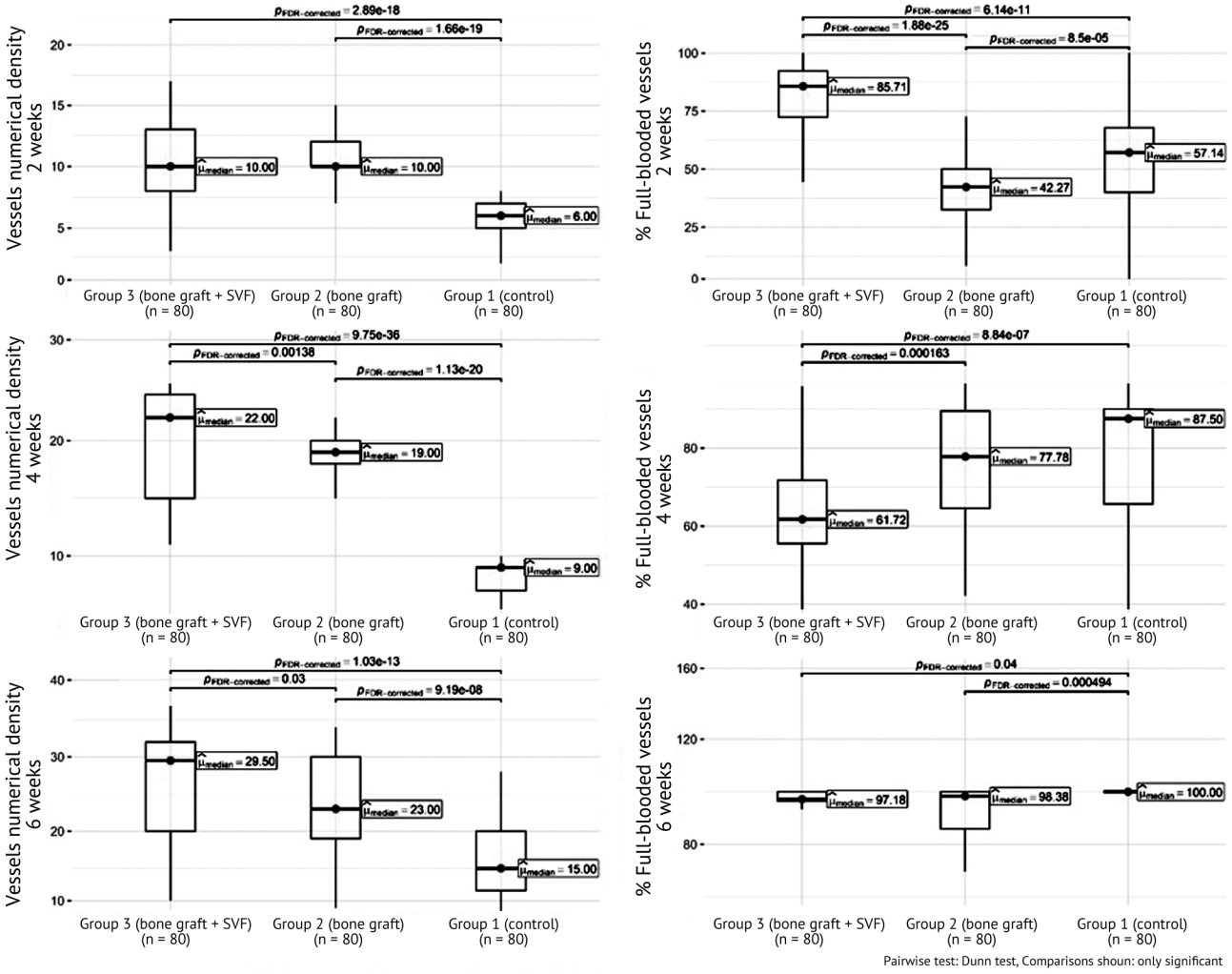

Fig. 1 Vessels numerical density and full-blooded vessels percentage for each group of the study at 2, 4 and 6 weeks

The value of the newly formed bone tissue volume density progressively increased from the 2nd to the 6th week of the study in the 1st group – 1.6 times, in the 2nd group – 2.2 times and in the 3rd group – 1.7 times (Fig. 3 and 4). During all periods of observation, the newly formed bone volume density in the 3rd group was on average 2.1 times higher than in the 1st study group ($p < 0.001$), and on average 1.36 times higher than in the 2nd group ($p < 0.001$).

The mature bone tissue volume density in the study samples increased from the 2nd to the 6th week in all groups; in the 2nd and 3rd groups the indicators were greater than in the 1st group of the study during all period of study. The dynamics of changes in the mature bone tissue volume density was similar to those of the newly formed bone tissue. The studies indicated a more active process of differentiation of the newly formed bone tissue starting from the 4th week of the study.

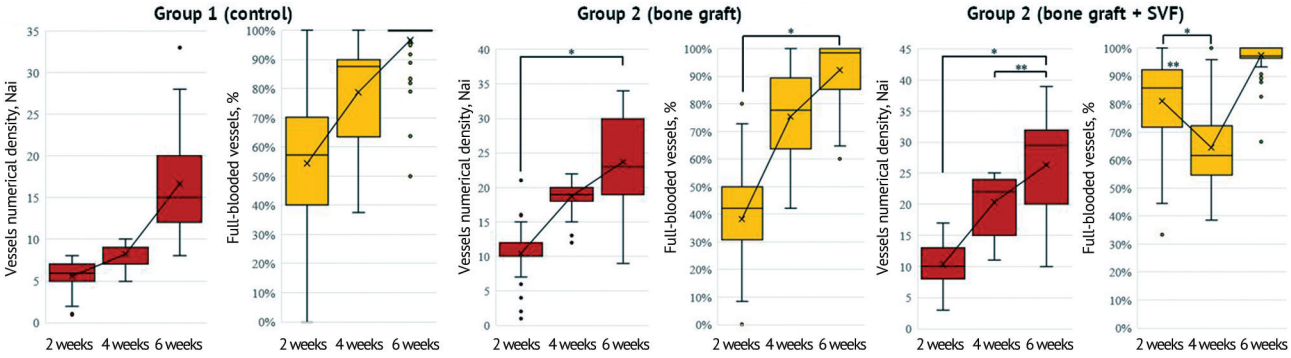

Fig. 2 Diagram showing vessels numerical density (left) and full-blooded vessels percentage (right) for each group of the study at 2, 4 and 6 weeks

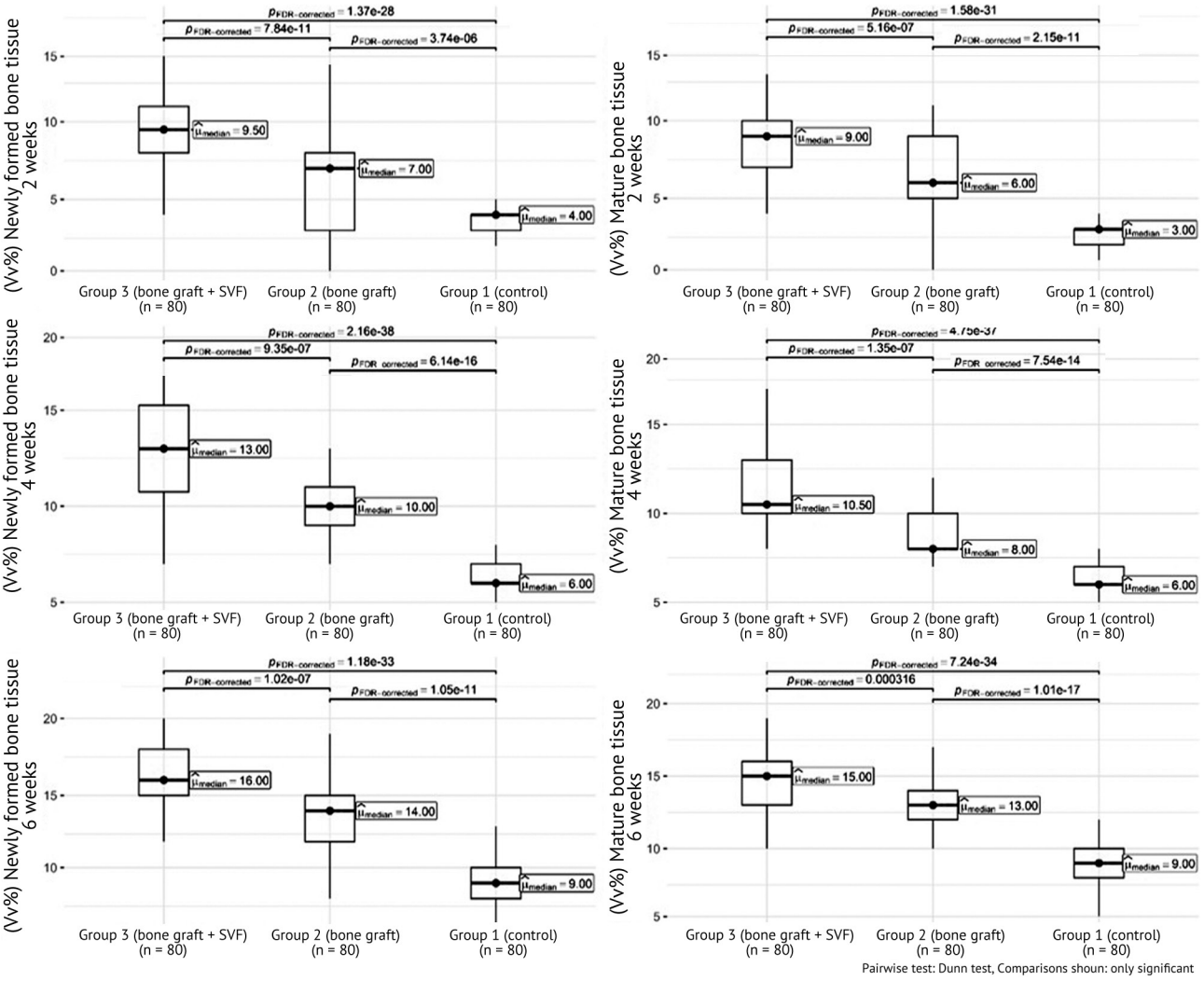

Fig. 3 Volume density of newly formed bone tissue (Vv%) and mature bone tissue (Vv%) for each group of the study at 2, 4 and 6 weeks

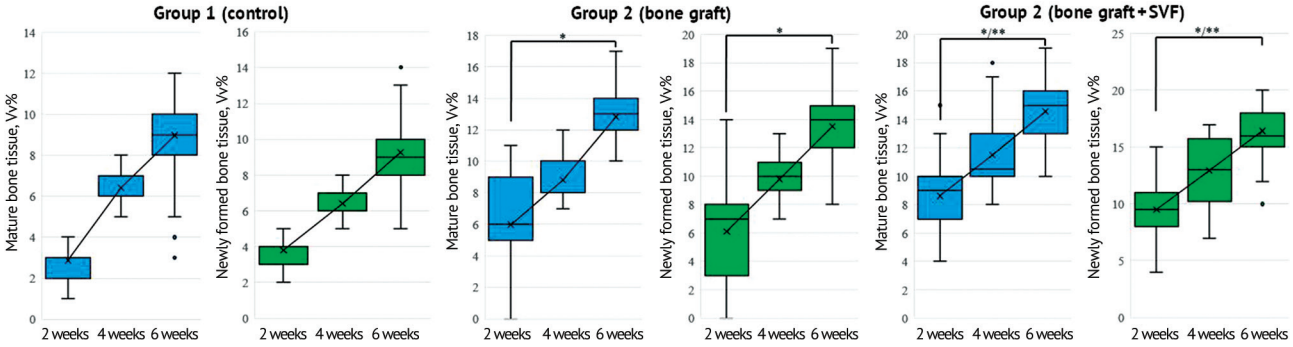

Fig. 4 Diagram showing volume density of newly formed bone tissue (right) and mature bone tissue (left) for each group of the study at 2, 4 and 6 weeks

DISCUSSION

The choice of the rabbit animal model for this study was justified by a similar type of reparative osteogenesis, the Haversian type in this species of mammals and humans [11, 17]. This allows the results of this study to be extrapolated to humans.

The choice of deproteinized cancellous human bone tissue as a bone matrix is justified by scientific literature data and the results of our previously conducted studies, which revealed the properties of this material, allowing it to be used as an independent bone-replacing material and considered as a bone matrix for creating efficient tissue engineering constructs [3, 18].

In the case of using a construct based on a deproteinized bone matrix containing an autologous material that can have impact on bone tissue regeneration, all four processes of bone tissue regeneration are switched on: osteoblastic, osteoinductive, osteoconductive, and stimulated osteogenesis (Fig. 5).

Despite the fact that in this study the bone matrices used are xenogenic for animals, starting from the 2nd week macro- and microscopic morphological signs of a local inflammatory reaction of soft tissues and rejection of bone matrices in areas of their heterotopic implantation were absent.

The adipose tissue stromal-vascular fraction, isolated and processed according to the standard method, was chosen as a biologically active component for creating a tissue-engineered construct based on a deproteinized cancellous bone matrix, enabling to exclude an additional experimental quantitative assessment of the cell composition of the obtained fraction [14, 15, 19].

This is justified by the cell composition of the fraction and the cells properties themselves – adipose tissue stem cells, endothelial and blood vessels smooth muscle cells and their precursors, fibroblasts, macrophages, T-lymphocytes, pericytes and other cells that cause a pronounced regenerative potential, anti-inflammatory effect and immunoregulatory activity. Also, the stromal-vascular fraction factors stimulate the formation of the vascular network, which contributes to the regeneration of bone tissue [2, 15, 20, 21, 22].

The comparative analysis of reparative processes using a deproteinized cancellous human bone tissue matrix and its combination with adipose tissue stromal vascular fraction revealed that the use of these bone substitute materials contributes not only to the early activation of reparative regeneration of the main structural elements of bone tissue at the site of bone defect, but also their timely differentiation.

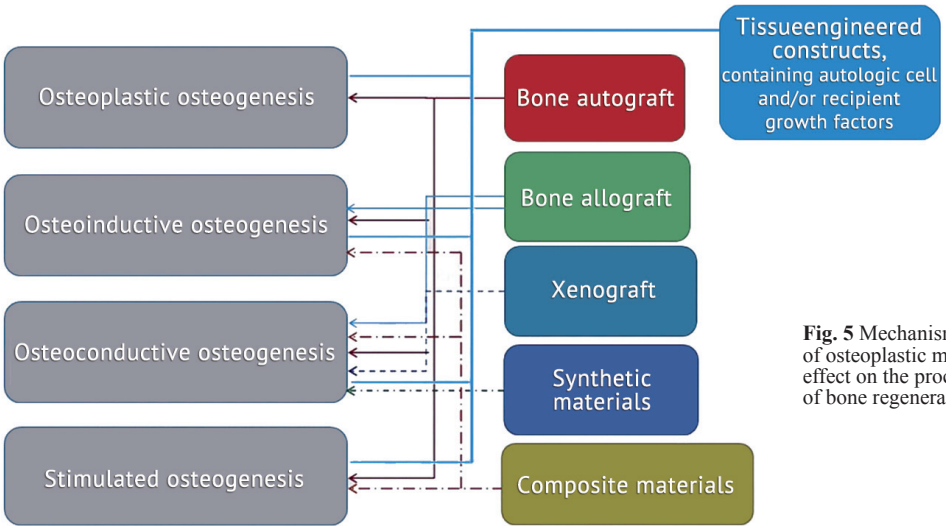

Fig. 5 Mechanisms of osteoplastic material effect on the processes of bone regeneration

CONCLUSION

According to the results of macro- and microscopic assessment at the deproteinized bone matrices heterotopic and orthotopic implantation sites, there were no signs of inflammation and destructive changes in the tissue. That fact is a sign of biological safety of deproteinized cancellous human bone tissue in relation to living tissues.

The use of deproteinized bone matrix in combination with stromal-vascular fraction to create a tissue-engineered construct may unleash several regeneration mechanisms and accelerate the process of bone defect site repair, compared to the situations with the use of a deproteinized bone matrix alone or without reconstruction of a bone defect.

Conflict of interest *The authors declare that they have no competing interests*

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Ethics approval *The study was approved by the institutional ethical committee at the FSBI Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics of the Ministry of Health of the Russian Federation, protocol №007/22 statement 029/22 dated 27.10.2022.*

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Information about the authors:

1. Evgeniya A. Anastasieva – graduate student, traumatologist-orthopedic surgeon, evgeniya.anastasieva@gmail.com, https://orcid.org/0000-0002-9329-8373;
2. Liliya A. Cherdantseva – Candidate of Medical Sciences, Head of the Laboratory, cherdanceff@yandex.ru, https://orcid.org/0000-0002-4729-3694;
3. Tatyana G. Tolstikova – Doctor of Biological Sciences, Professor, Head of Laboratory, tg_tolstikova@mail.ru, https://orcid.org/0000-0002-3750-2958;
4. Irina A. Kirilova – Doctor of Medical Sciences, Deputy Director for Scientific Work, irinakirilova71@mail.ru, https://orcid.org/0000-0003-1911-9741.

Contribution of the authors:

Anastasieva E.A.– concept and design of the study; performing the experiment; collection, analysis and interpretation of data, writing and editing the article.
Cherdantseva L.A.– collection, analysis and interpretation of data, editing the article.
Tolstikova T.G. – assisting with experimental part of the study, analysis and interpretation of data, editing the article.
Kirilova I.A. – analysis and interpretation of data, editing the article.

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Original article

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Biological fixation of customized implants for post-traumatic acetabular deformities and defects

V.A. Bazlov, A.A. Pronskikh, A.A. Korytkin, T.Z. Mamuladze, M.V. Efimenko, V.V. Pavlov✉

Tsivyan Novosibirsk Research Institute of Traumatology and Orthopedics, Novosibirsk, Russian Federation

Corresponding author: Vitaliy V. Pavlov, pavlovdoc@mail.ru

Abstract

Introduction The number of surgical interventions using additive technologies in medicine has been growing both in Russia and with every year. Due to the development of printing customized implants, the use of standard (imported) designs has decreased by an average of 7 % in the provision of high-tech medical care. However, the issue of the pore size of customized implants for management of post-traumatic defects in the acetabulum remains open. **Objective** To evaluate the results of the treatment of patients with post-traumatic acetabulum defects and deformities with the implementation in clinical practice of customized implants with structure and size porous surface that are optimal from the point of view of biological fixation. **Material and methods** Porous implants with different types of porous structure were produced by direct laser sintering using Ti-6Al-4V titanium alloy powders. Experimental work was carried out in vitro to determine the ability of living fibroblasts to penetrate the pores of different sizes. Next, the clinical part of this study was conducted in order to determine the signs of biological fixation of customized acetabular implants in a group of patients (n = 30). **Results** The results of this experiment performed to analyze the penetration of living fibroblasts into the porous structure of implants with different pore size demonstrated that metal structures with a pore size of 400-499 μm can be singled out from all others. **Discussion** Analysis of the literature data shows that there is no consensus on the structure and size of the pores of a customized implant. In our work, we investigated the ability of human living fibroblasts to penetrate into the surface structure of a customized implant, as a result of which we determined their optimal pore size of 400-499 microns. It should be noted that this study was conducted for a definite anatomical location: the acetabulum. However, it cannot be excluded that the data obtained are relevant for other anatomical locations. **Conclusion** Management of bone defects in the acetabulum area with customized implants featuring the surface pore size of 400-499 microns is a justified and relevant method. A prerequisite for the use of such implants is strict compliance with the indications for their use, careful preoperative planning and correct positioning.

Keywords: individual implant, porous surface, pore structure, 3D printing technology

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INTRODUCTION

The total incidence of acetabulum fractures among the adult population reported by literature sources ranges from 1 case per 50 thousand people to 3 cases per 100 thousand people per year. [1]. According to several authors, primary total hip arthroplasty may be a surgery of choice in the presence of risk factors for osteosynthesis. Such factors include multi-fragment fractures, massive damage to the articular cartilage in loaded parts, femoral head impaction [2, 3]. Due to untimely or incorrect treatment, there is a high risk of developing complications of acetabulum fractures such as aseptic necrosis of the femoral head, or degenerative ischemic damage to intra-articular cartilage [4-6]. The main difficulties faced by a surgeon in the treatment of post-traumatic coxarthrosis are primarily acetabulum bone tissue defects that do not allow achieving strong primary fixation of standard acetabulum components [7, 8]. Different autografts can be used to replace acetabulum defects; however, in case of the treatment of the consequences acetabulum fractures, the development of post-traumatic aseptic necrosis of the femoral head does not allow the use of autobone. In such cases, concomitant acetabulum deformation requires the use of augments and individual acetabulum components. The main issues during the planning of such surgical interventions are the following: absence of a common classification of pathological acetabulum changes [9], low reliability of the standard methods of preoperative planning using plain radiographs [10, 11]. Therefore, multispiral computed tomography is required, and the surgery is planned according to the developed 3D models. In several cases, when standard acetabular components and augments do not help in replacing the defect and achieving stable biological fixation, customized implants printed on a 3D printer are used [12].

One of the most important conditions for the implant surface formation is the possibility of the integration of bone tissue into the porous structure of a customized implant [12]. The size and structure of implant surface pores are essential for interacting with bone tissue in terms of primary and subsequent biological fixation of customized implants [13]. The absence of a unified approach to determining the size and geometry

of the porous structure of implants is primarily associated with the analysis of bone tissue in different anatomical zones – lower and upper extremities, bones of facial and cerebral skull – since bone tissue has different macro- and micro architecture due to its organo-specificity [14, 15]. In addition to providing stable biological fixation of the acetabular component, an important condition for achieving good functional results is the restoration of anatomical relationships in the affected joint that are close to a healthy countralateral side.

Objective To evaluate the results of the treatment of patients with post-traumatic acetabulum defects and deformities with the implementation in clinical practice of customized implants with the structure and size of their porous surface that are optimal from the point of view of biological fixation

MATERIALS AND METHODS

The study was carried out in two stages to solve the task. Stage 1 included the experiment to determine the optimal pore size and shape of titanium coating for implants. Samples of porous implants with different surface pore size were obtained by direct laser sintering using Ti-6Al-4V titanium alloy powder. Twenty samples with different sizes of porous surface were prepared for this experiment: 4 batches of 5 plates (10 mm × 10 mm × 5 mm) (Table 1).

Table 1

Description of test samples

Sample No.	Pore size, micron (μm)	Pore depth, mm	Sample size (L × W × H)
1	200-299	4	10 mm × 10 mm × 5 mm
2	300-399		
3	400-499		
4	500-599		
5	600-699		

An *in vitro* experiment was conducted to determine the ability of living fibroblasts to enter the pore structure of different size. This experiment was carried out together with the Novosibirsk Federal Research Center of Fundamental and Translational Medicine. 3D printed surgical hardware samples were colonized with fibroblasts (human living fibroblast culture), then stained with fluorescent stains: Hoechst 33342 (nuclei staining), DiOC6 (mitochondria staining) and Propidium Iodide (PI, nuclei of necrotic cells staining). Fluorescence intensity was registered using a LSM710 confocal microscope (Carl Zeiss); mean fluorescence intensity (mean RFU) for each section along the Z axis (depth, μm) was evaluated; the depth analyzed was up to 2 mm. At the end of the incubation period, the medium in chamber cells was replaced with FluoroBrite DMEM Media (Gibco, USA) that contains fluorescent stains: 5 μg/mL DiOC6, 5 μg/mL Hoechst 33342, 1 μg/mL Propidium Iodide (manufacturer: Sigma, Germany). Incubation continued 30 minutes. The medium was replaced with fresh FluoroBrite DMEM Media (Gibco, USA) and analyzed using a LSM710 confocal microscope (Carl Zeiss) in z-stack mode. Photo processing was performed using Fiji ImageJ software (NIH, USA) algorithms.

For detailed qualitative and quantitative description of such pathological acetabulum changes as deformation and bone defects, our clinic uses the method of layered 3D visualization. Choice of the tactic of implanting acetabuluar components is based on the original method developed at the Novosibirsk Research Institute of Traumatology and Orthopedics [16]. The method is carried out as follows. Based on the MSCT data, a volumetric 3D model is developed that helps to determine reference angles and lines, and the hemisphere of a healthy joint by mirror transfer of marks to the pathological side. The hemisphere is divided into three sectors that correspond to the pubic, ischial and supra-acetabular parts of the acetabulum. To determine the sector of the corresponding size, a geometric figure is selected from the pre-formed library at 1 mm intervals. The sector is spatially located in such way that at least 75 % of the surface of its base is in contact with the supporting dense bone tissue, and the apex matches the rotation center. After selecting a properly oriented sector with known values of volume and surface area, these parameters are described for each sector corresponding to the pubic, ischial and supacetabular surfaces of the acetabulum. Area difference in percentage is specified as the deformation of supporting bone tissue, and the volume difference – as the defect of the abnormal segment (Fig. 1)

In significant defects (bone deficiency of more than 40 %), it is essential to use customized acetabular components with a set polyaxial insertion of screws, or to use augments with a set direction of fixing screws.

The stage 2 of the study was carried out to determine the clinical efficacy of the proposed method: preoperative planning and surgical management of acetabulum defects with customized implants. A test group was formed that included 30 patients with significant post-traumatic acetabulum defects of grade III and IV according to AAOS classification. All patients underwent preoperative planning according to the developed algorithm [17]; customized implants with the pore size determined in the experiment (400-499 μm) were created, and surgical treatment of the defects was carried out. In addition to clinical and functional results, analyzed parameters included the state of bone tissue surrounding the customized implant, restoration of anatomical relationships in the hip joint, such as three-dimensional spatial displacement of the rotation center and change in femoral offset in relation to the healthy contralateral joint parameters. Gender and age composition as well as distribution according to the defect type are presented in Table 2.

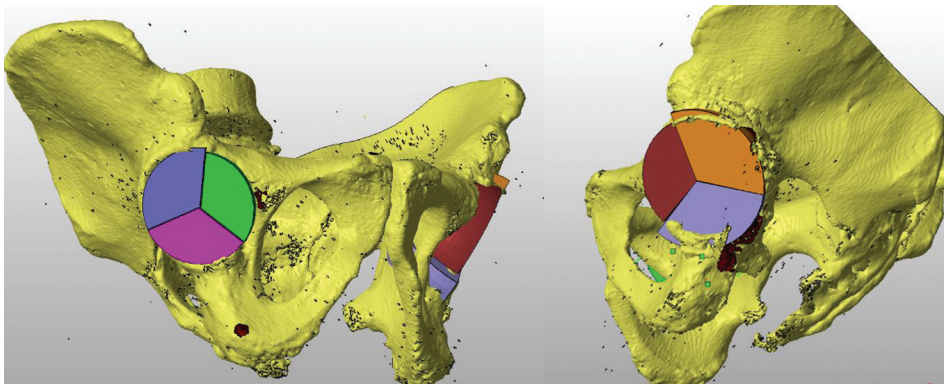

Fig. 1 Appearance of a 3D pelvic model with a sector-by-sector determination of acetabulum bone defect

Table 2

General characteristics of the test group

Parameters	Test group (n = 30)	
	Type III AAOS (n = 11)	Type IV AAOS (n = 19)
Average age, years	61 ± 24	49 ± 22
Gender (m)	6 (20 %)	10 (33 %)
Gender (f)	5 (17 %)	9 (30 %)

RESULTS

Together with the Research Institute of Experimental and Clinical Medicine (Novosibirsk), an *in vitro* experiment was conducted in order to analyze the ability of living fibroblasts to enter the pores of different sizes. The results obtained were subjected to statistical analysis (Table 3).

Figure 2a shows the results of implant surface confocal microscopy in the 3D mode: sample 3 (400-499 μm) – even stained, homogeneous arrangement of living fibroblasts at the depth of up to 2 mm; meanwhile, Figure 2b shows the results of the confocal microscopy of sample 2 (300-399 μm) – uneven, predominantly along pore edges, distribution of living fibroblasts at a depth of up to 2 mm in the structure of sample surface is observed.

Considering the results obtained during *in vitro* experiment, we observed the best penetration capacity of living human fibroblasts in sample 4 with a pore size of 400-499 μm. In the experiment, this sample was evenly colonized by living fibroblasts at a depth of up to 2 mm, while the cells remained viable with probability that was twice higher than in other samples.

In the clinical part of this study, the above approach was used in the test group (n = 30 clinical cases) for management of post-traumatic acetabulum defects grade III and IV according to AAOS with customized implants [18].

The average time of the surgery was 96.74 ± 43.57 minutes; intraoperative blood loss was 392.39 ± 198.6 mL. No revision interventions for component loosening or recurrent dislocation were required during 12 months following the surgery.

One year after the surgical treatment, the signs of biological fixation of customized implants were evaluated using the technique developed by Moore et al. [19]. Their method for assessing the biological fixation of the acetabular component of hip arthroplasty involves the analysis of five radiographic signs (Table 4).

Table 3

Results of the experiment performed to analyze the penetration of living fibroblasts into the porous structure of implants with different pore size structure

Sample No.	Pore size, μm	Maximum depth of implant colonization by culture, μm	Evenness of colonization at a depth of 200 μm	Staining of mitochondria with DiOC6, conditional unit	Conditional living/necrotic cell ratio (Hoechst/ Propidium Iodide)
1	100-299	50	even	1	1/2
2	300-399	50	uneven	1	1/1.8
3	400-499	under 250	even	fluorescence intensity is twice higher	1/1.3
4	500-599	300	even	1	1/16
5	600-699	under 400	uneven	1	1/1.7

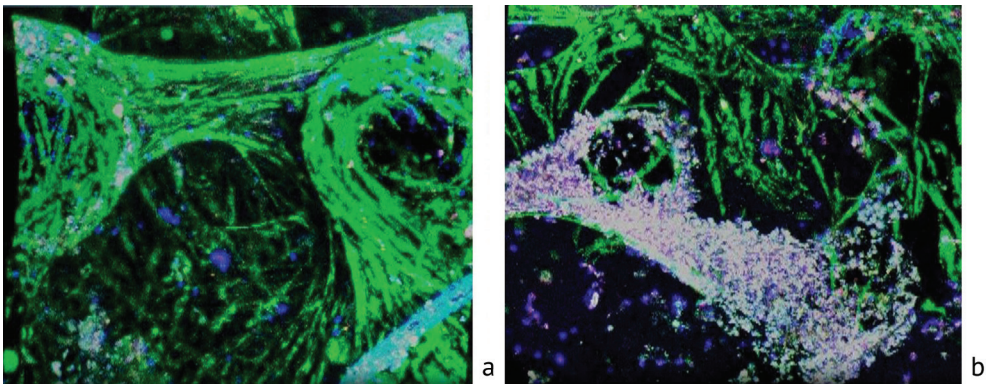

Fig. 2 Confocal microscopy in 3D mode: a – sample 3, fibroblasts are stained with green, 400-499 μm; b – sample 2, fibroblasts are stained with green, 300-399 μm

Table 4

Distribution of the number of radiographic signs of bone tissue change in the acetabulum region corresponding to the biological fixation of customized implants in the sample

Number of Rg signs of biological bone tissue fixation	Patients (n = 30)	%
5	4	13.4
4	10	33.2
3	12	39.8
2	2	6.8
1	2	6.8

Moore et al. in their work proved that implants with three or more biological fixation signs had no manifestations of loosening [19]. Thus, after one year following the surgery, 86.8 % of patients had 3 or more radiographic signs; this fact indicates that there was no loosening of the customized components in the acetabulum area.

