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# The effect of endodontic filling agents on the activity of the regeneration processes of bone tissue in the experiment

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**ABSTRACT**

**Introduction.** Highly important is to regenerate the inflammatory destructive processes of bone tissue outside the apical area of the tooth that would optimize reparative bone formation, mineralization of tooth tissues, restoration of periodontal function and stability of tooth to occlusive loads, especially with acquired extensive root apex. Low efficiency of endodontic treatment led to the searching of the new osteotropic drugs of osteoconductive action and biorevitalization to stimulate the repairing and regeneration of tissues outside the apical area and dense obturation of the apex of the tooth root.
**Aim.** of our research was to determine the dynamics of reparative processes in the bone tissue under the influence of drugs and compositions for endodontic treatment based on an analysis of indicators of a mineral metabolism, marker enzymes and an activity of antioxidant system and lipid peroxidation.
**Material and Methods.** An experiment was conducted on 120 white rats. It was created a bone defect, which was filled with studied biomaterials or left with a blood clot. From the experiment the rats were taken out on 14 and 90 day. In homogenates of bone tissue it was examined the activity of lysosomal enzymes — alkaline (ALP) and acid (AP) phosphatases and the content of microelements — calcium and phosphorus. In the blood of rats it was studied the concentration of general protein, lipid peroxidation products — malonic dialdehyde (MDA) and the enzyme activity of antioxidant system — catalase (CAT) and superoxide dismutase (SOD).
**Results and Conclusions.** Results of conducted experimental research of the effect of endodontic filling materials on the activity of the regeneration processes of bone tissue show that proposed compositions based on hydroxyapatite and beta-tricalcium phosphate, due to their osteoconductive and biorevitalization qualities, promote more active stimulation of bone tissue regeneration processes compared to generally accepted drugs.

**Keywords:** experiment, biomaterials, regeneration, alkaline phosphatase, acid phosphatase, calcium, phosphorus, malonic dialdehyde, catalase, superoxide dismutase, general protein.

## Introduction

Nowadays the problem of regeneration of bone tissue and searching of methods of influence on the regeneration processes of bone is one of the actual problems of modern medicine. The essence of reparative regeneration consists in restoring the cells, tissues or organs after experiencing various pathological processes [1]. Highly important is to regenerate the inflammatory destructive processes of bone tissue outside the apical area of the tooth that would optimize reparative bone formation, mineralization of tooth tissues, restoration of periodontal function and stability of tooth to occlusive loads, especially with acquired extensive root apex [2]. Low efficiency of endodontic treatment led to the search-

ing of the new osteotropic drugs of osteoconductive action and biorevitalization to stimulate the repairing and regeneration of tissues outside the apical area and dense obturation of the apex of the tooth root [3, 4].

## Aim

The aim of our research was to determine the dynamics of reparative processes in the bone tissue under the influence of drugs and compositions for endodontic treatment based on an analysis of indicators of a mineral metabolism, marker enzymes and an activity of antioxidant system and lipid peroxidation.

