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Histological changes in the experimentally damaged bone tissue of rabbits following injection of stem cells

T. L. Savchuk*, A. Y. Mazurkevich*, M. O. Malyuk*, I. O. Kharkevych*, R. R. Bokotko*, L. V. Kladnytska*, Y. S. Masalovych*, Y. V. Paramonova*, Y. V. Zhuk*, R. O. Dymko*, O. V. Kruchynenko**

*National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
**Poltava State Agrarian University, Poltava, Ukraine

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National University of Life and Environmental Sciences of Ukraine, Heroiv Oborony st., 15, Kyiv, 03041, Ukraine. Tel.: +38-097-897-48-40. E-mail: t_sav4uk@nubip.edu.ua

Poltava State Agrarian University, Poltava, 36003, Ukraine. Tel.: +38-099-062-64-96. E-mail: oleg.kruchynenko@pdau.edu.ua

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Bone defects are the results of pathological factors that disrupt the integrity of the bones and cause losses of the bone tissue or its absence. Disruption or hindering of the regeneration processes of the damaged bone tissue due to complications occur at a quite high rate and are the main problems in the bone tissue engineering. An effective treatment option is mesenchymal stem cells of mammals. In fact, they are considered the most promising type of autoimmune and allogeneic material in the cell regeneration therapy. We conducted a histological analysis of the damaged tibia of the rabbits following the injection of allogeneic mesenchymal stem cells. For this purpose, we used the mesenchymal stem cells from the bone marrow of the rabbits. The cells were cultivated in a CO_2 incubator using standard methods. The injury of the bone tissue was modeled using a surgical drill on the three-month-old rabbits of the chinchilla breed, in the middle third part of the diaphysis of the tibia. The animals were locally injected with allogeneic mesenchymal stem cells. The tissue samples from the defect region for histological studies were collected on days 3, 7, 14, 21, 28, and 42. The obtained histological sections from the injured area had been stained with hematoxylin-eosin and were analyzed under a microscope. The histological analysis of the experimentally damaged tibia revealed that the injection of allogeneic mesenchymal stem cells expedited the formation of fibrous connective tissue and fibrocartilage callus, stimulated osteogenesis, and promoted a consolidation of the bone tissue. At the same time, we observed the healing of the defect, which completed almost on day 28 of the study in the experimental animals, in contrast to day 42 in the control animals. We assume that mesenchymal stem cells – as multipotent stem cells – have immunomodulating properties and the capacity to osteogenically differentiate. Also, we think that allogeneic mesenchymal stem cells intensified the regeneration processes and enhanced the phases of reparative osteogenesis in the defect zone of the tibia.

Keywords: reparative osteogenesis; fibrocartilage callus; bone tissue; bone marrow; histological section; osteocyte; osteoblast; fibroblast.

Introduction

Despite the advancements in veterinary traumatology and orthopedics, the number of complications associated with impaired and slowed regeneration processes in the bone tissue remains quite high. Therefore, the development of new methods of enhancing the regeneration of damaged bone tissue is crucial (Fung et al., 2012; Calixto et al., 2023). Traumatized bone tissue repairs through the mechanisms of physiological and reparative regeneration based on the general biological patterns (Ankrum & Karp, 2010; Herrmann et al., 2019). At the same time, reparative regeneration is to some degree enhanced physiologically (Zhang et al., 2010; Li et al., 2022).

Complete repair of the bone tissue often occurs with impaired consolidation of bone fragments (Seitz et al., 1992; Venkataiah et al., 2021). Even if the bone fragments are accurately aligned and fixated in the necessary position, regeneration can happen in a number of morphological variants and bone healing can take different amounts of time due to high sensitivity of the bone tissue to changes in its environment (Alegre et al., 2012; Thormann et al., 2014). This results in slowed union or even nonunion of the bone fragments and formation of false joints, and the inability of large defects to heal spontaneously (Grassi & Isaksson, 2015; Al-Sharabi et al., 2023).

The aforementioned factors encourage the researchers to seek modern methods and means to stimulate the regeneration processes in a damaged bone particularly at the first stages to ensure the fastest possible consolidation of the fragments of the bone tissue.

Cell regeneration therapy using stem cells is increasingly being used in the hope of successfully treating wounds and traumas that cannot be effectively addressed with modern methods (Broxmeyer et al., 2006; Harrell et al., 2021).

A subject of significant interest is using cellular biotechnology to treat animals with traumas of the locomotor apparatus. Application of bone precursor cells in order to optimize the course of reparative processes in cases of fractures and their complications has been pathogenetically substantiated (Dimarino et al., 2013; Murayama et al., 2024).

Mesenchymal stem cells (MSCs) of mammals are considered the most promising type of autogenic and allogeneic material in cell regeneration therapy (Clines, 2010; Paramonova et al., 2024). One of the orthodox pathways of differentiation of mesenchymal stem cells of an adult organism is osteogenic (Chanda et al., 2010; Avnet et al., 2021). In certain conditions, mesenchymal stem cells differentiate into cells of the cartilage and bone tissues. Likewise, effective clinical use of mesenchymal stem cells in treatment of animals with pathologies of the locomotor apparatus has been reported in numerous studies, which underpin the scientific rationale for this method and indicate its scientific significance (Koch et al., 2008; Gutierrez-Nibeyro, 2011; Elahi et al., 2020; Savchuk et al., 2022).

A number of authors have confirmed the possibility of using MSCs *in vitro* to induce osteogenesis (Bruder et al., 1994; Impieri et al., 2024). During cultivation of MSCs, elements of the fibrillar intercellular matter were synthesized – collagen matrix, on which mineral components settled, forming mineralization nodes, which is a characteristic feature of the osteoblastic phenotype (El Hawary et al., 2021). In recent years, it has become known that mesenchymal stem cells have some other unique peculiarities – low immunogenicity and immunosuppressive properties, and also play a role of modulators of

lymphocyte–lymphocyte interactions. These properties of MSCs became broadly utilized in cell therapy (English, 2013; Katagiri et al., 2022). The literature sources provide a number of reports about using stem cells (SCs) to activate osteogenic repair (Godwin et al., 2012; Zou et al., 2023). Experiments conducted on rats demonstrated the properties of MSCs that promote complete union of the transplant and bone (Zhang et al., 2010; Thurairajah et al., 2017). Thus, bone defects in the skulls of the mice were filled with a gelatin implant with genetically labeled allogeneic MSCs (Ankrum & Karp 2010; Luby et al., 2019). In two weeks, the defect was filled with the bone tissue that comprised 99% of the donor-derived cells, while no regeneration occurred in the control. Moreover, using SCs in transplantation of xenogeneic matrix led to inhibition of the reaction of rejecting foreign material (Kittaka et al., 2015; Shen et al., 2022).

