



Microscopic changes in experimentally damaged rabbit bone following intravenous administration of stem cells

Taras Savchuk*

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-7351-5684>

Mykola Malyuk

Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0003-3019-6035>

Iurii Kharkevych

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-7877-8272>

Roman Bokotko

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-6217-5266>

Yuliia Paramonova

PhD, Assistant
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0002-7197-3075>

Abstract. The search for methods and means of stimulating reparative osteogenesis is a pressing issue in modern veterinary traumatology that requires further study, and the use of stem cells

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*Corresponding author



is considered one of the most promising approaches in this area. The study aimed to investigate microscopic changes in experimentally damaged bone tissue of rabbits under the influence of intravenously administered allogeneic mesenchymal stem cells on reparative osteogenesis. Mesenchymal stem cells were obtained from rabbit bone marrow and cultured in a nutrient medium according to standard protocols. The injury was simulated using a 2.5 mm diameter surgical drill on the medial surface of the middle third of the tibial diaphysis. After 24 hours, the animals in the experimental group were given a single intravenous injection (into the jugular vein) of 3.5×10^6 stem cells. The defect in the animals in the control group healed naturally. Histological studies were performed on days 3, 7, 14, 21, 28 and 42 of the experiment. The samples were stained with haematoxylin-eosin and examined under a microscope. Intravenous administration of allogeneic mesenchymal stem cells to animals stimulated regenerative processes and accelerated the stages of reparative osteogenesis at the site of injury. The study determined that in the experimental group, already on the third day, there were no blood clots and bone tissue fragments in the damage area, and the growth of fibrous connective tissue and intensive osteogenesis were recorded. The formation of bone callus and consolidation of bone tissue proceeded faster than in the control group. By day 21, the bone marrow had already acquired a normal structure, while in the control group, a similar result was observed only on day 28. Almost complete restoration of the defect after the introduction of stem cells was observed on day 28, while in the control group, a similar result was achieved only on day 42. The results of the study can be used to stimulate reparative osteogenesis and further study the processes of the influence of stem cells on the restoration of damaged tissues

Keywords: reparative osteogenesis; bone tissue; bone callus; bone marrow; allogeneic mesenchymal stem cells

Introduction

One of the key problems in veterinary surgery remains the improvement of methods for treating bone fractures in animals. Serious damage to bone tissue can threaten the life of an animal or impair the functionality of bone structures. Despite significant progress in this area, the mechanisms that promote accelerated reparative osteogenesis have not yet been sufficiently studied. A promising direction in bone tissue regeneration is the transplantation of allogeneic mesenchymal stem cells (MSCs) obtained from bone marrow or adipose tissue. This method has been successfully used to stimulate the regeneration of other tissues (Uğurbaş *et al.*, 2021).

A successful solution to this problem requires mastery not only of the techniques of

connecting and fixing bone fragments, but also a basic awareness of the dynamics of changes occurring during reparative regeneration. As noted by L. Impieri *et al.* (2024), successful restoration of damaged bone requires proficiency in methods of stimulating osteogenesis and means of avoiding complications. According to Y. Zhang *et al.* (2024), autologous bone tissue and alloplastic materials are widely used to treat bone tissue damage. Despite their advantages, practicality and popularity, these methods are not universal due to several significant issues. The main problems include the risk of nerve damage, the occurrence of pain syndrome, the development of infections, and the limited availability of donor material.

At the same time, V. Kutsevlyak & O. Lyubchenko (2024) proposed an alternative approach that involves the use of biologically active substances and stem cell transplantation to regenerate damaged bone tissue. J. Zou *et al.* (2023) proved that mesenchymal stem cells, due to the ease of their isolation and cultivation, have proven to be a particularly promising source for the treatment and regeneration of bone defects, as well as for maintaining bone tissue homeostasis. A substantial prerequisite for the successful functioning of mesenchymal stem cells in osteogenesis is the selection of an adequate cell culture medium. Studies conducted by W. Pan *et al.* (2024) and T.L. Savchuk *et al.* (2025) demonstrated that autologous cell injections are already being used for various pathologies and may be a promising alternative for accelerating the healing process of bone fractures.

Stem cells are increasingly applied for bone tissue regeneration. F. Li *et al.* (2022) proposed innovative strategies for restoring lost bone tissue using MSCs, growth factors, and special bone tissue scaffolds. This approach aims to overcome the shortcomings of traditional treatments, such as the use of autologous grafts or artificial bone substitutes. In addition, M. Murayama *et al.* (2024) demonstrated that a matrix based on hydroxyapatite-tricalcium phosphate populated with allogeneic mesenchymal stem cells provides effective repair of femoral bone defects in large dogs. At the same time, the use of immunosuppressive therapy was not necessary.