## Material and Methods

An experiment was conducted on 120 white rats of Wistar line of herd breeding at the age of 9–10 weeks. Studies on the laboratory animals were conducted following the principles of bioethics in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), the Law of Ukraine № 3447-IV "On the Protection of Animals from Brutal Treatment" and were appropriately approved by the local ethics Committee. To create a bone defect intervention was performed under general anesthesia (0.5 ml of 4% solution of sodium thiopental into the peritoneum). On the left side of the lower jaw of rats it was made a trapezoid-shaped cut with a scalpel in the area between the incisor and right molar and mucous membrane was separated. It was created a bone defect of 3 mm in diameter and of 2.5 mm in depth by spherical and fissure burs under constant irrigation with saline 0.9%, which was filled with studied biomaterials or left with a blood clot and covered with mucous membrane and the stitches were put in. All animals were divided into 6 research groups and 20 individuals in each: first group — intact animals that served as a control; second comparative group — animals, on which it was created a bone defect without entering the biomaterial; third group — animals, on which it was used material Nano Gen to fill the defect; fourth group — animals on which it was entered mineral trioxide aggregate (MTA) in the created defect; fifth group — animals, whose defect was filled with the composition based on beta-tricalcium phosphate (β-TCP); sixth group — animals, which created defect in a bone was entered with the composition made on the basis of calcium hydroxyapatite (HA) (Utility model patent number 95974, Ukraine, 2015). The animals, except the control group, were held on a bland diet on the first 3 days after the beginning of an experiment, and henceforth — on a standard diet and according to the sanitary norms in the vivarium. From the experiment the rats were taken out on 14 and 90 day by their decapitation under anesthesia. A blood sampling was provided and the bone defect area of an alveolar bone of the lower jaw of a rat was carved for the future biochemical research. In homogenates of bone tissue it was examined the activity of lysosomal enzymes — alkaline (ALP) and acid (AP) phosphatases and the content of microelements — calcium and phosphorus. In the blood of rats it was studied the concentration of general protein, lipid peroxidation products — malonic dialdehyde (MDA) and the enzyme activity of antioxidant system — catalase (CAT) and superoxide dismutase (SOD).

## Results

On 14th day in the second group of animals with created bone defect without entering the biomaterial it was established that MDA had increased by 2.3 times compared to the control ($p < 0.001$) (**Table 1**). Similar changes in the number of MDA were observed in the third and fourth groups of animals, destruction of bone tissue of which was filled with the biomaterials MTA and Nano Gen ($p < 0.001$). In the fifth and sixth groups of animals where were used compositions based on β-TCP and HA it was marked an increase in MDA — by 2 and 1.9 times, respectively ($p < 0.001$). At the same time changes were observed in the enzyme activity of an antioxidant system. Thus, in the second group of animals the concentration of SOD and catalase was decreased only by 1.6 time ($p < 0.001$) and in the third and fourth groups of animals the enzyme activity was decreased by 1.47 and 1.5 time, respectively ($p < 0.001$). In the fifth and sixth research groups it was marked a reduction of SOD and catalase only by 1.25 and 1.21 time respectively compared to intact animals ($p < 0.001$). The imbalance in the system POL/AOS caused the reduction of antioxidant-proox-

**Table 1.** Indicators of POL products, AOS enzymes and general protein concentration in serum of rats' blood on 14 day (M ± m)

| Groups of animals Experimental drugs | MDA kmol/L | SOD mkat/L | Catalase mkat/L | API | General protein g/L |
|---|---|---|---|---|---|
| I. Control (n = 10) | 2,35 ± 0.03 | 7,46 ± 0,27 | 0,263 ± 0.012 | 3,29 | 71,6 ± 01,2 |
| II. Comparison group (n = 10) | 5,43 ± 0.06 ***°°° | 4,68 ± 0,22 ***°°° | 0,164 ± 0.003 ***°°° | 0,89 | 100,3 ± 1,6 ***°°° |
| III. Nano Gen (n = 10) | 5,38 ± 0.05 *** | 4,97 ± 0,14 *** | 0,175 ± 0.003 ***° | 0,95 | 95,0 ± 1,3 ***° |
| IV. MTA (n = 10) | 5,35 ± 0.06 *** | 5,08 ± 0,16 *** | 0,178 ± 0.003 ***°° | 0,98 | 93,5 ± 1,1 ***°° |
| V. Composition based on β-TCP (n = 10) | 4,63 ± 0.04 ***°°° | 5,98 ± 0,21 ***°°° | 0,212 ± 0.003 ***°°° | 1,34 | 86,3 ± 1,5 ***°°° |
| VI. Composition based on HA (n = 10) | 4,48 ± 0.06 ***°°° | 6,17 ± 0,19 **°°° | 0,217 ± 0, 003 **°°° | 1,43 | 84,6 ± 1,3 ***°°° |

Note: * — an indicator of the likelihood difference compared to a control at p < 0.01, *** — p < 0.001; ° — indicator of the likelihood difference compared to the comparison group at p < 0.05, °° — p < 0.01, °°° — p < 0.001.

idant index (API) [5]. Thus, in the second group of experimental animals API decreased by 73%, and in the third and fourth groups — by 71% and 70%, respectively (p < 0.001). In the fifth and sixth study groups API index has decreased by only 59% and 56% respectively compared to the intact animals (p < 0.001).

