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HISTOLOŠKA PROCENA ODGOVORA KOŠTANOG TKIVA NA ENDODONTSKI MATERIJAL NA BAZI SILIKONA

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HISTOLOGICAL EVALUATION OF BONE TISSUE RESPONSE TO SILICON-BASED ENDODONTIC MATERIAL

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HISTOLOGICAL EVALUATION OF BONE TISSUE RESPONSE TO SILICON-BASED ENDODONTIC MATERIAL

Successful endodontic treatment implies that the materials for obturation remain in the tissue, if possible forever. It is therefore essential to know a long-term effects of materials on tissue. The aim of this study was to evaluate histological response of bone tissue to the implanted dimethylpolysiloxane-based material in the artificially prepared defect. The sample comprised 20 Wistar rats. The defect was formed in the mandible of rats by sterile stainless steel burs. Dimethylpolysiloxane-based sealer (Roeko Seal) was implanted in the defects of the experimental group while the defects of the control group were left to heal spontaneously. Half of the animals from both groups were put down after thirty days, whereas the other half was euthanized after ninety days. Microscopic preparations were analyzed by light microscope. Fibrous callus and a young bone were observed thirty days after the implantation. Ninety days after the implantation, the bone around the unabsorbed material was completely healed. Roeko Seal does not decelerate the healing of bone tissue, it enables complete healing tissue around the material.

Key words: sealer, obturation, bone healing

HISTOLOŠKA PROCENA ODGOVORA KOŠTANOG TKIVA NA ENDODONTSKI MATERIJAL NA BAZI SILIKONA

Uspešan endodontski tretman podrazumeva da materijal za opturaciju ostane u tkivu, po mogućnunosti zauvek. Zbog toga je neophodno poznavati dugoročne efekte materijala na okolno tkivo. Cilj ove studije je bila histološka procena odgovora koštanog tkiva na materijal na bazi dimetilpolisiloksana implantiran u artificijelni preparisani defekt. Uzorak je obuhvatio 20 Wistar pacova. Defekt je formiran u mandibulama pacova sterilnim svrdilima od nerđajućeg čelika. Siler na bazi dimetilpolisiloksana (Roeko Seal) je implantiran u defekte eksperimentalne grupe, dok su defekti kontrolne grupe ostavljeni da spontano zarastu. Polovina životinja iz obe grupe je žrtvovana nakon trideset dana dok je druga polovina žrtvovana nakon devedeset dana. Mikroskopski preparati su analizirani na svetlosnom mikroskopu. Fibrozni kalus i mlada kost su uočeni trideset dana nakon implantacije. Devedeset dana nakon implantacije, kost oko neresorbovanog materijala je u potpunosti zaceljena. Roeko Seal ne usporava zarastanje koštanog tkiva, omogućava potpuno zaceljenje tkiva oko materijala. **Ključne reči:** siler, opturacija, zarastanje kosti

INTRODUCTION

After the removal of canal contents and treatment of the complete canal system by irrigation follows obturation as the final stage of endodontic treatment (1). The aim of hermetic obturation is to enable healing processes in the periapical region.

There are a lot of techniques for obturation of canal system, and the majority imply various ways of condensation of gutta-percha in combination with obturation paste (2). The role of the paste in this combination is to make suppressed gutta-percha fill the imperfections of canal system, fill accessory canals if any, and be the bond between gutta-percha and the wall of root canal (3).

The border of canal filling can influence the outcome of endodontic treatment. It is considered that the material should not go over an apical foramen. However, some think that a small amount of a sealer over the apical foramen may have a positive effect on healing processes (3, 4). Obturation material often goes over the apical foramen, given that despite modern achievements, endodontic procedure is mostly "groping in the dark". Nevertheless, even in cases where the filling was done up to the wanted limit, sealer stays in contact with periapical tissue via apical foramen, for a long period of time (for decades) (5,6).

This fact stresses the importance of biological characteristics of obturation materials (7). A great deal of research has shown that most materials in freshly mixed state show a certain degree of toxicity and cause the reaction of surrounding tissue in which they have been implanted after a short period of time (1,8,9). Successful endodontic treatment means that the obturation material stays incorporated in the tissue, if possible, forever, and that the tooth is functional, which is the reason why it is necessary to know how a certain material behaves in the tissue after a longer period of time, as well as the interaction between the tissue and the material.