R.D. Calixto *et al.* (2023) demonstrated the ability of injected stem cells to actively migrate to the bone defect area and differentiate into specific cells, such as osteoblasts and chondrocytes. In turn, studies by Y. Guo *et al.* (2024) demonstrated that in the bone microenvironment, allogeneic MSCs can originate from the peripheral walls of stromal vessels located on the surface of trabecular bone and fill intertrabecular spaces for tissue regeneration. According

to A.M. Theodosaki *et al.* (2024), MSCs can stimulate cell proliferation and angiogenesis, reducing inflammation, and producing significant amounts of bioactive molecules involved in tissue regeneration processes.

Therefore, the use of cell therapy methods to restore the structure and functions of damaged tissues in animals is becoming increasingly relevant. Modern orthopaedics emphasises the use of stem cells, which creates prospects for solving the problem of stimulating reparative osteogenesis. The study aimed to evaluate microscopic changes in the course of reparative processes in experimentally damaged bone tissue and the stimulating effect of intravenously administered allogeneic mesenchymal stem cells on it.

Literature Review

Musculoskeletal injuries in animals are quite common, and their nature depends significantly on the mechanism of occurrence, severity, and treatment methods. Bone tissue can be damaged both by external factors, such as trauma, and by internal processes in the body, such as tumour formation. According to H. ElHawary *et al.* (2021), the body of an animal is not capable of independently regenerating critically large bone defects. Bone tissue repair is often accompanied by impaired consolidation of bone fragments, as noted by researchers A.I. Alford *et al.* (2021). F. Shen & Y. Shi (2022) identified that difficulties in regenerating damaged bone can cause delayed union, non-union of fragments, or even the formation of false joints.

In modern medicine, various methods of clinical and tissue therapy are becoming increasingly relevant, demonstrating potential in the treatment of complex wounds and injuries. As noted by S. Avnet *et al.* (2021), many of these cases cannot be effectively treated using traditional methods. According to O. Wittig *et al.* (2016), cell biotechnologies are notable, as they create new opportunities, particularly in

the regeneration of bone tissue in conditions of its deficiency. M. Jayankura *et al.* (2021) suggest local implantation of allogeneic bone marrow MSCs as a treatment option for animals with delayed union or non-union of damaged bones, which promotes the replacement of defective or missing osteoblastic cells. Mesenchymal stem cells are a type of cell with a characteristic immunophenotype that has the ability to differentiate into cells of various organs and tissues. This process depends on microenvironmental factors, as noted by A.O. Luby *et al.* (2019) and Y. Ren *et al.* (2021). According to studies by V. Venkataiah *et al.* (2021), the use of MSCs demonstrates significant advantages, as it minimises the risks associated with the immunological and microbiological safety of the recipient.

Since the early 2000s, the therapeutic potential of mesenchymal stem cells has received considerable attention, as evidenced by O. Rister *et al.* (2020) and R.R. Bokotko *et al.* (2021), in an analysis of their effect on various pathological processes in the body. Experimental studies by M.P. Benavides-Castellanos *et al.* (2021) suggest that mesenchymal stem cells from bone marrow and adipose tissue have comparable potential for bone formation in vitro and in vivo. A study conducted by A.M. Dimarino *et al.* (2013) on rats demonstrated that MSCs can promote complete graft-to-bone union. Accordingly, bone grafts should be implanted with bone tissue stimulators or pre-treated with stem cells to ensure regeneration.

S.P. Bruder *et al.* (1998) determined that the use of a porous ceramic scaffold saturated with mesenchymal stem cells promoted a faster regenerative process in dogs with segmental femoral defects compared to cases where a scaffold without cells was used. In turn, E. Kon *et al.* (2000) demonstrated the positive effect of implanting a porous hydroxyapatite carrier enriched with MSCs on the course of the regenerative process in sheep. The use of MSCs in the process of xenogeneic matrix transplantation

contributed to the suppression of the foreign material rejection reaction, as indicated in the study by F.M. Elahi *et al.* (2020). In addition, the results of C.R. Harrell *et al.* (2021) demonstrate that stem cells can facilitate the intracellular recurrent response of the transplant and effectively reducing the risk of rejection in kidney transplantation in rats.

Following M. Ullah *et al.* (2019), Intravenous administration of MSCs within the first 24 hours after femoral fracture in mice promoted significant accumulation of these cells in the injury site after 7 days. This mechanism is key to the regeneration process, as it indicates the involvement of progenitor cells, which significantly contribute to bone tissue healing. In addition to their high differentiation potential, MSCs are also characterised by potent immunomodulatory and angiomodulatory properties, as highlighted by R. Otsuka *et al.* (2020) and Y. He *et al.* (2022).

An analysis of the current state of treatment of animals with bone regeneration disorders has shown that, despite a significant number of studies, there are still controversial and unresolved issues. Among them, the question of improving the process of reparative osteogenesis is relevant. In this regard, there is a need to introduce new effective methods that would improve the conditions for bone tissue regeneration. Therefore, research into the properties of animal stem cells and their use in experimental bone tissue damage is a substantial and relevant area that will contribute to the development of scientifically sound and effective approaches to cell therapy in veterinary medicine.

