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

Marija Nikolić^{1,2}, Jelena Popović^{1,2}, Aleksandar Mitić^{1,2}, Aleksandar Petrović³, Radomir Barac^{1,2}, Nenad Stošić^{1,2}, Antonije Stanković⁴, Aleksandra Milovanović⁴

Successful endodontic treatment implies that the materials for obturation remain in the tissue, if possible forever. It is therefore essential to know the long-term effects of materials on tissue. This study aimed to evaluate the 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. A 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 of tissue around the material.

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Key words: sealer, obturation, bone healing

¹University of Niš, Faculty of Medicine, Department of Dental Diseases and Endodontics, Niš, Serbia

²Dental Medicine Clinic, Department of Dental Diseases and Endodontics, Niš, Serbia

³University of Niš, Faculty of Medicine, Department of Histology and Embryology, Niš, Serbia

⁴University of Niš, Faculty of Medicine, doctoral studies, Niš, Serbia

Contact: Marija Nikolić 52 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia E-mail: makinis80@yahoo.com

Introduction

After the removal of canal contents and treatment of the complete canal system by irrigation, obturation follows 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 the canal system, fill accessory canals if any, and be the bond between the gutta-percha and the wall of the 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 researchers 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, the 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 (for decades) (5, 6).

This fact stresses the importance of the biological characteristics of obturation materials (7). A great deal of research has shown that most materials in a 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 (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. It is important to understand how a specific material behaves in the tissue over time and its interaction with the tissue.

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 compositions even in freshly-mixed state (5, 8). According to implantation tests, siliconbased 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.

Aim

The aim of this paper was to investigate tissue reaction to bone implantation of endodontic material Roeko Seal in an artificially prepared defect in the mandible of rats after a long period (30 and 90 days).

Material and Methods

Twenty male Wistar rats with an average weight of 160–180 grams were used for the experimental procedure (the experiment was approved by the Ethic Committee of the Faculty of Medicine in Niš, No. 01 3797). The preparation of experimental animals involved the administration of an anesthetic, namely intraperitoneal injection of ketamine hydrochloride (0.1 ml/100 g). The experimental procedure involved the preparation of bone defect unilaterally (1.4 x 1.6 mm) (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 the anaesthetic (ketamine hydrochloride).

Samples of tissue were collected by resection of the 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 moulded in paraffin wax. Cutting was performed by microtome 2 mm glass knives (Historange). Staining was done by the 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 ANOVA and Kruskal–Wallis ANOVA).

Results

Experimental Group

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

Thirty-Day Findings in the Experimental Group (Roeko Seal)

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

Ninety-Day Findings in the Experimental Group (Roeko Seal)

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



Figure 1. Indicated curved borderline (BL) newly formed hypercellular immature bones (NB) and mature bones (MB) (the degree of fibrovascular proliferation—moderate (2)) (HE, x200)



Figure 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)

Control Group

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

Thirty-Day Findings in the Control Group

On the thirtieth day after the preparation of the defect, the osteosynthetic activity of osteoblasts and the defect filled with newly formed bone tissue could be observed. Endosteal communications were highly developed based on Volkmann and Haversian canal types. Osteocytes were situated in the enlarged lacunae with the rims of intensified basophilia. Changes could 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 (Figure 3).

Ninety-Day Findings in the Control Group

Ninety days after the preparation of the defect *restitutio ad integrum* was observed, as well as the complete filling of the 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 (Figure 4).

Statistical Analysis (Tables 2—10)



Figure 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)

Roeko Seal	
Component A	Component B
Dimethylpolysiloxane	Zirconium dioxide
Paraffin oil	Hexachloroplatinic acid
Silicone oil	

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

Level of	30 days Frequency %		90 da	ys
reaction			Frequency	%
0	5	83.33	6	100.0
1	1	16.67	0	0
2	0	0	0	0
3	0 0		0	0

EXPERIMENTAL GROUP

Friedman ANOVA (N = 6, df = 1)

Chi Sqr. = 1.00; p = .317

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

Level of	30 days		90 da	ys	
reaction	Frequency	%	Frequency	%	
0	3	75.0	4	100.0	
1	1	25.0	0	0	
2	0	0	0	0	
3	0	0	0	0	

CONTROL GROUP

Friedman ANOVA (N = 4, df = 1)

Chi Sqr. = 1.00; p = .317

Table 4. Differences in INFLAMMATORY RESPONSE between the groups (Kruskal—Wallis and ANOVA tests)

Measurings		Experimental	Control	Н	р
		group	grop		
30 days	Σ ranks	32	23	0.09	.760
90 days	Σ ranks	33	22	0.00	1.000

H—values of Kruskal—Wallis and ANOVA tests; p—p-value of probability

 Table 5. Differences in the degree of FIBROVASCULAR PROLIFERATION between the initial and final state

Level of	30 days		90 d	ays
reaction	Frequency	%	Frequency	%
0	0	0	6	100.0
1	0	0	0	0
2	6	100.0	0	0
3	0	0	0	0

EXPERIMENTAL GROUP

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

Level of	30 days		90 days	
reaction	Frequency %		Frequency	%
0	0	0	4	100.0
1	1	25.0	0	0
2	3	75.0	0	0
3	0	0	0	0