Tendency in the recovery of anatomical relationships were registered according to radiographs and MSCT before surgery and 12 months after it (Table 5).

The results obtained prove that due to the rational preoperative planning and use of customized implants, the most accurate restoration of anatomical relationships in the replaced joint was achieved in relation to a healthy contralateral joint.

Table 5

Shift of anatomical parameters in regard to healthy contralateral joint in mm

Parameter	Value before surgery	Value 12 months after surgery
Vertical shift	12.89 ± 12.42	3.72 ± 3.69
Horizontal shift	11.09 ± 12.93	5.87 ± 3.96
Anterior-posterior shift	8.41 ± 7.81	2.09 ± 1.21
Offset	7.37 ± 8.54	4.20 ± 2.85

In the test group (n = 30), VAS parameters were evaluated, as well as the results of Harris and SF-36 questionnaires over time, i.e. before surgical treatment, at discharge and a year after surgical intervention. Results are shown in Table 6.

In the test group (n = 30), there was a significant decrease in VAS score from 7.4 to 2.7 twelve months after surgery with the use of customized implants (on average by 47 %); this fact demonstrates an effective reduction in pain syndrome. Assessment of the changes in Harris Hip Score demonstrates that the average value increased from 48 to 75 points over 12 months (on average by 23 %), so, it can be characterized as excellent and good results.

Table 6

Evaluation of Harris and VAS scores and SF-36 questionnaire in the test subgroup (n = 30) over time

Parameter		Before surgery	12 months after surgery	Intragroup comparison, Mann – Whitney U test	
		Me [Q1; Q3]	Me [Q1; Q3]	Difference [95 % CI]	p value
VAS, points		8 [7; 8]	2.5 [2; 3]	0-1: -3.5 [-4.0; -3.0] 0-2: -5 [-5.5; -4.5] 1-2: -1.5 [-2.0; -1.0]	0-1: < 0.001* 0-2: < 0.001* 1-2: < 0.001*
Harris, points		48 [38.2; 52]	75 [73.2; 78]	0-1: 57 [48.0; 61.5] 0-2: 56.5 [46.0; 60.5] 1-2: -1.5[-6.0; 4.5]	0-1: < 0.001* 0-2: < 0.001* 1-2: < 0.469
SF-36, %	PH	27.5 [24;29.7]	65.5 [61;71]	0-1: 26 [22.0; 29.0] 0-2: 39.5 [32.0; 43.0] 1-2: 12.5 [8.5; 15.5]	0-1: < 0.001* 0-2: < 0.001* 1-2: < 0.001*
	MH	31.5 [29.2;35]	67 [65; 69.7]	0-1: 27.5 [25.0; 30.0 0-2: 33.5 [28.0; 39.0] 1-2: 8.5 [4.5; 11.5]	0-1: < 0.001* 0-2: < 0.001* 1-2: < 0.001*

SF-36 questionnaire also showed a significant increase in the quality of physical and mental health: average PH value increased by 46.7 %, MH value – by 38 %.

The pore size of a customized implant surface equal to 400-499 μm is optimal from the point of view of expected biological integration of bone tissue into implant surface up to 2 mm depth and, as a result, it determines good subsequent fixation of the implant that is confirmed by the X-ray signs of the changes in bone tissue of the acetabulum region. These data were also supported by an *in vitro* experiment conducted with the use of confocal microscopy.

Assessment of social and clinical adaptation parameters (VAS, Harris, SF36 questionnaires) confirmed the high effectiveness of customized implants with set surface structure over time. However, it should be mentioned that this study was carried out within a narrow anatomical location, i.e. the acetabulum, accordingly, it cannot be ruled out that the data obtained are also relevant for other anatomical areas.

DISCUSSION

Laser selective sintering technology enables to manufacture implants with pore size control up to 20 microns [20, 21, 22].

However, the authors note that the optimal pore size of a customized implant surface has not been determined. The lack of a unified approach to determining the size and geometry of the porous structure of the implant is primarily due to the study of bone tissue of various anatomical zones – the lower and upper extremities, the bones of the facial and cerebral skull – because bone tissue, depending on organ specificity, differs in its macro- and microarchitectonics [15, 23]. Taniguchi Naoya in his work examined three samples of porous titanium implants (with an estimated porosity of 65 % and a pore size of 300, 600 and 900 microns), designated as implants P300, P600 and P900 [23].

Accordingly, the P600 implant (632 microns) demonstrated significantly higher fixation ability after 2 weeks than the other implants. After 4 weeks, all models showed a sufficiently high fixation ability in the detach test.

Ran Qichun et al. work studied the effect of the pore size of implants on biological characteristics (in particular osseointegration), conducted a number of experiments on implants with a pore size of 500-699 and 700-900 microns, both in vivo and *in vitro* [24, 25, 26]. According to the study, implants printed on a 3D printer with a given pore size up to 600 microns outperform the other groups in terms of osseointegration of bone tissue into the porous structure of the implant surface.

Yuhao Zheng, Jing Zhang et al. in their study of the porous surface of customized implants noted that the issue of the implant surface with a pore size of less than 300 microns was not well investigated at the moment. Yuhao Zheng, examining the average pore sizes of cylindrical implants 542, 366, and 134 microns, indicated that with a porosity of more than 60 %, the optimal pore size is 366 microns; however, they did not describe the pore geometry [27, 28, 29, 30].

The pore size of 400-499 microns of the surface of a customized implant is optimal for managing post-traumatic acetabular defects from the point of view of the predicted biological fixation of the bone. This approach determines a good subsequent fixation of the implant, which is confirmed by the presence of radiological signs of changes in the bone tissue of the acetabulum area. The findings are also confirmed by an *in vitro* experiment conducted using confocal microscopy. However, it is worth noting that this study was conducted for a definite anatomical location – the acetabulum. However, it cannot be excluded that the data obtained are relevant for other anatomical zones.

CONCLUSION

The analysis of porous structure size in this experimental work led to conclusion that the optimized parameter of implant surface porous structure for better osteogenic result is 400-499 μm. Too small or too large pore size may more or less interfere with cellular behavior and bone regeneration. Thus, the management of bone defects in the acetabulum region using customized implants with the surface of a mesh porous structure (400-499 μm) is a justified method that is also relevant and socially significant due to the increasing number of patients requiring such surgical interventions. A mandatory condition for using such implants is strict compliance with the indications for their use, careful preoperative planning, and correct positioning.

Conflict of interest *All authors declare no conflict of interest.*

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Ethical approval *Ethical expertise conducted. Extract No. 005/23-5 from the protocol of the meeting 002/23 dated 16 February 2023 Informed consent All patients singed informed consents for conduction of the study and treatment.*

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Information about authors:

1. Vyacheslav A. Bazlov – Candidate of Medical Sciences, researcher, orthopedic traumatologist, sbazlov@yandex.ru, https://orcid.org/0000-0001-8982-5516;
2. Aleksandr A. Pronskikh – Candidate of Medical Sciences, researcher, orthopedic traumatologist, Proal_88@mail.ru, https://orcid.org/0000-0003-1197-556X;
3. Andrey A. Korytkin – Candidate of Medical Sciences, Director, andrey.korytkin@gmail.com, https://orcid.org/0000-0001-9231-5891;
4. Tariel Z. Mamuladze – researcher, orthopedic traumatologist, gromadina@inbox.ru, https://orcid.org/0000-0001-8891-535X;
5. Maksim V. Efimenko – researcher, orthopedic traumatologist, gromadina@inbox.ru, https://orcid.org/0000-0003-1516-7877;
6. Vitaliy V. Pavlov – Doctor of Medical Sciences, Head of the Department, pavlovdoc@mail.ru, https://orcid.org/0000-0002-8997-7330.

Contribution of the authors:

Bazlov V.A. – development of the concept and design of the study, interpretation and analysis of the data obtained, editing.
Pronskikh A.A. – analysis and interpretation of the obtained data, editing.
Korytkin A.A. – research coordination, interpretation and analysis of data obtained, editing.
Mamuladze T.Z. – collecting and processing material, conducting research, preparing text.
Efimenko M.V. – research coordination, interpretation and analysis of data obtained, editing.
Pavlov V.V. – development of the concept and design of the study, interpretation and analysis of the data obtained.

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Bioactive biodegradable polycaprolactone implant for management of osteochondral defects: an experimental study

A.V. Popkov, E.S. Gorbach✉, E.N. Gorbach, N.A. Kononovich, E.A. Kireeva, D.A. Popkov

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Evgenij S. Gorbach, gorbach.evg@mail.ru

Abstract

Introducrion Repair of the affected articular surface still remains an unsolved problem. **The purpose** of this study was to assess the efficacy of a biodegradable polycaprolactone implant coated with hydroxyapatite on the healing of an osteochondral defect of the femoral condyle in rats. **Materials and methods** An osteochondral defect of the medial femoral condyle was modeled in 76 Wistar rats divided into 2 groups. In the experimental group, the defect was replaced with a biodegradable polycaprolactone membrane coated with hydroxyapatite. In the control group, the defect remained untreated. The results were assessed within a year. **Results** In the experimental group, the animals had a significantly better range of motion at all stages of the experiment than the control animals. The implant ensured the integrity and congruence of the articular surface. On day 180, a newly formed area of the articular surface of the organotypic structure was observed in the defect. Biomechanical properties of the repaied zone restored after 60 days while in the control one they remained lower by 27-29 %. **Discussion** Filling the defect with an elastic implant made of polyprolactone with hydroxyapatite provided early functional load on the joint. The structure of the implant, simulating the extracellular matrix, promoted the growth, proliferation and directed differentiation of cells in the area of the osteochondral defect. The moderate rate of biodegradability of the material provided gradual replacement of the implant with organ-specific tissues. **Conclusion** A biodegradable polycaprolactone implant impregnated with hydroxyapatite particles might be effective for experimental osteochondral defect repair.
Keywords: articular cartilage, osteochondral defect, biodegradable implants, polycaprolactone, hydroxyapatite

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INTRODUCTION

Lesions of joint articular cartilage are a common pathology of the musculoskeletal system. An analysis of more than 30,000 arthroscopic interventions associated with injuries and diseases of large joints showed pathological changes in the cartilage of varying severity in 63 % of cases [1, 2]. Focal disorders of the articular cartilage and subchondral bone of the femoral condyles mostly result from injuries or diseases of the knee joint. Their delayed or inadequate treatment could lead to the development of degenerative joint disorders [3].

Osteochondral defects of large joints frequently require surgical treatment [4]. Despite the fact that a wide range of surgical techniques for the treatment of articular cartilage has been introduced into clinical practice, the search for methods of articular surface repir remains a very urgent and unsolved problem at the present stage of medicine and biotechnology [5].

The low ability of the cartilage to regenerate has been proven by many researchers and is associated with the lack of blood supply and innervating components in it. Therefore, a lot of research has been and is being carried out to develop new methods, which, as a rule, are aimed at stimulating the repair and articular cartilage recovery [6, 7].

To date, one of the most efficient and cost-effective methods is the microfracturing method in its various modifications. However, the regenerate thus formed in the subchondral defect area frequently undergoes lysis in patients older than 35 years [8, 9].

Mosaic chondroplasty contributes to the successful long-term repair of the damaged site with bone and cartilage autografts taken from unloaded areas of the articular surface [10, 11], but it can cause pain and degenerative changes in those areas, or they need to be filled with other implant materials, which requires additional costs and increases the time of the surgery.

In recent years, cell technologies have been successfully applied such as autologous chondrocyte implantation (ACI) into the area of the cartilage defect or a combination of autologous chondrocytes and collagen matrices (MACI) [12]. The shortcomings of these methods is complexity and high costs.

An alternative method for treating osteoarthritis is matrix-induced technologies (autologous membrane-induced chondrogenesis collagen, AMIC), when native bone marrow cells and poorly differentiated perivascular cells penetrate into the defect area as a result of preliminary microfracturing and further populate the implanted biocompatible biodegradable matrices. Current publications are mainly devoted to the results of replacement of cartilage defects with collagen matrices. However, natural collagen matrices are quite expensive and not always effective in the long term [13-15].

There are recent publications on the successful use of synthetic polymeric biodegradable implant materials, which are much cheaper and do not cause immune reactions [16-21]. However, complex experimental studies with a long-term follow-up period are required to objectively substantiate the use of such materials and technologies before introducing them in clinical practice.

The aim of our study was to investigate the safety and efficiency of the application of a biodegradable implant produced from polycaprolactone with the method of electrospinning and coated with hydroxyapatite and its impact on healing of an intra-articular defect in the loaded zone of the knee joint in rats.

MATERIALS AND METHODS

The study was conducted on 76 Wistar rats, whose age was 7 months at the beginning of the experiment. Osteochondral defects of the articular surface of the medial femoral condyle were modeled in all the animals divided into 2 groups, 38 rats each. The knee joint was approached and an osteochondral defect of the medial condyle of the femur, 1.5-2 mm wide and 2 mm deep, was modeled using a 2-mm cutter. In the experimental group, the defect was filled with a biodegradable polycaprolactone (PCL) membrane with hydroxyapatite (a biodegradable elastic PCL matrix produced by electrospinning and containing hydroxyapatite). In the control group, the defects were left untreated. Surgical interventions were performed in a sterile operating room under general anesthesia (Rometar 2 % – 1-2 mg/kg, Bioveta, Czech Republic; Zoletil 100 – 10-15 mg/kg, Virbac Sante Animale, France). Five rats were intact to calculate reference normal values.

Clinical methods

Clinical observation of all animals was carried out throughout the experiment. Their general condition and physical activity were assessed. Body weight was measured using electronic scales; general and local temperature in the area of surgical intervention and in the similar area of the contralateral limb was measured with a remote medical infrared thermometer (BWell Swis AG, Switzerland). The external condition of the lower leg soft tissues in the surgical area and the functional state of the limb were assessed. The circumference of the lower leg in its upper third was measured with a centimeter tape. The angles of passive knee extension and flexion were measured with a standard goniometer.

Defect healing was studied using microanatomical and histological methods. The animals were euthanized by an overdose of barbiturates after premedication with conventional pharmacological preparations on experiment days 14, 60, 180 and 360.

Anatomical methods

After euthanasia, the femur of the involved limb was isolated and the femur soft tissues were dissected. In the area of the metaphysis, its distal articular end was sawn off with a cutter. The features of the articular surface defect and the whole articular surface were examined, paying attention to the restoration of its congruence. The macroscopic evaluation standards of the International Cartilage Restoration Society (ICRS) were used [23]. The ICRS macroscopic evaluation of osteochondral repair has been widely used to study the repair of an osteochondral defect *in vivo* [24, 25, 26]. Two orthopedic surgeons and a histology researcher performed a blind evaluation of the effect of defect repair. Photographic digital documentation of anatomical preparations was carried out.

Biomechanical methods

To analyze the biomechanical properties of the articular surface of the regenerate formed in the area of the osteochondral defect, its compliance (P) was determined on fresh unfixed anatomical preparations (measuring the magnitude of the applied force as a result of the forced introduction of the indenter into the tissue under study). To do this, we used a pointer indicator with measurement steps from 0 to 10 mm and a division value of 0.01 mm (GOST 577-68) with a spherical shape of the indenter. The curvature of the cartilaginous surface was determined by a radius meter in the radius range of 0.5-10 mm. The measurements were performed as follows: the curvature of the cartilaginous surface was measured with a radius meter, setting the measuring device on a template corresponding to the radius of the cartilage surface curvature (the steady indicator reading is Mo), similarly, the value of penetration of the indenter into the cartilage was measured (M – according to the indication of the indicator arrow). The contact time of he indenter with the cartilage was 3-5 seconds. Each of the measurements was repeated three times. Average values were calculated. According to the formula $P = 10.5 M + 70$ (where P is the force of the spring mechanism, measured in gram-force (gf)) were determined the magnitude of the force P, with which the movable leg of the device penetrated into the cartilage.

Having determined the value of the indenter penetration (d) (in accordance with the readings of the digital indicator, where $d = Mo-M$), the compliance (P) was calculated: $P = d/P$ in mm/gf, given that 1 m/N = 0.102 mm/gf.

Histological methods

Fragments of the distal articular end of the femur dissected from soft tissues were fixed in 10 % neutral formalin solution for 3-5 days. Samples were demineralized in a decalcifying solution based on EDTA by constant shaking and changing solutions every day for 7-10 days. For dehydration of bone and cartilage

fragments, alcohols of ascending strength (from 70° to 100°) were used. The samples were then impregnated and embedded in paraffin.

Histological sections, 5-7 μm thick, were prepared using a sledge microtome (Reichert, Germany), placed on glass slides and dried. Deparaffinized preparations were stained with hematoxylin and eosin, as well as alcian blue – safranin-O. An immunohistochemical reaction to CD34 antibodies was carried out by additional staining with hematoxylin and eosin (protocol and antibodies from Abcam PLC, UK).

Light microscopic study and digitization of histological preparations were performed using an AxioLab.A1 microscope and an AxioCam digital camera (Carl Zeiss MicroImaging GmbH, Germany). The thickness of the subchondral bone trabeculae was measured with VideoTesT Master-Morphology-4.0 (NPK Zenit Ltd, Russia). The thickness of the cartilage tissue in the defect zone was measured after 180 and 360 days on digitized images of histological preparations of the experiment series and intact animals. The areas of tissue components in the regenerate formed in the defect zone were measured and their portion in the total area of the regenerate at different time-points of the experiment was calculated. Blind histological assessment was carried out according to the system proposed by O'Driscoll et al [27].

Statistical methods

Statistical analysis was performed using Attestat software 9.3.1. The values were presented as medians (Me) and quartiles (Q1-Q3). The significance of differences was determined by the Mann-Whitney test. At $p < 0.05$, the differences were considered statistically significant.

Ethics Approval was obtained from the institutional ethics committee before the experiment (Ethics Committee of the Ilizarov National Medical Research Center for Traumatology and Orthopedics, Kurgan, Russia (protocol code 1(71), date of approval 28.04.2022). Interventions, animal care, and euthanasia conformed to the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 18.03.1986), principles of laboratory animal care (NIH publication number 85-23, revised 1985), and the national laws.

RESULTS

Clinical assessment

After 14 days, the rats actively used the involved limb. Their behavior, food intake and physical activity did not differ from intact animals. Tissue swelling persisted for 7 days post-surgery. Measurements of the circumference of the upper third of the leg showed slightly lower volume of soft tissues of involved limbs in the control group in comparison to the experimental group (Fig. 1).

Neither weight loss nor critical changes in the body temperature were observed in the animals of both groups (Table 1). There was a slight increase in body temperature within 30 days after surgery in both groups. The local temperature in the joint area within a month after surgery was higher than the reference values by 2.6-2.7 °C in both groups.

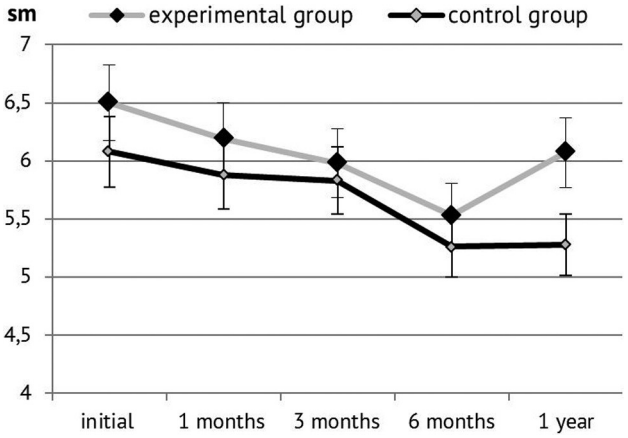

Fig. 1 Soft tissue circumference of the lower leg

Table 1

Physiological parameters: weight, local and body temperature

Experiment time-point	Animal group	Parameters		
		Body weight [gr]	General body temperature [°C]	Local temperature [°C]
Reference norm	–	397.0 (342-406)	34.6 (34.5-35.1)	31.1 (30.1-31.8)
14 days	Control	402.3 (331-409)	36.9 (36.4-36.9)*	33.7 (31.0-33.5) *
	Experiment	403.8 (344-409)	37 (36.7-36.9) *	33.8 (32.4-33.8) *
30 days	Control	408 (328-410)	36.2 (36.1-36.4) *	32.2 (31.2-32.2)
	Experiment	408 (344-416)	36.4 (35.8-36.5) *	31.2 (30-31.4)
60 days	Control	408.4 (343-412)	35.1 (34.7-35.4)	28.8 (28.5-28.9) *
	Experiment	409.0 (348-419)	35.6 (34.7-35.5)	31.1 (30.4-31.5)
90 days	Control	410.0 (338-410)	34.9 (33.5-35.05)	30.2 (28.85-31.2)*
	Experiment	410.0 (350-434)	35.03 (34.4-35.1)	31.3 (30.8-31.3)
180 days	Control	393(391-395)	35.1 (34.9-35.3)	30.8 (29.8-31.8)
	Experiment	413 (411-415)	34.9 (34.7-35.1)	30.7 (30.1-31.8)
360 days	Control	401 (398-404)	35.6 (35.5-35.7)*	30.4(29.7-31)*
	Experiment	418 (410-426)	35.1 (34.9-35.3)	30.9(30.4-31.4)

* significant difference, $p < 0.05$

At any time-point of the study, range of knee motion in experimental and control animals did not significantly differed from each other and from intact rats (Fig. 2). But at the 1-year point, the values of knee extension in both groups were reduced relative to the previous mesurements by 8.1 % in the experimental group and by 16.5 % in the control group. At the same time, in the experimental group, the values did not have significant differences from intact animals and were higher than in the control group by 11 %.

Knee flexion values in both groups did not differ from those in intact animals. However, a slight decrease was noted after 30 days in both groups (by 3 % and by 1.5 %, in the experimental and control groups, respectively) and after a year in the control group (by 1.5 %). At the same time, from 90 days to 360 days, the indicators of the experimental group exceeded those in the control one by 2 %-2.9 %. So, there was no significant difference in ROM between the groups.

Results of anatomical and histological study

The study of anatomical preparations of the distal articular end of the femur showed that in the animals of the experimental group, a smooth shiny articular surface was seen and preservation of the anatomical relief of the condyle already after 14 days due to the implanted material filling the defect gap. The defect was covered with a layer of transparent tissue under which the implant material was visualized filling the defect (Fig. 3 i). There were no signs of implant rejection, inflammatory reaction of surrounding tissues and cavities around it. On day 60, the layer of tissue on the implant surface was more pronounced, and the surface congruence was maintained (Fig. 3 j). throughout the study including long-term, the anatomical relief of the surface in the area of damage was preserved in experimental group (Fig. 3 k, l). Visualization of the implant at each subsequent study time-point was less expressed (Fig. 3 j, k, l). At six months post-surgery, the defect site was completely replaced by specific tissues. The lateral femoral condyle retained its anatomical shape without erosion. The color and gloss of the surface was similar to the norm (Fig. 3 l).

In the control group, the bottom of the defect was lined with a smooth tissue layer in all periods, denser and less transparent along the perimeter of the edge of the defect (Fig. 3 a-d). However, the defect was not filled even after 180 days of the experiment (Fig. 3 c). After 360 days, the defect was unevenly filled with a connective tissue substrate (Fig. 3 d). The congruity of the surface was disturbed at all time-points of the experiment (Fig. 3 a-d). In the intact lateral condyle, areas with erosions on the cartilage surface were visualized starting from day 60 of the experiment (Fig. 3 b, c, d).

At all time-points of the experiment, the indicators of macroscopic assessment according to the ICRS standards in the experimental group were significantly higher than in the control group (Table 2). The maximum scores were noted in the experimental group after six months and a year of the experiment. By those time-points, cartilage recovery was close to normal and corresponded to the grade II of healing while in the control group, the result corresponded to grades III and IV, which are characterized as abnormal and extremely abnormal recovery. The results obtained are consistent with those of descriptive morphology.

Table 2

Results of macroscopic study of osteochondral defect recovery (ICRS standards)

Group	Number of points, Me (Q1-Q3)		
	60 days	180 days	360 days
Control	2.5 (2.3-2.6)*	4 (3.9-4.2)*	4.5 (4.3-4.7)*
Experimental	10 (9.5-10.5)	10.5 (10.3-10.7)	11 (10.8-11.4)

*significant difference ($p < 0.01$)

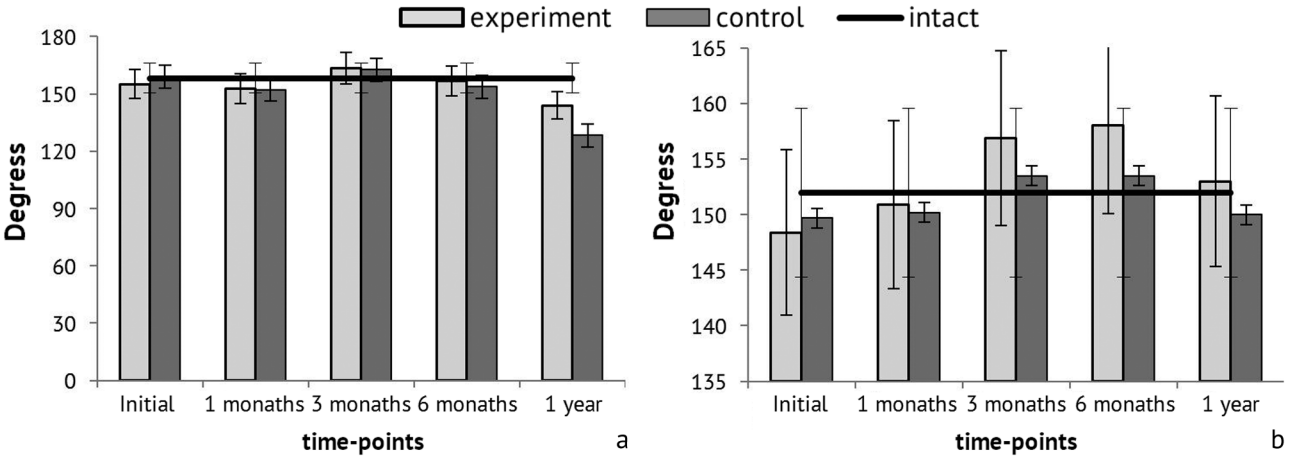

Fig. 2 Range of motion in knee joint: a – extension, b – flexion

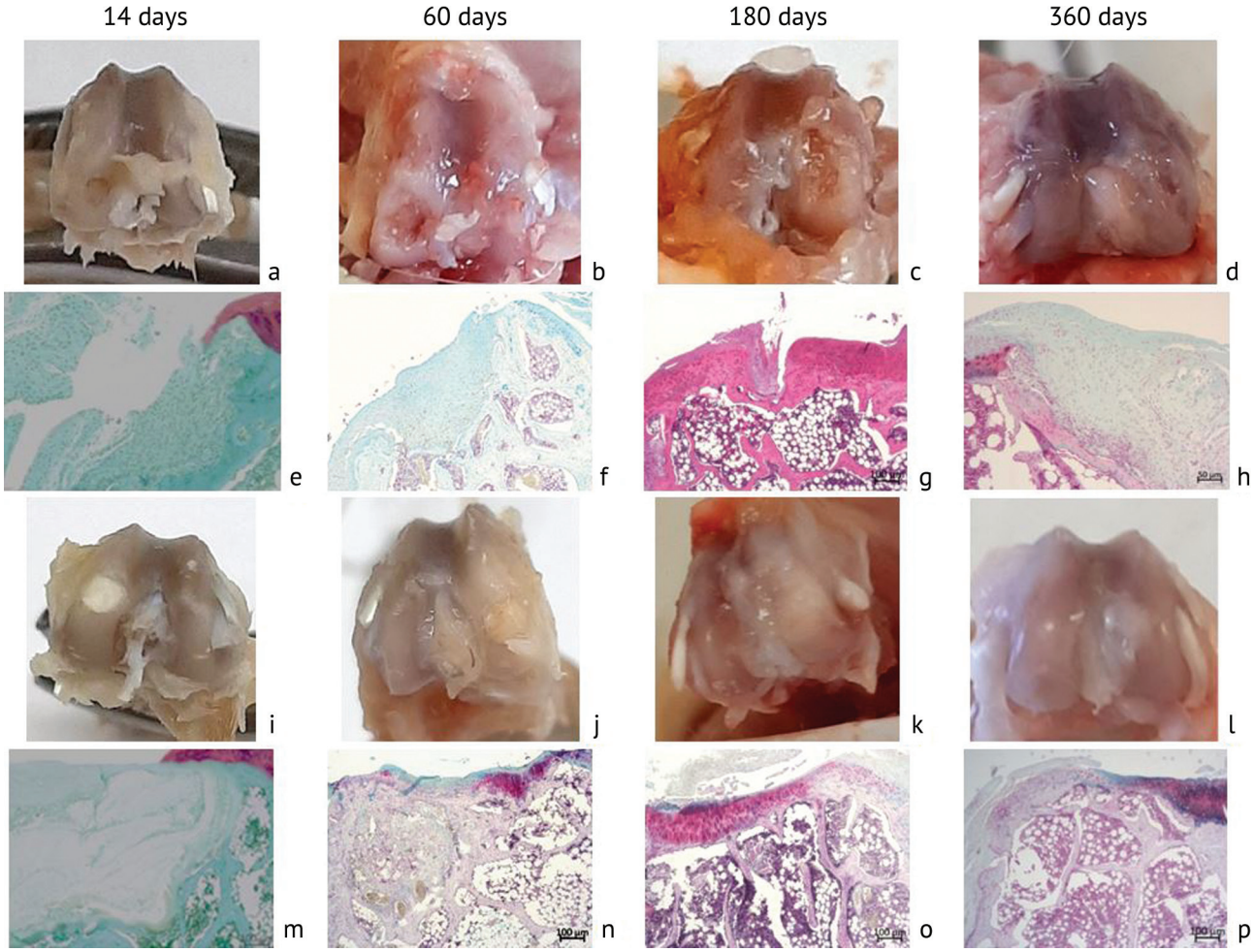

Fig. 3 Anatomical and histotopographic macro- and microphotos of the osteochondral defect at different time-points of the experiment. Anatomical preparations of the distal articular end of the femur in the control group (a-d); histotopographic sections of the area of the osteochondral defect of the femoral condyle in the control group (e-h); anatomical preparations of the distal articular end of the femur in the experimental group (i-l); histotopographic sections of the area of osteochondral defect of the femoral condyle in the experimental group (m-p); f, g, h, p – Staining with hematoxylin and eosin (e, f, g, m); alcian blue-safranin staining (h, n, o, p). Magnification (e-h, m-p):50×

Histological methods showed that the area of the defect in the experimental group was densely filled with implant material after 14 days, around which granulation and loose fibrous connective tissue and microvessels were detected (Fig. 3 m). Bands of loose fibrous connective tissue containing microvessels, an accumulation of poorly differentiated fibroblast-like cells, cells of the monocyte-macrophage series and lymphocytes grew from the side of the surrounding subchondral bone into the spaces between the structures of the implant (Fig. 4 b). Among them, there were cells in a state of division (Fig. 4 c). Inflammatory infiltrates around the structures of the implant material were not observed. In the intertrabecular spaces of the subchondral bone adjacent to the defect, predominantly red (hematopoietic) bone marrow was seen. Outside, small areas of hyaline-like cartilage crawled onto the implant filling the defect from the side of the preserved hyaline cartilage, connected with avascular fibrous connective tissue with small areas of granulations, covering most of the surface of the defect (Fig. 3 m, Fig. 4 a).