Materials and Methods

The experimental study was conducted during 2021-2022 at the educational and scientific laboratory "Centre for Cell Technologies in Veterinary Medicine", which operates based on the Department of Veterinary Surgery named after

Academician I.O. Povazhenko at the National University of Life and Environmental Sciences of Ukraine (NULES), Kyiv, Ukraine. Thirty-six three-month-old chinchilla rabbits, each with an average body weight of 3 kg, were used in the study. The conditions of keeping and feeding were the same for all animals and fully complied with the standard requirements for this species. The use of rabbits in experiments was conducted in compliance with the requirements of Directive 2010/63/EU (2010), Law of Ukraine No. 3447-IV (2006) and the permission of the bioethics committee of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Protocol No. 80-1 of 27 October 2020).

Mesenchymal stem cells were obtained from the bone marrow aspirate of the femur of clinically healthy donor rabbits. The extracted cell mass was cultured in a standard medium consisting of 80% DMEM (Gibco™, USA) and

20% calf foetal serum (Sigma, USA), with the addition of 10 µl/cm⁵ of antibiotic-antimycotic medium (VioWest, France). Cell isolation and cell material manipulation procedures were performed in a Class II biological safety cabinet (ESCO). Cultivation was conducted in a CO₂ incubator (HERA CELL, Germany) at 37°C and 5% CO₂ concentration. At the same time, MSCs settled, adhering to the surface of Petri dishes and spreading out. The suspended culture of haematopoietic cells was removed, after which only those cells that had adhesive properties were further cultivated. Cell passage was performed at a ratio of 1:2 (from one Petri dish to two) at a seeding density of 5 × 10⁴ cells/cm². Microscopic analysis of the culture was performed using a PrimoVert inverted microscope (Germany). Cells were harvested at the third passage, counted in the obtained substrate, and doses were prepared for administration (Fig. 1).

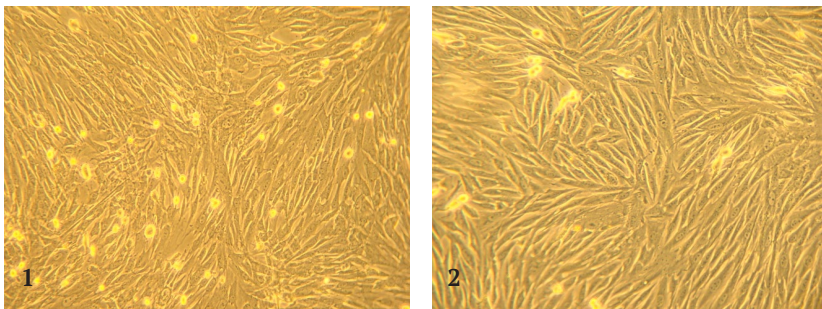


Figure 1. Unstained live culture of mesenchymal stem cells from rabbit bone marrow

Note: 1 – zero pass; 2 – third pass. ×100

Source: compiled by the authors

Two groups of animals were formed for the experiment: a control group and an experimental group (18 animals in each). At the initial stage of the study, animals in both groups were artificially subjected to mechanical damage to the tibia. To minimise trauma to healthy structures, the damage was created in the middle third of the tibial diaphysis from the medial surface in the form of a rounded defect using

a 2.5 mm diameter surgical drill. The operation was performed under general anaesthesia (Zoletil at a dose of 0.05 mg/kg of animal weight). Before this, infiltration anaesthesia was performed in surgical intervention with a 0.5% solution of Novocaine. Before the start of the operation, the 2 × 2 cm surgical field was vibrated and treated twice with a 5% iodine solution to ensure sterility. All stages of the surgical

intervention were performed in accordance with the rules of asepsis and antisepsis. The wound was sutured after forming a defect 0.5 mm deep and 2.5 mm in diameter, and the animal was brought out of anaesthesia. After modelling the experimental bone tissue damage, the animals in the experimental group were administered a single intravenous (into the jugular vein) injection of 3.5×10^6 allogeneic mesenchymal bone marrow stem cells in 0.5 mL of phosphate-buffered solution using a syringe. The animals in the control group did not receive treatment, and wound healing occurred naturally.

From each group, three animals were selected on days 3, 7, 14, 21, 28, and 42 of the experiment to obtain bone tissue samples, which were used for histological analysis. The selected tibia samples were labelled and fixed in a 10% aqueous solution of neutral formalin for 7 days. After that, the tissue was decalcified in a 5% nitric acid solution for 72 hours. Pieces 2-3 mm thick were cut from the decalcified bone, washed with tap water, dehydrated in solutions of ethyl alcohol with increasing concentration, and sealed with celluloidin.