Roeko Seal sealer belongs to the group of silicon-based materials. According to the studies published on cell culture silicon-based obturation materials showed good biological characteristics, which was not the case in sealers of different chemical composition even in freshly-mixed state (5, 8). According to implantation tests, silicon-based sealers show satisfactory biocompatibility (9, 10, 11). Obturation materials are expected to stay in an organism for a long time, therefore it is of utmost importance to check tissue reaction to them after longer periods of time.

AIM

The aim of this paper was to investigate tissue reaction to bone implantation of endodontic material *Roeko Seal* in artificially prepared defect in the mandible of rats after a long period of time (60 and 90 days).

MATHERIALS AND METHODS

20 male, wistar rats, average weight of 160-180 grams were used for experimental procedure (the experiment was approved by the Ethic Commute of Medical Faculty in Nis, No.01 3797). The preparation of experimental animals involved administration of anesthetic, namely intraperitoneal injection of ketamine hydrochloride. (0,1ml/100g). The experimental procedure involved preparation of bone defect unilaterally (1, 4 x 1,6mm) (left side) between the medial line and the mental foramen using a sterile stainless steel dental burs.

Roeko Seal sealer (Roeko, Germany) was implanted in the formed defects of the experimental group (n = 12) according to the manufacturer's instructions (material composition is shown in Table 1). Prepared defects of the control group (n = 8) were left to heal spontaneously without any implants. One half of the animals from the experimental (n = 6) and half of the animals from the control group (n = 4) were put down after 30 days, the other half after 90 days. The animals were put down by the excessive administration of anesthetic (ketamine hydrochloride).

Samples of tissue were collected by resection of mandible and consisted of the area of the defect and the surrounding bone. Tissue samples were fixed in 10% buffered formalin, demineralized in 10% formic acid, dehydrated in alcohol and molded in paraffin wax. Cutting was performed by microtome 2mm glass knives (Historange). Staining was done by H&E technique. Microscopic analysis was performed by the light microscope BX50 (Olympus, Japan).

The following parameters were examined: the degree of cell inflammatory response, the degree of fibrovascular proliferation and the reaction of the distal bone. Obtained data were classified according to a modified semiquantitative scale: 0 – absence, 1 - poorly, 2 – moderate and 3 – pronounced (12) - The obtained results were added to a specially created data base, and were analysed afterwards (*Friedman's ANOVA i Kruskal-Walis ANOVA*).

RESULTS

EXPERIMENTAL GROUP

The remainder of used material for the obturation of terpanation cavity was observed microscopically within the defects of all the samples of experimental group. During the proces of treating the sampled bone and making histological preparations in the majority of cases the obturation material fell out or remaind in traces, and the experimental defects appeared as empty spaces by the light microscope.

Experimental group (Roeko Seal) - 30 days

On the thirtieth day after the implantation, callus and newly formed bone tissue can be observed. The replacement of fibrous callus with a young immature bone can be observed (Picture 1). The bone distal to the defect has the structure of basophilically prominent border lines of osteon and partially with greater amount of extracellular matrix. The borders of osteon are cracked, partially widened of fine-grained or amorphous look of basophilic reaction.

Experimental group (Roeko Seal) - 90 days

Ninety days after the implantation a defect can be observed and partially retained material during the completion of preparations. The bone around unabsorbed material is repaired and completely healed (Picture 2). The boundary of a newly deposited mature bone can be partially observed. Bone mineralization in the area is relatively even, osteons are of smaller diameters, with a small number of concentric lamellae, and cement lines are of prominent basophilic reaction.

CONTROL GROUP

Besides the recorded morphological characteristics of healing on experimental damage, a series of morphological changes can be observed at the maximal distance of 3 mm from the edge of the defect in the control group as well. These changes depend upon chronological stages of the experiment.