In the control group, the defect was predominantly filled with loose fibrous tissue with granulation foci (Fig. 3 e, Fig. 4 d, e). In the intertrabecular spaces, inflammatory infiltrates, loose fibrous connective tissue, and foci of hematopoiesis were noted (Fig. 4 e).

After 60 days of the experiment, the volume of the implant material significantly decreased due to its biodegradation and replacement with tissue components; in the projection of the subchondral bone with reticulofibrous bone and loose fibrous connective tissues with numerous microvessels, and in the projection of the cartilaginous lining from the side of intact hyaline cartilage with small foci of hyaline-like tissue, and in the middle part with fibrocartilaginous tissue (Fig. 5 a).

Bone trabeculae along the periphery of the defect area were more mature and mineralized but slightly mineralized in the middle part (Fig. 5 c). The cell composition was characterized by cells of epithelial, fibroblastic, osteogenic and monocyte-macrophage differons. Osteoclasts were determined (Fig. 5 d).

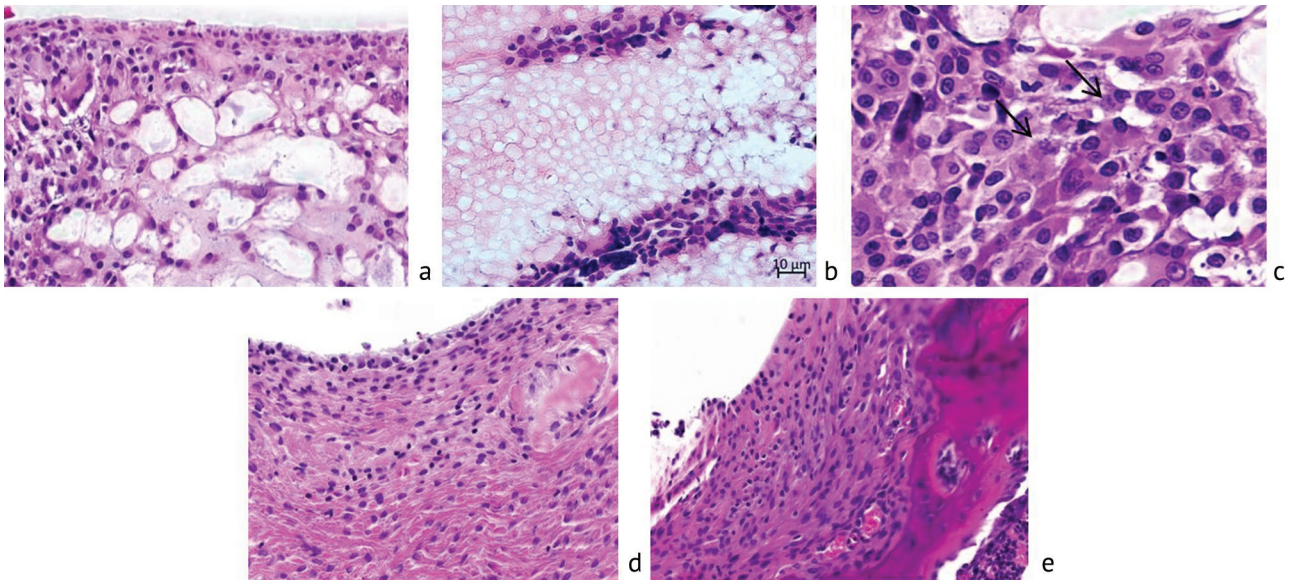

Fig. 4 Features of the regenerate in the area of the osteochondral defect after 14 days of the experiment. Experimental group: articular surface in the area of the defect replaced by the implant (a); ingrowth of loose fibrous connective tissue into the structure of the implant (b); (c) mitotically dividing cells in the regenerate formed in the area of the osteochondral defect (arrows). Control group: articular surface in the area of the defect (d); loose fibrous connective tissue that fills the defect and trabecular subchondral bone that forms the bed of the defect (e). Staining with hematoxylin and eosin. Magnification: a, b, d, e – 400×; c – 1000×

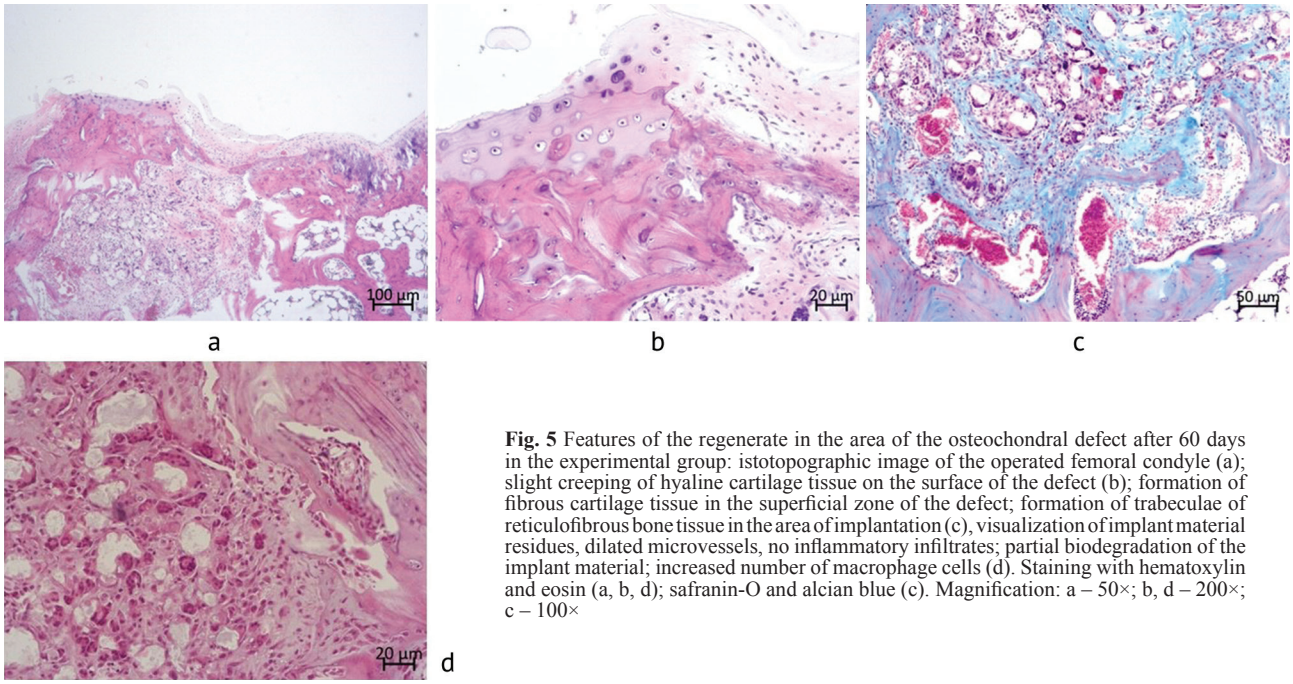

Fig. 5 Features of the regenerate in the area of the osteochondral defect after 60 days in the experimental group: istotopographic image of the operated femoral condyle (a); slight creeping of hyaline cartilage tissue on the surface of the defect (b); formation of fibrous cartilage tissue in the superficial zone of the defect; formation of trabeculae of reticulofibrous bone tissue in the area of implantation (c), visualization of implant material residues, dilated microvessels, no inflammatory infiltrates; partial biodegradation of the implant material; increased number of macrophage cells (d). Staining with hematoxylin and eosin (a, b, d); safranin-O and alcian blue (c). Magnification: a – 50×; b, d – 200×; c – 100×

Giant cells of foreign bodies were not found. Microvessels in the area of the defect and adjacent parts of the subchondral bone were plethoric and dilated. In the projection of the cartilage, the vessels were not found. There was no rarefaction of the subchondral bone beyond the defect area. Vessels and hematopoietic-fatty bone marrow were visualized in the intertrabecular spaces.

In the control group after 60 days, the defect was partially covered by the trabecular bone from the side of the subchondral bone (Fig. 6 a) and with fibrous tissue in the central part (Fig. 6 a, b). Surface congruence was not achieved and a crescent cavity was noted (Fig. 3 b, f. Fig. 6 a). From the side of the cartilage lining, no creeping of hyaline cartilage tissue into the defect area was detected (Fig. 6 b). The vessels in the intertrabecular spaces were dilated. Lymphocytic infiltrates were determined along their periphery (Fig. 6 c). A thickening of the subchondral compact plate was noted at the edges of the defect (Fig. 6 b). The bone marrow in the intertrabecular spaces was predominantly hematopoietic-fatty.

After 180 days in the experimental and control series the subchondral defect was replaced by trabecular bone of hematopoietic-fatty marrow in the intertrabecular spaces (Fig. 3 o, g, Fig. 7 a, c, Fig. 8 a, d).

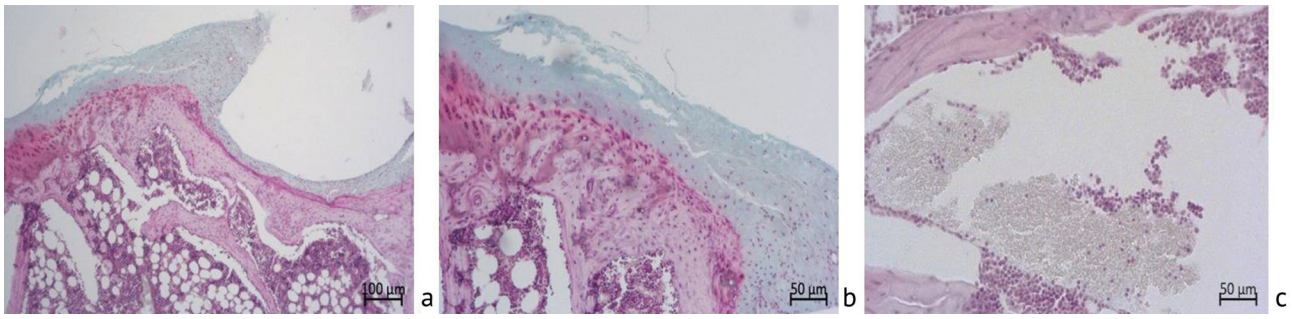

Fig. 6 Features of the regenerate in the area of the osteochondral defect after 60 days of the experiment. Control group: histotopographic image of the operated femoral condyle (a); thickening of the compact plate of the subchondral bone at the edges of the defect (b) and filling the defect with fibrous connective tissue; dilated sinusoidal capillaries with lymphocytic infiltrate in the perivascular region (c). Staining: safranin-O and alcian blue. Magnification: a – 50×; b, c – 100×

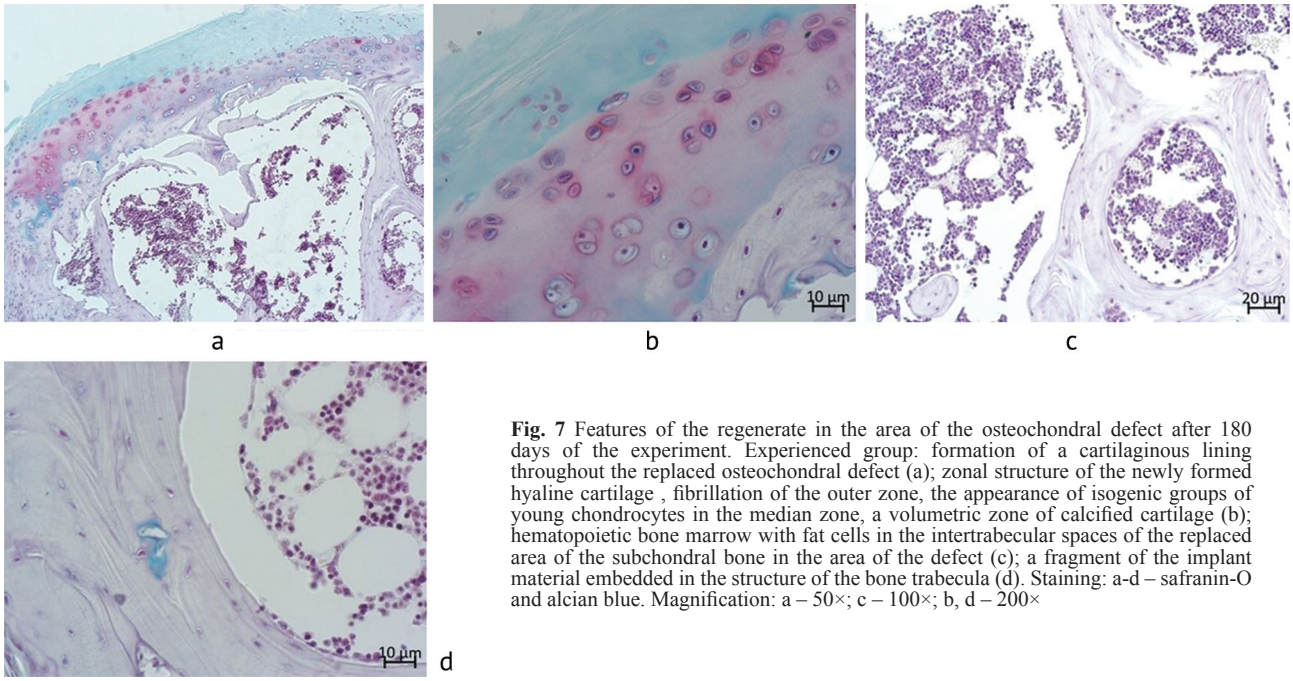

Fig. 7 Features of the regenerate in the area of the osteochondral defect after 180 days of the experiment. Experienced group: formation of a cartilaginous lining throughout the replaced osteochondral defect (a); zonal structure of the newly formed hyaline cartilage , fibrillation of the outer zone, the appearance of isogenic groups of young chondrocytes in the median zone, a volumetric zone of calcified cartilage (b); hematopoietic bone marrow with fat cells in the intertrabecular spaces of the replaced area of the subchondral bone in the area of the defect (c); a fragment of the implant material embedded in the structure of the bone trabecula (d). Staining: a-d – safranin-O and alcian blue. Magnification: a – 50×; c – 100×; b, d – 200×

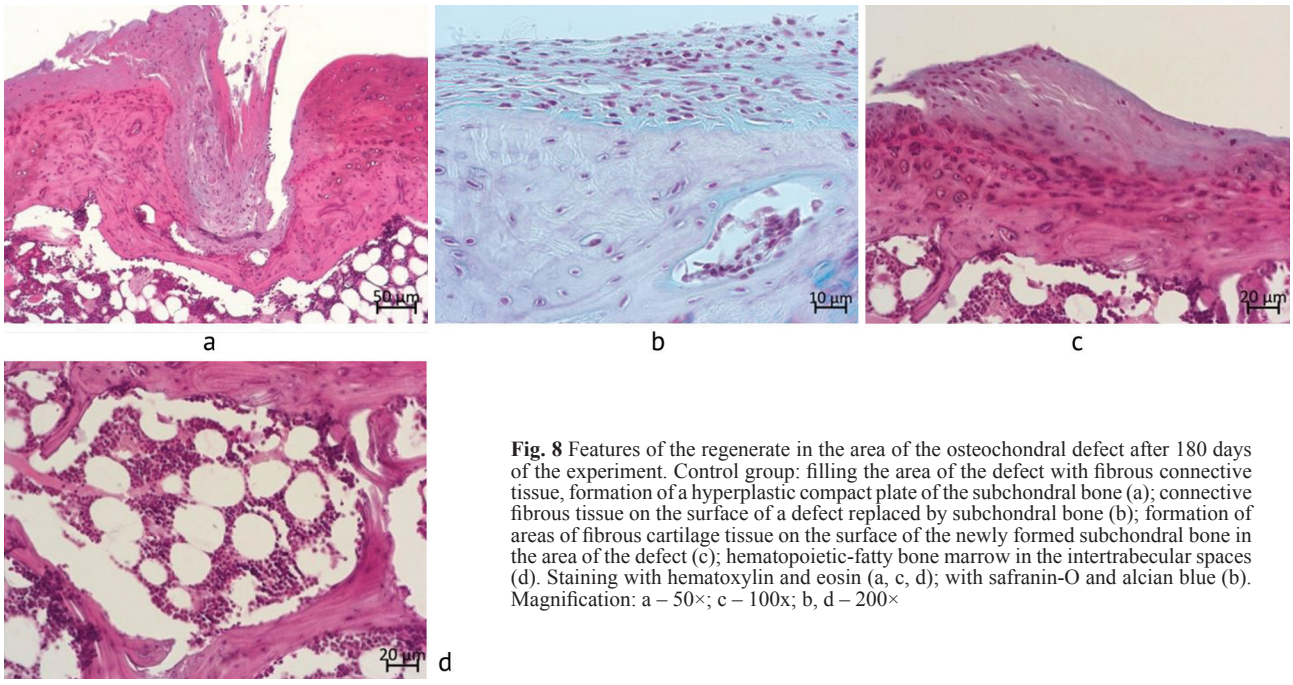

Fig. 8 Features of the regenerate in the area of the osteochondral defect after 180 days of the experiment. Control group: filling the area of the defect with fibrous connective tissue, formation of a hyperplastic compact plate of the subchondral bone (a); connective fibrous tissue on the surface of a defect replaced by subchondral bone (b); formation of areas of fibrous cartilage tissue on the surface of the newly formed subchondral bone in the area of the defect (c); hematopoietic-fatty bone marrow in the intertrabecular spaces (d). Staining with hematoxylin and eosin (a, c, d); with safranin-O and alcian blue (b). Magnification: a – 50×; c – 100x; b, d – 200×

In the control group, compacted bone conglomerates were formed closer to the outer surface of the condyle, similar to osteophytes (Fig. 3 g). In the projection of the articular cartilage, the area of the defect in the experimental series was replaced by a layer of hyaline cartilage tissue (Fig. 3 o, Fig. 7 a, b) while in the control group by a layer of fibrous connective tissue (Fig. 3 g; Fig. 8 a, b), in a separate case in combination with small fragments of fibrous connective tissue (Fig. 8 c). The remnants of the implant material in the area of the defect in the animals of the experimental series were not found what indicated its complete biodegradation by this period. In one field of vision, there was an area with a small fragment of the implant material embedded in the structure of bone trabecula (Fig. 7 d).

Immunohistochemical staining for CD 34 revealed newly formed vessels in the superficial connective tissue layer in control animals (Fig. 9 b). In the experimental group in the newly formed cartilage tissue formed in the area of the defect, the test was negative (Fig. 9 a).

After a year in the experimental group, the preservation of the integrity of the cartilage lining was noted. The newly formed cartilage acquired a zonal structure. It contained superficial (more voluminous than in intact animals), intermediate with small isogenic groups, and deep zones (Fig. 3 a, Fig. 10 a). In the control animals, the superficial area of the defect was filled with fibrous tissue, sometimes with small areas of fibrous cartilage (Fig. 3 h, Fig. 10 b). The subchondral bone in the experimental group practically did not differ from that in intact animals (Fig. 3 p, Fig. 10 d, f). In the controls, it was slightly sparse, with a thickened compact plate in the area of the defect (Fig. 3 h, Fig. 10 e).

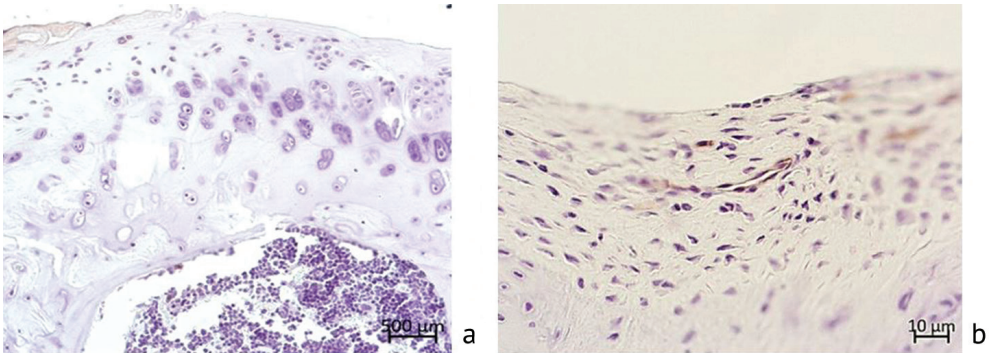

Fig. 9 Immunohistochemical staining to detect vessels in the surface lining of the regenerate of an osteochondral defect; vessels were not identified in the experimental group (a); microvessels were detected in the control group (b, brown staining). Staining is an immunohistochemical reaction using antibodies to CD34. Magnification – 400×

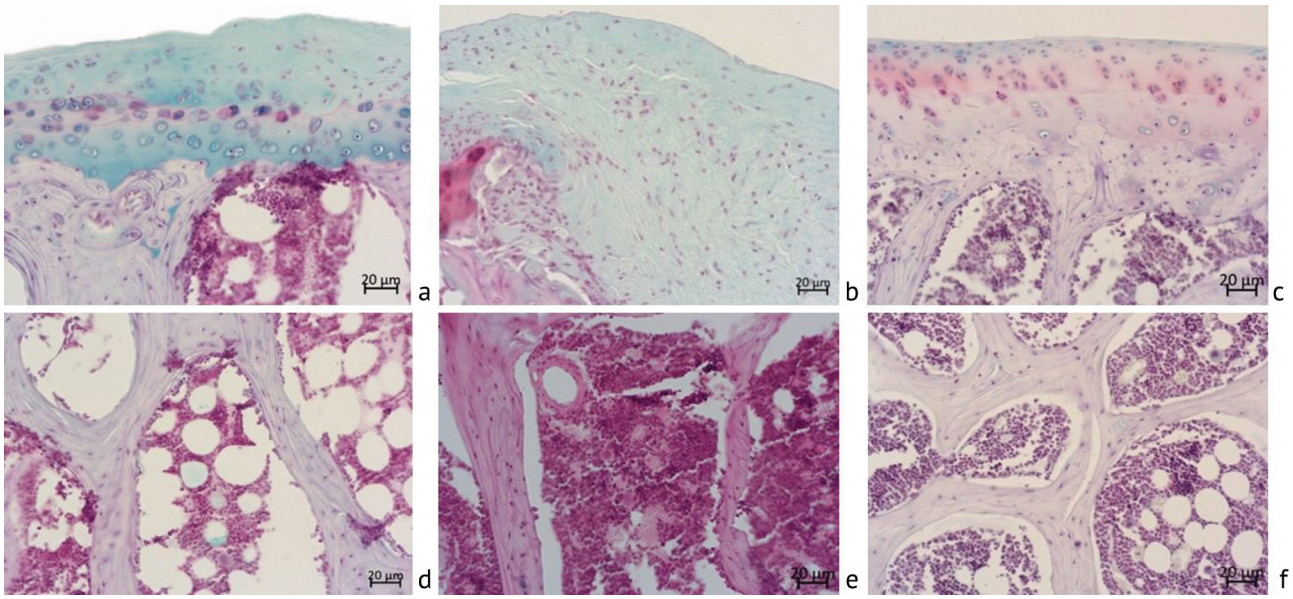

Fig. 10 Regenerate features in the subchonral defect area after 360 days of the experiment: a – formation of hyaline cartilage tissue of zonal structure in the experimental group; b – filling the defect area with fibrous connective tissue in the control group; c – structure of the articular cartilage in intact animals of the same age; d-f – structure of subchondral bone (d – experimental group, e – control group, f – intact animals). Staining: a-e – safranin-O and alcian blue. Magnification: a-e – 200×

A semi-quantitative assessment of the completeness of replacement and recovery of tissues in the area of the osteochondral defect showed that in all periods of the experiment, a more complete recovery was observed in the experimental group (Table 3). After 60 days, the median of the experimental group was 1.7 times higher than that in the control group, after 180 days by 3.12 times, after a year of the experiment by 3.25 times. Since the maximum number of points for this assessment system is 28 points, it can be said that after 180 days the healing of the articular surface defect in the experimental group was almost complete, and after a year of the experiment it was complete. In the control group, the healing of the defect was very poor even after a year.

The analysis of morphometric data showed significant differences in the fractional components of the tissue components of the regenerate in the defect area in the experimental and control groups in almost all periods of the experiment (Table 4). In the control series, there was no implant material and in none of the periods was the formation of hyaline cartilage tissue detected. Its fraction in the total structure of the regenerate was equal to zero. However, the fractions of bone tissue and bone marrow in the regenerates of the control and experimental groups did not have significant differences after 2 months and after 1 year of the experiment.

Table 3

Results of a semi-quantitative histological assessment of the completeness of osteochondral defect filling according to O'Driscoll (modified)

Group	Number of points at experiment time-points, Me (Q1-Q3)		
	60 days	180 days	360 days
Controls	4.4 (4.2-4.6)	7.5 (6.7-8.2)	8 (7.7-8.3)
Experimental	7.6 (7.3-8.1)	23.4 (23.3-23.7)	26 (25.8-27.3)

Notes: the maximum possible number of points is 28; $p < 0.01$ – differences between the groups are significant at all time-points

Table 4

Fractions of tissue components in the regenerate filling the osteochondral defect

Experiment time-point	Series	Fractions of tissue components (%). Me (Q1-Q3)					
		Bone marrow	Bone tissue	Fibrous connective tissue	Fibrous cartilage tissue	Hyaline cartilage	Implanted material
14 days	Control	0 $^{2}p = 4.26E-0.5$	9.9 (8.7-11.1) $^{1}p = 0.035$ $^{2}p = 0.008$	90.1 (88.3-91.9) $^{1}p = 5.85E-07$ $^{2}p = 0.0006$	0 $^{1}p = 0.011$	0 $^{2}p = 0.011$	0 $^{1}p = 0.00018$ $^{3}p = 0.000182$
	Experimental	0 $^{2}p = 4.26E-0.5$	6.2 (5.9-6.5) $^{1}p = 0.035$ $^{2}p = 0.056$	13.5 (11.2-14.1) $^{1}p = 5.85E-07$ $^{2}p = 0.004$	3.2 (2.6-3.8) $^{1}p = 0.011$ $^{2}p = 0.004$	0 $^{2}p = 0.011$	77.1 (75.3-78.9) $^{1}p = 0.00018$ $^{2}p = 2.5E-07$
2 months	Control	24.6 (23.3-27.1) **$^{1}p = 0.11$** $^{2}p = 0.016$ $^{3}p = 0.019$	43 (42-47) **$^{1}p = 0.78$** $^{2}p = 0.027$ $^{3}p = 0.00026$	28 (24.7-30) $^{1}p = 0.0023$ $^{3}p = 0.00026$	0 $^{1}p = 0.0014$ $^{2}p = 0.031$ $^{3}p = 4.69E-0.6$	0 $^{1}p = 0.0017$ $^{2}p = 0.011$ $^{3}p = 4.69E-0.6$	0 $^{1}p = 0.0014$
	Experimental	21.7 (21.3-22.2) **$^{1}p = 0.11$** $^{3}p = 00.17$	44.1 (43.7-45.7) **$^{1}p = 0.78$** $^{3}p = 0.00027$	21 (43.7-45.7) $^{1}p = 0.0023$ $^{3}p = 0.003$	2.8 (2.5-3.3) $^{1}p = 0.0014$ **$^{3}p = 0.43$**	3.5 (3.4-3.7) $^{1}p = 0.0017$ $^{3}p = 0.0016$	6.1 (7.1-5.6) $^{1}p = 0.0014$ $^{3}p = 0.0002$
6 months	Control	29.2 (28.9-33) $^{1}p = 0.08$ $^{2}p = 0.0033$ $^{3}p = 0.035$	57.2 (53.7-62.7) $^{1}p = 0.008$ $^{2}p = 0.0025$ $^{3}p = 0.02$	8.1 (7.4-9.9) $^{1}p = 0.013$ $^{2}p = 0.0084$ $^{3}p = 0.0016$	**0.68 (0.51-1.74)** $^{1}p = 0.013$ $^{2}p = 0.0084$ $^{3}p = 0.0015$	0 $^{1}p = 0.08$ $^{2}p = 0.0015$	0 **$^{1}p = 0.42$**
	Experimental	45.1 (44.5-45.3) $^{1}p = 0.08$ $^{2}p = 3.18E-0.5$ $^{3}p = 4.06E-07$	38 (36-45) $^{1}p = 0.008$ **$^{2}p = 0.24$** **$^{3}p = 0.22$**	5.9 (5.7-6.1) $^{1}p = 0.013$ $^{2}p = 0.000025$ $^{3}p = 2.98E-0.5$	0 $^{1}p = 0.013$ $^{3}p = 0.007$	9.6 (3.8-13.1) $^{1}p = 0.08$ **$^{2}p = 0.63$** $^{3}p = 0.043$	0.46 (0.36-1.38) **$^{1}p = 0.42$** **$^{2}p = 0.42$** $^{3}p = 0.022$
1year	Control	39.8 (36-50.7) **$^{1}p = 0.16$** **$^{2}p = 0.165$** **$^{3}p = 0.12$**	34 (33-89.1) **$^{1}p = 0.31$** **$^{2}p = 0.45$** **$^{3}p = 0.78$**	26.4 (10-40.4) **$^{1}p = 0.1$** $^{2}p = 0.01$ **$^{3}p = 0.19$**	0 $^{3}p = 0.013$	0 $^{1}p = 0.02$ $^{2}p = 0.0015$	0
	Experimental	52.7 (46.71-54.43) **$^{1}p = 0.16$** **$^{2}p = 0.398$** $^{3}p = 3.18E-05$	30.28 (16.58-32.3) **$^{1}p = 0.1$** **$^{2}p = 0.21$** $^{3}p = 0.247$	0 **$^{1}p = 0.1$**	0	12.86 (9.07-16.65) $^{1}p = 0.02$ **$^{2}p = 0.17$** $^{3}p = 0.63$	0 $^{3}p = 0.022$
Intact		54.1 (53.1-54.2)	36.8 (30.63-37.43)	0	0	10.4 (9-12.6)	0

Notes: ^{1}p – significance of differences between the groups; ^{2}p – significance of difference compared with experimental animals; ^{3}p – significance of difference as compared with the previous experimental time-point. Differences are significant at $p < 0.05$; **bold typed** are values that do not have significant difference ($p > 0.05$)

The content of connective tissue in regenerates filling osteochondral defects in animals of the control group was significantly higher than in the experimental group: after 14 days by 95 %, after 60 days – by 25 %, after 180 days – by 27.2 %, and after 360 days – by 73.6 %. In both groups, the maximum content of fibrous connective tissue in the composition of the regenerate was observed by day 60. By day 180, this component decreased by 71-72 % in both groups, while in the experimental group its portion was very small, only 5.9 %. After a year of the experiment, there was no fibrous connective tissue in the regenerate of the experimental group animals. In the control group, it occupied almost one third of the regenerate. Hyaline cartilage tissue in the experimental group was detected after 60 days of the experiment. Its content significantly increased by 36.5 % after 180 days of the experiment, and by another 25.3 % after a year (360 days). The content in those periods corresponded much to the indicators in the intact rats. After 360 days of the experiment, the regenerates of the experimental group animals contained only bone tissue, bone marrow and hyaline cartilage tissue. Their fractional content was similar to that in intact animals.

