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STRUCTURAL ORGANIZATION OF PERIODONTIUM AND MORPHOLOGICAL CHANGES IN ITS COMPONENTS IN MODELED PERIODONTITIS AND ITS CORRECTION IN RATS

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Abstract

Introduction. The aim of our research was to study peculiar features of morphological changes in the components of the periodontium in the modeled acute periodontitis and its correction by stem cells.

Materials and Methods. The results of histological study of periodontal tissues of the animals with acute experimental periodontitis, which was corrected using human mesenchymal stem cells as well as rat muscle stem cells, were analysed. Depending on the treatment, the animals were divided into 4 groups: the 1st group – the animals without pathology (intact); the 2nd – the animals with modeled acute periodontitis; the 3rd – the animals with periodontitis corrected by human mesenchymal stem cells, the 4th – the animals with periodontitis corrected using rat muscle stem cells.

Results and Discussion. According to the results of the research it was established that correction by human mesenchymal stem cells and rats muscular stem cells had positive effect on a course of the modeled pathological process. The morphological changes characteristic of periodontitis gradually reduced, i.e. the normal blood supply to the tissues restored, the arteries were less full-blooded, the tone of their walls decreased. Consequently, the swelling of the tissues gradually decreased becoming of original size. The polymorphonuclear infiltration fields decreased and gradually disappeared. Osteoblasts promoted restoration of the bone structure of the jaw alveolar process. The size of the periodontal pocket decreased, and in some areas it fully closed as a result of complete adhesion of the mucous membrane of the gums to the tooth, which rose on its surface upwards.

Conclusions. In the experimental modeling of acute periodontitis the destructive changes in the epithelium and connective tissue stroma, impaired blood flow, inflammatory response in the gum tissue were present. The correction by means of human mesenchymal stem cells as well as rat muscle stem cells had positive effect on the modeled pathological process: the morphological changes characteristic of periodontitis gradually reduced, normal blood supply to tissues restored, polymorphonuclear infiltration fields gradually decreased and disappeared. The bone structure of the jaw alveolar process restored owing to osteoblasts. The size of the periodontal pocket decreased; in some cases, they closed completely as a result of complete adhesion of the mucous membrane of the gums to the tooth, which rose on its surface upwards.

Key words: acute experimental periodontitis; stem cells; regeneration.

Introduction

Periodontal disease is one of the urgent issues of contemporary dentistry and its frequency is the second after only caries [1, 3, 15]. The current knowledge allows considering the etiopathogenesis of periodontal disease as a multifactorial model, including the presence of microbial invasion (bacterial periodontal pathogens), inadequate protective response of the immune system or its absence, the impact of negative local factors of the oral cavity [14, 15].

At present, the development and course of inflammatory periodontal disease in different age groups is not only of medical but also of social significance due to environmental degradation, malnutrition, chronic stress [3]. The urgency and importance of the problem is determined by a steady increase in morbidity, untimely early diagnosis,

refractory process, significant difficulties in achieving stable remission, presence of a close relations with the general state of the human body, etc. [14].

In contemporary periodontology, the use of agents that stimulate the regenerative potential of periodontal tissues, especially bone tissue, is of great importance [2, 4, 9, 10]. Thus, the importance of study of mesenchymal stem cells (MSC) for treatment of periodontal pathology is increasing. Such treatments are promising for more effective recovery of the lost bone tissue in various diseases, especially periodontal tissues [11, 12, 13, 16].

Hence, the **aim** of the research was to study the morphological changes in the components of periodontitis in cases of modeling of acute periodontitis and its correction by stem cells.

Materials and Methods

The study was performed on white outbred male rats weighing 180-200 g from the Vivarium of I. Horbachevsky Ternopil National medical University, following the "Recommendations for Care and Use of Laboratory Animals" [7]. The animals were on a complete diet of the vivarium with free access to water. The effectiveness of the correction by stem cells was studied on a model of periodontitis caused by introduction of lipopolysaccharide (LPS) into the gum tissue, 40 microliters (1 mg/ml) every day for 14 days. In 1, 7, 14 and 21 days after the last administration of LPS the rats were decapitated under thiopental anaesthesia (50 mg/kg). The animals were divided into 4 groups: the 1st – the animals without modeled pathology (intact); the 2nd – the animals with modeled acute periodontitis; the 3rd – the animals with periodontitis corrected by human mesenchymal stem cells, the 4th – the animals with periodontitis corrected by rat muscle stem cells.