When employing a ceramic porous carrier with mesenchymal stem cells, the regenerative process occurred faster in the dogs with segmental defect of the tibia, compared with the animals of the control group on which the ceramic carrier was used without MSCs (Bruder et al., 1998; Poliwoda et al., 2022).

One of the methods to optimize surgical treatment after traumatic disturbances of the union of bone fragments is the method of autotransplantation of the bone marrow cells (Griffin et al., 2011; Wittig et al., 2016). Good results were demonstrated by using autogenic bone marrow in combination with crystalline chymotrypsin (Grottkau et al., 2013; Riester et al., 2020). Transplantation of cultivated osteogenic cells of bone marrow in collagen gel caused up to 30% increase in the share of the bone tissue in the regenerated area, compared with control group of animals, as early as on day 120 of the experiment (El Tamer & Reis, 2009; Xu et al., 2021).

Also, research has been conducted on the effects of implantation of a porous hydroxyapatite carrier with MSCs on the course of the regenerative process in a bone defect in sheep. The yielded data were consistent with the data of other studies on using stem cells (Kon et al., 2000). Experiments on dogs revealed that using the matrix with hydroxyapatite tricalcium phosphate inhabited with allogeneic stem cells allowed repairing the defects of the femur without immunosuppression (Arinzeh et al., 2003; Pan et al., 2024).

Therefore, using the achievements of cell biology and cell technologies is one of the relevant experimental clinical directions in the development of treatment of patients of traumatologic and orthopedic profiles (Griffin et al., 2011). This is a relatively new approach in medicine that has been developing for only several decades (Zhang et al., 2024).

Thus, the analysis of the modern state of the problem of treating patients with disorders of bone regeneration revealed that despite numerous studies, there are a number of controversial and unsolved issues. The most important of them is the problem of optimizing the reparative bone formation, i.e. creating the most favorable conditions for the organism to realize its own osteogenic capacities. Therefore, there is a necessity for searching for new factors that improve the optimal conditions of bone regeneration. The methods of cell therapy for repairing the structure and functions of pathologically altered tissues in animal organism are gaining popularity, and stem cells can be the factor that can solve the problem of stimulating reparative osteogenesis.

Accordingly, research on the capacities of stem cells of animals and using them for treatment of experimental damage to the bone tissue is a quite relevant and timely task, which could aid in developing scientifically substantiated and effective methods of cellular therapy in veterinary medicine.

Thus, our objective was conducting a histological assessment of the course of reparative process in the bone tissue in case of experimental damage and studying how allogeneic mesenchymal stem cells of the bone marrow stimulate the healing.

Materials and methods

In total, we used 36 three-month-old chinchilla rabbits, each weighing about three kg. Maintenance and feeding was the same for all the animals and corresponded to all of their requirements. The animals were used in the experiments with adherence to the requirements of the directive of the European Parliament and the European Council (No. 2010/63/EU as of September 22, 2010) and the permit from the Bioethical Commission of the Faculty of Veterinary Medicine of the National Universoty of Life and Environmental Sciences of Ukraine (No. 80-1 as of October 27, 2020). On all the animals, an experimental trauma of the tibia (2.5 mm in diameter and 0.5 mm deep) was inflicted. There were used 18 animals as the transplantation group that were singly administered allogeneic mesenchymal stem cells, and the rest 18 comprised the control.

The mesenchymal stem cells were obtained from an aspartate of the tibial bone marrow of the clinically healthy donor rabbits. The obtained cellular mass was cultivated in a standard medium: 80% DMEM and 20% serum of calf embyos (manufactured by Stigma, USA), with addition of 10 μL/cm^3 of the medium of antimycotic antibiotic.

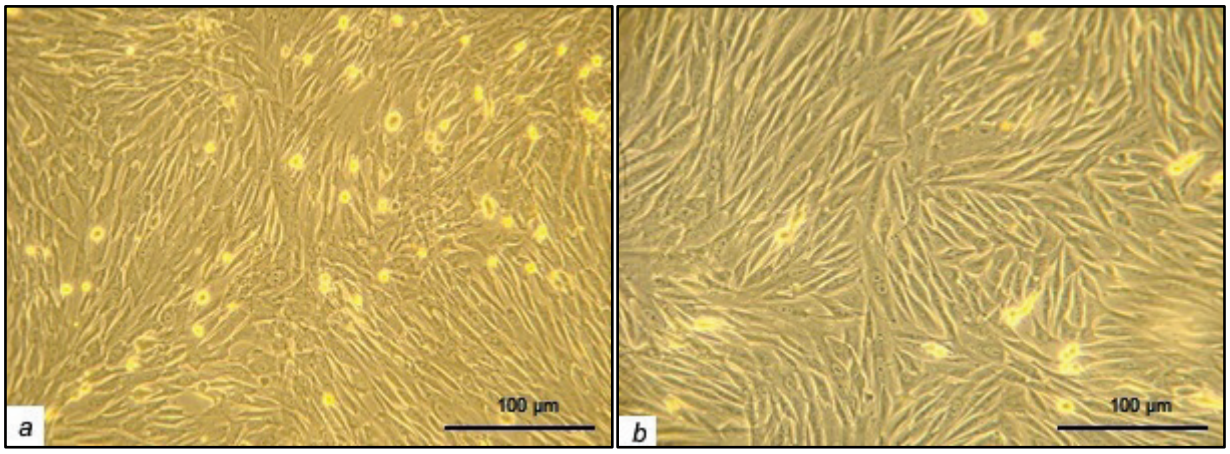

Fig. 1. Live non-stained culture of mesenchymal stem cells of the rabbit bone marrow: *a* – zero passage; *b* – third passage

The cultivation was conducted in a CO_2 incubator at 37 °C and in 5% concentration of CO_2. During the procedure, MSCs settled, attaching to the surface of Petri dishes, and proliferated. The suspended culture of hematopoietic cells was removed, and only those cells were further cultivated that had adhesive properties. The passage of the cells was performed in a 1:2 ratio (from one Petri dish they were inoculated into two), the inoculation dose measuring 5x10^4 cells/cm^2 (Mazurkevych et al., 2016; Bokotko et al., 2021). The microscopic analysis of the culture was carried out using an inverted microscope Axiovert 40 (Carl Zeiss). After the third passage, the cells were removed and counted in the obtained substrate and doses for injection were prepared (Fig. 1).