At the same time it was detected an increase of the concentration of the general protein in the second group of animals by 1.4 times compared to the intact animals (p < 0.001), and in the third and fourth groups of animals — by 1.3 times compared to the control group (p < 0.001). In the fifth and sixth research groups the concentration of the general protein was increased by 1.2 compared to the intact group (p < 0.001).

CF is a biomarker of the bone tissue, which characterizes the activity of osteoclasts and the intensity of osteolysis [6, 7]. In the second, third and fourth groups of the animals it was observed an increase in the lysosomal enzyme AP by 1.5 times compared to indicators in the intact bone (p < 0.001) (**Table 2**). However, in the fifth and sixth research groups content of AP grew up only by 1.2 and 1.3 time, respectively (p < 0.001). Simultaneously, changes of the content and the other biomarker of bone fabric — ALP were observed In the second group of animals the enzyme activity increased by 1.25 time (p < 0.001), and in the third and fourth groups of animals — by 1.3 times compared to the control group of animals (p < 0.001). In the fifth and sixth research groups ALP concentrations increased by 1.9 time compared to the intact bone (p < 0.001). Activity of processes of osteogenesis and osteolysis during the bone regeneration characterizes the index of mineralization, which is defined by ALP / AP. In the second, third and fourth groups of animals the index decreased by 18%, 16% and 14% respectively compared to the intact animals. However, in the fifth and sixth groups of animals it was observed an increase in the index by 44% and 47% respectively, demonstrating a prevalence of bone regenerative processes of osteogenesis over destruction of tissues.

On 14th day of study calcium content in the second and third groups of animals decreased by 1.8 time, in the fourth — by 1.6 time, in the fifth — by 1.5 time and in the sixth — by 1.3 time compared to indicators of the control group (p < 0.001) (**Table 3**). Simultaneously, it was observed a reduction of content of phosphorus ions in the second, third and fourth groups of animals by 1.8; 1.7 and 1.5 time, respectively, in the fifth group —

**Table 2.** Indicators of ALP mkat/L and AP mkat/L activity in homogenates of rats' bone tissue (M ± m)

| Groups of animals Experimental drugs | Terms of observation | | | | | |
|---|---|---|---|---|---|---|
| | 14 day | | | 90 day | | |
| | AP | ALP | ALP/AP | AP | ALP | ALP/AP |
| I. Control (n = 5) | 1,58 ± 0.02 | 20,2 ± 0,3 | 12,8 | 1,58 ± 0.02 | 20,2 ± 0,3 | 12,8 |
| II. Comparison group (n = 10) | 2,41 ± 0.02 *** | 25,3 ± 0,3 *** | 10,5 | 1,69 ± 0.03 ** | 21,1 ± 0,3 * | 12,5 |
| III. Nano Gen (n = 10) | 2,39 ± 0.01 *** | 25,8 ± 0,2 ***° | 10,8 | 1,65 ± 0.03 | 20,6 ± 0,3 | 12,5 |
| IV. MTA (n = 10) | 2,38 ± 0.02 *** | 26,2 ± 0,3 ***° | 11,0 | 1,64 ± 0.03 | 21,3 ± 0,3 ** | 13,0 |
| V. Composition based on β-TCP (n = 10) | 1,98 ± 0.03 ***°°° | 36,6 ± 0,2 ***°°° | 18,5 | 1,61 ± 0.03 ° | 22,7 ± 0,4 ***°° | 14,1 |
| VI. Composition based on HA (n = 10) | 1,97 ± 0.03 ***°°° | 37,2 ± 0,4 ***°°° | 18,9 | 1,60 ± 0.02 °° | 23,1 ± 0,3 ***°°° | 14,5 |

Note: * — an indicator of the likelihood difference compared to a control at p < 0.05, ** — p < 0.01, *** — p < 0.001; ° — indicator of the likelihood difference compared to the comparison group at p < 0.05, °° — p < 0.01, °°° — p < 0.001.