Control group 30 days

On the thirtieth day after the preparation of the defect, osteosynthetic activity of osteoblasts and the defect filled with newly formed bone tissue can be observed. Endosteal communications are highly developed based on Volkmann and Haversian canal types. Osteocytes are situated in the enlarged lacunae with the rims of intensified basophilia. Changes can be observed on the cement lines in the wider region of the experimental defect in the shape of lacunar enlargement of extracellular matrix between osteons and interstitial lamellae (Picture 3).

Control group - 90 days

Ninety days after the preparation of the defect *restitutio ad integrum* is observed, as well as complete filling of experimental cavity with bone tissue composed of numerous osteons of smaller diameter, with a certain number of concentric lamellae with the outer boundary characterized by the cement line of intensified basophilic reaction (Picture 4).

STATISTICAL ANALYSIS

(Tables 2-10)

DISCUSSION

For the investigation into biocompatibility of endodontic materials both *in vitro* (on cell culture) and *in vivo* tests (subcutaneous, intramuscular and intraosseous implantation) can be used (13*).* Implantation techniques are considered to be more superior because of the greater similarity to clinical conditions and the possibility of monitoring the healing process. Materials can be directly injected or implanted via Teflon, silicone or polyethylene tubes into tissues of rats, rabbits, guinea pigs and other experimental animals (14, 15, 16). Subcutaneous implantation is simpler and widely used (17), however, intraosseous implantation can imitate a clinical situation of close contact between an endodontic material and a bone. The implantation test is an unspecific *in vivo* test of tissue response to materials and as such implies pathohystological analysis after the implantation of tested materials in tissues of different animals. Complete healing of moderate size defect in rats is expected to be completed within 35 days (18), which is why similar time frame was chosen for the first stage of euthanasia.

Inflammatory response of low intensity could be observed in only one experimental animal thirty days after the implantation while it was absent from other animals. Roeko Seal cannot

be considered the cause of inflammation in observation periods. The degree of fibrovascular proliferation also decreased in the course of time, which was expected during the process of healing. Thirty days after the implantation of the material, a callus and newly formed bone tissue could be observed. Young bone tissue of lamellar structure completely filled the space between the material and the unaffected bone tissue until the ninetieth day.

Prepared defect is an extreme stimulus which requires bone remodeling, whereby the bone can repair itself, which led to the reaction of bone tissue 3 mm from the edge of the defect. Besides the established morphological healing characteristics, a series of morphological changes were observed in osteocytes and their lacunae, cement lines and the existing endosteal canal system, Volkmann and Haversian canals in all the experimental animal groups as well as the control group. Morphological changes in cement lines and endosteal canal system were observed in the thirty-day group, however, their disappearance and return to normal bone morphology was observed later in the ninety-day group.

RoekoSeal did not lead to the extension of reparation period, nor did it lead to alterations of bone tissue. Discrepancies in histomorphological characteristics in the implanted tissue were slight in comparison to the control group for all the observed parameters (the degree of inflammatory cell response, the degree of fibrovascular proliferation and the reaction of distal bone) for both periods of time. Roeko Seal proved to be non-biodegradable until the ninetieth day, therefore the defect was not closed as in the control group, however, the bone was repaired and completely healed with the aid of RoekoSeal.

Dimethylpolysiloxane-based material- Roeko Seal can initially cause inflammation after subcutaneous implantation, which is reduced in the course of time and then completely disappears (9, 19). Subcutaneous injection of Roeko Seal into the rat tissue causes a mild to moderate inflammatory reaction within 24 hours and 7 days, but the reaction slows down and becomes chronic by the 30th day with the implant being covered by a fibrous capsule. (19) .

The reduction in inflammation intensity was also described by Derakhshan et al. who analyzed biocompatibility of Roeko Seal in subcutaneous implantation, in rats that were put down after 7, 14 and 60 days. Roeko Seal showed biocompatibility despite the inflammatory reaction after 7 and 14 days since fibrous capsule was formed which the authors considered

to be a good sign because the inflammation was not strong enough to prevent fibroblasts from forming the capsule (9).

The tendency of the degree of inflammatory response to drop was observed in the present study, with a weak inflammatory response present in only one animal on the thirtieth day and absent in other cases.