In the control group, the content of bone tissue and bone marrow did not have significant differences from the experimental and intact groups at that time-point. At the same time, the portion of fibrous connective tissue was about 30 %, and the hyaline tissue was absent. The content of the implant material in the experimental group significantly reduced as the experiment period increased. Within the time points of 14 to 60 days, its content decreased by 92 %. By day 180 of the experiment, there was no implant material in the regenerate in the area of the osteochondral defect. It completely degraded by that time-point. The thickness of the cartilaginous lining in the area of the osteochondral defect after 180 days was significantly higher than that in the norm (Fig. 11). A year later, the thickness of the articular cartilage was comparable to that in intact animals of the same age. Morphometry of the subchondral bone trabeculae thickness showed that they were thicker in the experimental group than in the control group. Their thickness after a year did not differ from that in intact rats (Fig. 12).

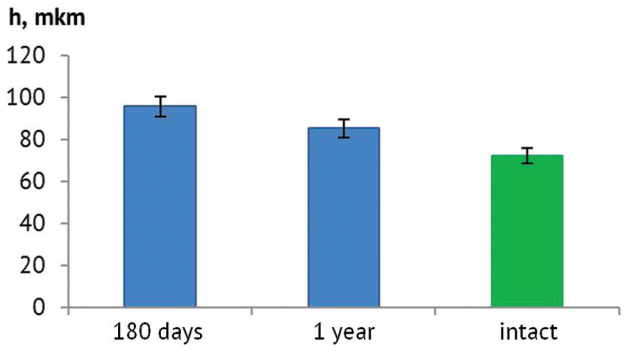

Fig. 11 Thickness of the articular cartilage in the experiment group after 180 days and a year relative the intact animals

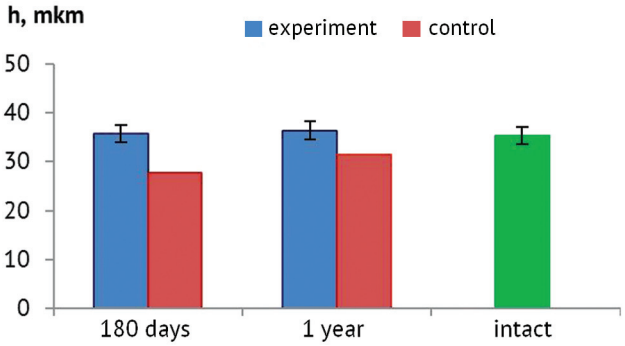

Fig. 12 Thickness of the subchondral bone trabeculae in the area of the osteochondral defect after six months and in a year of the study

Biomechanical study

Biomechanical methods established that the values of regenerate compliance in the area of the osteochondral defect relative to the control group were reduced by 29.2 % and by 18.5 % in the experiment group by day 60 of the experiment, but did not have significant difference compared to similar indicators in intact animals (Table 5). The values of the experimental group exceeded those in the control group by 15 %. After 180 days of the experiment, the biomechanical properties increased in both series: insignificantly in the control group by only 3 %, and by 11.9 %in the experimental group. In the experimental group, the values did not significantly differ from those in intact animals; in the control they remained lower by 26.2 %.

Table 5

Biomechanical properties of the regenerated articular surface in the osteochondral defect

Experiment time-point (days)	P – compliance, 10 mm³/g*cm	
	Control group	Experimental group
60 days	1.956 (1.597-1.982)*	2.250 (2.167-2.628) #
180 days	2.009 (1.580-2.250)*	2.553 (1.607-2.727)
360 days	1.997 (1.898-2.210)*	2.599 (2.408-2.691)#
Intact	2.762 (2.221-2.978)	

* – $p < 0.05$ – significant difference with intact animals; # – $p < 0.05$ significant difference with the control group.

After 360 days, the values of compliance of the regenerate formed in the area of the osteochondral defect did not differ from the previous period in the experimental group. In the experimental group, they slightly increased by 2 %, while in the control group they decreased by 1 %. As in the previous time-point, the animals of the experimental group did not show significant differences in the values of articular surface compliance from those in the intact animals while in the control animals they were significantly lower (by 27.2 %).

The study showed that the mechanical properties of the cartilage recovered to the level of the intact animals by day 60 in the experimental group and persisted up to 360 days, while in the control group they did not recover even after 360 days.

DISCUSSION

A lot of researchers worldwide have been searching for the ways to solve the problem of treating irreparable osteochondral defects. Many options have been proposed, including those using bioengineering technologies [30]. However, a perfect method of tissue repair in the area of an osteochondral defect, the effect of which could be preserved for a long period, has not been developed so far [18]. Therefore, the search for the ways to successfully manage irreparable articular surface defects continues and is aimed at prevention or delay of joint replacement procedures. The greatest preference would be given to less expensive and one-step techniques [17].

Previously, it was found that the most effective regeneration of hyaline-like cartilage is possible only with the use of collagen matrices populated with autologous cartilage cells in combination with microfracturing [31]. There are data on the positive use of cell-free collagen scaffolds [32, 33]. Some studies have shown that collagen matrices did not contribute to the restoration of joint surface congruence [34].

An alternative to collagen matrices has recently been polymeric implants. Polycaprolactone (PLC) is one of the most commonly used polymers in tissue engineering to restore the loss of bone and cartilage tissue [26, 35, 36]. One of its shortcomings is its low adhesive ability [37]. Many authors compensate for this drawback by adding hydroxyapatite particles to its composition or apply them to the surface of PLC products. Thus, this composite biomaterial has induction properties, is able to enhance proliferation and have effect on cell differentiation [38, 39].

It is known that the hydroxyapatite nanocomposite has greater biocompatibility and better adhesion properties of its surface compared to the microcomposite [40, 41]. The method of obtaining an elastic implant by electrospinning contributes to the creation of a voluminous fibrous framework with a fiber diameter and interfiber gaps that are optimal for cell and vessel migration [42]. It was revealed that post-traumatic changes in the joints and the development of osteoarthritis are associated with the temperature of the skin in the joint area. At the same time, the severity of the pain syndrome does not correlate with pain sensations of the patient [43].

In our study, skin temperature in the area of the knee joint was increased in the early postoperative period and persisted up to 2 weeks of the experiment, what was associated with the post-traumatic state of tissues after surgery [44]. In the control series, a significantly increased temperature was noted after one year of observation and was associated with an aggravation of the arthritic changes, what was confirmed by anatomical and histological methods [44-46]. A slight increase in the skin temperature in the animals of the experimental series, compared with the control one, we associate with the improvement of microcirculation in the restored tissues in the area of damage. It was observed in the works of other researchers [47, 48].

Throughout the experiment, the volume of limb soft tissues in the animals of the experimental series was higher than in the animals of the control series. MRI used by several studies revealed a decrease in limb circumference in patients with gonarthrosis, associated with reduction in the muscle diameter and replacement of muscle tissue with adipose tissue [49].

The range of joint motion in the series with replacement of an osteochondral defect with a PLC matrix impregnated with hydroxyapatite at all stages of the experiment, especially in long-term periods (six months and a year of observation) was significantly better than in the animals of the control series. We attribute this to the progression of osteoarthritis signs in the control series. The reduced range of motion in the knee joint can be explained by pain and discomfort by walking and joint loading [50-52]. Restoration of the anatomical integrity and complete organotypicity of the newly formed regenerate in the area of the osteochondral defect in the experimental series already after 180 days of observation, proven by anatomical and histological methods, in our opinion, is associated with the structure of the implant, its good adhesive ability, sufficient porosity and elasticity. Starting from the moment of surgery, the elastic implant filled the area of the osteochondral defect, ensuring the integrity and congruence of the articular surface, withstanding the functional load, that is providing factors that are considered important in the healing of osteochondral defects [53, 54].

Early functional loading contributed to the ingrowth of microvessels into micropores between the fibers of the implant. Perivascular cells and bone marrow cells attached to the structured surface of the implant, thanks to the hydroxyapatite nanoparticles deposited on them. Hydroxyapatite acted as an inducer of osteogenesis and promoted cell differentiation along the osteogenic pathway [55]. Since the vessels grew into the implant from the side of the intact subchondral bone, the formation of bone tissue occurred in the projection of the cancellous bone surrounding the defect, which is necessary as a base and nourishing factor for the formation of cartilage tissue on the surface. There were no vessels in the surface layers of the implant. Under such conditions, undifferentiated bone marrow cells penetrating the surface in the projection of the articular cartilage surrounding the defect differentiated along the chondrogenic pathway. Cell differentiation into chondroblasts and chondrocytes continued throughout the experiment with sufficient nutrition from the synovium, which produced a sufficient volume

of synovial fluid, because animals actively used the limb.

These mechanisms contributed to the complete replacement of the biodegradable implant with tissues of a typical structure by experiment day 180. By that period, cancellous bone with a cartilaginous hyalin-like lining was formed on the surface of the defect in the projection of the subchondral bone.

It was found that with an increase in the osteoarthritis severity according to the classification of the International Cartilage Restoration Society (ICRS), cartilage stiffness decreases, which is primarily affected by the integrity of the extracellular matrix. Therefore, methods to study stiffness, such as impression, can be used to characterize cartilage in all stages of OA [56].

The study of stiffness in patients after the treatment of osteochondral defects by implantation of autologous chondrocytes (ACI) into the area of damage to the cartilage surface showed the restoration of the stiffness of the newly formed tissue regenerate 2 years after implantation and a further increase in this indicator within 5 years [57]. In our study, the restoration of biomechanical compliance (analogous to stiffness) in the experimental group reached the parameters of intact animals after 60 days of the experiment and persisted throughout the year of observation. It remained lower by 27-29 % during the entire observation period in the control group.

In this experiment, we applied a strategy based on the fact that an elastic PCL implant with hydroxyapatite imitated the extracellular matrix, which promoted the growth, proliferation, and differentiation of cells in the area of the oseochondral defect, using the self-regenerative potential of cells and its interaction with biomolecules [58]. Our study showed that the mechanical properties of the cartilage in the experimental group restored to the level of the intact animals by day 60 and persisted up to 360 days, while in the control group they did not restore even after 360 days.

CONCLUSION

A biodegradable elastic implant produced with the method of electrospinning from polycaprolactone and impregnated with hydroxyapatite could potentially be used to treat osteochondral defects. This type of implant provided repair of the tissues in the osteochondral defect area and regeneration of both the articular cartilage and subchondral bone.

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Data Availability Statement *Data are available upon a demand to corresponding author (D.P.).*

Conflicts of Interest *The authors declare no conflicts of interest.*

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Information about the authors:

1 Arnold V. Popkov – Doctor of Medical Sciences, Professor, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989;
2 Evgenii S. Gorbach – traumatologist-orthopedist, gorbach.evg@mail.ru, https://orcid.org/0000-0002-0682-1825;
3 Elena Gorbach N. – Candidate of Biological Sciences, Leading Researcher, gorbach.e@mail.ru, https://orcid.org/0000-0002-9516-7481;
4 Natalia A. Kononovich – Candidate of Veterinary Sciences, Leading Researcher, n.a.kononovich@mail.ru, https://orcid.org/0000-0002-5990-8908;
5 Elena A. Kireeva – Candidate of Biological Sciences, Junior Researcher, ea_tkachuk@mail.ru, https://orcid.org/0000-0002-1006-5217;
6 Dmitry A. Popkov – Doctor of Medical Sciences, Professor of the Russian Academy of Sciences, Corresponding Member of the French Academy of Medical Sciences, Head of the Clinic, dpopkov@mail.ru, https://orcid.org/0000-0002-8996-867X.

Contribution of the authors:

Popkov AV – Conceptualization, supervision, project administration.
Gorbach ES – Conceptualization, formal analysis, investigation, visualization.
Gorbach EN – investigation.
Kononovich NA – validation, writing–original draft preparation.
Kireeva EA – data curation.
Popkov DA – formal analysis, writing–review and editing.
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Original article

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Resorbable implants in paediatric orthopaedics and traumatology

Pierre Lascombes[1,2], Pierre Journeau[3], Dmitry A. Popkov[4]✉

[1] University of Nancy, France
[2] University of Geneva, Switzerland
[3] Centre Hospitalier Universitaire de Nancy, Nancy, France
[4] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Dmitry A. Popkov, dpopkov@mail.ru

Abstract

Background Development of resorbable implants for paediatric orthopaedics is promising as there is no need for implant removal. **The aim** of this paper is to present our experience in resorbable implants in paediatric traumatology, and to make an overview of the recent literature. **Material and methods** In our department of paediatric traumatology and orthopaedics, we have operated 7 children with fractures of long bones with resorbable screws (ActivaScrew™). The inclusion criteria were intra-articular and juxta-articular fractures in children with an indication for screw fixation. To prepare the review, we searched for information sources at the scientific platforms such as PubMed, Scopus, ResearchGate, RSCI, as well as other published products (Elsevier, Springer). **Results** The cohort is represented by 7 patients, 4 girls and 3 boys, aged from 5 to 14 years old. The 7 fractures were 3 at the elbow and 4 at the ankle joint. In the immediate postoperative period, no patient presented with abnormal swelling, redness, or tissue reaction. Pain disappeared at day 7 in all cases. Weight-bearing and return to sport activities were allowed in normal delay. Radiological bone union was obtained between 3 and 6 weeks. Range of motion in adjacent joints was comparable to the opposite non-fractured side at 3 months. There were no cases of complications, no infection, and no need for a reoperation. **Discussion** The use of resorbable implants, either co-polymers or magnesium, solves the problem: removal of implants is not anymore necessary. Resorbable implants are becoming safer as they have good solidity allowing bone union of fractures and osteotomies before their eliminating. **Conclusion** Main indications of resorbable implants in pediatrics remain fractures and osteotomies fixed with screws. The development of plates and intramedullary nails will enlarge the indications. Level of evidence: IV.

Keywords: paediatric fracture, resorbable implants, co-polymer, magnesium

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INTRODUCTION

The surgical treatment of paediatric fractures usually requires their fixation with metallic implants, made of stainless steel or titanium. Main indications of internal fixation are intraarticular fractures classified as Salter III and IV types and diaphyseal fractures which cannot be treated with conservative methods. The former need most often screw fixation via an open or a closed approach. On many occasions, diaphyseal fractures are treated with intramedullary fixation. The first choice is the flexible nailing technique in forearm and humerus fractures, and in femur and tibia fractures before the end of growth. The advantages of metallic implants are well-known, but among their disadvantage, one question is permanent: removal or not? In one hand, the future is uncertain in case of a need for orthopaedic surgery. To remove implants after a long term may be a nightmare. But on the opposite, the removal of implants needs a second surgery, and complications are not negligible. In addition, the use of those devices can entail complications as hematomas, healing problems, local sepsis.

For these reasons, the development of resorbable implants is promising as there is no need for implant removal. But resorbable implants must be safe. This means they must have a comparable efficiency with classic metallic implants to stabilize the fractures: firstly, stability and resistance of the implants are mandatory till bone union is obtained; secondly, absence of free debris which might damage some tissues like brain, liver, ganglions, lungs. For that reason, some resorbable implants used in the past have been withdrawn from the market due to some complications as intraarticular synovitis and spread of debris.

Interference screws [1] and absorbable anchors are currently used in tendons and ligaments reconstruction. In adult trauma, some fractures of the ankle are treated with absorbable plates and screws. The biomechanical stability of these implants has shown excellent qualities and biological properties.

One category of resorbable or biodegradable materials is a polymer that has been used for around 40 years in many surgical applications. Initially used just for sutures, resorbable plates and screws are commonly used in maxillofacial surgery, such as for osteotomy syntheses or mandibular fractures. A second group of resorbable implant is the new generation of magnesium implants: experimental studies demonstrate their good tolerance and nice results. The first clinical studies show good results too.

The main goal of this paper is to present our experience with resorbable implants in paediatric traumatology, and to make an overview of the recent literature.

MATERIAL AND METHODS

In our level I department of paediatric traumatology and orthopaedics, we operated on 7 patients with fractures of long bones with resorbable screws, partially threaded ActivaScrew™ LAG (Bioretec). The decision to select these new implants was taken under following consideration: absence of debris and urine elimination of the polymer polylactide-co-glycolide (PLGA), strong fixation ability till bone union (https://bioretec.com/products).

The inclusion criteria were intra-articular and juxta-articular fractures in children with an indication for screw fixation, and acceptation of the patient and the family for a resorbable implant after given precise information. The exclusion criteria were pathological fractures.

Surgical technique Reduction of the fractures was obtained either by closed method and mini-invasive approach or by open approach with the same technique as in fixation with conventional metallic screws. One hole by screw was performed strictly into the epiphysis with a drill smaller than the diameter of the screw under the C-arm control to be parallel to both physis and joint (Fig. 1), thus a drill of 2.5 mm diameter was used for a screw of 3.5 mm diameter. Then, the cortex was enlarged with a countersink to bury around 50 % of the screw head. The adapted tap, 3.5 mm for 3.5 mm screws, is mandatory to be used. Just before insertion, the screw was dipped in a contrast fluid expecting to be visible on the C-arm control, but unfortunately it was not always visible. Finally, the screws were inserted with an adapted cap. At that moment, the first screw was not yet tightened. A second screw, of the same size, same technique was inserted almost parallel to the first one. Then, we tightened both screws: we just turn the screwdriver till the adapted system pulled out. It is not recommended to continue tightening the screws themselves as their head may break, leading to a complete loosening of the compressive effect on the fracture.

Imaging control should demonstrate the fracture line to disappear completely, proof of an excellent reduction. An immediate arthrogram of the joint confirmed the satisfactory anatomical reduction of the fracture (Fig. 2). An immediate immobilization was done with a splint plaster cast, replaced three days later with a resin cast.

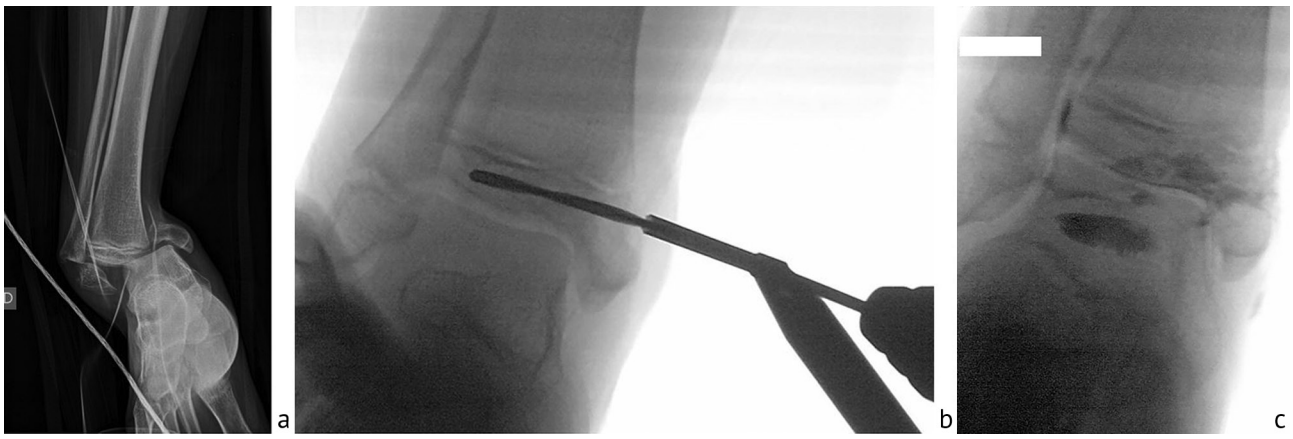

Fig. 1 Surgical technique: a – fracture with dislocation; b – reduction of the fracture and drilling of the epiphysis; c – screw with contrast fluid is visible on the C-arm control

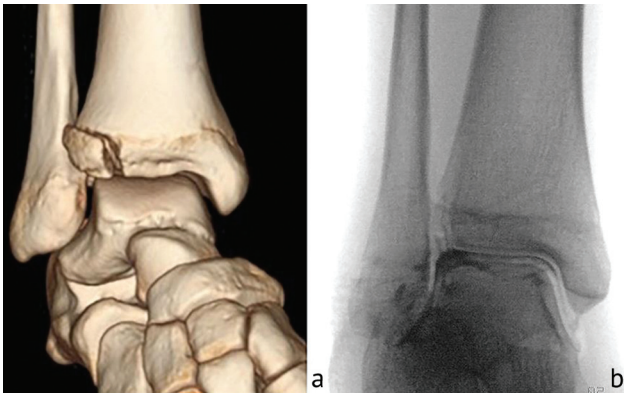

Fig. 2 Control of reduction:
a – Tillaux fracture;
b – fracture line disappeared, an immediate arthrogram confirms satisfactory anatomical reduction of the fracture

Assessed criteria were duration of the surgical procedure; presence or not of swelling, redness, tissue reaction, pain on day 1, day 7, and day 30; time for bone union, date of full weight-bearing, and range of motion at 3 months; date of return to sport activities; complications and reoperation rate.

RESULTS

The cohort is represented by 7 patients, 4 girls and 3 boys, aged from 5 to 14 years old. The 7 fractures were three at the elbow (Fig. 3) and four at the ankle joint. All of them were classified as intra-articular displaced fractures requiring anatomical reduction and stable screw fixation. The used resorbable screws were partially threaded, 3.5 mm diameter and of different length from 20 to 45 mm.

In the immediate postoperative period, day 1, 7 and 30, no patient presented with abnormal swelling, redness, or tissue reaction. Pain disappeared at day 7 in all cases. Weight-bearing and return to sport activities were allowed in normal delay. Radiological bone union was obtained between 3 and 6 weeks. Range of motion in adjacent joints was comparable to the opposite non-fractured side at 3 months. There were no cases of complications, no infection, and no need for a reoperation (Table 1).

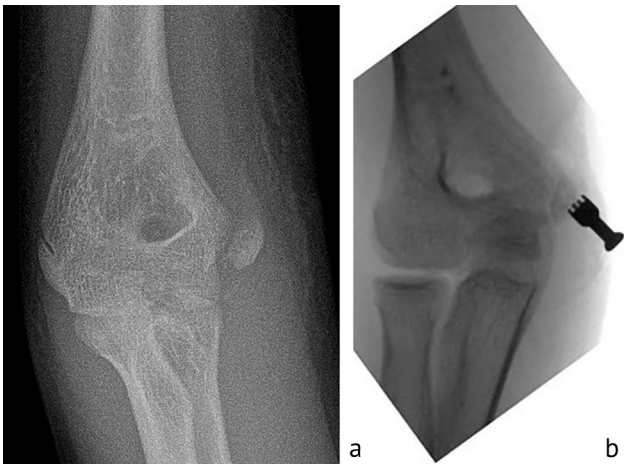

Fig. 3 Medial epicondyle fracture: a – radiograph at admission; b – reduction and osteosynthesis

Table 1

Cases included in the study

Sex	Age (years)	Fracture	Implants diameter/ length	Duration of surgery (minutes)	Delay of bone union	Delay of weight bearing	Delay sport return	Range of motion at 3 months	Complication	Follow-up
Girl	13	bimalleolar, ankle dislocation	2 screws 3.5/45 mm 3.5/45 mm	57	6 weeks	6 weeks	3 months	comparable to opposite side	no	1 year
Boy	14	proximal first metatarsal bone Salter III	1 screw 3.5/30 mm	S4	6 weeks	6 weeks	3 months	comparable to opposite side	no	1 year
Boy	5	olecranon longitudinal	2 screws 3.5/20 mm 3.5/20 mm	87	4 weeks	N/A	3 months	comparable to opposite side	no	9 months
Girl	14	distal tibia Salter III (Tillaux')	1 screw 3.5/40 mm	54	6 weeks	6 weeks	3 months	comparable to opposite side	no	1 year
Girl	10	medial epicondyle	1 screw 3.5/40 mm	47	4 weeks	played piano at 4 weeks	3 months	comparable to opposite side	no	3 years
Girl	12	medial malleolus	1 screw 3.5/20 mm	35	4 weeks	4 weeks	3 months	comparable to opposite side	no	6 months
Boy	10	elbow dislocation lateral condyle fracture	2 screws 3.5/24 mm 3.5/40 mm	47	6 weeks	N/A	2 months	comparable to opposite side	no	6 months

DISCUSSION

Different polymers of lactic acid (PLA) and glycolic acid (PGA) have been proposed for orthopedic surgery both in adults and children [2, 3]. PLA implants are known as responsible of local reactions during absorption and rapid degradation which may cause instability before bone union. On the other hand, poly-(L)-lactic acid (PLLA) implants have a slow resorption and may lead to bone cavity filled with either fibrous tissue or fluid [4]. They may also cause local reaction as foreign-body reactions. As for others, the PLLA/PGA interference screw completely degraded, and no remnant was present 3 years after

implantation for a bone-patellar tendon-bone graft ACL reconstruction [1].

In reconstruction of nerves, implantation of poly (DL-lactic acid) PDLLA film did not alter liver or renal functions. Pathologic examinations showed that implantation of PDLLA film did not cause pathologic changes to the rat liver, kidney, pancreas, or spleen. Taken together, these results suggest that PDLLA films have excellent biocompatibility and no obvious toxicity *in vivo*, and may be used to prevent nerve adhesion, thereby promoting nerve regeneration [2].

Poly lactic-co-glycolic acid (PLGA) has been developed as another component. This new resorbable implant slowly degrades while it reacts with water in the human body. It adds advantages of a mid-term resorption, with a good stiffness enough to obtain bone union, and without any type of inflammatory reaction [5, 6]. The degraded components are exhaled and excreted, the total elimination occurs within approximatively 2 to 3 years. Since theses implants do not include any metal, there is neither X-ray, ultrasound nor MRI disturbances [6, 7, 8].

In clinical practice, the advantages to avoid removal of implants have been widely emphasized [9, 10]. We had a previous experience with resorbable screws, 2.8 mm diameter made of poly-L-lactide- poly-D-lactide acid and trimethylene carbonate. Among 24 patients, we observed some complication like a loose screw head, joint stiffness and joint effusion [11].

Our short series demonstrate that resorbable screw osteosynthesis is justified in paediatric traumatology as this approach does not modify either the surgical technique or the follow-up care. The follow-up is comparable with classic metallic implants. We did not observe any abnormal mobility during the resorption period between the head and the body of the screw.

In a retrospective study, Kassai et al. [12] compared the fixation of the medial humeral epicondyle fractures with either a biodegradable poly L-lactide-co-glycolic acid (PLGA) or a traditional metallic implant. They observe normal delay for bone union, comparable results between both groups, and no specific complications with resorbable implants.

Varga et al. [13] published a comparative study of the treatment of 94 distal fractures of the radius, associated or not with ulna fractures. One group treated with one or two stainless steel Kirschner wires which were buried under the skin (n = 40), a second group with K-wire left outside (n = 24) and the third group with an original technique of distal radial elastic nailing with bioresorbable PLGA pins (n = 30). There was no difference between the complication rates of both K-wires groups, while the complication rate of the bioresorbable group was significantly lower.

In 2013, Sinikumpu et al. [14] from Finland published a preliminary technical report of a mini-invasive technique for pediatric diaphyseal forearm fractures with bioabsorbable elastic stable intramedullary nailing. They developed a two-stage surgical technique in children between 5 and 15 years old. The first stage was identical to the Nancy method [15, 16, 17]: mini-invasive approach, reduction of the fractures, introduction of metallic implants into the medullary canal of both bones through a metaphyseal small hole, and fixation of the radius and ulna. The second part of the technique was a careful removal of each metallic implant, each of them being immediately replaced by an absorbable rod (made of poly lactide-co-glycolide (PLCG) developed by the Bioretec company, first in the radius and second in the ulna, or reverse.