**Table 3.** Indicators of calcium mmol/L and phosphorus mmol/L content in homogenates of rats' bone tissue (M ± m)

| Groups of animals | Terms of observation | | | | | |
|---|---|---|---|---|---|---|
| | 14 day | | | 90 day | | |
| | Calcium | Phosphorus | Calcium/ Phosphorus | Calcium | Phosphorus | Calcium/ Phosphorus |
| I. Control (n = 5) | 13,31 ± 0,11 | 5,70 ± 0,13 | 2,33 | 13,31 ± 0,11 | 5,70 ± 0,13 | 2,33 |
| II. Comparison group (n = 10) | 7,14 ± 0,07 *** | 3,13 ± 0,11 *** | 2,28 | 8,02 ± 0,13 *** | 3,81 ± 0,12 *** | 2,11 |
| III. Nano Gen (n = 10) | 7,29 ± 0,09 *** | 3,20 ± 0,11 ***°°° | 2,28 | 8,19 ± 0,11 *** | 3,88 ± 0,12 *** | 2,11 |
| IV. MTA (n = 10) | 8,27 ± 0,13 ***°°° | 3,61 ± 0,09 ***°°° | 2,29 | 10,01 ± 0,12 ***°°° | 4,57 ± 0,10 ***°°° | 2,19 |
| V. Composition based on β-TCP (n = 10) | 8,85 ± 0,09 ***°°° | 3,85 ± 0,09 ***°°° | 2,30 | 11,43 ± 0,14 ***°°° | 4,99 ± 0,10 ***°°° | 2,29 |
| VI. Composition based on HA (n = 10) | 9,82 ± 0,12 ***°°° | 4,27 ± 0,11 ***°°° | 2,30 | 11,76 ± 0,14 ***°°° | 5,09 ± 0,13 ***°°° | 2,31 |

Note: * — an indicator of the likelihood difference compared to a control at $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$; ° — indicator of the likelihood difference compared to the comparison group at $p < 0.05$, °° — $p < 0.01$, °°° — $p < 0.001$.

by 1.4 time, and in the sixth experimental group — by 1.3 time compared to indicators of the control group ($p < 0.001$). Decrease of index of calcium and phosphorus correlation points to calcium deficiency compared to phosphorus.

On 90th day after modeling the destruction it is observed an increase in the concentration of MDA in the second group of animals by 1.45 time compared to control ($p < 0.001$), in the third and fourth groups of animals — in 1.3 time ($p < 0.001$) and in the fifth and sixth experimental groups it is noticed the returning of indicators of processes of lipid peroxidation to those of the control group (**Table 4**). Simultaneously, the positive dynamics is observed in the functioning of the antioxidant system. The activity of catalase and SOD in all experimental groups approached to standard indicators, although SOD concentration in the second group of experimental animals has not reached the control indicators and was lower by 1.1 time ($p < 0.04$). In addition, to indicators of the control group approached the indicator of general protein concentration, except the one of the second group of experimental animals, where its content remained increased by 1.2 time compared to the control group of animals ($p < 0.001$).

Positive dynamics was also observed in the indicators of biomarkers of bone tissue. The concentration of the enzyme ALP in the second group of animals has increased by 1.2 time ($p < 0.04$), and in the third and fourth groups of animals — by 1.1 time compared to the control group of animals ($p < 0.02$ and $p < 0.3$ respectively). Simultaneously, the content of lysosomal enzyme AP remained increased — in the second group of animals by 1.2 time ($p < 0.01$), while in the third and fourth groups of animals — in 1.1 time compared to indicators in the intact bone ($p < 0.1$) (**Table 2**). In the fifth and sixth research groups alkaline the content of enzymes ALP and AP closed to indicators of the control group. This shows an absence of inflammation in the bone tissue and reconstruction of balance processes of osteogenesis and osteolysis.