Other authors have observed that there is a lack of inflammatory response on the fourteenth day after the implantation. Silva-Herzog et al. came to the conclusion that Roeko Seal is biocompatible when implanted subcutaneous into the tissue. Fibrous scar tissue with no signs of inflammation was observed on the 14th day (20). In the same study, spectrophotometric analysis showed that RoekoSeal caused the smallest amount of inflammatory exudate that was significantly different from other investigated materials (AH Plus i Sealapex) and control group (20).

On the other hand, there are authors who observed inflammation even 30 and 90 days after the implantation with Roeko Seal. Dammaschke et al. noticed persistence of previously caused inflammation 30 days after the molar filling in rats. They explained the results by the fact that persistent inflammation could be the consequence of irritable nature of the used sealer (21) Low/ moderate inflammatory infiltrate could be detected in Roeko Seal even 90 days after the tooth filling which was regarded as favorable by Tanomaru-Filho et al. Roeko Seal induced periapical reparation with results similar to AH Plus and Resilon/Epiphany which were also tested in this experiment. Positive results were also obtained in case of reparation-deposition of mineralized tissue on the apical foramen which covered at least half the surface of apical aperture (22).

Results obtained in this research do not correspond to the described results since there were no signs of inflammation after 90 days. Roeko Seal showed the qualities of a biocompatible material and therefore the tissue around it gradually recovered and regenerated in the course of time. Experimental procedure in which teeth of animals were filled was significantly different from bone implantation applied in this experiment which could be the reason why there was a discrepancy between the results.

Ghanaati et al. subcutaneously implanted dimethylpolysiloxane-based material Gutta Flow in rats. Sixty days after the implantation they observed that the material was well integrated in subcutaneous tissue by microscopic analysis. Unlike AH Plus based on plastic resin, which

was also tested in this research Gutta Flow did not succumb to biodegradation. Gutta Flow remained encapsulated in subcutaneous tissue as a foreign body. The given data showed that this material induced inflammatory response which lead to its isolation by fibrous capsule within a living organism since the inflammatory cells of a host could not decompose. This may result in the retention of this material in periapical tissue as a foreign body in cases of overfilling. In conclusion the authors stressed out the fact that the use of biodegradable materials reduced the risk of infection and accelerated periapical healing (23). These results are concordant with the results of the present study where silicon-based material was not absorbed within 90 days, even though it did not cause chronic inflammation. The discrepancies in results could be attributes to different experimental model and tissue in which material was implanted.

Roeko Seal is most commonly defined as a nontoxic or low-level toxic sealer even when it comes to in vivo research. It showed high compatibility with L929 and HeLa cells (24). Silicon is considered to be biocompatible material, therefore these results are expected. Oztan et al. noted low toxic effect of AH Plus and Roeko Seal sealers on fibroblasts of rats (L929 cells) after experimental periods of 24, 48 and 72 hours (25).

CONCLUSION

Roeko Seal does not hinder reparatory mechanisms, nor does it impair morphofunctional relationships in bone tissue.