Modeling pathological process in the bone tissue. In order to minimize the level of general traumatism, the damage to the bone tissue

was inflicted in the middle third of the diaphysis of the tibia, on the medial surface, in the form of a round defect using a surgical drill of a 2.5 mm diameter. The operation was performed under general narcosis (Zoletil in calculation of 0.05 mg/kg of animal weight). Furthermore, in the area of operative access, anesthesia was previously introduced with a 0.5% Novocain solution. An operative 2×2 cm field was shaved and treated twice with a 5% solution of iodine (the Filonchikov's method). All the procedures with surgical intervention were conducted with adherence with the rules of aseptic techniques and antiseptics. After the formation of a 0.5 mm deep defect of a 2.5 mm diameter, the surgical wound was stitched, and the animal was withdrawn from narcosis.

A day after the modeling of experimental damage to the bone tissue, the animals of the experimental group were singly injected with 3.5×10^6 allogeneic mesenchymal stem cells per 0.5 mL of phosphate buffer solution using an insulin syringe into the region of the damaged bone. The animals of the control group were singly injected with a 0.5 mL phosphate buffer solution in the area of damaged bone.

During 42 days, the experimental animals were monitored. From each group, three animals were withdrawn on days 3, 7, 14, 21, 28, and 42 of the experiment and samples of the bone tissue were collected for macroscopic study.

The histological studies were performed according to the manual. For this purpose, the collected samples of the tibia were labeled and fixated in a 10% aqueous solution of neutral formalin for 7 days. After fixation, a decalcification of the bone tissue was conducted in a 5% solution of nitric acid for 72 days. Then, from the decalcified bone, 2–3 mm thick samples were cut out. The sampled pieces were rinsed in tap water, dehydrated in aqueous solutions of ethyl alcohol of ascending concentration and were densified using celloidin. The 7–9 μm-thick histological sections were preparared using a sledge microtome, and were stained with Carazzi's hematoxylin and eosin. Under a Micros MSI 100 LED microscope, the stained histological preparations were evaluated, considering the superficial structure of the bone, structure of the newly formed tissue, and also the presence and pattern of positions of cellular elements in the damaged region.

Results

On day 3 of the experiment, in the rabbits of the experimental group, the defect region was filled with a newly formed fiborous connective tissue, which contained a relatively small quantity of bundles of collagen fibers. In addition, a relatively intensive osteogenesis was already observed in it (Fig. 2a). In the bone marrow, near the defect region, no blood cells or fragments of the bone tissues were observed, unlike the animals of the control group (Fig. 2d). In the region of the bone defect, the external surface was already covered by a thick layer of dense fibrous connective tissue. At the same time, within the bone marrow beside the defect and the fibrous connective tissue had extended further (Fig. 2c). Such changes following the injection of allogeneic mesenchymal stem cells promoted a significant increase in the mechanical resilience of the recovering region of the tibia.

Therefore, this indicates that following the injection of mesenchymal stem cells, the first phase of reparative regeneration has developed, taking place in the form of the beginning of the third stage of inflammation. Meanwhile, the animals of the control group were undergoing only the first and second stages of inflammation (alteration and vascular reaction with exudation), and were observed to have fragments of the bone tissue in a state of destruction (Fig. 2b), blood clots in the region of the defect, and enlargement of the blood vessels.

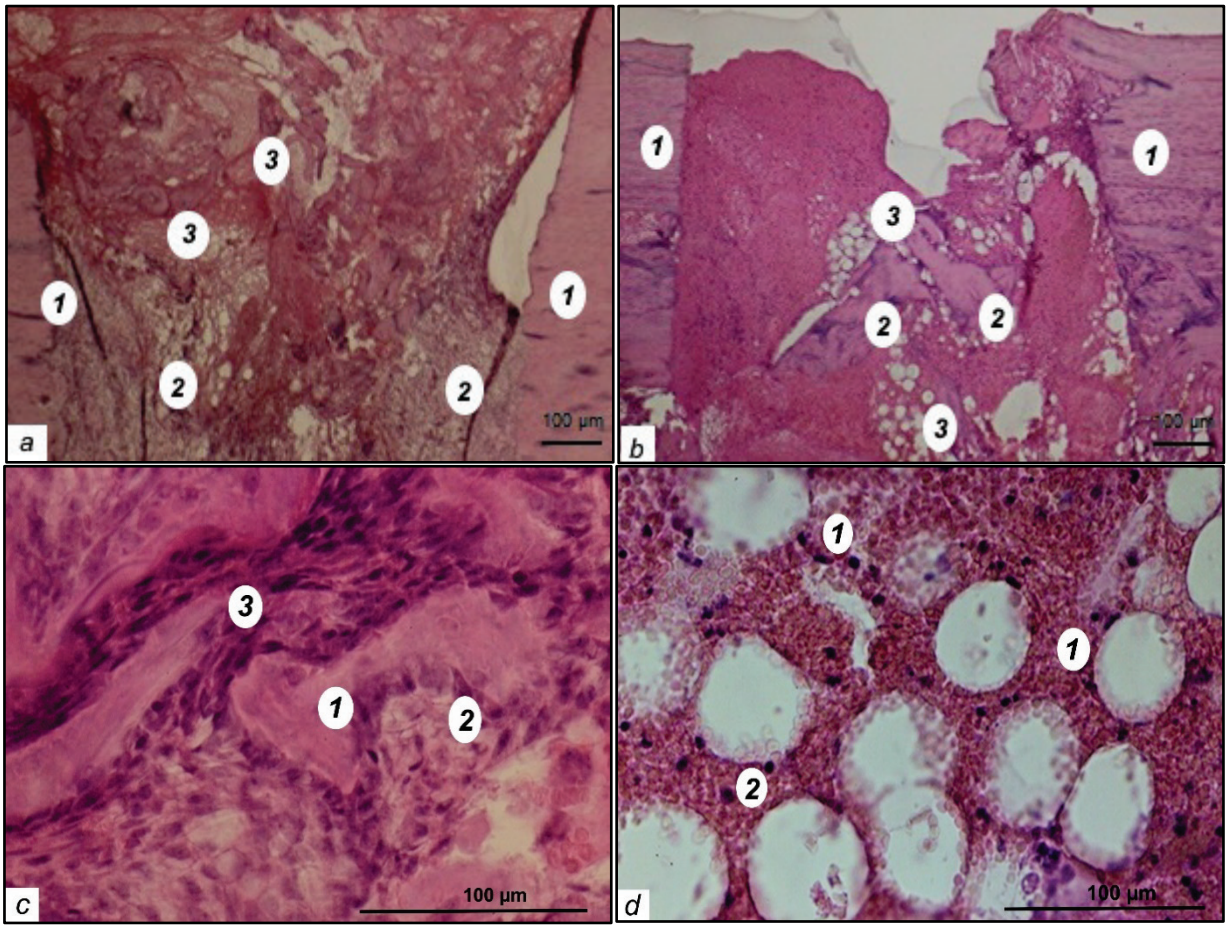

Fig. 2. The defect region of day 3 of the experiment: *a* – the bone tissue around the defect region (*1*), expansion of the fibrous connective tissue (*2*), the newly formed bone tissue (*3*); *b* – the bone tissue around the defect (*1*), bone fragments (*2*), extension of the bone marrow into the defect region (*3*); *c* – the bone tissue (*1*), osteoblasts (*2*), the fibrous connective tissue (*3*); *d* – infiltration with erythrocytes (*1*), non-differentiated cells (*2*); hematoxylin and eosin