The material for histological study was obtained on the 1st, 7th, 14th and 21st days after taking the animals out of the experiment by decapitation under thiopental anaesthesia. Periodontal tissues of the animals were used as the material for morphological studies.

For histological study, the material was fixed in a 10% solution of buffered neutral formalin. Further histological preparations were performed according to generally accepted methods [6]. Production of serial paraffin sections, 4-6 μm thick, was performed on a sled microtome. Staining of the preparations was performed with haematoxylin and eosin, which were used to study the structure of periodontal tissues in the norm as well as the nature of morphological changes after periodontitis modeling. For cytological study, the smears were stained according to Romanowsky-Giemsa staining (RGS). For photo documentation, the images of histological specimens were displayed on a computer monitor using a Nicon

Eclipse C microscope and a SCMOS Digital Camera by means of ToupWiev software at various magnification.

Obtaining MSCs was performed for pregnant females, approximately on the 21^{st}-24^{th} day of pregnancy. After thiopental euthanasia, umbilical cords were taken from the isolated foetuses, washed from the blood with sterile HBSS buffer solution adding 1% penicillin-streptomycin. After that, the enzyme method was used to dissociate the cell mass and obtain viable MSCs. For this the tissue samples were ground with a scalpel into 0.5-2 mm^3 fragments, transferred to centrifuge tubes with 2 ml of DMEM/F12 Advanced growth medium and 0.2 ml of collagenase I at a concentration of 0.075 mg/ml and mixed with no flotation. The tubes with primary material and collagenase were incubated in a thermal bath at 37 °C for 70 minutes stirring thoroughly every 15 minutes. After fermentation, 4 ml of growth medium was added to the tubes, pipetted and centrifuged for 5 min at 300 g. The procedure was repeated twice. The pellet obtained was resuspended in 7 ml of DMEM/F12 Advanced adding 10% of foetal calf serum (FCS) and plated into culture vials. Cultivation was carried out in a CO_2 incubator at a temperature of 37 ^oC and a CO_2 concentration of 5%. For introduction of MSCs, the conditioned medium was selected from a culture vial with a 90% confluent of myogenic MSCs and the cells were washed with HBSS buffer solution (Gibco). To separate the cells from the bottom of the culture vial, the Tryple enzyme preparation (Gibco) was used and incubated for 5 min at 37 °C. The action of the dissociating solution was neutralized with a conditioned medium. After that, the cell suspension was transferred into a centrifuge tube and precipitated the MSCs for 8 min at 1700 rpm. The precipitate obtained was dissolved in 1 ml of HBSS solution and the cells were centrifuged again under the same conditions. The precipitate was dissolved in saline, the obtained cell suspension was sieved, a pore diameter of 100 μm; the number of isolated cells was counted by means of a haemocytometer using a vital dye – trypan blue. The rats were injected with stem cells into the gums by a single injection of 1 million cells per 1 kg of body weight. To maximize the viability of the cells, the introduction of MSCs was carried out within 30 min after the suspension had been obtained.

Results and Discussion

The analysis of histological sections from oral cavity of the intact and control rats, which were a complex of a tooth and surrounding tissues, established a typical periodontal structure. The structure of such a complex was characterized by the presence of the tooth (cement, pulp chamber and the pulp represented by vessels and nerves), a periodontal

ligament (loose connective tissue filled with collagen and elastic fibres), a bone of the jaw alveolar process and gingival mucosa (Fig. 1).