For histological analysis, 7-9 μm thick sections were prepared using a microtome and stained with Karatsy's haematoxylin and eosin. The prepared specimens were examined under a Micros MSI 100 LED microscope (Germany). The histological sections were evaluated considering the surface structure of the bone, the characteristics of the newly formed tissue, and the presence and location of cellular elements in the damaged area.

Results and Discussion

The studies showed that on the third day of the experiment, in contrast to the control group (Fig. 2), no clots with blood cell elements were found in the experimental group animals in the created defect or in the adjacent bone marrow. In addition, no bone tissue fragments formed as

a result of bone defect modelling were recorded. At this stage of observation, the defect itself was filled with reticular tissue (Fig. 3).

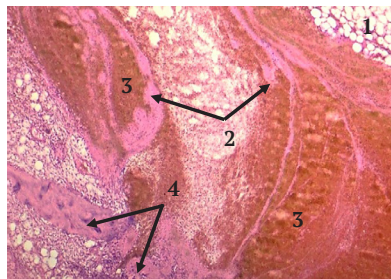


Figure 2. Condition of the defect on the third day of the experiment in animals of the control group

Note: 1 – bone marrow; 2 – fibrin strands; 3 – blood clots; 4 – bone fragments. Haematoxylin, Karatsi, and eosin, $\times 100$

Source: compiled by the authors

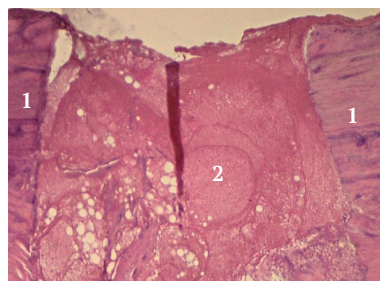


Figure 3. Condition of the defect on the third day of the experiment in animals of the experimental group

Note: 1 – bone tissue around the defect; 2 – reticular tissue at the site of the defect. Haematoxylin, Karatsi and eosin, $\times 50$

Source: compiled by the authors

The bone tissue located near the defect site in the experimental group animals had a typical microscopic structure characteristic of healthy bone. In the defect area itself, an active process of fibrous connective tissue formation was observed, which served as a kind of basis for further regeneration (Fig. 4). New foci of bone tissue

appeared in the structure of the bone marrow, indicating the activation of reparative mechanisms. However, this newly formed tissue had not yet reached the full morphological maturity characteristic of tubular bones. Osteocytes within this tissue were arranged chaotically, indicating the initial stage of new structure formation. In addition, the orientation of the newly formed osteons remained unregulated, which is natural for the early stages of bone regeneration.

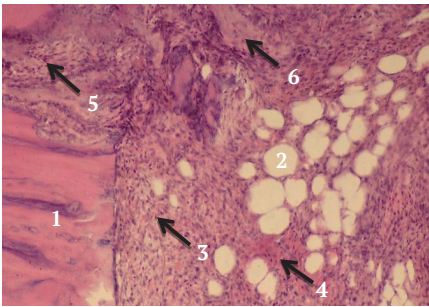


Figure 4. Condition of the defect on the third day of the experiment in animals of the experimental group

Note: 1 – bone tissue on the side of the defect; 2 – newly formed bone tissue; 3 – accumulation of red blood cells; 4 – reticular tissue; 5 – fibroblast proliferation; 6 – proliferation of fibrous connective tissue. Haematoxylin, Karatsi, and eosin, $\times 100$

Source: compiled by the authors

The processes of fibrous connective tissue growth and bone tissue formation in the damaged bone marrow area are activated by the intravenous administration of allogeneic mesenchymal stem cells. This procedure not only contributes to a significant increase in the mechanical strength of the tibia as a whole, but is also significant in compensating for the mechanical weakness caused by the defect. Thus, on day 3 after intravenous administration of allogeneic mesenchymal stem cells, the first phase of reparative regeneration was observed, manifested as the onset of the third stage of inflammation proliferation. In contrast, only the

first and second stages of the inflammatory process, including alteration and vascular reaction with exudation, were observed in the control group animals. The data obtained are consistent with the findings of studies conducted by R. Otsuka *et al.* (2020) and T. Duangchan *et al.* (2021), which showed that the introduction of mesenchymal stem cells contributes to the manifestation of their immunomodulatory properties and affects the course of the inflammatory process that occurs when bone tissue is damaged.

On the 7th day of the experiment, the animals in the experimental group showed significant defect repair, which differed significantly from the animals in the control group (Fig. 5). In the defect area, partial filling with bone tissue was observed, extending to the entire depth of the affected area. In addition, an active process of osteogenesis was detected, as evidenced by a significant number of fibroblasts and osteoblasts, which demonstrated high activity, producing structural elements necessary for the formation of bone tissue. The dynamics of these cells emphasise enhanced regeneration in the damage zone (Fig. 6).