On day 7 of the experiment, in the rabbits of the experimental group, the defect region was overgrown by the bone tissue along the entire depth and was already covered by a notable fibrocartilage callus (Fig. 3a). In the defect region, intensive osteogenesis was observed (Fig. 3e). In the bone marrow, in the region of the modeled defect and near it, we observed enlarged capillaries, overfilled with blood, pronounced growth of the fibrous connective tissue, and a quite intensive osteogenesis (Fig. 3c).

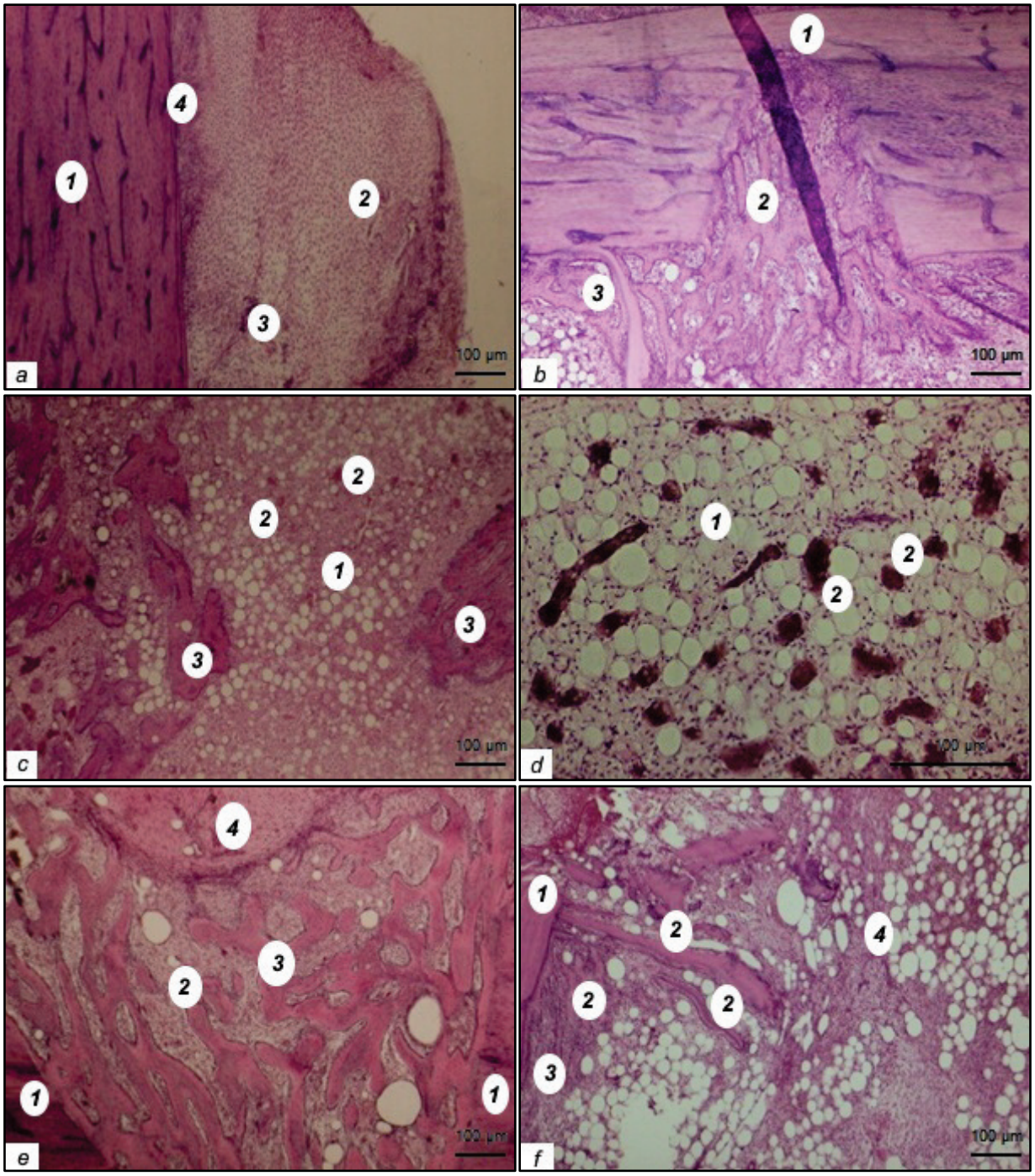

Fig. 3. The defect region on day 7 of the experiment: *a* – the bone in the defect region (*1*), the newly formed cartilage tissue of the fibrocartilage callus (*2*), the fibrous connective tissue (*3*), the newly formed bone tissue (*4*); *b* – partial replenishment of the defect by the newly formed bone tissue (*1*), osteogenesis in nonunion area of the defect region (*2*), osteogenesis beside the defect (*3*); *c* – the fibrous connective tissue (*1*), enlarged capillaries overfilled with blood (*2*), the newly formed bone tissue (*3*); *d* – immature cells (*1*), enlarged capillaries overfilled with blood (*2*); *e* – the bone tissue around the defect (*1*), the fibrous connective tissue (*2*), the newly formed bone tissue (*3*), dense fibrous connective tissue above the defect region (*4*); *f* – the bone tissue beside the defect region (*1*), the newly formed bone tissue (*2*), expansion of the fibrous connective tissue in the bone marrow beside the defect region (*3*), expansion of the fibrous connective tissue in the bone marrow on the opposite side of the defect region (*4*); hematoxylin and eosin

In the rabbits of the control group, the defect region on the side of the periosteum was only partly filled with the compact bone tissue, which did not attain a typical microscopic structure. In the bone marrow, beside and on the opposite side of the defect region itself, we noted growth of the fibrous connective tissue (Fig. 3b). In the region of the defect and on both sides of it, intensive osteogenesis was observed. In the bone marrow near the defect region, we saw expressed proliferation of immature cells and single megakaryocytes (Fig. 3f). From the defect region to the opposite part of the shaft, through the entire bone marrow, and perpendicular to the defect region, lay the

cords of dense fibrous connective tissue. Remote from the defect region, in the bone marrow, we observed enlarged blood capillaries, oversaturated with blood (Fig. 3d).