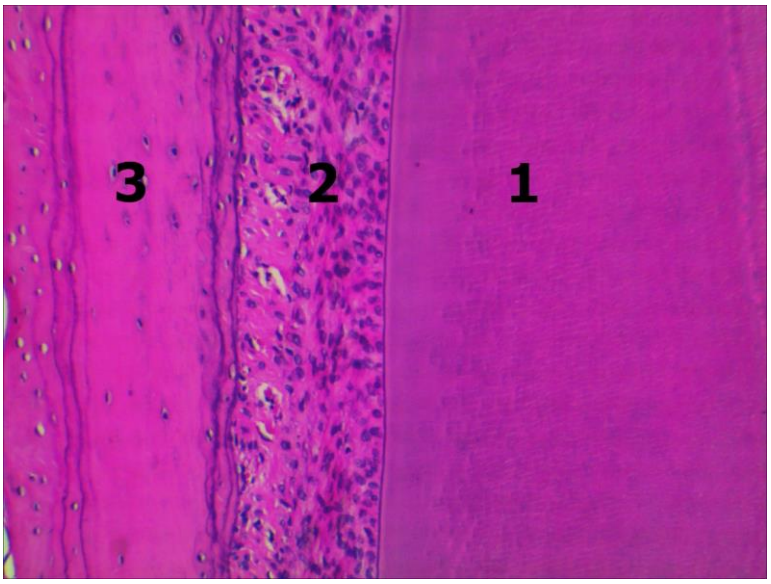

Fig. 1. Histological structure of the periodontium of an intact rat. Staining with hematoxylin and eosin. × 140.
Tooth tissue (cement) – 1, periodontal ligament – 2, bone of the jaw alveolar process – 3.

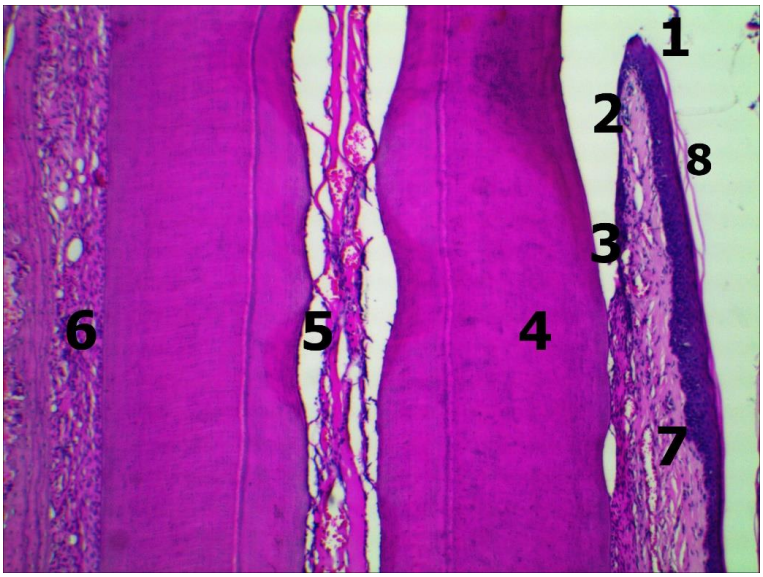

Fig. 2 - Histological structure of the periodontium of an intact rat. Staining with haematoxylin and eosin. × 140.
Oral epithelium of the oral cavity – 1, mucous epithelium at the periodontal ligament – 2, gingival furrow – 3, tooth tissue (cement) – 4, tooth canal filled with pulp elements – 5, bone of the jaw alveolar process – 6, gums submucosal connective tissue– 7, exfoliation of the keratinized epithelium layer – 8.

The mucous membrane of the gums was close to the bone of the alveolar process. The mucous membrane was also close to the tooth tissues. At the point of contact between the

mucous membrane and the tooth, only a shallow furrow was defined. Moreover, the epithelium turned to the oral cavity slightly differed from the epithelium turned to the tooth. The edge between the epithelium lining the gums surface at the oral cavity and the underlying connective tissue had wavy contours due to the alternation of protrusions of the submucosal connective tissue membrane with immersed mucous epithelium papillae, while the edge between the epithelium adjacent to the periodontal ligament (alveolar epithelium) and the ligament was more straight-lined (Fig. 2).

The gums epithelium of the oral cavity was a squamous keratinizing epithelium in which the following layers were distinguished: basal, prickly cell, granular cell and keratinized. In contrast, the alveolar epithelium did not have a keratinized layer.

The vascular bed was represented by small arteries and arterioles, as well as individual structural components that were part of the hemomicrocirculatory tract. Sometimes small clusters of formed elements of blood were present in the blood vessels lumen (Fig. 3).