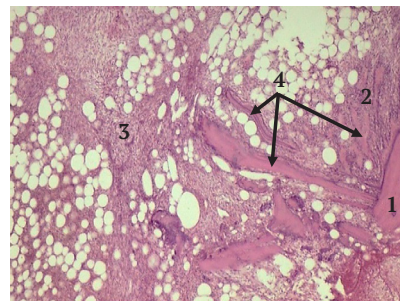


Figure 5. Condition of the defect on the 7th day of the experiment in animals of the control group

Note: 1 – bone tissue on the side of the defect site; 2 – proliferation of fibrous connective tissue in the bone marrow on the side of the defect site; 3 – proliferation of fibrous connective tissue in the bone marrow opposite the defect site; 4 – newly formed bone tissue. Haematoxylin, Karatsi, and eosin, $\times 50$

Source: compiled by the authors

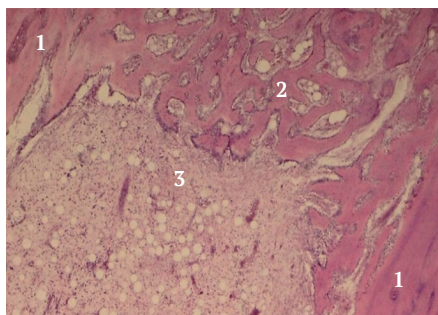


Figure 6. Condition of the defect on the 7th day of the experiment in animals of the experimental group

Note: 1 – bone wall; 2 – newly formed bone tissue; 3 – proliferation of fibrous connective tissue in the bone marrow. Haematoxylin, Karatsi, and eosin, $\times 50$

Source: compiled by the authors

In the area of the created bone marrow defect, active formation of fibrous connective tissue was observed, along with a pronounced process of osteogenesis. On the outside of the bone in the damaged area, small foci of bone formation could be seen, which were beginning to develop. At the same time, the osteon plates of the compact bone layer were in the early stages of differentiation, demonstrating a low level of structural maturity. On the outer side of the bone, in the defect area, active formation of bone callus was observed, consisting mainly of newly formed cartilage tissue. Within this tissue, there were occasional thin strands of fibrous connective tissue, which were insignificant in terms of quantity and structural density (Fig. 7). In addition, the initial formation of bone tissue was already noticeable, indicating the gradual restoration of the damaged area and the start of regeneration processes.

Similar results were recorded in studies conducted by S.P. Bruder *et al.* (1998) and S. Poliwoda *et al.* (2022), where a ceramic carrier enriched with mesenchymal stem cells was used. At the same time, the study showed that in animals of the experimental group, after the

introduction of stem cells, the process of cartilage and bone callus formation had already begun. Thus, on day 7, the animals in the experimental group showed the second phase of reparative regeneration differentiation. At the same time, the animals in the control group showed only the third stage of the inflammatory process, proliferation, which was accompanied by active growth of fibrous connective tissue in the defect area and a significant increase in osteogenesis.



Figure 7. Bone callus on the 7th day of the experiment in animals of the experimental group

Note: 1 – bone in the defect area; 2 – newly formed bone tissue; 3 – newly formed cartilage tissue of the bone callus; 4 – fibrous connective tissue. Haematoxylin, Karatsi, and eosin, $\times 50$

Source: compiled by the authors

On the 14th day of the experiment, the defect area in the experimental group animals was completely covered with newly formed bone tissue. According to the results of microscopic analysis, this tissue was characterised by a structure similar to cancellous bone (Fig. 8), which differed significantly from the condition in the control group animals (Fig. 9). In addition, active growth of fibrous connective tissue was observed in the bone marrow area, accompanied by an intense process of osteogenesis. Thanks to these regenerative changes, new bone tissue was formed in the

specified area, demonstrating a high level of regenerative capacity.

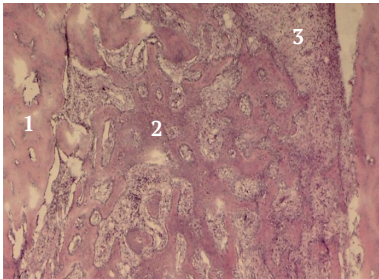


Figure 8. Condition of the defect on the 14th day of the experiment in animals of the experimental group

Note: 1 – bone wall; 2 – proliferation of fibrous connective tissue; 3 – newly formed bone tissue. Haematoxylin, Karatsi, and eosin, $\times 50$

Source: compiled by the authors

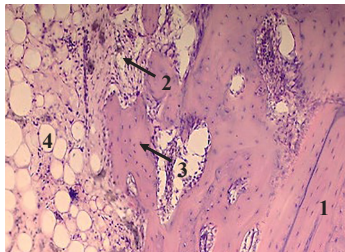


Figure 9. Condition of the defect on the 14th day of the experiment in animals of the control group

Note: 1 – newly formed bone tissue; 2 – fibrous connective tissue; 3 – osteogenesis in bone marrow; 4 – bone marrow. Haematoxylin, Karatsi, and eosin, $\times 100$

Source: compiled by the authors

On the surface of the newly formed bone tissue in the defect area in the experimental group, bone callus formation was observed. The bone callus was characterised by the presence of a thick layer of dense fibrous connective tissue (Fig. 10). Inside the bone callus, a fairly active process of osteogenesis was observed, indicating intensive regeneration and formation of bone structures in the studied area.