This means that on day 7 of the studies, the rabbits of the experimental group have been undergoing the second phase of reparative regeneration – differentiation, while the animals of the control group were in the third stage of inflammation – proliferation, with growth of the fibrous connective tissue in the region of the defect and intensive osteogenesis. On day 14 of the experiment, in the rabbits of the experimental group, the defect region was already completely overgrown by the newly formed bone tissue, the microscopic structure of which was similar to such of the short bone (Fig. 4a).

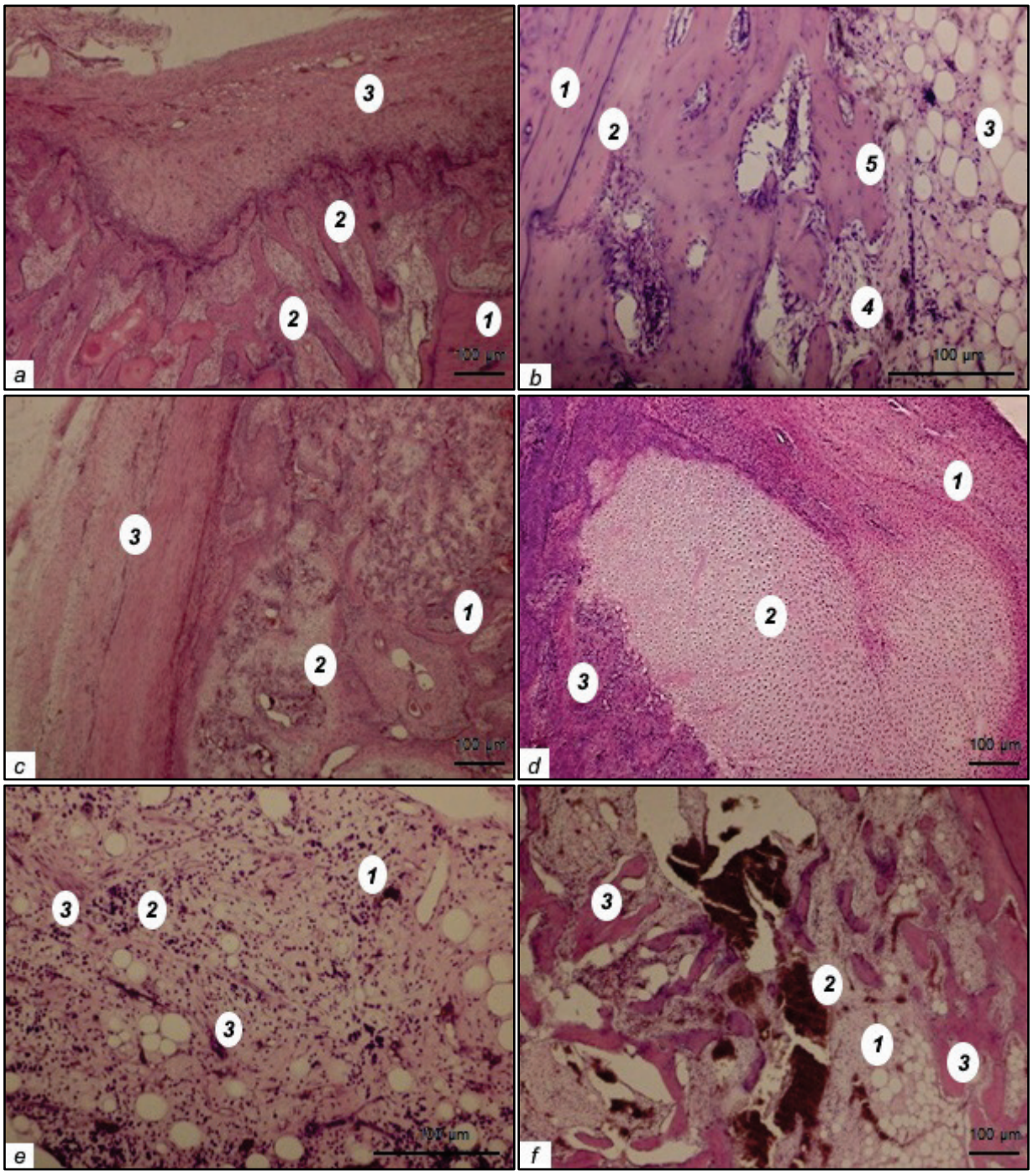

Fig. 4. The defect region of day 14 of the experiment: *a* – the bone tissue beside the defect (*1*), the newly formed bone tissue in the defect region (*2*), the fibrous connective tissue (*3*); *b* – the newly formed bone tissue (*1*), the surface of the newly formed bone region (*2*), the bone marrow (*3*), the fibrous connective tissue (*4*), osteogenesis in the bone marrow (*5*); *c* – the bone tissue (*1*), the cartilage tissue (*2*), the dense fibrous tissue (*3*); *d* – the external part of the fibrocartilage callus (*1*), a site of large round cells (*2*), the internal part of the fibrocartilage callus (*3*); *e* – enlarged capillary oversaturated with blood (*1*), cell proliferation (*2*), the fibrous connective tissue (*3*); *f* – the fibrous connective tissue (*1*), an enlarged vein overfilled with blood (*2*), the newly formed bone tissue (*3*); hematoxylin and eosin

On the surface of the newly formed bone tissue, in the place of the defect, fibrocartilage callus was observed, represented by a thick layer of dense fibrous connective tissue (Fig. 4c). In the bone marrow adjacent to the defect, we saw expansion of the fibrous connective tissue and an intensive osteogenesis with formation of the bone tissue in this area, stretching from the defect region to the bone wall on its opposite side (Fig. 4e).