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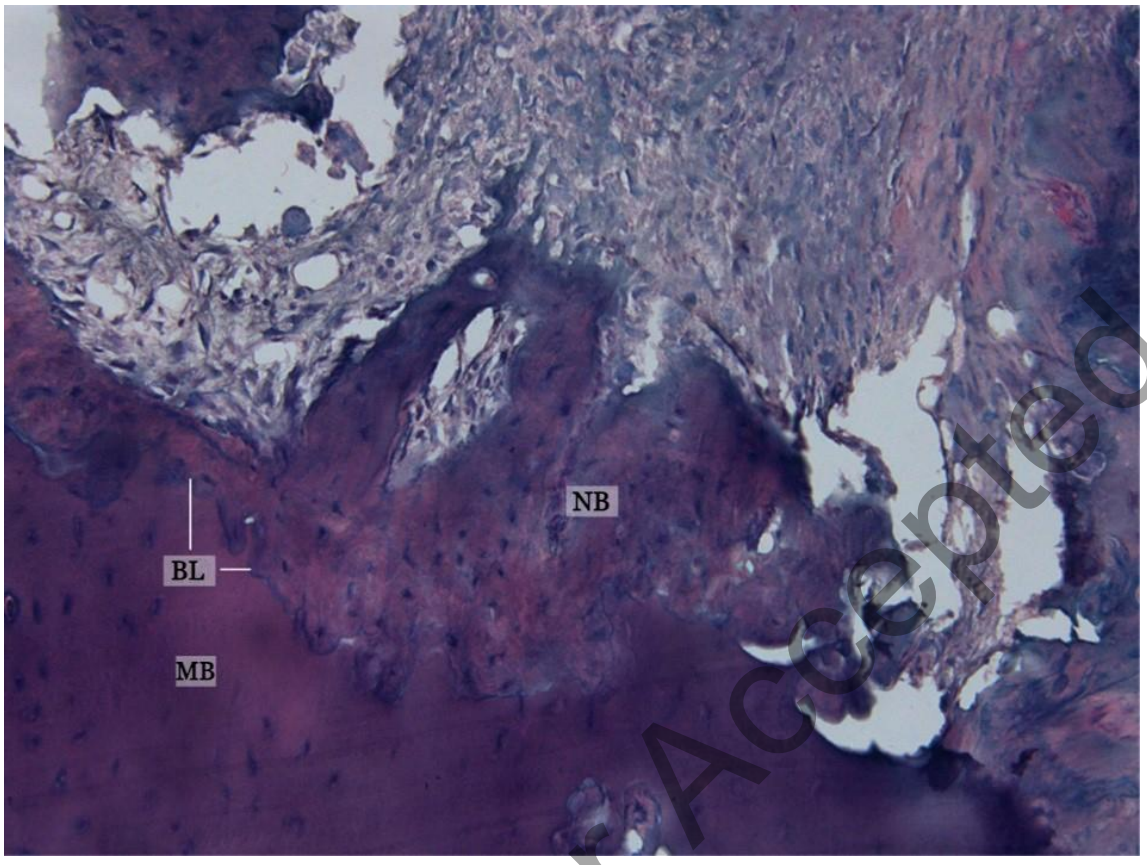