The content of calcium ions remains below normal in the second and third groups by 1.6 time, in the fourth group — by 1.3 time and in the fifth and sixth research groups — within the indicators of the control group. A content of phosphorus ions is below normal in the second and third groups of animals by 1.5 time, in the fourth group — by 1.3 time, and in the fifth and sixth research groups — by 1.1 time compared to indicators of the control group (**Table 3**).

## Discussion

Analysis of various scientific studies indicates that the speed and quality of regeneration of bone tissue destruction outside the apex depend on the use of endodontic filling materials that are used to stimulate and accelerate the process of osteo-regeneration [8, 9].

The goal of our research was to evaluate the influence of endodontic filling agents on the activity of bone regeneration processes by ana-

**Table 4.** Indicators of POL products, AOS enzymes and general protein concentration in serum of rats' blood on 90 day (M±m)

| Groups of animals Experimental drugs | MDA kmol/L | SOD mkat/L | Catalase mkat/L | API | General protein g/L |
|---|---|---|---|---|---|
| I. Control (n = 10) | 2,35 ± 0,03 | 7,46 ± 0,27 | 0,263 ± 0,012 | 3,29 | 71,6 ± 1,2 |
| II. Comparison group (n = 10) | 3,41 ± 0,04 ***°°° | 6,67 ± 0,21 *° | 0,246 ± 0,009 | 2,03 | 85,1 ± 1,1 ***°°° |
| III. Nano Gen (n = 10) | 3,11 ± 0,04 ***°°° | 6,85 ± 0,14 | 0,248 ± 0,01 | 2,28 | 74,8 ± 1,3 °°° |
| IV. MTA (n = 10) | 2,98 ± 0,04 ***°°° | 6,9 ± 0,12 | 0,252 ± 0,011 | 2,4 | 74,5 ± 1,2 °°° |
| V. Composition based on β-TCP (n = 10) | 2,41 ± 0,02 °°° | 7,51 ± 0,22 ° | 0,257 ± 0,013 | 3,22 | 72,8 ± 1,2 °°° |
| VI. Composition based on HA (n = 10) | 2,39 ± 0,02 °°° | 7,6 ± 0,26 ° | 0,269 ± 0,013 | 3,29 | 72,4 ± 1,0 °°° |

Note: * — an indicator of the likelihood difference compared to a control at $p < 0.05$, *** — $p < 0.001$; ° — indicator of the likelihood difference compared to the comparison group at $p < 0.05$, °°° — $p < 0.001$.

lyzing the indicators of mineral metabolism, marker enzymes and the activity of antioxidant system and lipid peroxidation.

We found that destruction of bone tissue is accompanied by excessive activation of processes POL and essential decrease of the expression of enzymes AOS in the bone as well as in the blood that is indicated by the growth of MDA content and the decrease of SOD concentration and catalase. Since POL is considered to be one of the basic mechanisms of cell membrane structures damage, the prevalence of lipid peroxidation processes and the relative lack of antioxidant system enzymes cause damage of cell membranes with further interruption of their functions [10]. Similar results were observed in the works of other authors who evaluated these indicators when the bone tissue was damaged. Thus, O.V. Denga found that trepanation of the tooth and its infection leads to the increase of MDA level and the decrease of activity of one of the major antioxidant enzymes of catalase [11], V.H. Fedirko showed that polytrauma in case of osteoporosis caused the increase in the level of POL, the decrease of activity of SOD and catalase in the bone tissue [12], O.V. Lubchenco found that the course of experimental periodontitis is accompanied by excessive inflammation due to the increase of lipid peroxidation level and the decrease of antioxidant protection [13].