Recently, an international Europe-based, multicenter, prospective, single-arm, open-label study has evaluated the elastic stable intramedullary nailing of forearm fractures in children between age 3 and 13 with a resorbable Activa IM-Nail™. Seventy-six patients with a mean age at inclusion of 8.9 ± 2.4 years have been operated on. The mean time of operation was 58.9 ± 22.9 minutes. Except one case of postoperative fall, no case of recurrent fractures was observed at a mean follow-up of 8.9 ± 5.1 months. The authors draw a conclusion of the safety and effectiveness of the resorbable Activa IM-Nails™ in the treatment of forearm fractures [18].

Hedelin et al. [19, 20] reported a series of 32 pelvic osteotomies: Salter osteotomy (n = 21) and triple osteotomy (n = 11), fixed with a 4.5 mm (55-70 mm) PLGA polymer screw Activa®. They demonstrated the good stability of pelvic osteotomies after fixation with PLGA screws. In all hips studied, the overall correction was maintained, and there were no complications related to the resorption of the screws. On the MRI performed 2 years after the surgery, there were no significant local reactions. In another paper, the same author reports a series of 21 cases of Salter osteotomy fixed with resorbable screws. Neither perioperative surgical complications nor local reactions occurred [19].

In 2018, Grün presented an excellent paper which describes all resorbable implants used in paediatrics, resorbable, ceramic and metallic, with a specific focus on the magnesium-based (Mg-based) implants [21]. Biodegradable Mg is more tensile, stable, and load bearing, compared to polymers and ceramics. Magnesium also shows favorable biomechanical properties able to support bone fracture healing.

Holweg et al. [22] reported a successful series of 20 fractures of the ankle joint, bi- and trimalleolar fixed with bioabsorbable Mg-based screws. These implants were composed of pure Mg alloyed with calcium and zinc. Blood analysis revealed that Mg and Ca were within a physiologically normal range. No loosening or breakage of screws was observed. Holweg et al. [23, 24] also demonstrated that specific

balance between zinc and calcium improved mechanical strength and a reduced *in vitro* degradation rate. In *in vivo* experiments with screws, all osteotomies completely consolidated after a maximum of 12 weeks. But screws were resorbed at a mean of 2.5 years after medial malleolus fracture fixation [25]. Marek et al. demonstrated no influence of Mg-Zn-Ca screws on physis and longitudinal bone growth throughout their degradation [26, 27]. Wiktor et al. stated only good and excellent outcomes for humeral capitellum fractures in 6 immature patients aged 10.6-15.3 treated with internal fixation with bioabsorbable nails [28].

CONCLUSION

As the major problem of metallic implants, stainless-steel or titanium, is to remove them or not, the use of resorbable implants, either co-polymers or magnesium-based, solves the problem: removal of implants is not anymore necessary. Year after year, new components are available on the market. These components are becoming safer as they show good solidity allowing bone union of fractures and osteotomies. The implants decompose and are eliminated from the body. Main indications for resorbable implants in paediatrics remain fractures and osteotomies fixed with screws. The development of plates and intramedullary nails will enlarge the indications.

Conflict of interest *Authors declare no conflict of interest.*

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Ethical approval *The study was conducted in accordance with the Declaration of Helsinki, and approved by the Local Ethics Committee of the CHRU of Nancy.*

Informed consent *All patients or their legal representatives provided informed consent.*

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Information about authors:

1. Pierre Lascombes – MD, PhD, Honorary Professor of Orthopaedics & Anatomy, Past Professor, pierrelascombes@gmail.com, https://orcid.org/0000-0003-3348-5385;
2. Pierre Journeau – MD, PhD, Professor, p.journeau@chru-nancy.fr, https://orcid.org/0000-0003-7847-3524;
3. Dmitry A. Popkov – Doctor of Medical Sciences, Professor of the Russian Academy of Sciences, Corresponding Member of the French Academy of Medical Sciences, Head of the Clinic, dpopkov@mail.ru, https://orcid.org/0000-0002-8996-867X.

Contribution of the authors:

Lascombes P. – conceptualization, methodology, writing – original draft preparation, writing – review and editing.
Journeau P. – methodology, validation, data curation, visualization.
Popkov D.A. – validation, writing – review and editing.

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Combined osteosynthesis for tibial shaft fracture treatment

Konstantin A. Klyshnikov, Natalia V. Sazonova, Arnold V. Popkov✉

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Arnold V. Popkov, apopkov.46@mail.ru

Abstract

Introduction Widespread use of intramedullary and extramedullary implants, as well as external fixation devices, has demonstrated that current surgical methods are not always successful. **The study aimed** to assess the efficiency of a combination of transosseous osteosynthesis with intramedullary reinforcement using elastic titanium hydroxyapatite-coated rods (HA-rods) in long bone fracture treatment. **Material and methods** Medical records of 40 patients aged from 18 to 55 years with closed diaphyseal tibia fractures of A1-A3 type (AO/ASIF) treated with the Ilizarov transosseous osteosynthesis method combined with intramedullary elastic HA-coated wires were analysed. **Result** Ilizarov fixator removal was performed on average 45.3 ± 14.7 days after surgery. Radiological signs of bone union (immature callus, patterns of periosteal and endosteal stratifications overlapping the fracture line) were visible by week 3 to 4. **Discussion** Combination of the external fixator and intramedullary elastic HA-coated wires overcomes shortcomings of both external and internal means of fixation. External osteosynthesis provides advantages of the Ilizarov method: preservation of blood supply, absence of soft tissue injury, joint function and early weightbearing. Elastic intramedullary wires do not injure a. nutricia and mechanically stimulate endosteal and periosteal bone formation. **Conclusion** The advantages of combined osteosynthesis provide reduction of Ilizarov apparatus fixation time, reduction in the number of wires and half-pins in the frame assembly, stimulation of bone callus formation and prevention of secondary bone fragment displacement.

Keywords: Ilizarov, flexible intramedullary nailing, hydroxyapatite

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INTRODUCTION

The problem of urgent care for patients with injuries of the locomotor system is becoming increasingly important every year due to a growing number of severe injuries and following disability, especially in people of working age [1-4].

Widespread use of intramedullary and extramedullary implants, as well as external fixation devices, has demonstrated that current surgical methods are not always successful. The failure of treatment is related to iatrogenic lesion of surgical intervention, long-lasting duration of external fixation, delayed bone union, the lack of comfort when using external fixation devices [5, 6].

It is recognized that the use of bioactive implants (elastic titanium rods and degradable intraosseous implants) is a promising approach to solve the problems of bone regeneration reducing the treatment time [7-9].

The study was aimed to assess the efficiency of a combination of transosseous osteosynthesis with intramedullary reinforcement using elastic titanium hydroxyapatite-coated rods (HA-rods) in long bone fracture treatment.

MATERIAL AND METHODS

This retrospective study was conducted at the Ilizarov National Medical Research Center for Traumatology and Orthopaedics (Kurgan, Russia) from April 2015 to December 2020. Medical records of 40 patients aged from 18 to 55 years old with closed tibial shaft fractures of A1-A3 Types (AO/ASIF classification) were analyzed.

The criteria for inclusion in the study were adults of working age operated on with a combined technique. We excluded from the study patients of other ages, open or complicated fractures.

After obtaining institutional review board approval, the data were collected about clinical and radiological features of fractures, postoperative period, bone healing and functional recovery. Incidence, severity of complications and outcomes were assessed as well.

Surgical technique The surgery was performed under epidural anesthesia in all cases. The standard skeletal traction allowed reducing bone displacements. The intramedullary nailing was performed simultaneously at the time of fixator placement using two bent nails. Two oblique tunnels toward the medullary canal were formed in the metaphysis (proximal or distal, closest to the fracture) using a drill of 4-mm diameter [10]. Bent nails with bioactive coating (HA-coated elastic nails) were

easily inserted through the holes into the medullary canal under fluoroscopic control. We used 1.8-mm diameter titanium alloy nails with hydroxyapatite coating (Orthopediatrics nails modified by Metis Ltd, Tomsk, Russia). The nails were 20-40-μm thick and had 2-8 % porosity, obtained by the method of anodic oxidation in arc mode [11]. This type of coating is presented by ultraporous system consisting of macro- and micropores from 50-100 nm to 1-2 μm in diameter.

The intramedullary nailing was followed by application of the Ilizarov fixator (Experimental Plant of Russian Ilizarov Scientific Center "Restorative traumatology and orthopaedics", Kurgan, Russia) according to the technology of transosseous osteosynthesis. It should be emphasized, that intramedullary nails did not interfere with wires or half-pins of the external fixation device.

Radiography in two standard views was taken before surgery, on the date of surgery, then every 2 weeks until bone union. The radiographs after frame removal were also evaluated.

Statistical analysis was conducted with AtteStat 12.0.5 software. Means and standard deviations were used to describe continuous variables.

RESULTS

Forty patients (9 females, 31 males) with a mean age of 29.6 years (range, 18-55 years) were included. Thirty three patients (82.5 %) underwent antegrade intramedullary nailing while seven patients (7.5 %) underwent ante- retrograde nailing (Figure 1). In seventeen cases (42.5 %) bent intramedullary nails enabled complete fracture reduction thus there was no need to insert additional olive wires in fractured bone ends at the level of intermediate external fixator rings. From the first days after the surgery, all patients were encouraged to walk independently with partial then total weight bearing using crutches. A postoperative edema of the injured leg disappeared within 2 to 3 weeks. The postoperative pain related to fracture was moderate and persisted until the end of the second week. It responded to simple nonsteroidal anti-inflammatory drug treatment.

Fourteen days after the surgery, radiographic check revealed that the contours of the fragmental ends were blurred. The endosteal bone callus became visible and well-expressed in the medullary canal parts close to the fracture line and along the intramedullary implant. Periosteal reaction was clearly visible and defined on bone fragments on both views. It was 2.8 ± 0.2 mm thick and 14.7 ± 1.3 mm long. In 24 cases, the periosteal callus was uninterrupted and overlapped the fracture line uniting proximal and distal fracture ends two weeks after the surgery.

After four weeks, radiographs showed signs of bone union: blurred image of fracture ends, a barely visible fracture line, high optical density of uninterrupted periosteal bone in antero-posterior and lateral views. In that period, 32 patients started to walk with 50 % or even full loading on the injured leg without assistive devices.

Two weeks later, bone union was noticed in all cases: the fracture line was barely defined; the image of the periosteal callus was large dense and compact. Weight-bearing walking was not associated with pain. We noticed a satisfactory recovery of ankle range of motion in all cases. Thus, an indication for frame removal was justified and the procedure was performed within the period of 32-62 days (in average, 45.3 ± 14.7 days) after the surgery in all patients without plaster cast immobilization. A month after frame removal, radiographs demonstrated bone callus remodeling with permeability of the medullary canal, anatomical alignment of the segment. There were no cases of intramedullary nail migration. The nails were removed 4 to 7 months after fixator removal in all cases without any difficulties.

Regarding complications, pin site infection observed in 9 cases (22.5 %) were treated successfully by local care in 7 cases. Antibiotics and wire removal were necessary in 2 patients. The preoperative range of motion in the knee or in the ankle joint recovered in all patients by the latest follow-up control. There were no neurological or vascular complications in this series.

Case report Male patient, 47 years old, was admitted with closed spiral shaft fracture of the distal third of the right tibia (Fig. 1 a). The surgery consisted of osteosynthesis of leg bones with the Ilizarov fixator and intramedullary reinforcement of the tibia using HA-coated elastic nails (Fig. 1 b). Radiological signs of bone union were evident by 30th day (Fig. 1 c). Thereby the fixator was removed without subsequent immobilization (Fig. 1 d). The patient was allowed full weight-bearing 2 weeks after frame removal. A follow-up control (1 year and 3 months after frame removal) demonstrated bone callus remodeling and normal radiological parameters of the injured tibia (Fig. 1 e, f).

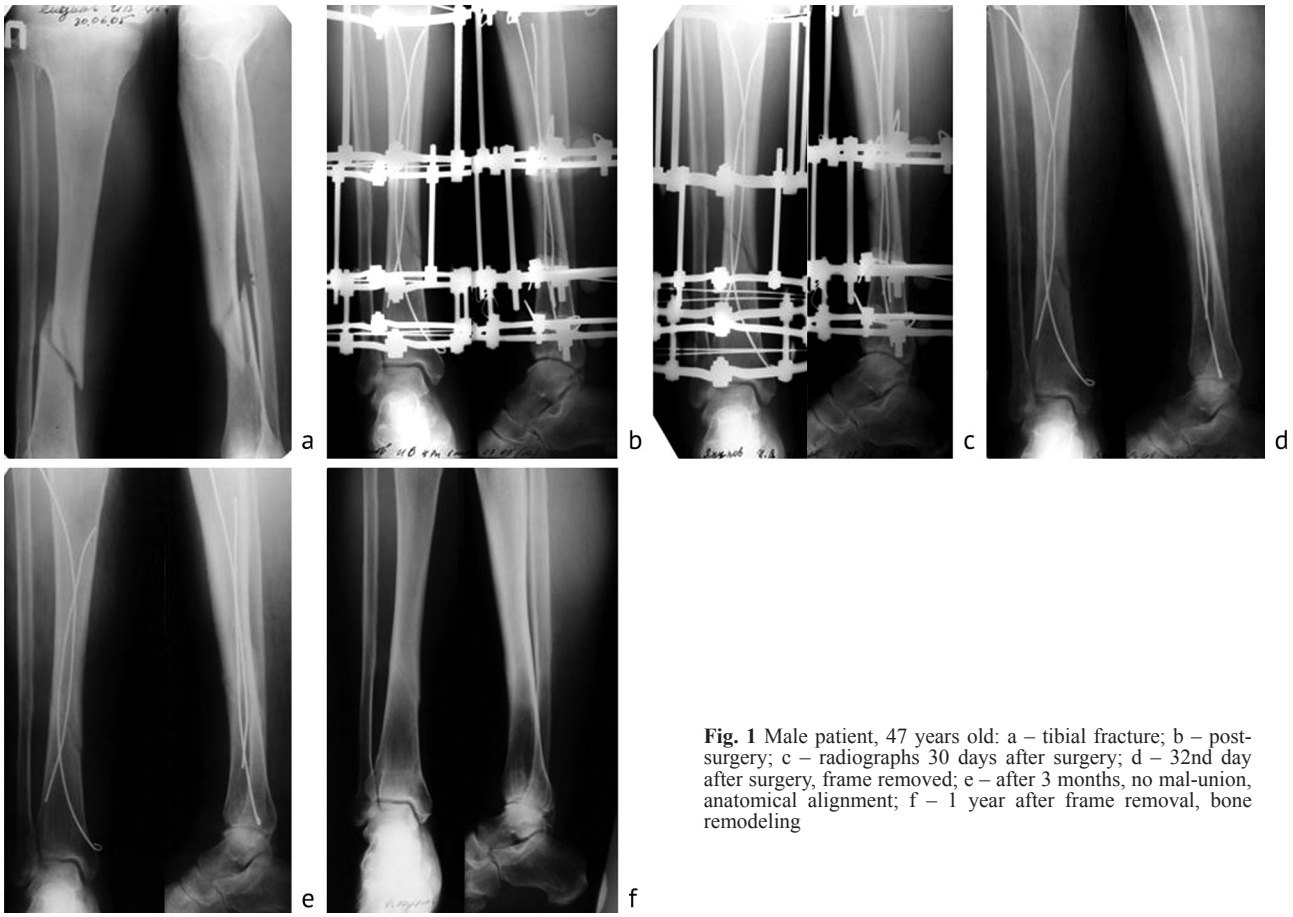

Fig. 1 Male patient, 47 years old: a – tibial fracture; b – post-surgery; c – radiographs 30 days after surgery; d – 32nd day after surgery, frame removed; e – after 3 months, no mal-union, anatomical alignment; f – 1 year after frame removal, bone remodeling

DISCUSSION

Tibial shaft fractures are one of the most common long-bone fractures and their incidence is estimated to occur in 4 percent of the senior population, representing 11.3 to 41.2 % of all skeletal fractures [12, 13]. Despite improvement of surgical methods, we can face delayed bone union and poor outcomes in tibial fractures. Obviously, the results of treatment are conditioned by both the fracture pattern and the method of treatment [14-17].

The extramedullary osteosynthesis can require large approach resulting in increased blood loss and additional lesion of surrounding the fracture soft tissues, worsens the blood supply to the fracture zone [14-16]. The main disadvantage of intramedullary osteosynthesis is significant damaging to intramedullary circulation, destruction of the bone marrow and endosteum. It potentially decreases bone regeneration [16, 17].

Ilizarov external fixation method is recognized for surgical treatment of tibial fractures. But its application is associated with long wearing (3 to 9 months) of external frame and related to this fact high incidence of pin-site infection. It remains uncomfortable for patients [18-20].

The combination of the external frame and HA-coated intramedullary elastic nails reduces inconveniencies of both external and internal devices. External osteosynthesis provides all advantages of the Ilizarov method: preservation of circulation, little soft tissue damage, early joint function and weight-bearing [19-21]. Elastic intramedullary nailing does not injure a. nutricia and mechanically stimulates endosteal and periosteal bone formation [22]. We suppose that HA-coating enables biological support for bone union [23]. Morphological experimental studies performed at the Ilizarov Medical Research Institute of Traumatology and Orthopedics revealed bone tissue structure around intramedullary nails and along their entire length, which persists until the end of the experiment and ensures complete stability of bone fragments [24]. Thin elastic nails do not interfere with spongy bone tissue in the medullary canal. Bundles of osteoid collagen fibers are firmly fixed to the rough, nanostructured surface of the nails coating and connected to the endosteal surface of the bone improving stability of bone fragments. This coating of a nanostructured highly porous

hydroxyapatite layer provides high biocompatibility and osteointegration of implants into the surrounding bone preventing the development of fibrous connective tissue [25, 26]. Our small series demonstrated that this combination of stability of bone fragment fixation and biological bone regeneration stimulation enables satisfactory outcomes of tibial fracture repair along with reduced external fixation time.

CONCLUSION

The results of the study revealed effectiveness of external fixation associated with elastic HA-coated intramedullary nailing. It is assumed that this combined technique ensures mechanical and biological favorable environment for bone union. The outcomes allow recommending this minimally invasive technique for treatment of tibial shaft fractures which are considered as having compromised bone regeneration.

Conflict of interest *Authors declare no conflict of interest.*

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Information about authors:

1. Konstantin A. Klyshnikov – postgraduate student, klyshnikov.kostyan@mail.ru;
2. Natalia V. Sazonova – Doctor of Medical Sciences, Head of Department, traumatologist-orthopedist, nv.sazonova@yandex.ru.;
3. Arnold V. Popkov – Doctor of Medical Sciences, Professor, traumatologist-orthopedist, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989

Contribution of the authors:

Klyshnikov KA – retrospective analysis of clinical material, assessment of treatment results.
Sazonova NV – assessment of traumatism in the Russian Federation, level of disability due to fractures of the tibia.
Popkov AV – development of technology for combined osteosynthesis, surgical treatment of patients.

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Preliminary experience with bioabsorbable intramedullary nails for paediatric forearm fractures: results of a mini-series

Gergő Jozsa[1]✉, Tamas Kassai[2], Marcell Varga[2]

[1] University of Pécs, Pécs, Hungary
[2] Manninger Jenő National Trauma Center, Budapest, Hungary

Corresponding author: Gergő Józsa, dr.jozsa.gergo@gmail.com

Abstract

Introduction Forearm fractures are common injuries in childhood. Completely displaced and unstable fractures require surgical intervention. Elastic Stable Intramedullary Nailing (ESIN) is widely used in treating these fractures. Although stainless steel and titanium implants are the most widely used, resorbable nails are becoming an option. **Aim** To present our initial experience in treating forearm fractures in children with Resorbable Stable Intramedullary Nailing (ReSIN). **Methods** The authors present several cases treated with ReSIN, their summarry and describe the techniqual steps. **Results** The series included 4 patients operated on with ReSIN. Bone union with anatomic and functional recovery was stated in all cases within the period of 5-7 months after surgery. **Discussion** More and more paediatric fractures can be treated with absorbable implants and result in good outcomes. It can be said that the new methods enabled similar stable fixation as with metal implants, which is considered the gold standard. A distinct advantage over metal implants is that there is no need to remove the implant, thus avoiding a second operation and reducing the risk of surgical complications. Another positive thing is that absorbable implants can be sunk the level of the cortical layer of the bone, they can easily be dropped under the skin. The only drawback of the method is the price of the implants. **Conclusion** The management of paediatric diaphyseal forearm fractures with bioabsorbable intramedullary nails is a promising emerging alternative to the gold standard ESIN technique.

Keywords: paediatric, forearm, fracture, bioabsorbable, resorbable, implant, PLGA

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INTRODUCTION

The current gold standard method in the treatment of paediatric diaphyseal forearm fractures requiring operative treatment is the elastic stable intramedullary nailing (ESIN). Such fractures include displaced and or unstable fractures, where conservative treatment with a cast would be insufficient to achieve satisfactory results after bone healing. The ESIN or flexible intramedullary nailing (FIN) is minimally invasive, in contrast to an open approach using plate and screw fixation, and leads to less soft tissue damage upon later implant removal. Standard nails are titanium elastic nails (ESIN) which are generally removed in a second surgery after sufficient bone healing has occurred [1-9].

However, in recent years bioabsorbable intramedullary nails have been developed for and used to manage paediatric diaphyseal forearm fractures. Using a bioabsorbable material presents multiple benefits, such as eliminating the need for a second surgery to remove the nail, thereby reducing soft tissue damage. Moreover, it decreases anaesthesia-related risk, exposure to radiation, and potential irritation which is usually caused by a protruding titanium elastic nail tip [10-12].

The Activa IM-Nail™ developed by Bioretec Ltd. has shown promising results in Finnish pediatric forearm diaphyseal fracture treatment clinical studies. This implant material is a PLGA (poly-L-lactide-co-glycolide) polymer with a radiopaque tricalcium phosphate (β-TCP) tip. The Pécs University Hospital Department of Paediatrics and Department of Pediatric Traumatology, Péterfy Hospital, Manninger Jenő National Trauma Center have been involved in an ongoing prospective multicentre clinical study analysing the treatment of paediatric diaphyseal forearm fractures with the Activa IM-Nail™ since 2021 [13].

The authors will explore the advantages of using bioabsorbable intramedullary nails in treating paediatric diaphyseal forearm fractures.

Epidemiology and Aetiology of Forearm Fractures

Fractures are prevalent in the paediatric population, accounting for approximately 25 % of all childhood injuries [1]. Radial and ulnar fractures have the highest incidence, making up 36 % of all childhood fractures [2, 3]. The mechanism of injury is mainly accidental trauma resulting from sports or leisure activities. A 1996 Welsh study found that 36.1 % of subjects sustained fractures while participating in sports or leisure activities [2]. Team ball and wheel sports such as cycling, rollerskating, and skateboarding were the most common, making up 42.4 % and 34.9 %, respectively. That study found distal radius fractures to have the highest incidence and soccer and rollerskating

to be the most common sports and leisure activities causing the injury [3, 14-17]. The study also observed that of the fractures that occurred in schools, 45 % happened on the playground. Of these, three-quarters occurred while the child was running, and half resulted from falling on a hard surface [2, 5-7].

The mechanism of distal radial fractures is typically a fall on an outstretched arm (FOOSH). Forearm shaft fractures often occur this way or due to a direct blow to the forearm. Studies show that protective equipment such as wrist guards can effectively prevent distal radius fractures during activities such as rollerskating. These guards prevent the hyperextension motion, which can occur in a FOOSH, absorb shock, and facilitate sliding of the guard along a surface to divert the direction of the kinetic force [14-16].

Diagnosis and Classification of Paediatric Forearm Fractures

The gold standard approach to diagnosing a forearm fracture is X-ray imaging. Patient's history and physical examination consistent with the clinical picture of a fracture can be sufficient for diagnosis. However, X-ray imaging will confirm the diagnosis and provide information influencing the treatment plan. Both anterior-posterior and lateral view images should be ordered. Orthogonal films enable the clinician to examine for pathologies that may not be visible at certain angles. The clinician should also consider imaging the elbow or wrist joint to check for Monteggia or Galeazzi injuries.

Treating a multi-fragmentary fracture includes careful analysis of X-ray images in preoperative planning. In the case of highly complex fractures, CT may be indicated to provide a more detailed image of the fracture morphology and soft tissue involvement. The clinical approach to the diagnosis of fractures in children, however, differs slightly from that in adult patients.

If a clinician were to be confident in their professional opinion that a paediatric forearm fracture was, for example, a minimally or non-angulated radial greenstick fracture, an X-ray could be omitted and opted for conservative therapy. Furthermore, examining fracture crepitation and excessive palpation should be avoided to minimise the child's pain and negative psychological response.

Paediatric diaphyseal forearm fractures are classified by the AO (Arbeitsgemeinschaft für Osteosynthesefragen) Paediatric Comprehensive Classification of Long-Bone Fractures. They are classified according to the fracture morphology, complexity, and involvement of one or both forearm bones. The classification code comprises two main parts describing the fracture location and morphology. Paired forearm bones are collectively defined as '2'. Fracture locations are further labelled as proximal '1', diaphyseal '2', or distal '3'. A lowercase letter specifies which bone is broken in fractures affecting only one bone belonging to a set of paired bones. For example, the code for an isolated radius fracture would include an 'r'. The fractured subsegment, i.e. epiphysis, metaphysis, and diaphysis, is labelled 'E', 'M', or 'D', respectively. The second part of the code describes the fracture morphology. Fracture pattern and severity are described with numbers.

Finally, a degree of displacement can be described with roman numerals. A simple transverse nondisplaced fracture of the radial diaphysis, when no ulnar fracture is present, is therefore described with the code 22r-D/4.1. A both-bone forearm fracture, e.g. a simple transverse nondisplaced fracture of the radial and ulnar diaphysis, is described with the code 22-D/4.1. This classification system helps to describe fractures clearly and concisely and is used internationally [4].

Aim To present our initial experience in treating forearm fractures in children with Resorbable Stable Intramedullary Nailing (ReSIN).

METHODS

Bioabsorbable Intramedullary Nailing Technique

Bioabsorbable intramedullary nails such as the Activa IM-Nail™ from Bioretec Ltd. are introduced similarly to the aforementioned traditional ESIN technique, with some differences due to the different material of the nail. Intraoperative imaging with C-arm fluoroscopy is also used in bioabsorbable intramedullary nail insertion. However, only the radiopaque tricalcium phosphate (β-TCP) tip is visible on the film, not the entire nail as with TENs. The PLGA material is radiolucent. Also, unlike the ESIN procedure, the medullary canal should be prepared with an implant-specific dilator tool when using a bioabsorbable intramedullary nail. This is done to decrease the risk of implant breakage upon insertion against resistance. The dilator tool prepares a space for the implant within the canal. Furthermore, surgeons often bend titanium elastic nails prior to insertion to create a curvature of the nail. It should not be performed with the Activa IM-Nail™, as the current PLGA material is too brittle and could be damaged if bent with force [8-10].

Perhaps of most clinical significance and in contrast to traditional treatment with ESINs, postoperative immobilisation with a cast is recommended for bioabsorbable intramedullary nails such as the Activa IM-Nail™. Cast options include a long arm cast for two weeks followed by a short arm cast for two to four weeks or a long semicircular arm cast

with volar support for 4-6 weeks. Unfortunately, cast immobilisation typically leads to joint stiffness and decreased range of motion after cast removal. This is a primary cause of hesitation for surgeons considering the bioabsorbable intramedullary nailing method when comparatively, the standard TEN technique does not require plaster casting and therefore avoids such complications entirely [10-13].

RESULTS

Case 1 An eight-year-old female patient with a displaced both-bone diaphyseal forearm fracture was treated using the Activa IM-Nail™. Preoperatively the fracture underwent closed reduction. The patient experienced no surgical or postoperative complications. A long arm cast was applied for three weeks, followed by a short arm cast for the subsequent two weeks. The clinical outcome was highly satisfactory; the patient did not experience functional impairment or decreased range of motion. X-rays taken pre-, intra-, and postoperatively illustrate the success of the treatment (Fig. 1). Unlike children treated with titanium elastic nails, this patient does not require a second surgery for hardware removal. The outcome of this patient is representative of the vast majority of the preliminary outcomes recorded in this study.

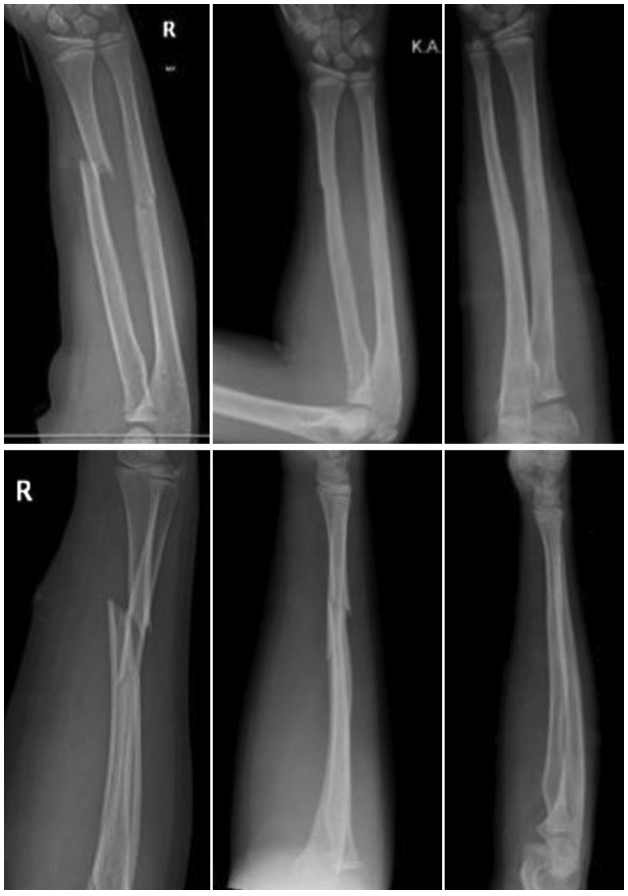

Fig. 1 Case 1. From left to right: preoperative, same-day postoperative control, six months postoperative control X-rays

Case 2 The 11-year-old girl fell while playing; her right forearm was injured and deformed, and she reported pain when moving the wrist and elbow joints. During her physical examination, we noticed a deformity of the left forearm and a dorsal deviation proximal to the middle third. However, sensation, circulation and movement were preserved in the fingers. The X-ray confirmed the right radius and ulna's incomplete (subperiosteal) fracture with axis deviation (Fig. 2 a). The patient was admitted to the pediatric surgery department for surgical treatment.