In rabbits of the control group, the defect region had already been completely filled with newly formed bone tissue, the microscopic structure of which was similar to the microscopic structure of the

compact bone (Fig. 4b). Unlike the typical osteons of the compact bone, lamellae in the osteon-like structures of the newly formed bone were poorly differentiated, the osteocytes were located unevenly, and the orientation of the newly formed osteons had no organized arrangement. On the external surface of the tubular bone, in the defect region, fibrocartilage callus has formed, which contained a large amount of intercellular matter, non-differentiated cells, and fibroblasts (Fig. 4d). In the bone marrow, we observed enlarged capillaries oversaturation with blood, expansion of the fibrous connective tissue, and formation of the bone tissue (Fig. 4f).

This means that while the rabbits of the experimental group have been undergoing the third phase of reparative regeneration, reorganization, the animals of the control group were in the second stage of reparative regeneration – differentiation, i.e. differentiation of cells and formation of tissue-specific structures in the region of trauma.

On day 21 of the experiment, the defect region in the rabbits of the experimental group was completely filled with newly formed bone tissue, with a microscopic structure similar to that of compact bones, in which osteon-like structures were already observed. However, this newly formed bone tissue contained large cavities of different forms. On the surface of the bone, fibrocartilage callus was seen, the microscopic structure of which was similar to such of the spongy bone (Fig. 5a). The microscopic structure of the bone marrow in the defect region was similar to the intact bone marrow (Fig. 5c). Furthermore, the newly formed bone matrix contained quite numerous sites of heightened concentrations of calcium salts.

In the rabbits of the control group, on the bone surface in the defect region, fibrocartilage callus was seen. Its external layer was presented by dense fibrous connective tissue. In the internal area of the fibrocartilage callus, which was adjacent to the external surface of the newly formed bone, intensive osteogensis processes were observed (Fig. 5b). In the bone marrow, near the defect region, we saw single small sites of proliferation of immature cells and osteogenesis processes (Fig. 5d).

In our opinion, such changes indicate a notable enhancement of the processes of calcification of the bone matrix through the influence of allogeneic mesenchymal stem cells on reparative osteogenesis. The development of the fourth phase of reparative regeneration – remodeling – was observed in the animals of the experimental group, while only the third phase of reparative regeneration – reorganization: reorganization of the tissue structures and their mineralization – was observed in the animals of the control group.

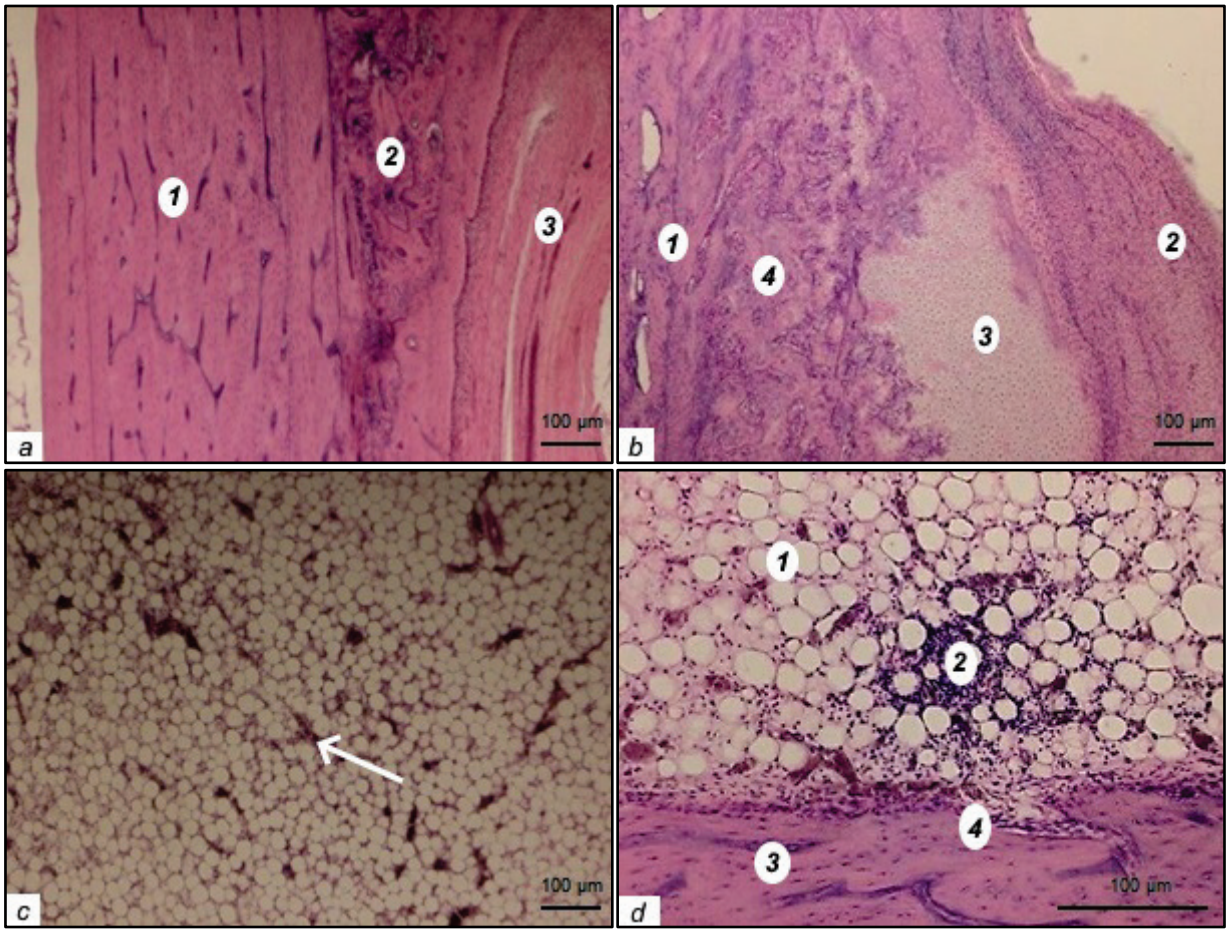

Fig. 5. The defect region on day 21 of the experiment: *a* – the bone tissue beside the defect region (*1*), the fibrocartilage callus (*2*), the dense fibrous connective tissue (*3*); *b* – the newly formed bone tissue (*1*), the external part of the fibrocartilage callus (*2*), a site of large rounded cells (*3*), the internal part of the fibrocartilage callus (*4*); *c* – a hematopoiesis site (indicated by the arrow); *d* – the bone marrow (*1*), a site of proliferation of immature cells (*2*), the newly formed bone tissue (*3*), osteoblasts (*4*); hematoxylin and eosin

On day 28 of the experiment, in the rabbits of the experimental group, the defect region was completely filled with the compact bone tissue that was quite similar to the typical structure of the compact bone, although still contained quite broad channels and had non-ordered microscopic structure (Fig. 6a). In contrast to the animals of the control group, the newly formed bone tissue in the defect region was more mature. Fibrocartilage callus has notably decreased and had densified into bone (Fig. 6c).