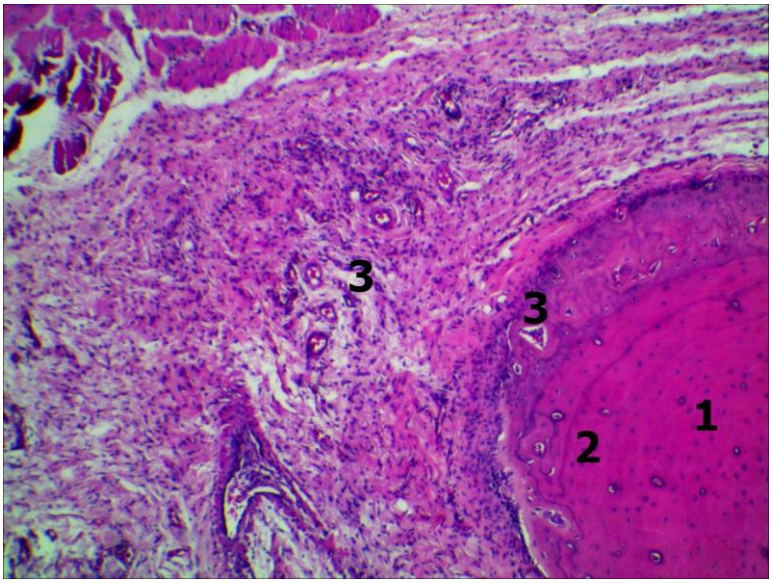

Fig. 3. Histological structure of the periodontium of an intact rat. Staining with hematoxylin and eosin. × 120.
Bone of the jaw alveolar process – 1, intraosseous vessels – 2, vessels of the periodontium soft tissues – 3.

The periodontal ligament was represented by fibrous connective tissue. Morphometric examination revealed that the thickness of the periodontal ligament in rats varied between 30-50 μm.

In the structure of the alveolar braid, two layers were also distinguished: the denser one, which was directed to the periodontal ligament; and the spongy part, which was located in the thickness between the two dense layers. In the outer layer of the bone, the osteoblasts

together with collagen fibres formed the periosteum. In the inner parts of the bone, the endosteum formed islet zones, which were also similar to the periosteum. The thickness of the alveolar bone at the apex of the tooth root was 250-350 μm. Closer to the crown, the thickness of the alveolar bone decreased gradually.

In periodontitis modeling, clear signs of inflammation were evidenced mainly in soft tissues on the 7th day of the experiment (Fig. 4).

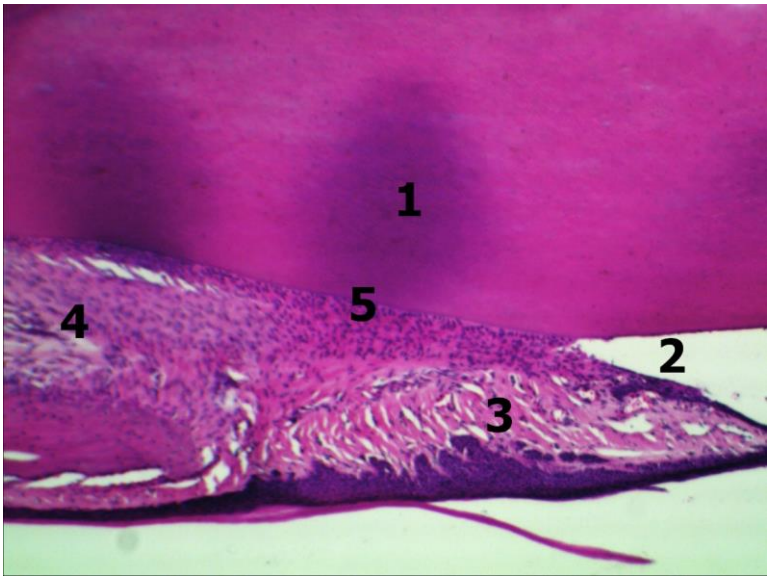

Fig. 4. Histological structure of the periodontium of rats in 7 days after periodontitis modeling. Staining with hematoxylin and eosin. × 140.
Tooth tissue – 1, periodontal pocket formation – 2, submucosal oedema – 3, periodontal ligament oedema and thickening – 4, polymorphocellular inflammatory infiltrate – 5.