Closed reduction was performed under general anaesthesia, after which the fracture of the radius and ulna were stabilised with absorbable 3.2 mm diameter medullary nails. After the operation, an additional long arm cast was applied. The postoperative control X-ray showed the fracture in a good position; the tricalcium-phosphate marking is visible in the metaphysis of the proximal radius and distal ulna (Fig. 2 b).

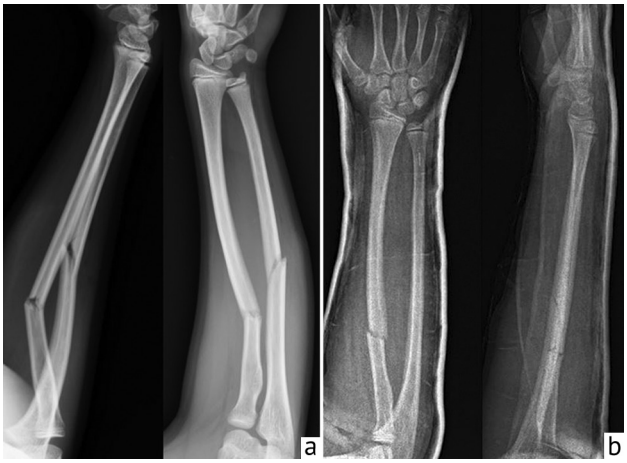

Fig. 2 Case 2: a – preoperative subperiosteal right forearm fracture; b – IM nails stabilise forearm postoperatively

Case 3 An 8-year-old boy was playing in the yard, running, and then fell on his left forearm. According to him, he heard a crack. During his physical examination, the child reported tolerable pain under the effect of the Fentanyl given in the ambulance, and there were no neurovascular abnormalities in the fingers. However, significant swelling and deformity were observed in the middle third of the right forearm. The X-ray confirmed a middle-third forearm fracture with displacement (Fig. 3 a).

Closed reduction was performed under general anaesthesia; the ulna reposition was done with a wire inserted percutaneously into the fracture gap. Stabilisation of the forearm bones was done with absorbable IM nails. A long arm cast immobilised

the left upper limb for four weeks. The postoperative control radiogram showed the fracture in a good position (Fig. 3 b).

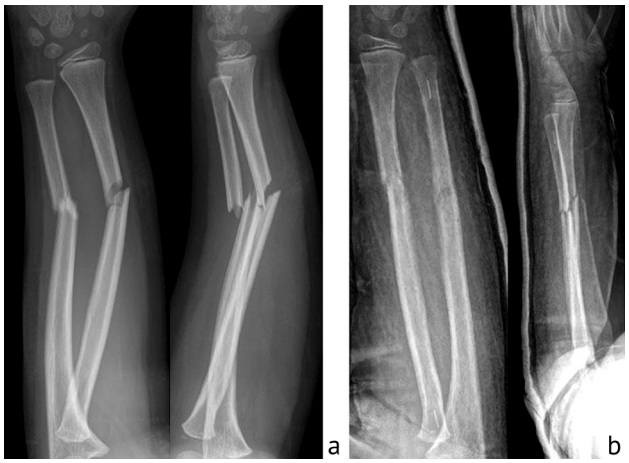

Fig. 3 Case 3: a – preoperative X-rays; b – postoperative control X-rays

Case 4 A 7-year-old girl fell while riding a horse; her right forearm was injured and deformed, and she complained of severe pain. X-rays confirmed the patient had a both-bone diaphyseal forearm fracture (Fig. 4 a) of the right arm. The fractures were reduced, and both bones were fixed with Activa IM-Nail™. Postoperative imaging showed good alignment (Fig. 4 b).

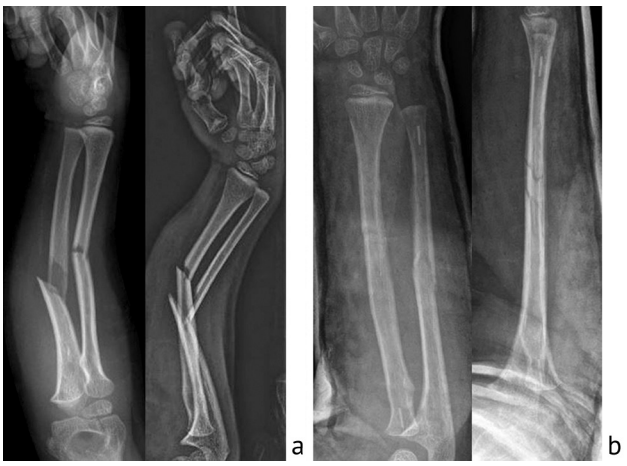

Fig. 4 Case 4: a – preoperative radiograms; b – postoperative excellent alignment

DISCUSSION

More and more paediatric fractures can be treated with absorbable implants and result in good outcomes. The authors described the most frequently occurring types of forearm fractures, in which the same results can be achieved with absorbable implants as with metal and titanium implants, which are currently considered the gold standard. Based on the clinical results so far (short- and medium-term follow-up of the patients), it can be said that the new methods enabled similarly stable fixation as the fixation with metal implants, which is considered the gold standard. A distinct advantage over the procedures with metal implants is that there is no need to remove the implant, thus avoiding a second operation and reducing the risk of surgical complications [10, 11, 12, 13, 18]. It is also a positive thing that absorbable implants can be sunk the level of the cortical layer of the bone, they can easily be dropped under the skin, so they do not cause soft tissue irritation. The health care system is also not burdened by the second operation (metal removal) and the associated hospital care costs [19, 20]. The only drawback of the method is the price of the implants.

CONCLUSION

The management of paediatric diaphyseal forearm fractures with bioabsorbable intramedullary nails is a promising emerging alternative to the gold standard ESIN technique. Research suggests that patient outcomes are comparable to those treated with traditional ESIN. However, large-scale and long-term studies are still needed, as well as further research into bioabsorbable polymers and other potential alternative biomaterials.

Conflict of interest *Authors declare no conflict of interest.*

Ethical statement *Clinical application of the technique was accepted and permitted in 2010 by our medical review board, the Hungarian Pediatric Trauma Committee, and the Hungarian Pediatric Surgery Committee.*

The work was performed in Pécs and Budapest. Surgical Division, Department of Paediatrics, Medical School, University of Pécs, 7 József Attila Street, Pécs, H7623, Hungary and Department of Paediatric Traumatology, Péterfy Hospital, Manninger Jenő National Trauma Center, 1081, 17 Fiumei Street, Budapest, Hungary.

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Information about authors:

1. Gergo Jozsa – MD, PhD, Head of the Department, dr.jozsa.gergo@gmail.com, https://orcid.org/0000-0003-3348-5385;
2. Tamas Kassai – MD, Medical Doctor, kassai.tamas@obsi.hu;
3. Marcell Varga – MD, PhD, Medical doctor, drvmarcell@gmail.com, https://orcid.org/0000-0002-1293-9918.

Contribution of the authors:

Jozsa G. – conceptualization, methodology, validation, data curation, writing–original draft preparation, writing–original draft preparation.
Kassai T. – methodology, visualization.
Varga M. – validation, writing–review and editing.
All authors have read and agreed to the published version of the manuscript.

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Original article

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Tibial lengthening over a bioactive degradable intramedullary implant: a case report

A.V. Popkov✉, E.S. Gorbach, U.F. Mamedov, R.V. Stepanov

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

Corresponding author: Arnold V. Popkov, apopkov.46@mail.ru

Abstract

Introduction Long duration of distraction osteosynthesis remains an unsolved problem. One of the promising ways to stimulate reparative regeneration of bone tissue is the technology of combined osteosynthesis with intramedullary elastic reinforcement with titanium wires coated with hydroxyapatite. A significant drawback of this combined distraction osteosynthesis is the planned removal of intramedullary wires several months after disassembling the Ilizarov apparatus. **The purpose of this work** is to demonstrate the possibility of stimulating reparative regeneration and reducing the duration of distraction osteosynthesis using an intramedullary degradable implant with bioactive filling. **Methods** We present the first in clinical practice case of surgical leg lengthening in a female 10-year-old patient using the Ilizarov apparatus an intramedullary degradable implant made of polycaprolactone (PCL) saturated with hydroxyapatite to stimulate reparative regeneration in the tibia. Monthly radiographic monitoring of the process of reparative regeneration of bone tissue was supplemented by computed tomography after disassembling the Ilizarov apparatus. **Results** The process of lengthening the tibia was accompanied by pronounced formation of a bone "sleeve" around the implant, which was directly connected to the endosteum of the tibia. The density of bone substance in the medullary canal reached 496.6 HU. The cortical layer of the tibia in the elongation zone increased to 4 mm, and its density was equal to 1288.8 HU. **Discussion** Leg lengthening of 4 cm was achieved along with simultaneous correction of valgus recurvatum bone deformity at IO = 15 days/cm, that is two times shorter than the generally accepted excellent IO in distraction osteosynthesis according to Ilizarov. **Conclusions** Biodegradable polycaprolactone implants saturated with hydroxyapatite might be not inferior to titanium wires coated with hydroxyapatite in regard to the degree of osteoinduction and do not require repeated surgical intervention to remove them.

Keywords: distraction osteosynthesis, Ilizarovs apparatus, biodegradable implant, hydroxyapatite

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INTRODUCTION

Distraction osteosynthesis, developed by G.A. Ilizarov, is a unique method of bone tissue bioengineering due to its ability to generate *in vivo* a vascularized bone tissue that features micro- and macrostructure of the native bone [1]. Moreover, the surrounding soft tissues are simultaneously exposed to regeneration and lengthening under the influence of tension stress [2]. The evolution of the distraction osteosynthesis resulted in the development of numerous technologies for lower and upper limb length discrepancy, bone defects and deformities [3-5]. Many orthopaedic surgeons, giving their due to the advantages of the Ilizarov method, point to significant duration of the external frame wearing that remains an unresolved problem [6-9]. The index of external fixation (IEF) varies from 0.7 to 5.9 months/cm and depends on the age, etiology, affected bone segment and amount of lengthening. We believe that such a long time of external fixation really increases the likelihood of pin-site infection. The research aimed to stimulate osteogenesis started at the Ilizarov Centre by the end of the XX and the beginning of the XXI century [2, 10, 11]. The technology of combined osteosynthesis with intramedullary elastic reinforcement with titanium nails coated with hydroxyapatite appeared to be one of the simple but very promising ways of stimulating reparative bone tissue regeneration [12]. According to experimental studies conducted at our institution, it does not contradict the principles of the Ilizarov method and does not interfere with intramedullary blood supply [13]. The average IEF of the femur using this technology in children was 20.3 ± 1.36 days/cm [10]. The only but a significant drawback of such combined distraction osteosynthesis is obligatory removal of intramedullary nails a few months after Ilizarov frame removal.

Our case report demonstrates a possibility to lengthen tibia over a biodegradable intramedullary nail that is filled with hydroxyapatite in order to stimulate bone union and avoid nail removal as the previous treatment protocol required [10, 12].

MATERIALS AND METHODS

We present a case of 4-cm tibial lengthening in 10-y.o. girl with congenital lower limb length discrepancy due to left tibia. Length discrepancy was complicated by 10° valgus recurvatum deformity with the deformity apex located at the junction of the proximal and middle third of the tibial shaft (Fig. 1 a).

At our institution, the parents of the child were proposed the method of Ilizarov limb lengthening over an intramedullary degradable polycaprolactone (PCL) implant saturated with hydroxyapatite (HA) for treatment (Fig. 1 b).

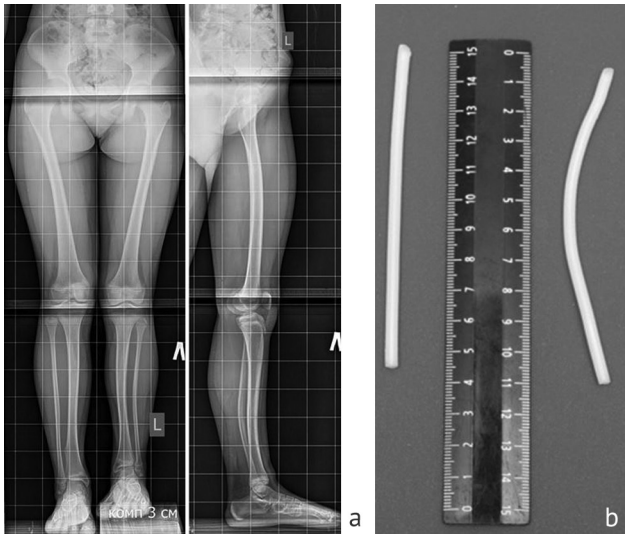

Fig. 1 Preoperative period: a – standing radiographs of lower limb; b – biodegradable implants

The implant materials were ε-polycaprolactone (Sigma-Aldrich, United States; Mn 80000) and hydroxyapatite (Fluidinova, Portugal; 10 ± 5 μm). PCL was dissolved in high purity acetone with a concentration of 15 wt %. Hydroxyapatite was pre-ground in a ball mill in a ceramic chamber with ceramic grinding media with added acetone in a mass ratio of 1.5:1 at a rotation speed of 72 rpm for 12 hours. The PCL solution was added and mixed with HA in the ball mill. The mixture was poured in a thin layer into a preheated fluoroplastic mold. After drying, the composite was crushed in a low-speed polymer crusher (Shini SG-1621N, Taiwan). Filabot EX2 single screw extruder (Filabot, USA) was used to obtain 4-mm wide filaments. Additionally, HA-particles were applied to the implant surface by dipping into a suspension of HA-powder in a solvent of known concentration, and then dried to remove the residual solvent. The implants have the following mechanical properties: ultimate tensile strength 18.3 ± 2.4 MPa (by stretching) and 32.0 ± 3.4 MPa (by pulling) and elastic modulus 425.7 ± 21.9 MPa (by stretching) and 213.9 ± 8.8 MPa (by pulling). For comparison, the titanium alloy nails demonstrate ultimate tensile strength 950 MPa (by stretching) and 1080 MPa (by pulling) and elastic modulus 113.8 MPa (by stretching) and 110 MPa (by pulling) [14]. The implant applied for treatment was 100 mm long and 4 mm wide.

The parents signed an informed consent on the treatment protocol of Ilizarov tibial lengthening and insertion of a PCL/HA intramedullary implant. Institutional ethics board approval for the study was obtained.

Surgery

The first stage of the operation was PCL/HA nail insertion into the medullary canal.

An oblique hole in proximal tibial metaphysis towards the medullary canal was formed using a 5-mm awl through 3 cm soft-tissue approach. Use of awl provided "reaming" for the implant in metaphyseal and proximal and middle diaphysis. The slightly bent implant was inserted manually through this hole, external part of implant cut and then the soft tissues were sutured tightly.

The Ilizarov frame assembly comprised three rings connected with rods and hinges. The positioning of hinges depended on deformity apex and CORA. Partial corticotomy was performed with a conventional chisel and completed with osteoclasis. Upon radiographic control, the frame systems were stabilized. It is important to emphasize that the implant in the medullary canal does not interfere with the insertion of wires but requires strict implementation of the corticotomy technique. There is a risk to cut PCL/HA implant if standard osteotomy would be used.

Post-operative period

Each patient was evaluated every 10-14 days during distraction and deformity correction phase and then monthly during the consolidation phase. Regular radiography (Shimadru Sonialvision 4, Japan) for immediate and every two-weeks bone regeneration control was supplemented by CT (Toshira Aquilion 64, Japan) upon Ilizarov frame removal.

Elongation phase was initiated on the 7th postoperative day at the rate of 1 mm/day divided into 4 times. Planned amount of lengthening was achieved in 42 days (Fig. 2 a). The fixation phase lasted 23 days. In three weeks of fixation phase the radiology revealed continuity of cortices in the lengthening zone and disappearance of the central fibrous zone of the bone regenerate. It enables frame removal (Fig. 2 b). After frame removal the patient was recommended to walk with progressive weight-bearing on the operated leg. Two months after the external fixator removal, the patient walked with full weight bearing, without additional means of support. The recovery of ROM in adjacent joints was noticed.

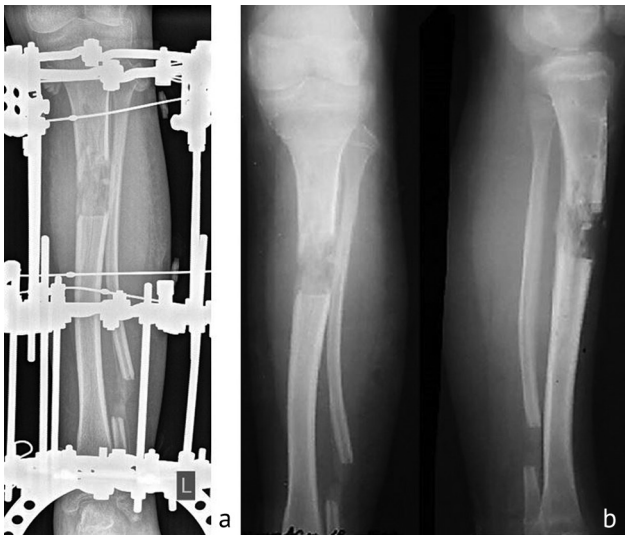

Fig. 2 Radiographs of the left tibia: a – by the end of the distraction period; b – radiographs at frame removal

Detailed description of radiographs and computed tomograms. From the first days of distraction the transverse corticotomy of the tibia at the apex of the deformity provided the separation of bone fragments in the absence of direct contact between them. However, the image of regenerated bone tissue got visible since 14th day after the onset of elongation. It could be described as heterogeneous, with separate "islands" of compactions. After one month, the bone regenerate image filled the entire diastasis between the bone fragments. Until the end of the distraction phase the continuity of longitudinally oriented trabeculae maintained. Optical density of the regenerated bone exceeded both optical density of the paraosseous tissues and the density of endosteal callus. Another feature was periosteal and endosteal reaction. Its first signs appeared two weeks after the beginning of lengthening. Throughout the distraction phase, on the image of newly formed bone there was no central zone so called "fibrous, non-mineralized zone of the distraction regenerate", which is typical in conventional Ilizarov lengthenings. Intramedullary osteogenesis was observed, particularly along the trajectory of the PCL/HA nail.

The radiological signs of bone union were observed in three weeks of fixation phase, it corresponded to continuity of cortices in the lengthening gap and disappearance of the central fibrous zone of the bone regenerate. The frame was removed at this stage. Thus, EFI was 18 days per cm.

Two months after the frame removal, radiographs showed remodeling of callus, increase in the density of newly formed bone (Fig. 3 a, b). There was no deformity neither fractures. Alignment remained normal.

Computed tomography (Fig. 3 c, d, e) performed after the removal of the Ilizarov fixator confirmed a three continuous cortices at lengthening site (their density was 1288.8 ± 141.2 HU), and mineralized central zone of the distraction regenerated bone. The cortical plate along the anterior surface of the leg was presented in the form of separate fragments.

Longitudinally oriented merging trabeculae in the structure of the endosteal part of the regenerated bone formed a bone "sleeve" (Fig. 3 c, d, e) around the implant with the density of 496.6 ± 20.9 HU. No destruction areas neither cysts in the tibia were revealed.

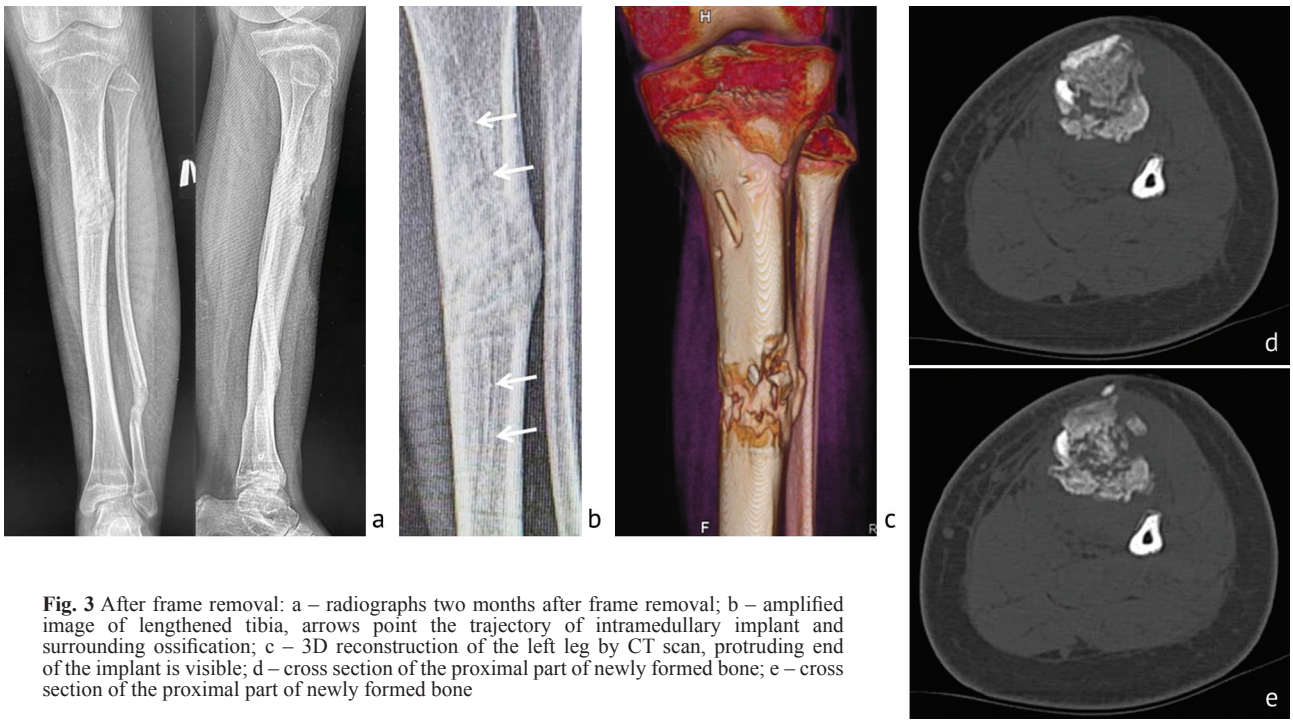

Fig. 3 After frame removal: a – radiographs two months after frame removal; b – amplified image of lengthened tibia, arrows point the trajectory of intramedullary implant and surrounding ossification; c – 3D reconstruction of the left leg by CT scan, protruding end of the implant is visible; d – cross section of the proximal part of newly formed bone; e – cross section of the proximal part of newly formed bone

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Thus, the consecutive radiographs demonstrated that lengthening with external frame and intramedullary implant made of hydroxyapatite-saturated polycaprolactone provided optimal conditions for bone regeneration. The increased activity of osteogenesis was manifested in the formation of the distraction regenerated bone without evident central fibrous non-mineralized zone, associated with developed periosteal reaction.

DISCUSSION

The use of biodegradable implants providing advantage of non-removal after is a promising approach [15-17]. Due to osteoinductive filling they induce bone formation around it and provide osteointegration stimulating osteogenic activity in the bone marrow canal ensuring stability of bone fragments until union throughout the time of implant resorbtion [18]. The features of implants made of polymers of lactic and glycolic acids for self-locking and auto-compression related to changing in structure under hydrolysis reaction is discussed [19]. There are no studies about possibility to apply intramedullary biodegradable nails for limb lengthening where applied force is traction one and request for bone metabolism is higher than for fracture union. The case presented in the article demonstrates the first experience in this combined technology.

Polycaprolactone (PCL) is a biodegradable thermoplastic used in a variety of medical applications, including bioprinting of hard tissues such as bones and cartilages. It is a polymer that provides improved control of the mechanical properties of ready-made 3D structures. In surgery the 3D implants made of polycaprolactone are used to fill in defects of skull bones and as elastic matrices to fill damaged cartilage tissue [20, 21]. In traumatology, pins and screws made of bioresorbable material are indicated for avulsion [22]. But all available absorbable implants are not bioactive without osteoinductive properties.

The principal difference of the nail that we used in the presented case is hydroxyapatite particles both on the implant surface and as a filling. It is well-known that hydroxyapatite possesses osteoinductive activity [18, 20, 21]. This feature could be favorable for bone lengthening especially in conditions of compromised bone regeneration [23].

This technology demonstrated by this case as excellent bone regeneration. The specific radiological signs were pronounced periosteal reaction both on adjacent bone fragments and at the level of diastasis and bone regenerate without apparent central fibrous zone, so-called “growth zone”. This active bone formation resulted in reduced time of external fixation with index of 18 days per cm.

We have a hypothesis, the result can be explained that bone tissue trabeculae are formed not only under the influence of longitudinal tension forces, but also due to surrounding nail osteoformation related to osteoinductive properties of hydroxyapatite. The elastic PCL/HA intramedullary nail elicits a controlled action and reaction to the host tissue environment with a controlled resorption to be replaced by stimulated regenerating bone tissue.

CONCLUSIONS

This case demonstrates effectiveness of bioactive degradable intramedullary nail in combination with external fixation for tibial lengthening. Intramedullary implant ensures mechanical stability and biological stimulation for bone regenerate and union. Resorbility and biocompatibility of the nail provide advantage to avoid a procedure to avoid implant in follow-up.

Conflict of interest *Authors declare no conflict of interest.*

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Information about authors:

1. Arnold V. Popkov – Doctor of Medical Sciences, Professor, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989;
2. Evgenii S. Gorbach – postgraduate student, gorbach.evg@mail.ru;
3. Ulvi F. Mamedov – postgraduate student, ulvi.mamedof@gmail.com;
4. Roman V. Stepanov – radiotherapist, stepanovrv@list.ru.

Contribution of the authors:

Popkov A.V. – Conceptualization, Writing – Review & Editin.
Gorbach E.S. – Methodology.
Mamedov U.F. – Visualization.
Stepanov R.V. – Formal analysis, Investigation.

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Original article

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Aneurismal bone cyst of the medial cuneiform bone: a case report of a new surgical approach and literature review

Sergey S. Leonchuk[1]✉, Tiwari Punit[2]

[1] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] Maharishi Markandeshwar University of Health Sciences and Medical College, Kumarhatti, Solan, Himachal Pradesh, India

Corresponding author: Sergey S. Leonchuk, leon4yk@mail.ru

Abstract

Introduction An aneurysmal bone cyst (ABC) is a rare, non-neoplastic, destructive, hemorrhagic, and expansile lesion accounting for 1 % of all bone tumors. ABC of the foot is very rare. Patients with foot ABC usually complain of pain and swelling of the affected area. Radiographs and MRI may be helpful in the diagnosis of ABC. No single surgical procedure has gained wide acceptance in the treatment of foot ABC. **Purpose** To show new effective surgical approach in the treatment of patient with ABC of the medial cuneiform bone. **Material and methods** We present the case of a 47-year-old woman with a 10-months history of pain and swelling in her right foot. Postoperative histopathological evaluation of resected tissues confirmed the diagnosis of ABC. An en bloc resection (total extraction of the remnant of the medial cuneiform bone) was performed and the defect was replaced with a fibular bone graft from the right leg. Allograft (Bio-Ost®) was placed along the autograft. Tibialis anterior tendon was attached to the fibular bone graft. We performed fixation of the foot and ankle using the Ilizarov original apparatus for prevention of bone graft instability and opportunity for early weight-bearing on the operated foot. **Results** The postoperative period was uncomplicated with complete healing of the bone defect without recurrence after 12 months of observation. AOFAS score increased significantly from 34 points preoperatively to 92 at 1-year follow-up. **Discussion** The optimal treatment of this lesion is still under discussion. Different treatment modalities have been described in the literature: wide resection, curettage with or without adjuvants, arterial embolization, intralesional sclerotherapy. Biological reconstruction using bone graft seems to be the best option, but fractures and nonunion are common complications of bone grafting. **Conclusion** The combination of Ilizarov external fixation and bone grafting provided favorable conditions for the healing of foot bone defect due to ABC without complications, allowed mobility and early weight-bearing of the patient. Recurrence was not detected radiologically. Harvesting of the fibular bone graft did not affect the position of the foot and its movements. Our surgical approach should be considered as a treatment option in similar cases.

Keywords: Aneurysmal bone cyst, Foot, Ilizarov, bone graft; external fixation, allograft, medial cuneiform bone

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INTRODUCTION

An aneurysmal bone cyst (ABC) is a rare, non-neoplastic, destructive, hemorrhagic, and expansile lesion accounting for 1 % of all bone tumors [1]. The etiology of this pathology is unknown, although it is now commonly accepted that benign bone cysts are caused by trauma or local circulatory disturbance, which results in an increase in venous pressure and the development of enlarged and dilated vascular components within the affected bone [2, 3]. Overall, ABC is diagnosed more commonly in the second decade of life and is more common in females than in males [4].

Midfoot ABC is very rare. Patients with foot ABC usually complain of pain and swelling in the affected area. Radiograph and magnetic resonance imaging (MRI) may be helpful in the diagnosis of ABC. A radiograph of the patient`s foot demonstrates a lytic lesion in the medial cuneiform and MRI shows cystic formations with typical fluid-fluid levels due to blood sedimentation. A histopathological examination is needed to evaluate the ABC.

The differential diagnosis associated with ABC includes giant cell tumor, giant cell reparative granuloma, Brown tumor arising from hyperparathyroidism, chondroblastoma, or telangiectatic osteosarcoma [1, 3, 5, 6].

Several classifications of ABC have been proposed based on natural history, activity, and morphological features [7-10]. There is a very useful classification of ABC according to Capanna et al [7], which is based primarily on the extent and size of the cyst and its proximity to the cortex and soft tissues, described five morphological subgroups (types) and three distinct stages.