In the rabbits of the control group, fibrocartilage callus was notably reduced and was made up of dense fibrous connective tissue. Furthermore, the dense fibrous connective tissue that covered this callus contained calcification sites (Fig. 6d). The defect region was completely overgrown by the bone tissue that had not yet attained the typical microscopic structure (Fig. 6b). The external and internal surface of the newly formed bone tissue in the defect area was somewhat uneven. A notable osteogenesis was still observed.

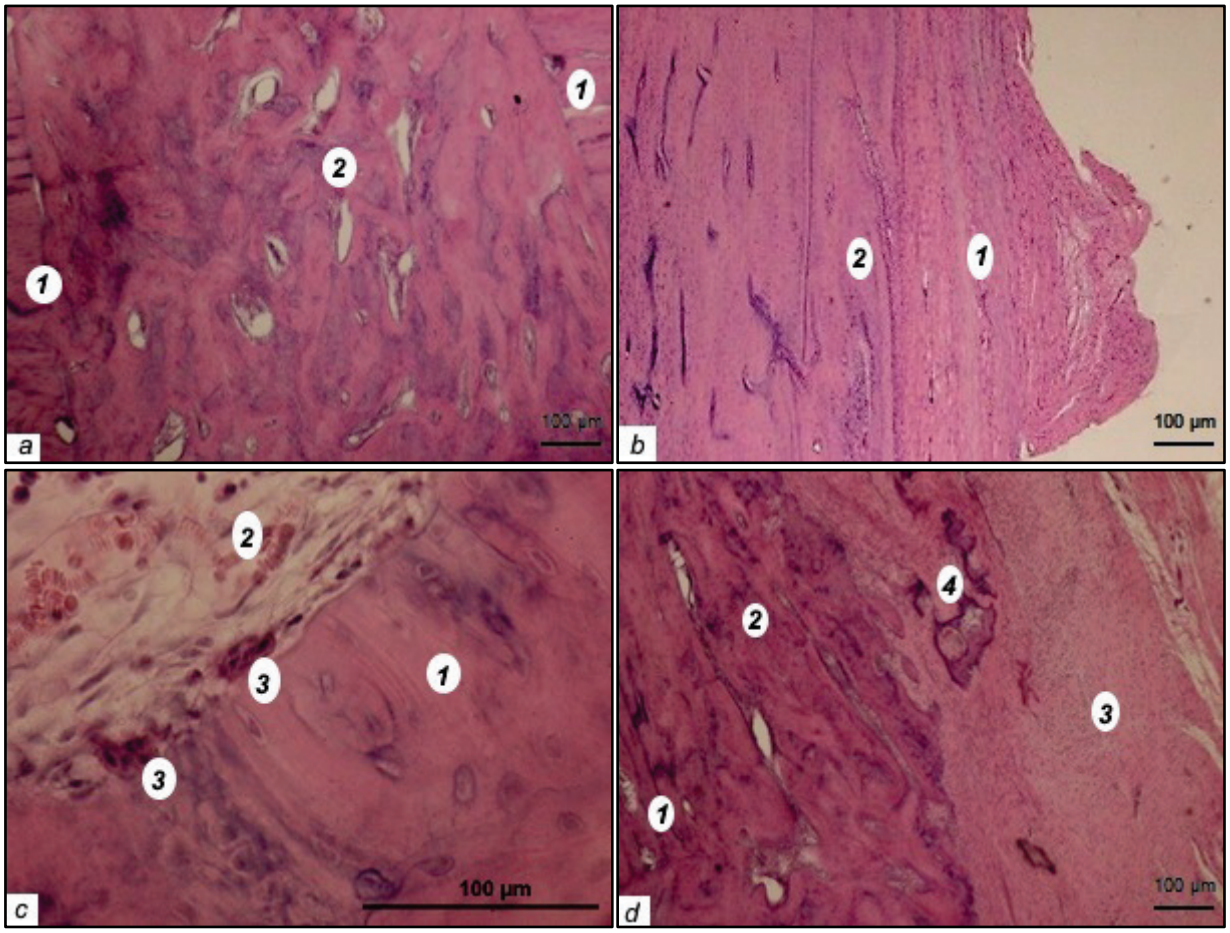

Fig. 6. The defect region on day 28 of the experiment: *a* – the bone tissue beside the defect (*1*), the newly formed bone tissue in the defect region (*2*); *b* – the fibrocartilage callus (*1*), the newly formed bone tissue (*2*); *c* – the bone tissue (*1*), the bone marrow (*2*), osteoclast (*3*); *d* – the bone tissue beside the defect region (*1*), the fibrocartilage callus (*2*), the dense fibrous connective tissue (*3*), a calcification region (*4*); hematoxylin and eosin

Such microscopic changes in the rabbits of the experimental group indicated the development of the fifth phase of reparative regeneration – completion: restoration of the form and function of the bone tissue. The animals of the control group were observed to undergo only the forth phase of reparative regeneration – remodeling: integration and adaptation of the replenished area.

On day 42 of the experiment, in the rabbits of the experimental group, fibrocartilage callus was gone and the newly formed bone tissue in the defect region was already quite similar to the typical structure of compact bone, with only single cavities noted in it (Fig. 7a).

In the rabbits of the control group, the fibrocartilage callus densified, becoming the bone, and the structure of the newly formed bone was similar to the typical microscopic structure of the compact bone. In it, there were single cavities where osteogenesis was still ongoing. In the bone marrow, near the defect region, the capillaries were enlarged and overfilled with blood (Fig. 7b). Thus, on day 42 of the experiment, no significant changes were observed in the rabbits of the experimental group. By contrast, the rabbits of the control group were undergoing the fifth phase of reparative osteogenesis – completion (restoration of the form and function of the bone tissue).