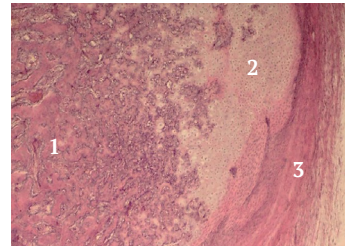


Figure 10. Bone callus on the 14th day of the experiment in animals of the experimental group

Note: 1 – dense fibrous connective tissue; 2 – cartilage tissue; 3 – bone tissue. Haematoxylin, Karatsi, and eosin, $\times 50$

Source: compiled by the authors

Thus, on day 14, the animals in the experimental group underwent the third phase of reparative regeneration reorganisation, while the animals in the control group underwent the second stage of this process differentiation with active formation of tissue-specific structures and cell differentiation in the damage area. Studies by Y. Ren *et al.* (2021) demonstrated similar changes with the use of mesenchymal stem cells for the regeneration of bone tissue defects in dogs. In subsequent works, R.D. Calixto *et al.* (2023) and Y. Guo *et al.* (2024) confirmed the ability of stem cells to undergo osteogenic differentiation, which is central in the process of bone defect consolidation during this period.

On the 21st day of the study, the defect site in the experimental group animals was filled with newly formed bone tissue. The structure of this tissue was similar to the microscopic structure of compact bone, where osteoid-like formations were already visible, indicating an active phase of regeneration (Fig. 11). Microscopic examination of the bone callus revealed that its structure corresponded to the characteristic features of compact bone. The outer layer of the callus was covered with dense fibrous connective tissue, which provided additional protection and insulation from the external environment.

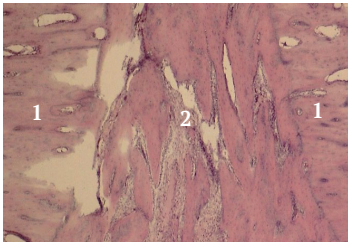


Figure 11. Condition of the defect on the 21st day of the experiment in animals of the experimental group

Note: 1 – bone tissue around the defect; 2 – newly formed bone tissue. Haematoxylin, Karatsi and eosin, ×50
Source: compiled by the authors

Compared to the control group animals (Fig. 12), the newly formed bone tissue showed a higher degree of maturity. This was evidenced not only by the presence of isolated large irregularly shaped cavities, but also by the formation of new osteons, which is a characteristic feature of mature bone structure. In addition, numerous areas of increased calcium salt concentration were observed in the newly formed bone matrix, indicating active processes of mineral saturation of the tissue.

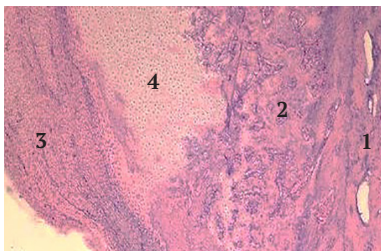


Figure 12. Condition of the defect on the 21st day of the experiment in animals of the control group

Note: 1 – newly formed bone tissue; 2 – inner part of the bone callus; 3 – outer part of the bone callus; 4 – large round cells. Haematoxylin, Karatsi and eosin, ×50
Source: compiled by the authors

According to the author's position, which is consistent with the data from studies by

M.P. Benavides-Castellanos *et al.* (2020) and W. Katagiri *et al.* (2022), there was an evident intensification of bone matrix calcification processes under the influence of allogeneic mesenchymal stem cells, which stimulated reparative osteogenesis. The structure of the bone marrow in the defect area was similar to the microscopic structure of intact bone marrow. There were isolated areas of newly formed bone tissue, and the level of cell proliferation in these areas was relatively low (Fig. 13).