No single surgical procedure has gained wide acceptance in the treatment of foot ABC. The predominant therapy for ABCs is an intralesional resection performing curettage with the use of adjuvants [11]. A wide resection, especially in the foot, is not easy to achieve and can cause complications depending on the dimension of the operation and the localization [11]. Medial cuneiform bone is an important cornerstone for medial arch continuity, structural integrity, and pathological fracture risks are the conditions that should be considered in the treatment of foot ABC [12].

Purpose: to show a new effective surgical approach to the treatment of patient with ABC of the medial cuneiform bone.

MATERIALS AND METHODS

We present a clinical case of aneurysmal bone cyst of the medial cuneiform bone in an adult woman with a 10-month history of pain and swelling of her right foot and the long-term result of using Ilizarov external fixation and bone grafting.

A female patient, 47 years old, was admitted to the Ilizarov Center with complaints of pain and swelling in her right foot (Fig. 1, a). The patient had a 10-month history of complaints and was treated conservatively without any relief. Pain and swelling in right foot were increasing in the last 3 months. The patient lived in the countryside and had no history of significant personal, ethnic, demographic, or life incidents. There was no history of trauma. No allergies were reported. Blood analysis and urine analysis were normal. Electrocardiogram, chest X-ray, and arterial blood gas were also normal. Functional condition according to the American Orthopedic Foot and Ankle Society (AOFAS) was 34 points preoperatively with a full range of ankle joint motion.

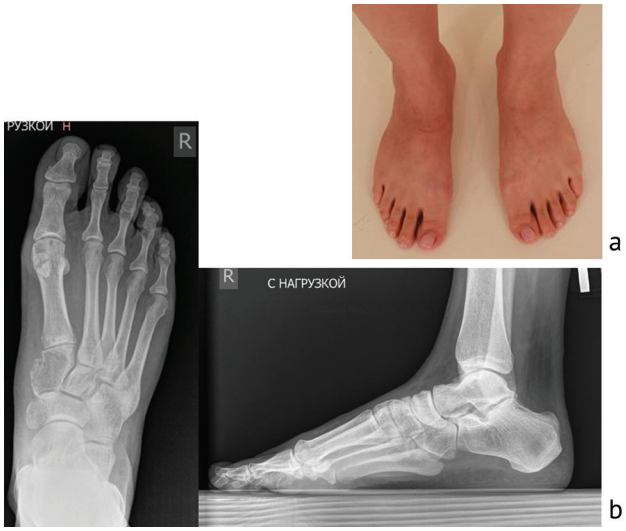

Fig. 1 Before surgery: a – photo; b – X-ray pictures in anterior-posterior (AP) and lateral views (demonstrating an osteolytic lesion of the medial cuneiform)

Radiographs and CT showed a lytic lesion in the medial cuneiform with subtotal bone defect (Fig. 1, b). MRI revealed a well-defined lesion with multiple thin septations and typical fluid-fluid levels due to blood sedimentation (Fig. 2).

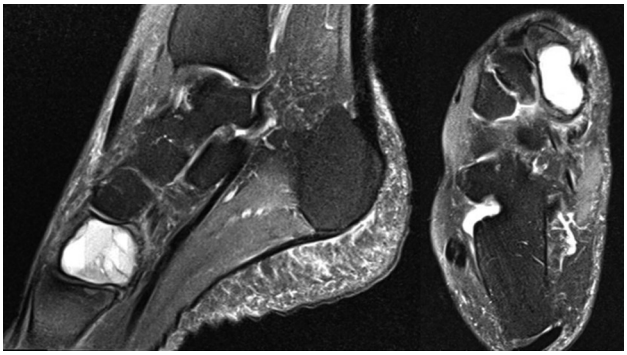

Fig. 2 MRI scans of the right foot showing a multiloculated expansile lytic lesion with multiple thin septations and typical fluid-fluid levels due to blood sedimentation

According to Capanna et al. classification, the ABC was type I (centrally located lesions that are well contained with no outline or slightly expanded) in the active stage.

Intraoperative biopsy aspirate was haemorrhagic; postoperative histopathological evaluation of resected tissues confirmed the diagnosis of ABC (Fig. 3).

Firstly, we applied the Ilizarov original frame on the right leg and foot with universal hinges (Fig. 4). At the level of the middle third of the lower leg, one wire and one half-pin were drilled, and in the lower third three wires were inserted (one was an olive wire through both bones); three wires were passed through the forefoot. We applied two full rings in the tibia and two half-rings in the forefoot.

In the 2nd step, we produced a medial longitudinal incision in the midfoot area. We cut the tibialis anterior tendon at its insertion and tagged its end with sutures. En bloc resection of the lesion (extraction of the remnant of medial cuneiform bone) was performed (Fig. 5, a, b, c). A fibular bone graft was harvested from the lower third of the right leg and the graft, about 4 cm long, was placed while preserving the medial arch of the foot (Fig. 5 d). The fibular bone graft was slightly tapered on both ends with about 3 mm bevelled edges and shaped so as to lock and fill in the defect between the first metatarsal and navicular bones. A prepared allograft (Bio-Ost®) was placed along the autograft (Fig. 5, e). The hole was drilled through the fibular bone graft and the tibialis anterior tendon was attached to it (Fig. 5, f).

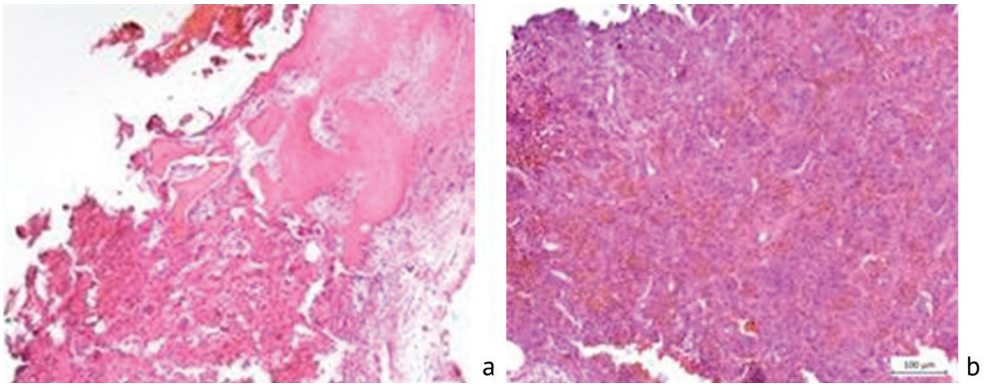

Fig. 3 HE-stained sections of resected tissues (histopathological evaluation): a –lamellae surrounded with congestion of small blood vessels, and blood-filled cystic spaces separated by fibrous septae (×40); b – multiply of osteoblast proliferation, fibrous connective tissue and multinucleated giant cell proliferation (×100). HE – hematoxylin-eosin

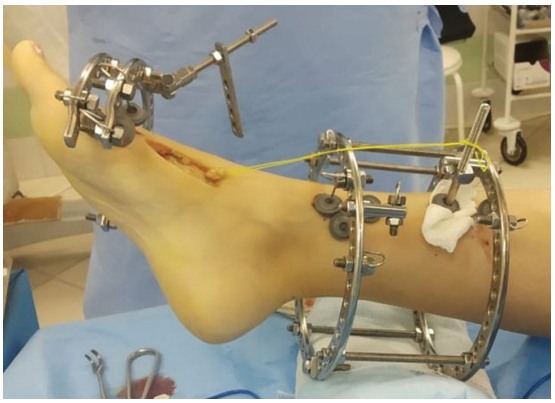

Fig. 4 Photo of the right leg and foot after partial Ilizarov frame assembly and medial longitudinal incision in the midfoot

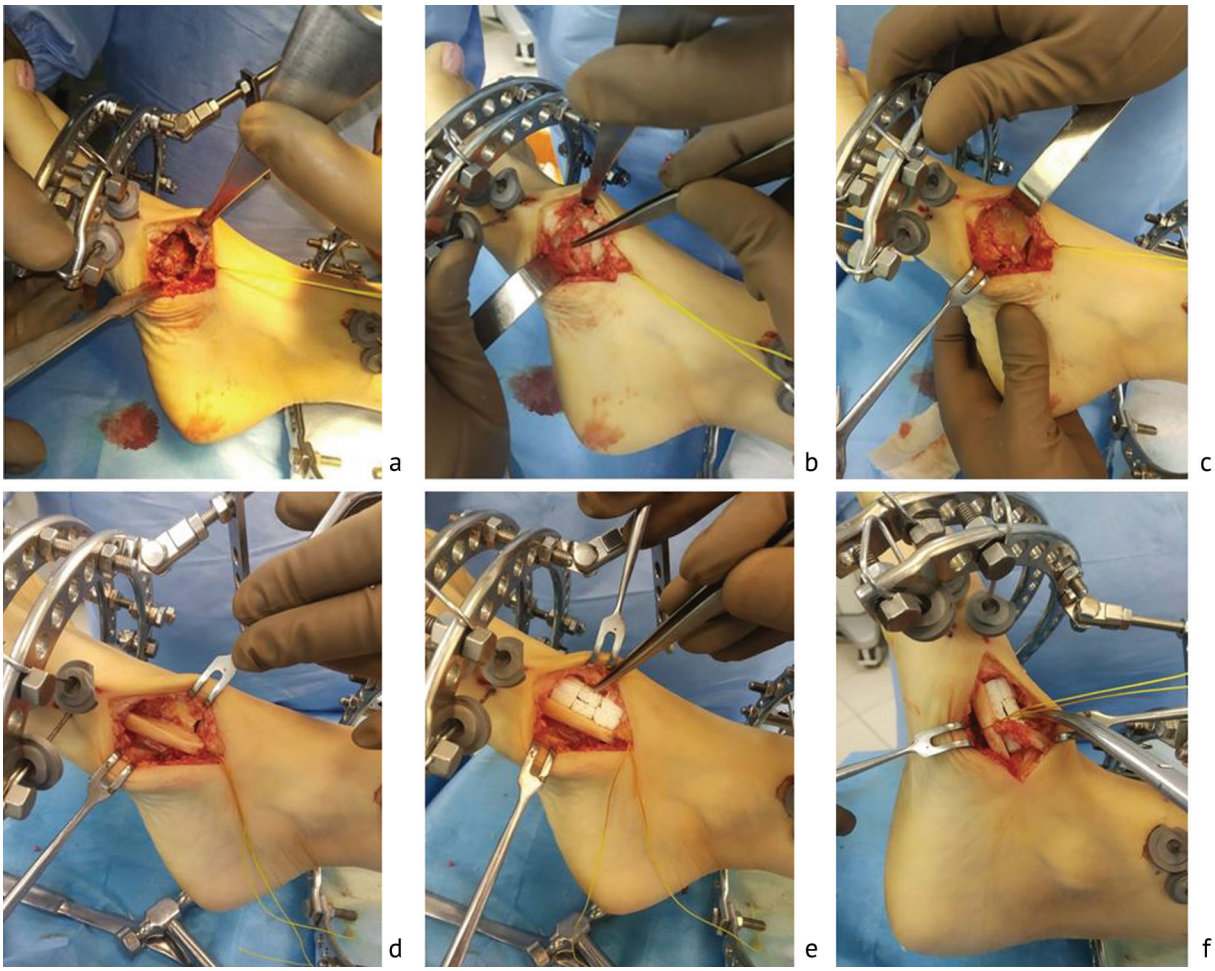

Fig. 5 Photos during the operation steps: a – view of the lytic bone process; b – zone of the defect after resection of the lesion; c – the size of the defect after resection of articular surfaces of 1st metatarsal, central cuneiform, and navicular bones; d – fibular bone graft shaped to fill the defect between the first metatarsal, central cuneiform, and navicular bones; e – placement of the allograft (Bio-Ost®) along the autograft; f – attaching the tibialis anterior tendon to the fibular bone graft

In the next step, we added two olive wires through the hindfoot (calcaneus) and one olive wire through the navicular bone (Fig. 6). The final Ilizarov frame consisted of two full rings in the leg, two half-rings in the forefoot and one half-ring in the hindfoot. Basic circular supports on the leg, forefoot, and hindfoot were connected by rods with hinges.

During the treatment, the patient was attended daily by a physiotherapist in our department. The patient started walking gradually increasing weight-bearing on the right foot on the third day after surgery with or without crutches. Dressings after surgery were changed daily for 3 days, and then weekly. The patient was discharged for outpatient treatment after 8 days. The postoperative period was uncomplicated. The period of fixation of the right foot and ankle with the Ilizarov apparatus on was 56 days.

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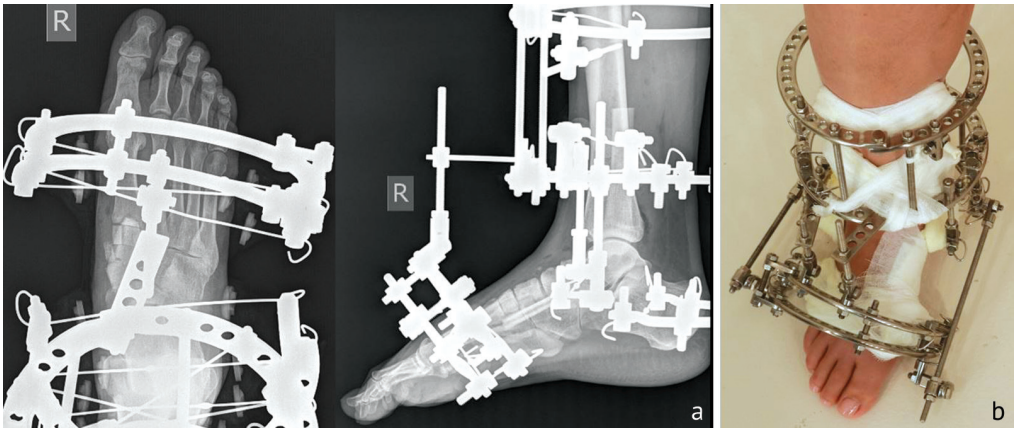

Fig. 6 Treatment: a – X-rays in AP and lateral views; b – photo of the right foot after the operation

RESULTS

Our treatment approach enabled to create favorable conditions for the healing of the defect zone without complications (Fig. 7, 8). One year after the surgery, the patient was satisfied with the result of treatment that provided free painless weight-bearing (Fig. 9). Recurrence was not detected radiologically. AOFAS score increased significantly from 34 points preoperatively to 92 postoperatively. The ankle range of motion recovered. The muscle strength of the tibialis anterior muscle was assessed as 5 points. Harvesting of the fibular bone graft did not affect the foot position and movements.

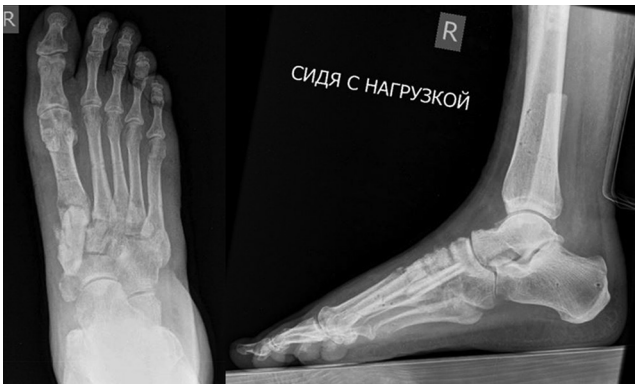

Fig. 7 X-ray pictures of the patient`s foot after Ilizarov frame removal (56 days after surgery)

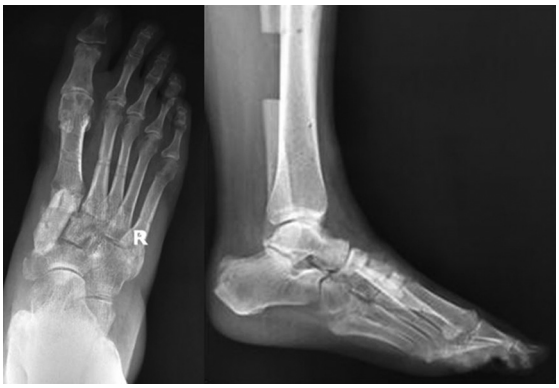

Fig. 8 X-ray pictures of the patient`s foot in AP and lateral views 90 days after surgery

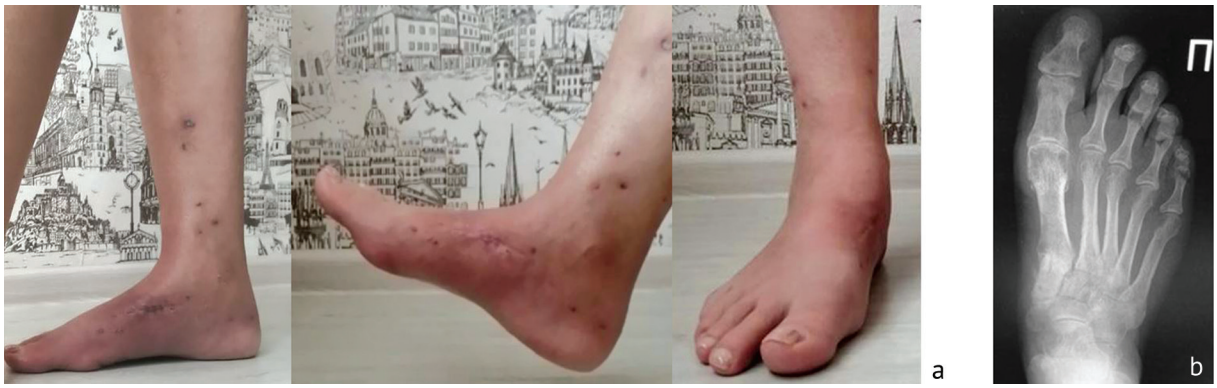

Fig. 9 A year after surgery: a – photos; b – X-ray picture in AP view

DISCUSSION

ABC is a lytic and benign but locally aggressive pseudotumor lesion. This pathology is found in long bones (tibia, femur, pelvis, or humerus) [3].

In the general population, the ABC is more frequent in children and young individuals, is diagnosed more commonly in the second decade of life, and has a male-to-female ratio of 1:1.16 [4]. ABC of the cuneiform foot bones is extremely rare [12]. CT and MRI scans may be helpful in the diagnosis of this lesion.

The optimal treatment of this lesion is still under discussion [11]. Different treatment modalities have been described in the literature: wide resection, intralesional resection such as curettage with or without adjuvants, arterial embolization, intralesional sclerotherapy using polidocanol or the systemic application of denosumab [14].

Due to the rarity of ABC of the foot, only case reports on the surgical treatment were published [1, 5, 6, 12, 15-17]. One study describes a case series treated by percutaneous instillation of polidocanol or intralesional curettage [11].

Once the cyst is removed, the cavity can be packed with either bone-graft, bone cement or other alternatives [16]. At present, biological reconstruction using bone graft seems to be the best option [16].

The adjuvant treatments such as argon beam coagulation, phenol, cryosurgery, and cement have complications such as postoperative fracture, skin necrosis/wound infection, and delayed bone healing [12]. In addition, treatment alternatives such as adjuvant radiotherapy, arterial embolization, and sclerotherapy do not contribute to the structural integrity of the bone and there could be recurrence too [12, 15, 18]. Inadvertent arterial embolization can have devastating effects, and its indications should be scrutinized accordingly [18].

The most preferred treatment option for most ABCs of the foot is curettage with bone grafting. But in our opinion, wide excision (en-block resection) of the lesion like in our ABC case might be preferable with the least chance of recurrence.

The medial cuneiform is an important cornerstone for medial arch continuity and structural integrity, and the risk of a pathological fracture should be considered in the treatment of ABC [12]. Restoring the structure and functions of the midfoot following resection is a challenging task because of its complex anatomy; the tibialis anterior tendon needs to be reattached to avoid functional disability [5]. Fractures and nonunion are common complications of bone grafting [16]. The application of fibular grafts in the reconstruction of bone defects caused by trauma, osteomyelitis, or tumor resection is an effective treatment option [19]. The use of the Ilizarov apparatus showed its effectiveness in the challenges of foot surgery [20, 21]. Due to these reasons, we decided to use a combination technique of Ilizarov external fixation and bone grafting providing an opportunity to start early weight-bearing instead of screw or plate fixation.

Kumar et al used K-wires to secure the graft in position after the excision of an ABC lesion of the medial cuneiform [5]. Bingol et al described compression screw fixation in the case of medial cuneiform ABC [12]. In our case, we used the Ilizarov original frame. This technique has not been published earlier in literature.

High recurrence rate was reported for ABC [3, 11], the incidence might be 10 percent and higher [11, 22, 23]. Deventer et al showed a local recurrence in 60 % of the curettage subgroup of patients and the disease after sequential instillations of polidocanol in the instillation subgroup persisted in 40 % [11]. It is important to note that no patient, out of the total five treated with polidocanol, could be managed with a single injection alone. The authors concluded that the less invasive character of the instillation justifies it as primary attempt of therapy.

Chowdhry described a local recurrence rate of 21 % after intralesional curettage in 14 patients with foot ABC [22].

Mankin et al in their review of 150 ABC cases treated with curettage and packing with bone grafting or polymethylmethacrylate, found a recurrence rate of 22 % [23].

Garg et al [24] and Dormans et al [25] described a reduction of local recurrence by the use of a high speed burr, phenol, and intralesional curettage of primary ABC in children and adolescents.

Complications associated with transosseous osteosynthesis and external fixation in foot and ankle surgery were reported by many authors [26, 27]. No complications related to vessels and nerves, and wire/pin-site infection were observed during and after our treatment, using Ilizarov external fixation.

Harvesting of the fibular bone graft might result in complications, including painful neuromas, vascular injury, long-lasting ankle pain, nerve injury, and ankle instability [19]. In our clinical case, harvesting of the fibular bone graft did not affect position and movements of the treated foot, did not cause pain and neurological problems.

The procedure of tibialis anterior tendon transfer can have complications such as re-rupture, loss of strength, instability by walking, gait disturbance with forefoot drop, and weak dorsiflexion of the ankle [12]. In our ABC case of an adult patient, we applied the Ilizarov original apparatus to create favorable conditions for healing the foot bone defect and reattached the tibialis anterior tendon with stable fixation and the possibility of early weight-bearing. The patient maintained a full range of motion in the ankle joint without pain and excellent muscle strength of the tibialis anterior at the final follow-up.

CONCLUSION

The combination of Ilizarov external fixation and bone grafting provided favorable conditions for the healing of foot bone defect due to ABC without any complications, patient's mobility and early weight-bearing. Our approach in the management of foot ABC should be considered as a treatment option in similar cases.

Conflict of interest *All authors declare no conflict of interest.*

Funding *The authors received no financial support for the research and/or authorship of this article.*

Ethics approval *The study was performed in accordance with the ethical standards of the Declaration of Helsinki (revised in October 2013) and was approved by the ethics committee of the Ilizarov Center.*

Informed consent *The patient has provided informed consent for the case report to be published.*

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Information about the authors:

1. Sergey S Leonchuk – MD, PhD, Head of Department, leon4yk@mail.ru, https://orcid.org/0000-0003-0883-9663;
2. Tiwari Punit – MS (Orthopaedics), Associate Professor, punit_tiwari28@yahoo.com, https://orcid.org/0000-0002-4174-8344.

Contribution of the authors:

Leonchuk SS – preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation); preparation, creation and/or presentation of the published work, specifically visualization/ data presentation.
Punit T – preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages.

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Review article

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Mechanical stimulation of distraction regenerate. Mini-review of current concepts

Alexander Cherkashin

Texas Scottish Rite Hospital for Children, Dallas, Texas, United States of America, alex.cherkashin@tsrh.org, https://orcid.org/0000-0002-0422-9158

Abstract

Introduction One of the key limitations of distraction osteogenesis (DO) is the absence or delayed formation of a callus in the distraction gap, which can ultimately prolong the duration of treatment. **Purpose** Multiple modalities of distraction regenerate (DR) stimulation are reviewed, with a focus on modulation of the mechanical environment required for DR formation and maturation. **Methods** Preparing the review, the scientific platforms such as PubMed, Scopus, ResearchGate, RSCI were used for information searching. Search words or word combinations were mechanical bone union stimulation; axial dynamization, distraction regenerate. **Results** Recent advances in mechanobiology prove the effectiveness of axial loading and mechanical stimulation during fracture healing. Further investigation is still required to develop the proper protocols and applications for invasive and non-invasive stimulation of the DR. Understanding the role of dynamization as a mechanical stimulation method is impossible without a consensus on the use of the terms and protocols involved. **Discussion** We propose to define Axial Dynamization as the ability to provide axial load at the bone regeneration site with minimal translation and bending strain. Axial Dynamization works and is most likely achieved through multiple mechanisms: direct stimulation of the tissues by axial cyclic strain and elimination of translation forces at the DR site by reducing the effects of the cantilever bending of the pins. **Conclusion** Axial Dynamization, along with other non-invasive methods of mechanical DR stimulation, should become a default component of limb-lengthening protocols.

Keywords: bone regeneration, mechanical stimulation, axial dynamization

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INTRODUCTION

Introduced by G.A. Ilizarov, the principles of distraction osteogenesis (DO) are now used to lengthen and reconstruct limbs to help treat multiple orthopedic conditions, both congenital and acquired [1-3]. However, several challenges remain during its clinical application, including long treatment duration. Extended time in an external fixator exponentially increases the risk of complications [3-8]. Due to long treatment time spent in a frame, "patients may have non-surgical problems, such as social, domestic, educational, and psychological problems, as well as problems that may be cared for by the nursing and physiotherapy staff" [9]. Treatment is often long because the distraction regenerate (DR) must mature enough to withstand weight-bearing. The process is often further prolonged due to delayed consolidation and/or the development of pathologic distraction regenerate [10, 11].

In an effort to decrease fixation time, multiple research efforts are currently focused on stimulating DR maturation utilizing different methods. Proposed solutions include biological stimulation of the regenerate, pharmacological stimulation, physical stimulation, and any combination of the above (Table 1). All these solutions can be performed using invasive (through various surgical interventions) and non-invasive approaches.

Mechanical stimulation is the foundation of the entire DO process. During the distraction phase of limb lengthening, tension stress affects all tissues inside and surrounding the distraction gap [44]. The mechanobiological phenomena of DR formation during the DO process essentially prolong the body's evolutionary-developed mechanism of fracture healing, where tension stress stimulates connective tissue proliferation, cell differentiation, and angiogenesis.

Table 1

Various modalities to stimulate distraction regenerate

Distraction Regenerate Stimulation		
Physical	**Biological**	**Pharmacological**
Mechanical (see below)	Grafts [12-14]	Vitamins [15-17]
Ultrasound [18-21]	Bone marrow and PRP [22, 23]	Biometals [24, 25]
Hyperbaric oxygen therapy [26, 27]	BMPs [28, 29]	Supplements [30, 31]
Electromagnetic 21, 32, 33]	Growth factors [34, 35]	Bisphosphonates [36-38]
Laser therapy [39, 40]	Cell therapy [41-43]	

Both angiogenesis and a proper mechanical environment are necessary for successful bone regeneration during DO [45, 46]. As the distraction forces are seized, bone resorption and remodeling take place to convert DR into a mature bone structure that is capable of bearing a physical load [47]. Known as the consolidation stage, this is the longest phase in the DO process, where different mechanical DR stimulation techniques are typically applied.

All known mechanical stimuli can be divided into invasive (surgical) and non-invasive techniques (Fig. 1).

Historically, mechanical stimulation techniques were applied following an abnormal formation of DR in an effort to fight the so-called delayed consolidation. However, there has recently been a shift towards a prophylactic application of mechanical stimulation to accelerate the consolidation and avoid delayed consolidation all together.

The goal of this work is to review the current methods of reducing treatment time during limb-lengthening procedures, with a particular interest on the use of mechanical stimulation to promote maturation of the distraction regenerate.

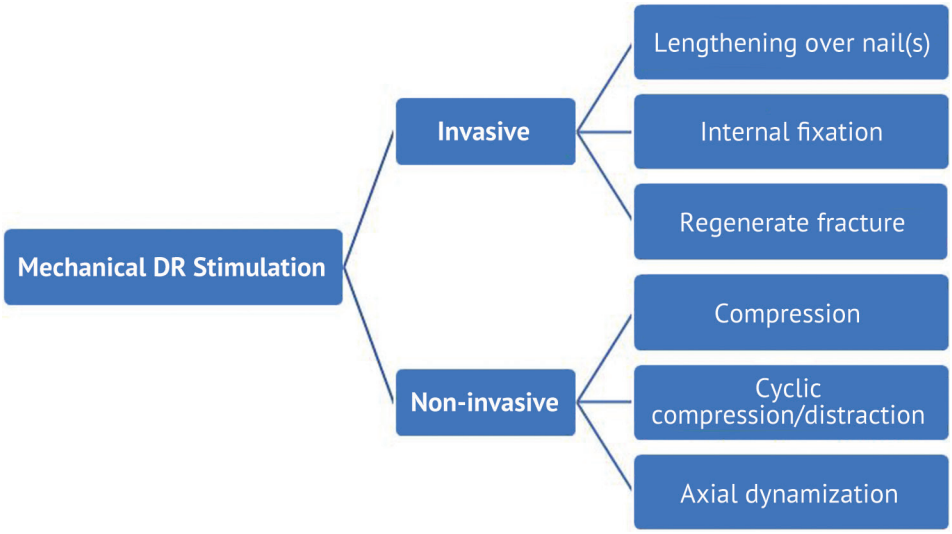

Fig. 1 Various techniques of mechanical stimulation of the distraction regenerate

MATERIAL AND METHODS

We summarize recently (no more than 30 years) published studies about definition, classification, indications and clinical application of methods for mechanical stimulation of bone healing in lengthening procedures. To prepare the review, we searched for information sources at the scientific platforms such as Web of Science, PubMed, Scopus, ResearchGate, RSCI, as well as other published products (Elsevier, Springer) using search words or word constructions: bone lengthening, Ilizarov method, mechanical stimulation of bone healing, dynamization, external frame, clinical translation.