The increase of AP concentrations in our study indicates on the higher activity of osteoclasts and shows an acuter course of the inflammatory process. However, a higher concentration of ALP in bone tissue homogenate confirms the increase of the number of osteoblasts and their functional activity that promotes more intense bone formation in the area of bone tissue destruction. The analysis of our results verifies that the increase of concentrations of bone tissue biomarkers — AP and ALP is accompanied with the decrease of mineralization index that is defined by ALP/AP correlation and describes the activity of osteogenesis and osteolysis processes. We have detected reduction of index of calcium and phosphorus correlation that points on calcium deficiency compared to phosphorus. Similar results were observed in the works of S.I. Palamarchuk and A.V. Borisenko that showed an increase of enzymes AP and ALP activity and a reduction of the of calcium soluble protein concentration [14, 15].

Applying of drugs Nano Gen, MTA and proposed compositions based on β-TCP and HA for medical purpose, processes of osteogenesis occurred with different intensity. The most expressed positive trend was observed by application of the proposed compositions whose compound included a means for revitalization and bioreparation, containing hyaluronic acid, modified by vitamin C and by amino acids — proline, lysine and glycine. This led fibroblasts to synthesize proteins of intercellular matrix — procollagen and collagen and stimulated tissue regeneration. Similar results were observed in experimental studies of Jung-ju Kim and co-authors who studied the regeneration of created bone tissue defect, which was filled with hyaluronic acid in combination with collagenous sponge and demonstrated osteoinductive effect and stimulated bone tissue reparative processes in the area of the defect [16].

Applying the compositions, the most expressed positive dynamics is confirmed by balancing of POL and AOS, activity of mineralization processes in the area of the bone defect and also returning of general protein concentration to indicators of the comparison group. Applying drugs Nano Gen

and MTA, processes of osteogenesis proceeded more slowly, imbalance remained between POL and AOS and a slight increase in general protein concentration was observed.

Therefore, we found that the proposed compositions have antioxidant and noticeable osteotropic qualities and better stimulate the regeneration processes of bone tissue.

## Conclusions

1. In case of experimental bone destruction, it is observed an imbalance between AOS and POL with a predominance of lipid peroxidation processes that leads to progress of inflammation as well as to violation of bone tissue mineralization and is confirmed by appropriate results of biochemical research both in serum of blood and in the bone tissue.
2. In the groups of animals, where the compositions based on hydroxyapatite and β-tricalcium phosphate were used, compared to groups of animals, where generally accepted drugs were used, it was observed less noticeable violations in the system POL / AOS. This stimulated a decrease of inflammatory activity and an earlier restoration of osteogenesis and osteolysis balance and accelerated calcification processes.
3. The proposed compositions, due to their osteoconductive and biorevitalization qualities, promote more active stimulation of bone tissue regeneration processes compared to generally accepted drugs.

## Summary

The article presents a comparative assessment of the dynamics of the reparative process of the bone tissue under the influence of drugs MTA and Nano Gen and compositions based on beta-tricalcium phosphate and calcium hydroxyapatite, containing organic biorevitalizants, by analyzing the indicators of mineral metabolism, marker enzymes and activity of antioxidant system and lipid peroxidation. The experiment was conducted on 120 white rats, on which was made a bone tissue defect filled with investigative biomaterials. From the experiment animals were withdrawn on 15 and 90 days by decapitation under anesthesia. A blood sampling was provided and the bone defect area of the alveolar bone of the lower jaw of a rat was carved for the future biochemical research. In homogenates of bone tissue it was investigated an activity of lysosomal enzymes — alkaline and acid phosphatase, content of calcium and phosphorus. It was explored a concentration of general protein, lipid peroxidation products — malonic dialdehyde (MDA) and the enzyme activity of antioxidant system — catalase and superoxide dismutase (SOD) in the blood of rats. The most expressed positive dynamics was observed by applying the proposed compositions that characterizes a balance of POL and AOS processes, returning of indicators of general protein concentration to indicators of the comparison group and activity of mineralization processes in the area of the bone defect.

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

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