They were manifested primarily by dilation of the lumen of blood vessels and their plethora, oedema of both epithelium and subepithelial connective tissues, as well as periodontal ligament, its thickness almost doubled. The gingival furrow deepened and expanded forming a periodontal pocket. Focal polymorphocellular inflammatory infiltrates were observed in the periodontal ligament, especially in the area directly adjacent to the gingival pocket. Infiltrates were sometimes evidenced in the thickness of the bone of the jaw alveolar process as well as formation of a lympho-leukocyte rolling around them and destruction of the bone tissue (Fig. 5).

As the process progressed, the inflammatory manifestations intensified with the layering of destructive processes on them and the simultaneous development of compensatory changes. Destructive changes were manifested by further detachment of the periodontal ligament, deepening of the periodontal pocket as well as focal resorption of the alveolar bone.

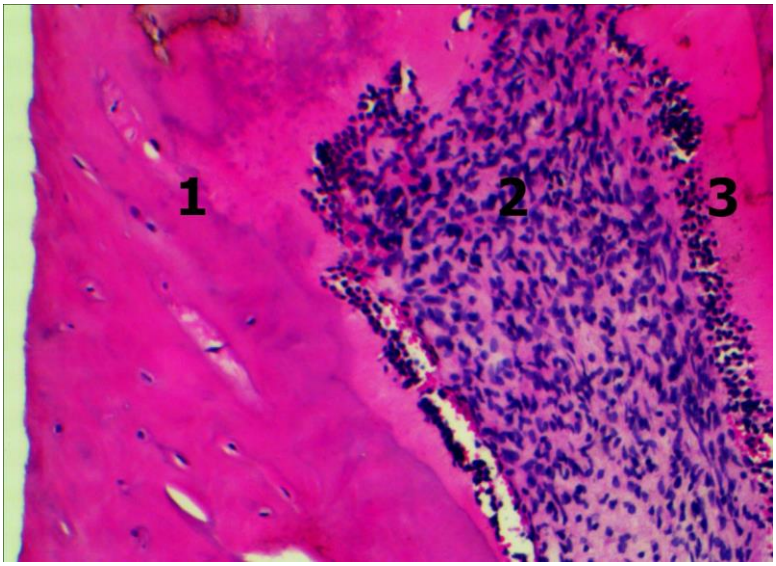

Fig. 5. Histological structure of the periodontium of rats in 7 days after periodontitis modeling. Staining with hematoxylin and eosin. × 180.
Bone of the jaw alveolar process – 1, polymorphocellular inflammatory infiltrate with destruction of bone tissue – 2, inflammatory lymphocytic leukocyte rolling – 3.

The dystrophic changes were also present in the epithelium manifested by its thinning at the oral cavity, a decrease in cell size and pyknosis of their nuclei. Compensatory manifestations were characterized by advance of granulation tissue in the gums, acanthosis foci of the epithelium and its thickening near the tooth and increase in the size of its papillae, which expanded into the underlying connective tissue as a result of proliferation. At the same time, the advance of connective tissue was observed (Fig. 6, 7).

Regarding the blood vessels, with longer duration of the modeled pathology their arterial walls thickened and the lumen narrowed. Thickening of the walls of the arteries occurred both due to oedema and due to hypertrophy of the smooth muscle cells that was confirmed by increase in their size. Such changes in the arteries were aimed at reducing the hemodynamic load on the hemomicrocirculatory tract. However, hemodynamic disorders led to development of perivascular oedema and thickening of vascular adventitia due to its defibering (Fig. 8).