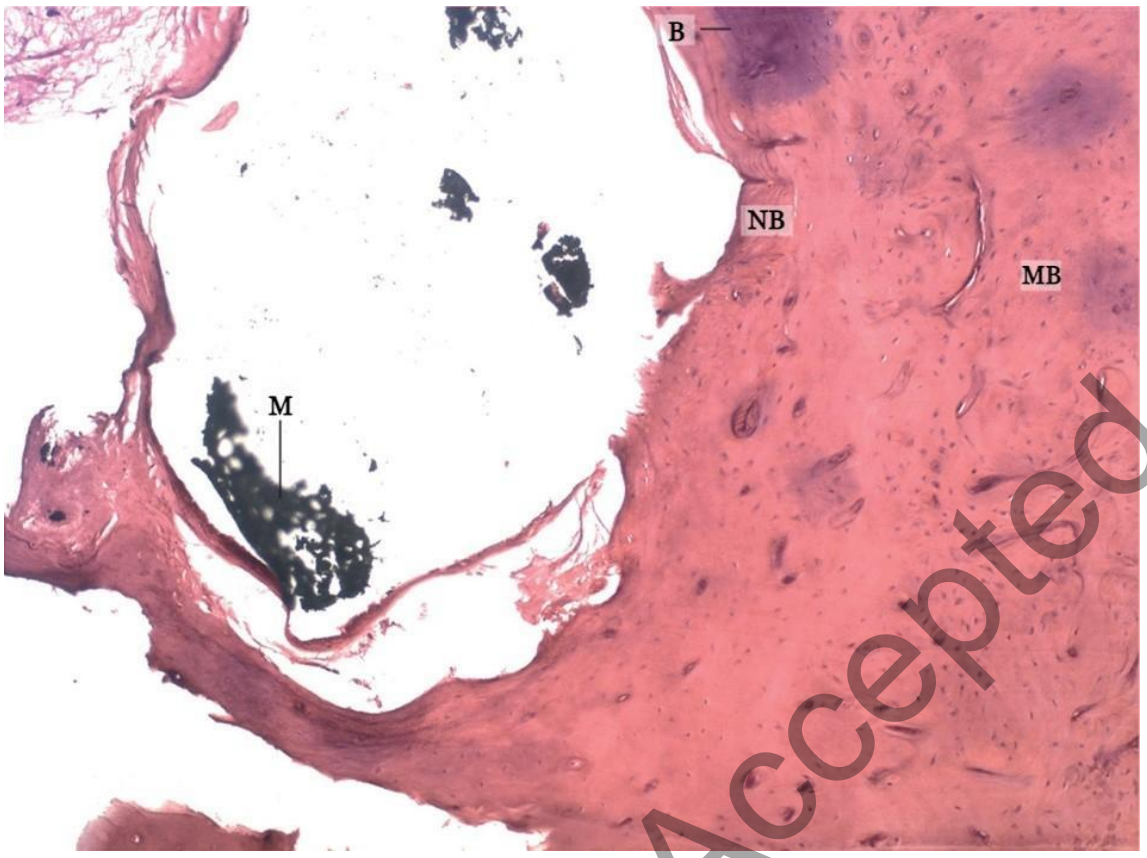
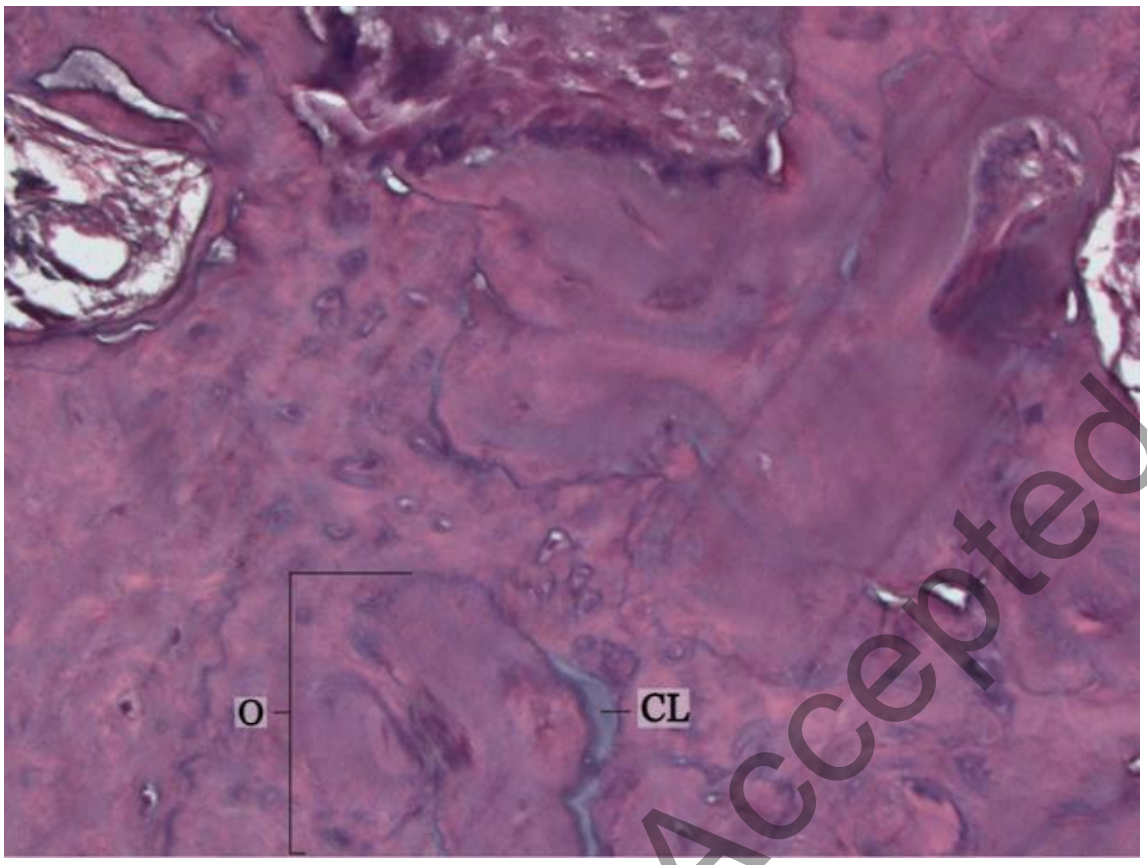
PICTURES