RESULTS AND DISCUSSION

Invasive (surgical) mechanical stimulation

Most surgical methods that involve a change to the mechanical environment are performed at the end of the consolidation stage as a response to delayed consolidation problems. These techniques include plating or intramedullary fixation after lengthening [4]. In most cases, these techniques are considered desperate measures to avoid a regenerate fracture after frame removal. Another desperate technique involving surgical stimulation of the pathologic distraction regenerate relies on performing a fracture through the DR site. The fracture helps re-stimulate fracture healing mechanisms, initiate additional angiogenesis, and re-introduce growth and biological stimuli supplied to the pathologic regenerate. A new development, introduced by Popkov et al. [48], uses a prophylactic placement of intramedullary devices during the initial surgery. This provides extra stability during distraction, as well as creates an environment to recruit additional biological factors for DR maturation. They also illustrated that the use of HA-coated implants increases the effect of DR stimulation [49].

Non-invasive mechanical modulation

Non-invasive mechanical stimulation can be performed in various ways: weight-bearing [46, 50], cyclic compression/distraction (accordion technique) [51-53], destabilization of the frame by releasing nuts on threaded rods, destabilization of the frame by removing fixation elements (wires and pins), and replacing threaded rods with dynamization devices.

Weight-bearing

Since the very first application of the Ilizarov circular fixator, lower limb lengthening has required

at least partial weight-bearing as part of the process. Ilizarov listed weight-bearing as a categorically required part of leg lengthening [2]. There are multiple papers emphasizing the positive effect of lower extremity loading during DO treatment for DR maturation and remodeling. It is also the least costly method to mechanically stimulate the regenerate. The only consideration must be patient education and compliance, as a majority of non-invasive DR stimulation techniques rely on patient weight-bearing to be effective [50, 54].

Compression

Compression of the DR is often another desperate measure to solve poor regeneration. It is usually performed during the lengthening stage, when the distraction interzone does not progressively display signs of mineralization on X-rays, or at the consolidation stage, when there are no signs of improvement at the lengthening site [55, 56]. There are two important points to consider. First, patient preparation and education are necessary as the planned amount of lengthening may not be achieved. Second, the shape of the pathologic regenerate must be considered when a fully mineralized cortex on one side of the bone is present [57, 58]. This is commonly known as a regenerate cyst. The cyst prevents any ability to compress the DR and can ultimately cause the development of a deformity, either during compression or later following frame removal. Similar problems can arise from the premature mineralization of the fibula in cases of tibial lengthening. This occurs when the tibial regenerate lags behind, resulting in the fibula acting as a strut that shields the tibia from necessary axial loading. In these cases, early surgical intervention may salvage the lengthening by breaking through the thin mineralized band of regenerate or the prematurely consolidating fibula along with the use of various grafting techniques. An acute compression performed at the end of distraction phase with compression tension of 5.6 N/cm^2 is considered as optimal for bone healing stimulation [59].

Cyclic compression/distraction

Ilizarov was the first to suggest the use of alternating cycles of distraction and compression to improve the quality of bone formation in the distraction gap [2]. Under the optimal frame stability, patient's weight-bearing creates alternating distraction/compression (ADC) forces at the lengthening site as part of the DO process. Therefore, it is logical that the ADC forces created on a fixation device might further improve regeneration. This practice was later named as an accordion maneuver [53] and widely reported as a treatment for poor regenerate [51, 60-63]. Liu et al. [52] performed impressive animal studies to uncover the underlying mechanisms of ADC. The studies showed an improvement of bone formation during DO, suggesting that better outcomes may be achieved by moderately increasing the amplitude and slowing down the rate of the ADC technique [52].

Axial Dynamization

For many years, rigid fixation with internal or external devices was the paradigm of fracture treatment. However, recent advances in our understanding of bone healing and mechanotransduction suggest that systematically altering the construct's stiffness throughout different phases of healing improves regeneration [64-66]. Dynamization has recently become a buzz word in multiple DO publications; however, there are some problems regarding terminology and definitions. Multiple terms that describe DR dynamization are ill-defined and ambiguous at the present. Starting with dynamization itself – multiple publications currently describe different techniques of bone healing stimulation under the same term.

The term dynamization is described as "the transfer of a progressive load to the fracture site at a given point in the healing cycle" [67]. Nowadays, dynamization encompasses many different methods of altering the fixation of fractures as the bone heals [68], such as decreasing the external fixator's stiffness during the healing process by removing stabilizing elements [69]. A new concept of "reverse dynamization" was also recently introduced by Glatt et al., where frame destabilization is performed during the early stages of fracture healing (during the first week after the initial fixation) to produce a larger volume of newly formed callus. The frame instability is reversed to a more rigid fixation after 3-4 weeks to, in theory, encourage blood vessel growth within the callus. Reverse dynamization somewhat contradicts the original Ilizarov idea that frame stability plays an important role in bone healing [1, 2]. In contrast to the intramembranous ossification described by Ilizarov, reverse dynamization generates a large volume of bone callus, possibly through endochondral and trans-chondral types of ossification.

Many other vague terms are often used in conjunction with dynamization to describe the mechanical stimulation of the distraction regenerate, including but not limited to stable fixation, rigid fixation, and micromotion. First, the term micromotion should be avoided in scientific literature. The physiologic load of an external fixator typical configuration can lead to an axial displacement of bone fragments away beyond 3 mm [70]. This amount of fragment displacement cannot be described as micro [71]. Secondly, we propose that rigid fixation be reserved to describe stabilization without any meaningful load on the bone healing site, essentially inhibiting the mechanobiological processes necessary for optimal bone regeneration as fixation is too rigid. In contrast, stable fixation of bone fragments minimizes the amount of shear and bending strains at the fracture or lengthening site, while still allowing for some axial loading to promote bone regeneration.

Dynamization should only describe and be used interchangeably with Axial Dynamization. We propose

to define Axial Dynamization as the ability to provide axial load at the bone regeneration site with minimal translation and bending strain. Shear and bending strains are both undesirable forces, whereas axial loading and unloading promote regeneration [2]. However, it remains doubtful that most modern external fixator assemblies will be able to entirely eliminate all instances of bending strain [70]. The original fixator developed by Ilizarov incorporates built-in Axial Dynamization with the use of thin wires only, which act as a fixed beam bending when under a load. As a result, the frame provides some axial displacement of bone fragments during weight-bearing [72]. Extended use of half-pins in modern external fixators has increased frame rigidity and replaced fixed beam bending with cantilever bending, which ultimately creates undesirable bending and translation forces.

There are many other methods of altering fixation stability that should not be considered dynamization, including removing stabilizing elements of the fixation device, destabilizing connecting elements of the fixator, or removing some of the external fixation pins and wires. These methods would be better named as partial fixation removal or fixator destabilization.

When applying dynamization, simply untightening the nuts of the fixator connecting rods, will not provide the proper conditions to eliminate shear and bending strains. Instead, the best way to dynamize is with spring-loaded devices or elastic washers to provide axial loading with a dampening effect. An example of such dynamization would involve mounting the original De Bastiani dynamization washer [67] or a spring-loaded device between the external fixator rings [70]. Use of such spring-loaded dynamization devices not only stimulates bone healing but also improves patient comfort, allowing better weight-bearing and indirectly improving the healing process [70].

Axial Dynamization works [73, 74] and is most likely achieved through multiple mechanisms: direct stimulation of tissues by axial cyclic strain and elimination of translation forces at the DR site by reducing the effects of the cantilever bending of the pins. However, it remains unclear when dynamization should be applied during limb lengthening. Frames are traditionally dynamized at the end of the consolidation period before the external fixator is removed. Nonetheless, we have started dynamizing frames earlier, at around 3-4 weeks after lengthening is complete. There is also an argument to initiate dynamization during the distraction period to mimic the effects of all-wire frames, which include properties of built-in dynamization as previously stated. Introducing dynamization during the early distraction period would likely result in a mechanical environment similar to the traditional all-wire fixator developed by Ilizarov and ultimately help develop better DR. However, it must be noted that dynamization also depends upon the patient putting weight on the treated extremity, which could be a challenge during the early stages of limb lengthening. Whereas late dynamization performed during the consolidation period would actually improve patient comfort by reducing the cantilever bending of the fixator pins and providing a dampening effect. This would allow for more weight-bearing and physiologic walking that will help stimulate DR maturation.

Advancements in automated distraction will possibly allow for a more frequent rhythm of distraction, plus the ability to use passive Axial Dynamization techniques alongside frequent patient-independent cycles of compression/distraction.

CONCLUSION

Mechanical stimulation is the most accessible and usually most affordable way to speed-up the mineralization of the distraction regenerate. Multiple publications prove the effectiveness of mechanical modulation techniques involved in DO for improving the conditions of bone healing. Non-invasive techniques of DR mechanical stimulation should become a default component of the limb-lengthening procedure, rather than reserved to rescue pathologic regeneration and delayed consolidation. Axial Dynamization using spring-loaded or elastic devices proves effective in achieving cyclic axial loading, while minimizing shear and bending forces on the regenerate. There is a need for a consensus on the definitions and protocols that surround Axial Dynamization. Therefore, additional research is needed to develop the protocols and process of Axial Dynamization, which will most likely involve incorporating a combination of early and late dynamization techniques into the treatment of limb lengthening.

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Information about the author:

Alexander Cherkashin – MD, PhD, Co-Director Center for Excellence in Limb Lengthening and Reconstruction, alex.cherkashin@tsrh.org, https://orcid.org/0000-0002-0422-9158.

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Review article

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Biocompatible implants in orthopedics: bone tissue engineering

Arnold V. Popkov[1], Dmitry A. Popkov[1,2]✉

[1] Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation
[2] University of Kragujevac, Faculty of Medicine, Kragujevac, Serbia

Corresponding author: Dmitry A. Popkov, dpopkov@mail.ru

Abstract

Introduction Technological advances in bone tissue engineering have improved orthopaedic implants and surgical techniques for bone reconstruction. This approach allows overcoming inconvenience of the paucity of autologous materials available and donor site morbidity. **Aim** To demonstrate advances of the past 30 years in the development of bioimplants providing alternatives to bone grafting in reconstructive orthopaedics. **Methods** Preparing the review, the scientific platforms such as PubMed, Scopus, ResearchGate, RSCI were used for information searching. Search words or word combinations were bioactive osteoinductive implants, bone grafting, bone reconstruction, hydroxyapatite, bone scaffolds. **Results** This review presents and discusses the experimental and clinical application of biotolerant, bioinert and bioactive materials for reconstructive bone surgery. **Discussion** Future generations of biomaterials are designed to be osteoconductive and osteoinductive. Properties of polycaprolactone (PCL) filled with hydroxyapatite (from 10 to 50 wt %) make this hybrid material with controllable absorption a promising strategy for reconstructive surgery in comparison to other materials. **Conclusion** The main trends in tissue engineering in the field of orthopaedics are represented by construction of three-dimensional structure implants guiding cell migration, proliferation and differentiation as well as mechanical support. Association with bone morphogenetic proteins, growth factors enables proliferation and differentiation of cell types of the targeted bone tissue. A promising advancement should be biodegradability with a controllable degradation rate to compliment cell/tissue in-growth and maturation in limb reconstruction.

Keywords: bone tissue engineering, reconstructive orthopedics, clinical translation

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INTRODUCTION

Methods for surgical treatment of fractures and bone diseases with the use of osteosynthesis technologies have spread globally over the past century. A great number of internal (intraosseous and extraosseous) and external (wire- and half-pin-based) fixators have been proposed to ensure the most reliable osteosynthesis of a broken bone and to provide favorable conditions for reparative bone tissue regeneration: accurate reduction of bone fragments, their stable fixation, sparing attitude to osteogenic tissues, optimal rate of elongation, good blood supply to the involved limb, and early functioning in the postoperative period [1, 2]. Most orthopedic surgeons believe that the Ilizarov method is one of the best methods to provide the above-mentioned conditions. Nevertheless, clinical practice shows that the duration of osteosynthesis with the Ilizarov frame lasts at least four months for closed long bone fractures. In conventional limb lengthening, the excellent external fixation index is about 30 days/cm, the good one is 45 days/cm, the fair one is 60 days/cm [3-9]. In 2004, Eralp et al. reported an index of 1.65 months/cm for lengthening of the tibia with the Ilizarov fixator [10]. It is obvious that long-lasting treatment cannot satisfy either the patient and his relatives or the health care institutions. Therefore, there is a necessity to reduce the period of external frame wearing and to stimulate osteogenesis, both with conservative and invasive methods [11, 12].

Autologous bone grafting and various bioactive products from the decalcified bone, biocomposite matrices, recombinant bone morphogenetic proteins, and biomaterials from ceramics were offered for this purpose. Alongside, experimental studies on cell technologies have intensified [11, 13]. Bioengineering in orthopedics aims at creating biomaterials that are suitable to replace the damaged ones such as skin, muscle tissue, blood vessels, nerve fibers, and bone tissue. Biomaterials are the materials designed to serve as interfaces with biological systems in order to augment or replace host tissue, organ, or body function [14].

This publication aimed to reveal the trends in experimental development and clinical application of advanced bioactive implants in limb reconstruction dedicated to replace bone grafts.

MATERIALS AND METHODS

We summarized the recently published studies on definition, classification, production, indications and clinical application outcomes for implants with osteoinductive and osteoconductive properties used in limb reconstruction. To prepare the review, we searched for information sources

at the scientific platforms such as PubMed, Scopus, ResearchGate, RSCI, as well as other published products (Elsevier, Springer) using search words or word constructions: bone tissue engineering, reconstructive orthopaedics, clinical translation, scaffolds, hydroxyapatite.

RESULTS AND DISCUSSION

This area of materials science in orthopedics is also called bioceramics. The name emphasizes the leading role of the ceramic component in implants for joint replacement, filling materials for dentistry, implants for maxillofacial surgery, and medical cosmetic products [15, 16]. Biomaterials must possess certain chemical properties (absence of undesirable chemical reactions with tissues and interstitial fluids, resistance to corrosion), mechanical characteristics (strength, resistance to breakdown, long-lasting mechanical support), biological properties (absence of reactions from the immune system, consolidation with bone tissue, stimulation of osteogenesis).

Biomaterials used as implants that replace a bone part or as temporary fixators for fractures are also classified by their biological activity on bone tissue regeneration:

• Biotolerant materials (stainless steel and cobalt-chromium alloys); a layer of fibrous tissue develops between the surface of those implants and the host bone; reparative regeneration of the injured bone occurs within conventional time and at some distance from the implant (distant osteogenesis);

• Bioinert materials (titanium and aluminum oxides) do not cause the formation of fibrous layer on the implant surface; reparative osteogenesis proceeds in direct contact with the implant surface (contact osteogenesis), but bone union occurs within usual terms;

• Bioactive materials (calcium phosphate ceramics and silicon-based bioglasses) are characterized by the formation of a chemical bond with the bone (bonding osteogenesis), enhance bone formation starting from the implant surface and induce the formation of a continuous bond from the tissue to its surface.

Metal implants occupy a large place in traumatology. Alloy steel is most frequently used to restore the integrity of a fractured bone (screws, locking intramedullary nails or bone plates). Internal fixation implants are made from materials that must primarily meet the objectives of providing reliable fixation of the fracture for functional treatment within a certain period, sometimes for 12-18 months. This is a rather long period of time. Therefore, materials must be chosen to resist fatiguing failure after fixation on the surface of bone fragments in order to maintain them in an anatomical position under loading until biological bone union.

All metals can be classified according to the effect on reparative osteogenesis into biotolerant materials (stainless steel and cobalt-chromium alloys) or bio-inert materials (titanium and aluminum oxides). There are no bioactive metals that would stimulate reparative osteogenesis. Chromium-nickel and chromium-nickel-molybdenum corrosion-resistant steels, alloys of cobalt, tantalum, titanium, and pure metals such as nickel, silver, and titanium are the most frequently used materials for production of surgical implants applied in current medicine. Thus, in dentistry, dental implants are made from titanium and its alloys, since titanium is a biocompatible and corrosion-resistant material. In fact, all metals corrode under the influence of human body fluids. And without exception, all metal implants get protected from corrosion by a passive layer consisting of insoluble products of their oxidation. Corrosion increases by about 100 times if the passive protective layer of a metal implant, which consists of insoluble products of their oxidation, is damaged eventually by friction [17]. Under these conditions, the implant will not be able to provide stable fixation for a long time period required for bone fracture union.

Undoubtedly, titanium is one of the most promising materials for the manufacture of surgical implants widely used in traumatology. Numerous experiments and clinical practice have confirmed that titanium and its alloys is the most optimal metal for implantation [18].

Typical bioactive materials include bioglasses. The most common composition is 24.5 % Na_2O, 24.5 % CaO, 45.0 % SiO_2, 6 % P_2O_5. By varying the composition, one can change their bioactivity and resorbability. Other materials are based on hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ (dense and porous ceramics) [15, 19].

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is one of the few known bioactive materials. It enables bone ingrowth and osseointegration of an orthopedic, dental, and maxillofacial implant due to its high biocompatibility. In recent years, a special term has appeared in the literature, biocompatible nanoceramics (where HA grains vary in size from one to several hundreds of nanometers), or nanostructured bioceramics, which defines a nanostructured ceramic material used in medicine to regenerate lost bone tissue [20].

The phenomenon of bioactivity is determined mainly by chemical factors, such as the crystalline phase and the molecular structure of the material, and by physical factors such as roughness and porosity of the material surface. Back in 1973, Hulbert et al. [21] proposed a new concept of the so-called biological fixation of skeletal implants by active bone growth (osteoconduction) on their surface. The materials were oxide ceramic and carbon compounds, as well as metals coated with stable oxide layers. Later, the concept of bioactivity of materials was defined as their ability to interact with the surrounding host bone tissue and to form a chemical bond with it [15, 22-24]. The ion-exchange reaction between the bioactive implant and the surrounding body fluids leads to the formation of a layer of carbonate apatite on it, which is chemically and crystallographically equivalent to the mineral composition of the bone. This ability of the implant to initiate the formation of calcium phosphate under *in vitro* conditions is interpreted as the first sign of possible bioactivity *in vivo*.

Multiple complex and interrelated processes take place on the surface of a bioactive implant. First, ions and proteins are adsorbed there, forming a biofilm on the surface of the implant. This process strongly depends on the physical and chemical characteristics of the surface topography (roughness, porosity, morphology), chemical composition, energy and charge. As a result, both the amount and functionality of adsorbed proteins are largely controlled by the surface of the biomaterial. The adsorbed biofilm promotes the adhesion of cells facilitated by specific transmembrane receptors, integrins [16, 25]. The surface of the material, its biocompatibility determines the degree of adhesion of osteogenic and mesenchymal stem cells on their surface [26-28]. The degree of adhesion and disposition of these cells determine their ability to proliferate and differentiate into osteoblasts upon contact with the implant. The latter is crucial in the development of a mechanically strong interface of complete fusion between the implant surface and bone tissue without a layer of fibrous tissue [29-33].

The traumatology science currently develops two fundamentally different approaches to address injuries and bone loss: 1) simple replacement of a damaged area of the bone with a massive implant, with or without bioengineered structure, that replaces the bone and adjacent joints, or 2) creating conditions for regeneration of the bone in the injured area with an osteoinductive (absorbable or non-absorbable) implant. An analysis of literature reveals that both directions are increasingly associated with bioceramics, the use of which in medicine has been expanding as the developments in the field of chemistry progress and technologies for production of materials with the properties that are close to bone tissue improve [34-36].

Among the synthetic materials that can be used for implantation, calcium phosphate-based ceramics are the most promising. Hydroxyapatite is not only biocompatible, but also the most bioactive. However, the main shortcoming of ceramics is its fragility. Therefore, bioinert metals and alloys with a calcium phosphate coating can be used for fabrication of orthopedic devices for the musculoskeletal system, which experiences significant mechanical loads [37-40]. The coating provides biological compatibility and expressed biological activity in the formation of bone tissue around the metal. There are two research trends:

1. Development of joint prosthetic devices, the bearing metal part of which is covered with ceramics for the purpose of osteoinduction and formation of an extensive bone coupling that ensures reliable contact of the metal with the bone tissue over the maximum area, thus eliminating the failure of fixing the elements of the joint on the bone for many years [37, 40].

2. Development of intramedullary implants that do not experience significant load, but their hydroxyapatite coating contributes to the filling of extensive bone defects after trauma or surgical bone resections [41, 42].

Such a coating is designed to induce reparative osteogenesis around the implant, thereby contributing to the filling of extensive bone defects. The coupling created in this way around the implant provides optimal conditions for consolidation of bone fractures or nonunion, the formation of a distraction regenerate [40].

The main biological advantage of HA coatings is enhanced bone formation, accelerated bonding between the implant surface and surrounding tissue, and a reduced release of potentially harmful metal ions [30].

Methods for applying a bioactive coating to implants are numerous. The basic technologies for the deposition of hydroxyapatite are microarc oxidation, magnetron sputtering, formation of composite polymer coatings, vacuum arc deposition under the conditions of short-pulse high-frequency plasma immersion ion implantation. The technology of coating determines mechanical properties of the coating and physicochemical characteristics of the implant surface [18, 28, 29, 43-47].

The inconvenience of metal implants with a bioactive coating includes the second surgery to remove them. A solution to this problem is found in using of an implant fabricated from a strong composite material which will gradually undergo resorption while the defect is filled with regenerating bone tissue.

The first fixation devices for osteosynthesis made from biodegradable materials have been available since the early 1980s [48, 49]. However, their use for fracture treatment has not been widely accepted yet due to a number of reasons. A few types of biodegradable orthopedic implants available are either not intended for management of fractures, or do not meet the requirements of the AO/ASIF principles in terms of their properties and application methods [50-52].

Nowadays, it has become possible not only to obtain biodegradable materials with strong mechanical properties and an optimal degradation profile for fracture management, but also to produce structures close to classical metal fixators in sizes, what enables to consider biodegradable fixators of the latest generations as their full alternative [53, 54]. However, most of the researches are still devoted to either products or materials of early generations not related to osteosynthesis of limb bone fractures. Very few publications describe practical aspects of their use [55, 56].

Materials that undergo degradation due to the physiological effects of body tissues can be conditionally designated as biodegradable, including bioabsorbable and bioresorbable. Biodegradable materials is a wide group and is defined as a community of materials that undergo decay due to the physiological effects of body tissues on them (*in vivo*), regardless of the removal of degradation products from the body [56, 57]

In 1966, Kulkarni et al. [58] reported the results of a study on the biocompatibility of polylactic acid (PLA) and its stereoisomer (Poly-L-Lactic Acid, PLLA). In 1971, the first result of a medical evaluation of the polymers in suture, rod, and film form was also presented [59]. The requirements for orthopedic fixators made of biodegradable materials were formulated

later: adequate fixation of bone fragments and/or soft tissues to the bone implant must retain mechanical properties within the estimated consolidation period; degradation period should not be too long to avoid the problems typical for metal fixators; implant must be made of materials that are completely safe for humans: non-toxic, non-antigenic, non-pyrogenic and non-carcinogenic [60-65].

The group of polymeric biodegradable materials for osteosynthesis includes polyesters based on lactic and glycolic acids, polycaprolactone, as well as their co-polymers, which can be characterized as bioinert bioresorbable. The degradation of these compounds proceeds mainly along the hydrolytic path. However, it also partly occurs enzymatically, mainly after the hydrolytic decomposition of the molecule into relatively small fragments due to the enzymes of phagocytes, macrophages and neutrophils, while the end products of decomposition are CO_2 and water [66-72]. As a material for the manufacture of orthopedic fixators, PLLA is of main interest. It has a high crystallinity, hydrophobicity and retains its properties for a long time, sometimes even too long (up to 5 years or more), is non-toxic and does not elicit an immune response [73].

The PLLA strength for compression is 80-500 MPa, the tensile strength is 45-70 MPa, the elastic modulus is 2.7 GPa, that are close to the values of the human bone tissue, which for the cortical bone are 131-224 MPa, 35-283 MPa and 17-20 GPa, respectively, and for spongy bone 5-10 MPa, 1.5-38 MPa and 0.05-0.1 GPa, relatively. PLLA products retain their original mechanical resistance for at least 3 months after implantation; degrade within 24 months. In some cases, after 4 years of implantation in the tibia, only initial surface signs of screw erosion were noted, what makes us consider that products made from pure PLLA are conditionally biodegradable [48, 71-74]. PLLA of high crystallinity degrades very slowly, while being inferior in strength to both polyglycolic acid (PGA) and biostable materials (metals). By combining PLLA and PGA, it was possible to solve the issue of relatively insufficient strength of the promising copolymer containing polylactic and glycolic acids [55, 56, 74-81].

Polylactic acid screws and pins are used in the clinical practice for fixation of small bone fragments in intra-articular fractures, fractures of the ankles and tibiofibular syndesmosis, bones of the wrist joint [75, 77]. In most fractures of the upper and lower extremities, it is not possible to ensure the stability of bone fragments only with such degradable implants. The economic effect of the use of biodegradable materials in fractures of various locations, including ankle fractures, was estimated by Böstman et al. and ranged from 410 to 903 US dollars due to minimization of repeated surgical activity needed for the removal of metal implants [81, 82]. The terms of fracture union remain standard [83, 84].

To obtain a real opportunity to stimulate osteogenic processes, a number of researchers propose to add special inductors (fillers) to the composite material as matrix for transplantation of stromal progenitor cells, native bone marrow cells [85, 86].

Several biocomposite materials containing bone collagen and bone sulfated glycosaminoglycans of animals and humans have been developed in Russia in order to restore bone defects: *Biomatrix* – bone xenocollagen and bone sulfated glycosaminoglycans; *Allomatrix-implant* – bone allocollagen and bone allosulfated glycosaminoglycans; *Osteomatrix* – biocomposition based on natural bone components xeno- or allocollagen, sulfated glycosaminoglycans and hydroxyapatite; *CollapAn* – a calcium-phosphate biocomposite material based on synthetic hydroxyapatite, collagen and an antibiotic [86-92].

These materials have porous and cellular structure corresponding to the architectonics of native cancellous bone. Such a structure provides not only volume maintenance in the defect due to elastic properties, but also an optimal opportunity for penetration and ingrowth of connective tissue cells, blood vessels and bone formation into the implant [88, 89, 92]. However, for all their advantages, they do not have the necessary mechanical characteristic of native bone tissue.

Osteomatrix is used in dentistry to replace bone defects formed after the removal of cysts and teeth. It was shown that 3 months after surgery the bone defects were actively filled with young bone tissue [91]. Good clinical results were also demonstrated for the *CollapAn*. In the area of the defects filled with *CollapAn*, the cortical layer and the medullary canal gradually formed by the 4-5th month, along with an increase in the intensity and uniformity of the callus. It was well tolerated; there were no cases of rejection and allergic reactions. In fracture treatment, an endosteal callus with a small periosteal component occurred by the end of the 4th week after the operation. The use of *CollapAn* in delayed fracture union and nonunion contributed to the formation of callus, mainly in its periosteal part, by the end of the 6-8th week after the operation. On average, by the end of the 8-9th month, bone consolidation was confirmed [92, 93].

Western European and American firms have developed a whole series of calcium-phosphate-collagen composites for filling bone defects or synostosis of vertebrae in order to replace autologous bone material in surgical practice. Thus, *Collagraft*®, a composite of collagen and biphasic calcium phosphate ceramic, contains highly purified type I collagen and biphasic calcium phosphate, which consists of 65 % hydroxyapatite and 35 % tricalcium phosphate ceramic [94].

Hydroxyapatite-poly-L-lactide (u-HA-PLLA) composites contain poly-L-lactide (PLLA). When u-HA-PLLA-composite rods were implanted into the subcutaneous layer, their bending strength retained 85 % of the original value after 8 weeks and 80 % after 25 weeks, while after 25 weeks the molecular weight of the rods decreased to approximately 20 % of original [95]. It was reported [96] that complete degradation of u-HA-PLLA composite rods for bone fixation happens approximately 4.5-5 years after implantation.

Beneficial properties of u-HA-PLLA composites enable to use bioresorbable devices made from them for internal fixation in bone fractures, orthopedic reconstructive and restorative operations. However, all degradable products based on PLLA have a significant drawback. In the course of degradation, the acidity of the environment of surrounding tissues increases, which negatively affects the processes of reparative regeneration of bone tissue and, consequently, the terms of fracture consolidation increase [97].

In order to eliminate this issue of implant degradation, the researchers at Tomsk Polytechnic University (Russia) together with researchers from the Ilizarov center (Russia) proposed to use polycaprolactone (PCL) as an implant matrix. Products from PCL with the inclusion of hydroxyapatite (from 10 to 50 wt %) were studied in the treatment of fractures of long bones in animals, treatment of bone defects and experimental limb lengthening. Experimental studies revealed a high biological activity of this new type of intraosseous implants: pH of the environment remained at the level of 6-7, bone union of tibial fractures occurred within 1 month, external fixation index for limb lengthening did not exceed 20 days/cm in monofocal procedures [98].

CONCLUSION

Thus, ceramic polymer composites are commercially available nowadays for treatment purposes. The combination of inorganic and organic components seems reasonable for designing in bone reconstruction surgery. Although autografts and allografts are still widely used due to the lack of artificial materials, some hydroxyapatite-polymer composites are attractive due to their similarity to the structure and properties of the bone tissue and osteoinductive activity. The use of materials depends both on medical and biological characteristics of a bone defect and particularities of underlying pathology. Variability of clinical problems requires a large range of biomaterials and implants on their basis.

The main objective of tissue engineering in the field of orthopaedics should be construction of implants serving as three-dimensional structures to guide cell migration, proliferation and differentiation along with mechanical support. Association with bone morphogenetic proteins, growth factors enables proliferation and differentiation of cell types of the targeted bone tissue. Tissue-engineered implants must be biodegradable with a controllable degradation rate to compliment cell/tissue in-growth and maturation.

The manufacture of implants should easily and efficiently reproduce various shapes and sizes. They have to ensure bone union in non-complicated fractures within three to four weeks and stimulate bone healing in lengthening procedure after two to three weeks of fixation phase. Osteoiductive implants should accelerate mineralization of newly formed organic matrix of a lengthened bone.

Conflict of interest *The authors do not have conflicts of interest.*

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Information about the authors:

1. Arnold V. Popkov – Doctor of Medical Sciences, Professor, Chief Researcher, apopkov.46@mail.ru, https://orcid.org/0000-0001-5791-1989;
2. Dmitry A. Popkov – Doctor of Medical Sciences, Professor of the Russian Academy of Sciences, Corresponding Member of the French Academy of Medical Sciences, Head of the Clinic, dpopkov@mail.ru, https://orcid.org/0000-0002-8996-867X.

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