CONTROL GROUP

Friedman ANOVA (N = 4, df = 1)

Chi Sqr. = 4.00; $p = .046^*$

* significance at the level of p < 0.05

ANOVA tests)

Measurings		Experimental group	Control group	Н	р
30 days	Σ ranks	36	19	1.50	.221
90 days	Σ ranks	33	22	0.00	1.000

H—values of Kruskal—Wallis and ANOVA tests; p—p-value of probability

Table 8. Differences in the degree of REMOTE BONE REACTION between the initial and final state

Level of	30 days		90 days	
reaction	Frequency	%	Frequency	%
0	0	0	6	100.0
1	0	0	0	0
2	6	100.0	0	0
3	0	0	0	0

EXPERIMENTAL GROUP

Friedman ANOVA (N = 6, df = 3)

Chi Sqr. = 6.00; p = .014*

* significance at the level of p < 0.05

Table 9. Differences in the degree of REMOTE BONE REACTION between the initial and final state

CONTROL GROUP

Level of reaction	30 days		90 da	ys
	Frequency %		Frequency	%
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. Differences in REMOTE BONE REACTION between the groups (Kruskal-Wallis and ANOVA

tests)

Measurings		Experimental	Control	Н	р
		group	group		
30 days	Σ ranks	36	19	1.50	.221
90 days	Σ ranks	33	22	0.00	1.000

H—values of Kruskal—Wallis and ANOVA tests; p—p-value of probability

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 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 (17), however, intraosseous widely used 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 cause of inflammation considered the 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 filled the space between the material and the unaffected bone tissue until the ninetieth day.

Prepared defect is an extreme stimulus which requires bone remodelling, whereby the bone can repair itself, which leads 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 were observed later in the ninety-day group.

Roeko Seal did not lead to the extension of the 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. Roeko Seal proved to be nonbiodegradable 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 Roeko Seal.

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. concluded that Roeko Seal is biocompatible when implanted subcutaneously into the tissue. Fibrous scar tissue with no inflammation was observed on the 14th day (20). In the same study, the spectrophotometric analysis showed that Roeko Seal caused the smallest amount of inflammatory exudate that was significantly different from other investigated materials (AH Plus and Sealapex) and control group (20).

On the other hand, some authors observed inflammation even 30 and 90 days after the implantation with Roeko Seal. Dammaschke et al. noticed the 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 the 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 the case of the reparation-deposition of mineralized tissue on the apical foramen which covered at least half the surface of the 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 days after the implantation, rats. Sixty microscopic analysis showed that the material was well integrated in subcutaneous tissue. 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 an inflammatory response which led 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 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 attributed to different experimental models 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 a biocompatible material, therefore these results are expected. Oztan et al. noted the 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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HISTOLOŠKA PROCENA ODGOVORA KOŠTANOG TKIVA NA ENDODONTSKI MATERIJAL NA BAZI SILIKONA

Marija Nikolić^{1,2}, Jelena Popović^{1,2}, Aleksandar Mitić^{1,2}, Aleksandar Petrović³, Radomir Barac^{1,2}, Nenad Stošić^{1,2}, Antonije Stanković⁴, Aleksandra Milovanović⁴

¹Univerzitet u Nišu, Medicinski fakultet, Katedra za bolesti zuba i endodonciju, Niš, Srbija
²Klinika za dentalnu medicinu Niš, Odeljenje za bolesti zuba i endodonciju, Niš, Srbija
³Univerzitet u Nišu, Medicinski fakultet, Katedra za histologiju i embriologiju, Niš, Srbija
⁴Univerzitet u Nišu, Medicinski fakultet, student doktorskih studija, Niš, Srbija

Kontakt: Marija Nikolić Bulevar dr Zorana Đinđića 52, 18000 Niš E-mail: makinis80@yahoo.com

Uspešan endodontski tretman podrazumeva da materijal za opturaciju ostane u tkivu, zauvek ako je to moguće. Stoga, neophodno je poznavati dugoročne efekte materijala na okolno tkivo. Cilj ove studije bila je histološka procena odgovora koštanog tkiva na materijal na bazi dimetil-polisiloksana implantiran u artificijelni preparirani defekt. Uzorak je obuhvatio 20 *Wistar* pacova. Defekt je formiran u mandibulama pacova sterilnim svrdilima od nerđajućeg čelika. Siler na bazi dimetil-polisiloksana (*Roeko Seal*) implantiran je u defekte pacova iz eksperimentalne grupe, dok su defekti pacova iz kontrolne grupe ostavljeni da spontano zarastu. Jedna polovina životinja iz obeju grupa žrtvovana je nakon 30 dana, a druga nakon 90 dana. Mikroskopski preparati su analizirani na svetlosnom mikroskopu. Fibrozni kalus i mlada kost uočeni su trideset dana nakon implantacije. Devedeset dana nakon implantacije, kost oko neresorbovanog materijala u potpunosti je zacelila. *Roeko Seal* ne usporava zarastanje koštanog tkiva i omogućava potpuno zaceljenje tkiva oko materijala.

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Ključne reči: siler, opturacija, zarastanje kosti

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