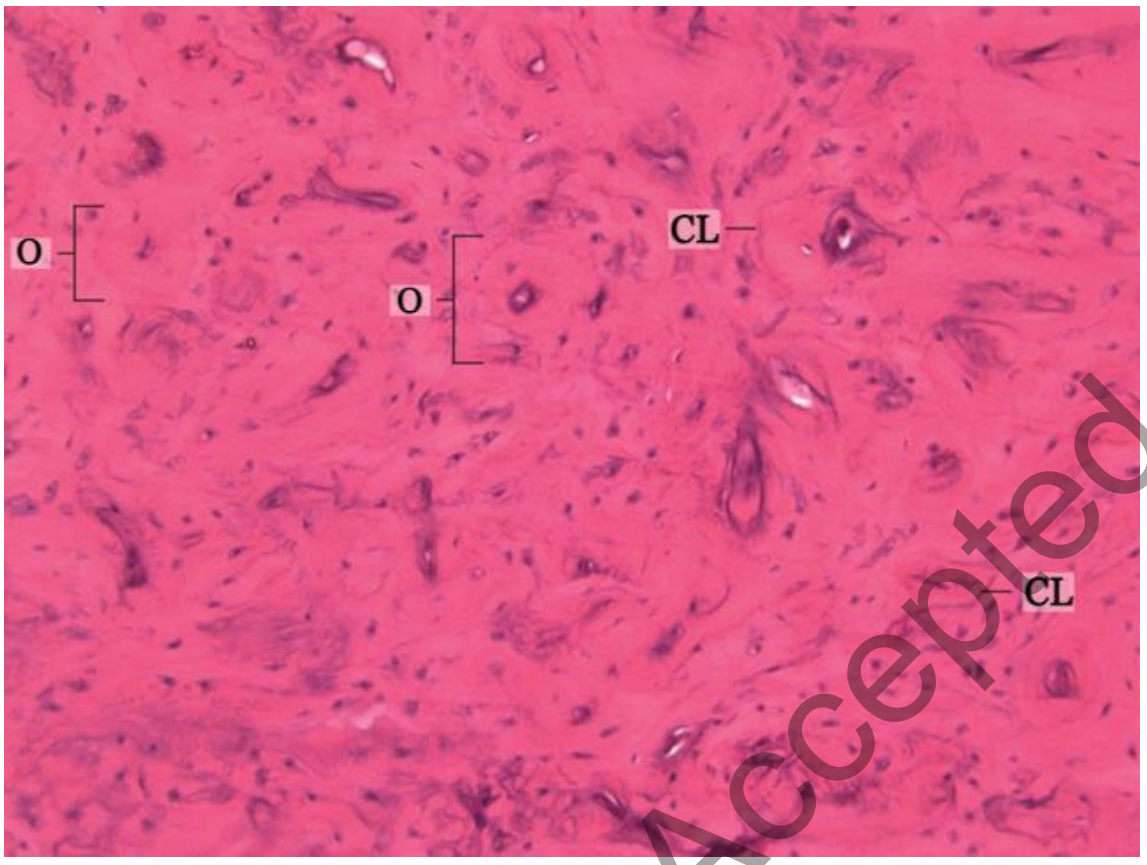
Picture 1. Indicated curved border line (BL) newly formed hypercellular immature bones (NB) and mature bones (MB) (the degree of fibrovascular proliferation- moderate (2)) (HE, x200).