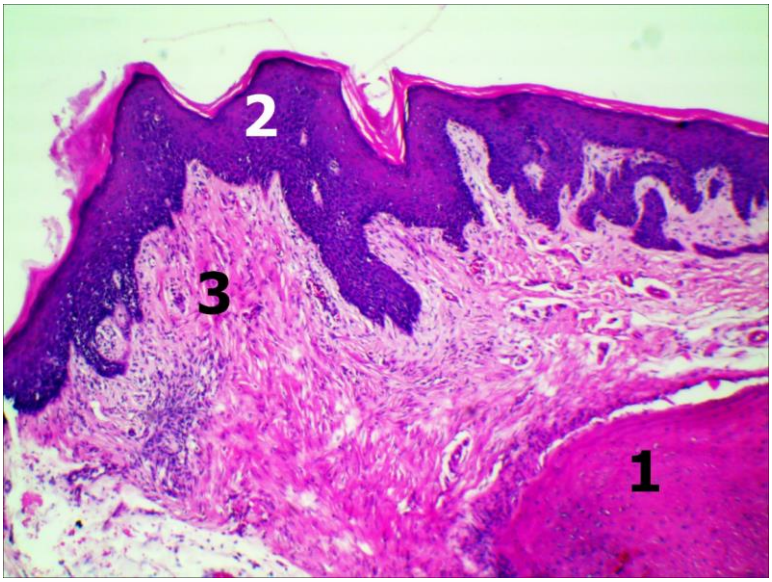

Fig. 6. Histological structure of the periodontium of rats in 14 days after periodontitis modeling. Staining with hematoxylin and eosin. × 140.
The bone of the jaw alveolar process – 1, acanthosis of the epithelium – 2, advance of connective tissue – 3.

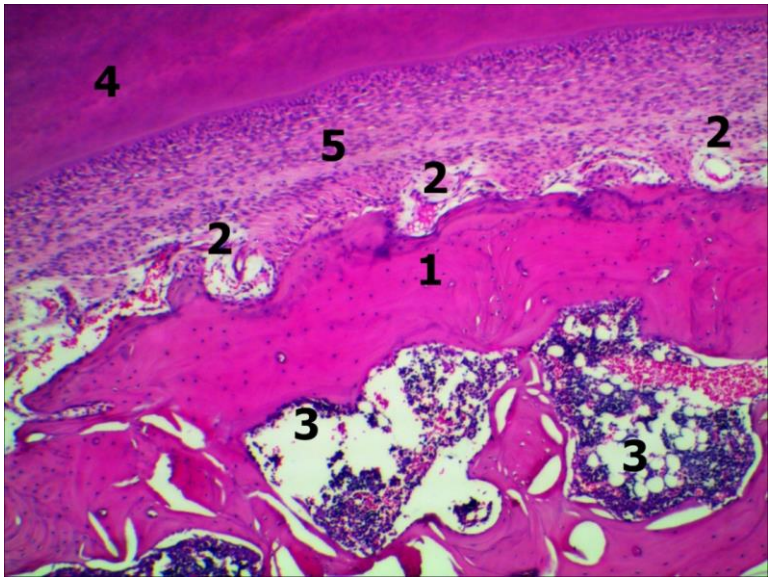

Fig. 7. Histological structure of the periodontium of rats in 21 days after periodontitis modeling. Staining with hematoxylin and eosin. × 140.
Bone of the jaw alveolar process – 1, resorption of the compact cortical part of the alveolar bone by osteoclasts – 2, destruction of the spongy part of the alveolar bone at the area of polymorphonuclear infiltrates – 3.

Occasionally there were arteries with particularly thickened walls resembling arteries of the "closing type" that proved their active functional load under different conditions of hemodynamics (Fig. 8).