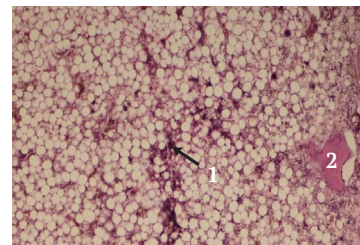


Figure 13. Bone marrow at the site of the defect on the 21st day of the experiment in animals of the experimental group

Note: 1 – cell proliferation; 2 – bone tissue centre. Haematoxylin, Karatsi, and eosin, ×100
Source: compiled by the authors

Thus, on day 21, the animals in the experimental group transitioned to the fourth phase of reparative regeneration remodelling. At that time, the animals in the control group were still in the third phase of this process, which involves the reorganisation of tissue structures and their gradual mineralisation. On day 28 of the experiment, the defect site in the experimental group animals was filled with compact bone tissue, the structure of which resembled the typical structure of compact bone. At the same time, its microscopic organisation remained somewhat disordered, and the channels remained quite wide (Fig. 14). Compared to the animals in the control group (Fig. 15), the bone callus in the experimental group was significantly smaller and showed signs of hardening

to bone. At the defect site, the newly formed bone tissue showed signs of greater maturity. The study noted that the surface of the bone callus was covered with a thick layer of dense fibrous connective tissue. Such changes may indicate a significant acceleration of bone tissue formation in the defect area due to the introduction of allogeneic MSCs.

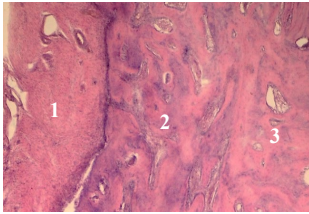


Figure 14. Condition of the defect on the 28th day of the experiment in animals of the experimental group

Note: 1 – dense fibrous connective tissue; 2 – bone callus; 3 – newly formed bone tissue at the site of the defect. Haematoxylin, Karatsi and eosin, $\times 50$

Source: compiled by the authors

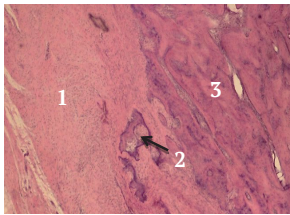


Figure 15. Condition of the defect on the 28th day of the experiment in animals of the control group

Note: 1 – dense fibrous connective tissue; 2 – bone callus; 3 – newly formed bone tissue at the site of the defect. Haematoxylin, Karatsi and eosin, $\times 50$

Source: compiled by the authors

Microscopic changes in bone tissue structure in animals of the experimental group, observed on day 28 of the experiment, indicate a transition to the fifth phase of reparative regeneration. This stage is characterised by the completion of recovery processes, which include not only

the return of tissue to its original form, but also the restoration of the functional properties of the bone structure. Similar early morphological changes at this stage may be an indicator of the activation of regeneration mechanisms, in particular the migration of stem cells to the site of bone damage and their influence on the process of osteogenesis, as noted by M. Ullah *et al.* (2019) and A. Theodosaki *et al.* (2024). At the same time, only the fourth phase of the regenerative process, which emphasises tissue remodelling, was observed in the control group animals.

On day 42 of the study, no bone callus was observed in the experimental group animals, unlike in the control group animals (Fig. 16). Newly formed bone tissue was observed in the defect area, which was already approaching the typical structure of compact bone. However, there were still some cavities in its structure, but their number was insignificant (Fig. 17). During the observation period, no other significant microscopic changes were found in the animals of the experimental group. At the same time, the animals in the control group underwent the fifth phase of reparative osteogenesis, which was characterised by the completion of the process: restoration of the structure and functional capacity of bone tissue.

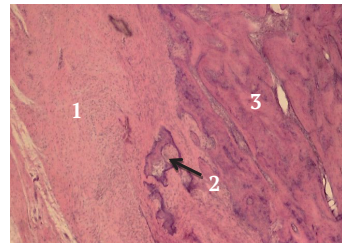


Figure 16. Condition of the defect on the 42nd day of the experiment in animals of the control group

Note: 1 – intact bone tissue on the side of the defect site; 2 – newly formed bone tissue; 3 – bone marrow; 4 – capillary hyperemia. Haematoxylin, Karatsi and eosin, $\times 50$

Source: compiled by the authors

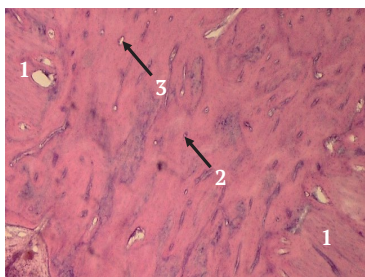


Figure 17. Condition of the defect on the 42nd day of the experiment in animals of the experimental group

Note: 1 – bone tissue on the side of the defect; 2 – osteon of newly formed bone tissue; 3 – osteon canal. Haematoxylin, Karasi and eosin, $\times 50$

Source: compiled by the authors

In general, the results obtained regarding the effect of allogeneic mesenchymal stem cells on the process of reparative osteogenesis showed a high degree of consistency with the data presented in the works of F. Shen & Y. Shi (2022) and J. Zou *et al.* (2023). These scientific works proved that mesenchymal stem cells can actively migrate to the sites of damage, thereby promoting accelerated restoration of damaged tissue areas and ensuring a more effective and rapid healing process. The current study provides a detailed analysis of the microscopic changes accompanying the process of bone tissue regeneration and investigates the effect of intravenous administration of allogeneic mesenchymal stem cells on the corresponding dynamics. The results indicate that the use of allogeneic MSCs accelerates the process of reparative osteogenesis, which highlights their therapeutic potential in the restoration of damaged bone tissue.