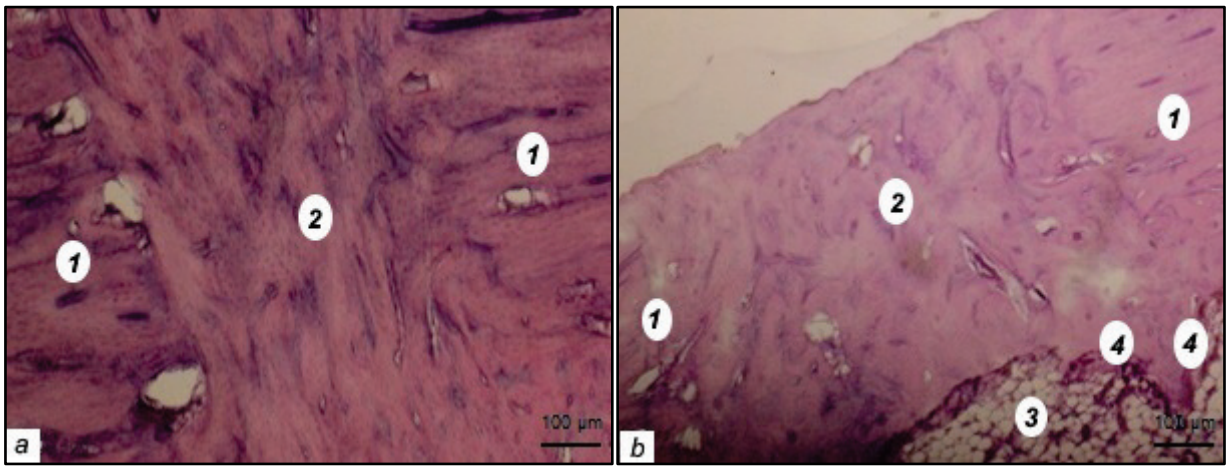

Fig. 7. The defect region on day 42 of the experiment: *a* – the bone tissue around the defect (*1*), the newly formed bone tissue (*2*); *b* – the intact bone tissue beside the defect region (*1*), the newly formed bone tissue (*2*), the bone marrow (*3*), a capillary hyperemia (*4*); hematoxylin and eosin

Discussion

In general, our results regarding the effect of allogeneic mesenchymal stem cells on the regeneration of the tissues of organism, the reparative osteogenesis, are consistent with the studies by a number of researchers (Grottkau Riester et al., 2020; Zou et al., 2023), in particular, regarding the ability of stem cells to expedite the repair of damaged bone region.

On day 3 after the injection of allogeneic mesenchymal stem cells, the rabbits of the experimental group were noted with the first stage of reparative regeneration. It manifested as the beginning of the third stage of inflammation – proliferation, with growth of the fibrous connective tissue in the defect region, intensive osteogenesis, and insignificant swelling of the soft tissues. In the animals of the control group, we observed only the first and second stages of inflammation (alteration and vascular reaction with exudation), presence of fragments of the bone tissue in the condition of destruction, blood clots in the defect region, and enlargement of the blood vessels. The data we yielded are in agreement with a number of studies conducted earlier. For example, stem cells exerted immunomodulating capacities (Mazurkevych et al., 2017; Katagiri et al., 2022) and affected the inflammatory process (Ren et al., 2021) that emerges as a result of injury of the bone tissue.

On day 7 of the study, in the rabbits of the experimental group, we observed the second phase of reparative regeneration – differentiation: formation of the tissue-specific structures in the region of injury, intensive osteogenesis with partial filling of the defect region with the bone tissue, and the formation of fibrocartilage callus. In the animals of the control group, we observed only the third stage of inflammation – proliferation, with growth of the fibrous tissue in the defect region and intensive osteogenesis. A similar picture was observed in the studies using a ceramic carrier with mesenchymal stem cells (Bruder et al., 1998; Poliwoda et al., 2022). At the same time, we already saw the formation of fibrocartilage callus, which has not been reported so far.

On day 14 of the study, the rabbits of the experimental group were observed to undergo the development of the third phase of reparative regeneration – reorganization: the formation of fibrocartilage callus, which completely covered the defect region, and decrease in the volume of the defect, which was filled with newly formed bone tissue. In the animals of the control group, we observed the second stage of reparative regeneration – differentiation, i.e. the cells have been differentiating and forming tissue-specific structures in the injured region. The authors assume that stem cells are able to osteogenically differentiate (Ding et al., 2011; Otsuka et al., 2020), affect the process of regeneration, and are significant in achieving the consolidation of bone defect.

On day 21 of the study, in the rabbits of the experimental group, the fourth phase of reparative regeneration – remodeling – was developing: the bone marrow was returning to its normal structure, the callus was decreasing, and its structure became similar to that of compact bone. In the animals of the control group, we saw only the third phase of reparative regeneration – reorganization: reorganization of the tissue structures and their mineralization. The provided explanations suggest the processes of calcification of the bone matrix through the action of allogeneic mesenchymal stem cells. Such changes were noted in the studies using mesenchymal stem cells for regeneration of the bone tissue defect in dogs (Arinzeh et al., 2003; Pan et al., 2024).

On day 28 of the study, the rabbits of the experimental group were observed to undergo the development of the fifth phase of reparative regeneration – completion: restoration of the form and function of the bone tissue, as indicated by the densification of fibrocartilage callus to the bone, and the newly formed bone in the defect region, similar to the compact bone. The animals of the control group were observed to undergo only the fourth phase of reparative regeneration – remodeling: integration and adaptation of the replenished bone. The data of the studies are consistent with the data of other studies regarding the effects of stem cells on osteogenesis (Shen et al., 2022; Impieri et al., 2024). This could be related to the effects of stem cells on the mechanism of acceleration of all phases of regeneration of the bone tissue.

On day 42 of the study, no significant changes were noted in the rabbits of the experimental group; the newly formed bone tissue was similar to such of the compact bone. In the animals of the control group, we observed the fifth stage – completion: restoration of the form and function of the bone tissue.

Thus, we described the microscopic changes and repair of the bone tissue and how allogeneic mesenchymal stem cells influenced those processes. We found that allogeneic mesenchymal stem cells affect the osteogenesis and accelerate the process of regeneration of the bone tissue.

Conclusion

The experimental modeling of the pathological process in the tibia by mechanical damage with distinctly determined parameters allows monitoring the phases of reparative regeneration of the bone tissue under natural conditions and yielding reliable results of studying the efficacy of allogeneic mesenchymal stem cells in stimulation of the processes of reparative osteogenesis in bone.

Injection of allogeneic mesenchymal stem cells increased the activity of regenerative processes and expedited the phases of reparative osteogenesis in the zone of experimental defect, where under these conditions the repair of the experimentally damaged bone tissue has almost completed on day 28 of the study, compared with day 42 in the animals of the control group.

We found that on day 3 after the injection of allogeneic mesenchymal stem cells in the region of experimentally damaged bone tissue, the defect was filled with mostly newly formed fibrous connective tissue. At the same time, the region of bone injury was covered by a quite thick layer of dense fibrous connective tissue. In addition, the stimulation of osteogenesis has been more intensive, which is significant in this particular period of repair of bone defect.

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