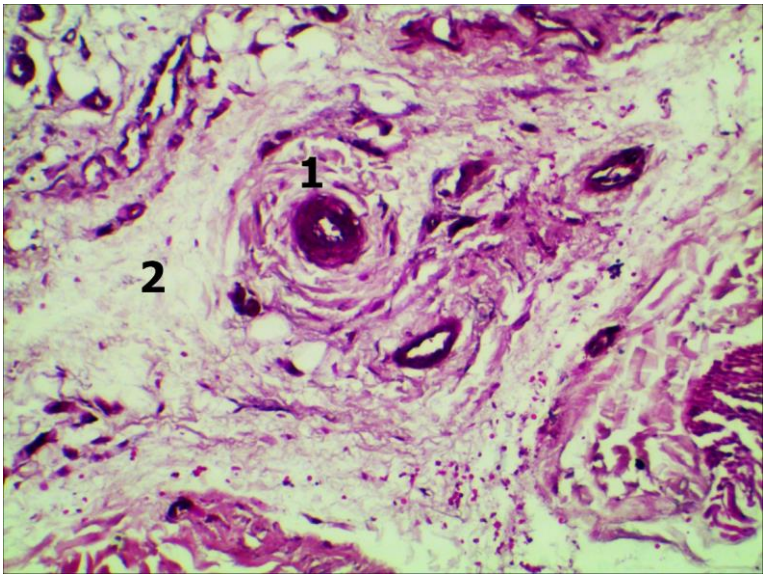

Fig. 8. Histological structure of the periodontium of rats in 21 days after periodontitis modeling. Staining with hematoxylin and eosin. × 180.
Artery with thickened wall, narrowed lumen and fibrous adventitia – 1, perivascular oedema – 2.

Such arteries were characterized by the presence of subintimally located bundles of smooth muscle of longitudinal direction, i.e. the so-called “polyp-like cushions”, which could have a particularly intense effect on the speed and volume of blood flow (Fig. 9). It was possible to distinguish bundles of longitudinal smooth muscles on the rounded form of sections of their nuclei as in a circular arrangement of fibres their nuclei were of a spindle-shaped form.

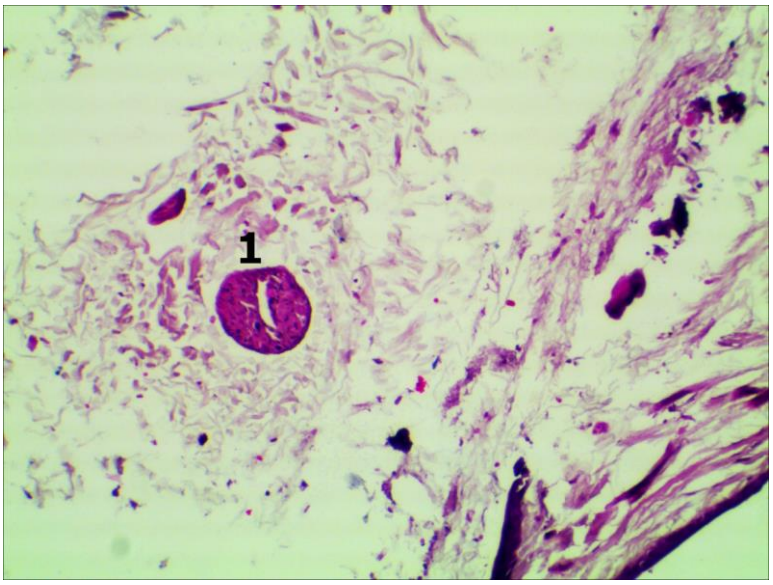

Fig. 9. Histological structure of the periodontium of rats in 21 days after periodontitis modeling. Staining with hematoxylin and eosin. × 140.
Artery with a thickened wall, a narrowed lumen and a longitudinal bundle of subintimally located smooth muscle – 1.

The correction methods by human mesenchymal stem cells as well as rat muscle stem cells had a positive effect on the course of the modeled pathological process. The morphological changes characteristic of periodontitis gradually reduced. That is, the normal blood supply to the tissues restored. The arteries were less full-blooded, the tone of their walls decreased. Thus, the swelling of the tissues gradually reduced, they were of original size. Polymorphonuclear infiltration fields decreased and gradually disappeared owing to osteoblasts. The bone structure of the jaw alveolar process restored. The periodontal pocket decreased in size, and they even completely closed as a result of full adhesion of a gums mucous membrane to a tooth which rose on its surface upwards.

However, in case of use of rat muscle stem cells, the acanthosis and sclerotic changes in the submucosal connective tissue were still evidenced in some areas even before the end of the observation (Fig. 10).