Conclusions

Histological studies conducted by experimentally modelling the pathological process in the tibia through mechanical damage with defined parameters can be used to track in detail the

stages of reparative regeneration of bone tissue. In addition, they provided reliable data on the effectiveness of intravenously administered allogeneic mesenchymal stem cells as a stimulator of reparative osteogenesis.

The study established that on the third day of the study, no blood clots or bone tissue fragments were observed in the bone tissue of the experimental group animals after intravenous administration of allogeneic MSCs in the created defect and in the adjacent bone marrow, unlike the animals in the control group. Intensive formation of new bone tissue and active growth of fibrous connective tissue in the created defect in the animals of the experimental group began on the third day of the experiment, while similar processes in the control group were recorded only on the seventh day. The process of cartilage callus formation in the experimental group began much more quickly and could be observed on the 7th day of the experiment, while in the control group, this process was delayed, appearing only on the 14th day of the study. The formation of bone callus in the experimental group was recorded on the 14th day of the experiment, while in the control group, the same process started much later and was recorded only on the 21st day of the experiment, which showed a significant difference in the rate of tissue regeneration. Bone marrow regeneration in the experimental group was established on the 21st day, while in the control group, similar changes were recorded only on the 28th day of the study.

Intravenous administration of allogeneic MSCs demonstrated the ability to significantly activate regenerative processes and accelerate the stages of reparative osteogenesis in the damaged area. Under these conditions, defect repair was practically completed by day 28 of the experiment, whereas in the control group animals, it was completed by day 42. The results obtained open up prospects for the introduction of new effective methods of

stimulating reparative osteogenesis, as well as creating a basis for more in-depth research into the effect of stem cells on the regeneration of damaged tissues. Accordingly, a promising direction for further scientific research is the use of allogeneic mesenchymal stem cells for the treatment of various clinical cases of bone tissue damage in animals.

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Conflict of Interest

None.

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Мікроскопічні зміни в експериментально ушкодженій кістці кролів за внутрішньовенного введення стовбурових клітин

Тарас Савчук

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-7351-5684>

Микола Малюк

Доктор ветеринарних наук, професор
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0003-3019-6035>

Юрій Харкевич

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-7877-8272>

Роман Бокотько

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-6217-5266>

Юлія Парамонова

Доктор філософії, асистент
Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0002-7197-3075>

Анотація. Актуальною проблемою сучасної ветеринарної травматології, котра потребує вивчення, є пошук способів та засобів стимулювання репаративного остеогенезу і одним із перспективних підходів у цьому напрямі вважається застосування стовбурових клітин. Метою досліджень було вивчити мікроскопічні зміни в експериментально ушкодженій кістковій тканині кролів за впливу на репаративний остеогенез внутрішньовенно введених аlogenних мезенхімальних стовбурових клітин. Мезенхімальні стовбурові клітини отримано з кісткового мозку кролів і культивовано у живильному середовищі згідно зі стандартними протоколами. Моделювання ушкодження виконували хірургічним свердлом діаметром 2,5 мм на медіальній поверхні середньої третини діяфіза великогомілкової кістки. Через добу тваринам дослідної групи одноразово внутрішньовенно (у яремну вену) вводили $3,5 \times 10^6$ стовбурових клітин. Відновлення дефекту у тварин контрольної групи відбувалось природним способом. Гістологічні дослідження проводили на 3, 7, 14, 21, 28 та 42 добу експерименту. Зразки забарвлювали гематоксиліном-еозином та вивчали під мікроскопом. Внутрішньовенне застосування тваринам аlogenних мезенхімальних стовбурових клітин стимулювало регенеративні процеси та прискорювало етапи репаративного остеогенезу в

місці пошкодження. Встановлено, що у дослідній групі вже на 3 добу в зоні ушкодження були відсутні згустки крові і уламки кісткової тканини та зафіксовано розростання волокнистої сполучної тканини й інтенсивний остеогенез. Формування кісткового мозоля та консолідація кісткової тканини проходили швидше ніж у контрольній групі. До 21 доби кістковий мозок набував вже нормальної будови, тоді як у контрольній групі аналогічний результат спостерігали лише на 28 добу. Практично повне відновлення дефекту за введення стовбурових клітин спостерігалось на 28 добу, а у контрольної групи аналогічний результат досягався лише на 42 добу. Результати дослідження можуть бути використані для стимулювання репаративного остеогенезу та подальшого вивчення процесів впливу стовбурових клітин на відновлення ушкоджених тканин

Ключові слова: репаративний остеогенез; кісткова тканина; кісткова мозоль; кістковий мозок; аlogenні мезенхімальні стовбурові клітини