Picture 2. Lamellar bone with formed osteon, trapped scraps of unabsorbed material (M) and partially noticeable boundary (B) of newly formed bone tissue (NB) towards mature bone (MB) (fibrovascular proliferation - absent (0)) (HE, x100).



Picture 3. In the wider region relative to the edge of the defect, especially on the edges of osteons (O), cement lines are pronounced (CL), cracked to irregular polygon shape, filled with fine-grained to amorphous material (degree of distal bone reaction- moderate (2)) (HE, x200).



Picture 4. Completely healed tissue in the former experimental defect. Bone structure of osteon type (O) which are numerous and with cement lines (CL). Intensified basophilic reactions (fibrovascular proliferation- absent (0), inflammatory response- absent (0)) (HE, x200).

TABLES

Table 1. *Roeko Seal* -composition

Roeko Seal	
Component A	Component B
Dimethylpolysiloxane paraffin oil silicone oil	zirconium dioxide hexachloroplatinic acid

Table 2. Differences in INFLAMMATORY RESPONSE between the inicial and final state–

EXPERIMENTAL GROUP

Level of reaction	30 days		90 days	
	Freque.	%	Freque.	%
0	5	83.33	6	100.0
1	1	16.67	0	0
2	0	0	0	0
3	0	0	0	0
Friedman ANOVA. (N=6, df=1) Chi Sqr = 1.00; p = .317				

TABLE

Table 3. Differences in INFLAMMATORY RESPONSE between the inicial and final state –

CONTROL GROUP

Level of reaction	30 days		90 days	
	Frequ.	%	Frequ.	%
0	3	75.0	4	100.0
1	1	25.0	0	0
2	0	0	0	0
3	0	0	0	0
Friedman ANOVA. (N=4, df=1) Chi Sqr = 1.00; p = .317				

Table 4. Differences between the groups–INFLAMMATORY RESPONSE (Kruskal-Wallis ANOVA)

Masuring		**Experimental group**	**Control group**	**H**	**p**
30 days	Σ ranks	32	23	0.09	.760
90 days	Σ ranks	33	22	0.00	1.000

H - Kruskal-Wallis ANOVA test value; p - the p-value of probability value

Table 5. Differences in the degree of FIBROVASCULAR PROLIFERATION between the initial and final state – **EXPERIMENTAL GROUP**

Level of reaction	30 days		90 days	
	Frequ.	%	Frequ.	%
0	0	0	6	100.0
1	0	0	0	0
2	6	100.0	0	0
3	0	0	0	0
Friedman ANOVA. (N=6, df=3) Chi Sqr = 6.00; p = **.014***				

* significance at the level of p<0.05

Table 6. Differences in the degree of FIBROVASCULAR PROLIFERATION between the initial and final state – **CONTROL GROUP**

Level of reaction	30 days		90 days	
	Frequ.	%	Frequ.	%
0	0	0	4	100.0
1	1	25.0	0	0
2	3	75.0	0	0
3	0	0	0	0
Friedman ANOVA. (N=4, df=1) Chi Sqr = 4.00; p = **.046***				

* significance at the level of p<0.05

Table 7. Differences between groups– FIBROVASCULAR PROLIFERATION (Kruskal-Wallis ANOVA)

Measuring		**Experimental group**	**Control group**	**H**	**P**
30 days	Σ ranks	36	19	1.50	.221
90 days	Σ ranks	33	22	0.00	1.000

H - Kruskal-Wallis ANOVA test value; p - the p-value of probability value

Table 8. Diferences in the degree of REMOTE BONE REACTION between the initial and final state– **EXPERIMENTAL GROUP**

Level of reaction	30 days		90 days	
	Frequ.	%	Frequ.	%
0	0	0	6	100.0
1	0	0	0	0
2	6	100.0	0	0
3	0	0	0	0
Friedman ANOVA. (N=6, df=3) Chi Sqr = 6.00; p = **.014***				

* significance at the level of p<0.05

Table 9. Diference in the degree of REMOTE BONE REACTION between the initial and final state – **CONTROL GROUP**

Level of reaction	30 days		90 days	
	Frequ.	%	Frequ.	%
0	0	0	4	100.0
1	1	25.0	0	0
2	3	75.0	0	0
3	0	0	0	0
Friedman ANOVA. (N=4, df=1) Chi Sqr = 4.00; p = **.046***				

* significance at the level of p<0.05

Table 10. Difference berween group – REMOTE BONE REACTION (Kruskal-Wallis ANOVA)

Measuring		**Experimental group**	**Control group**	**H**	**p**
30 days	Σ ranks	36	19	1.50	.221
90 days	Σ ranks	33	22	0.00	1.000

H - Kruskal-Wallis ANOVA test value; p - the p-value of probability value