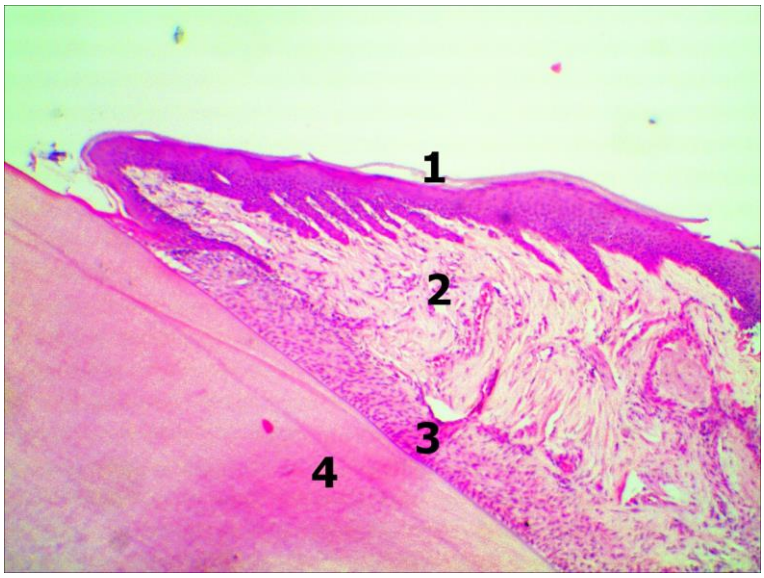

Fig. 10. Histological structure of rat periodontitis in 14 days after application of rat muscle stem cells in periodontitis modeling. Staining with hematoxylin and eosin. × 140. Epithelium with partial presence of acanthosis – 1, connective tissue – 2, periodontal ligament – 3, tooth tissue – 4.

It should be noted that if the described processes of periodontal tissue remodeling took place with both methods of correction, the results of our studies also proved that human mesenchymal stem cells were a little more effective than rat muscle stem cells (Fig. 11). The morphological organization of the structural components of the periodontium was restored more fully. As a result of hyperplasia, the mucosa covered the tooth often reducing periodontal pockets completely.

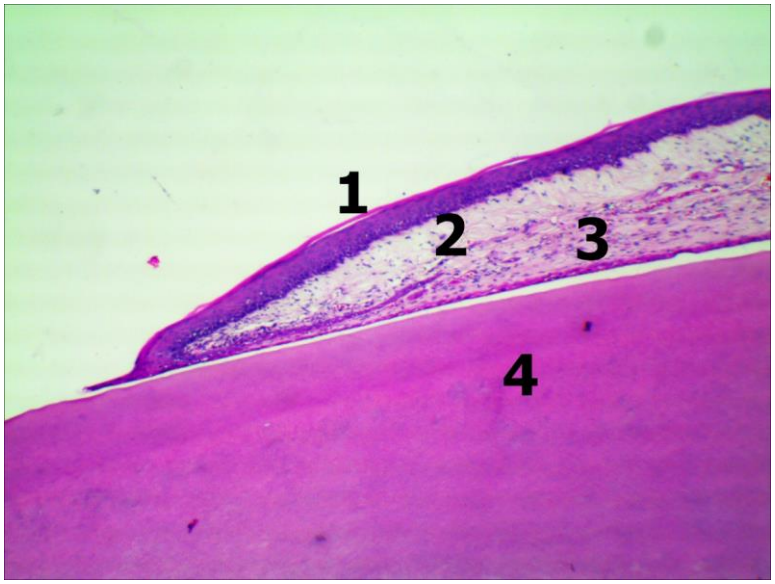

Fig. 11. Histological structure of rat periodontium in 21 days after application of human mesenchymal stem cells in cases of periodontitis modeling. Staining with hematoxylin and eosin. × 140.
Completely restored structure of the epithelium of the gingival mucosa – 1, moderately evidenced connective tissue – 2, usual form of periodontal ligament – 3, tooth tissue – 4.

Conclusions

In the experimental modeling of acute periodontitis destructive changes in the epithelium and connective tissue stroma, impaired blood flow, inflammatory response in the gum tissue are present.

The correction methods by human mesenchymal stem cells as well as rat muscle stem cells have a positive effect on the simulated pathological process: the morphological changes characteristic of periodontitis gradually reduce, normal blood supply to tissues restores, polymorphonuclear infiltration fields gradually decrease and even disappear. Osteoblasts promote restoration of the bone structure of the jaw alveolar process. The periodontal pocket decreases in size, and in some cases they fully close as a result of complete adhesion of the gums mucous membrane to the tooth, which rose on its surface upwards.

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