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Subcutaneous Tissue Response to Adseal and Sure-Seal Root Sealers in Rats: a Histopathological Study

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ABSTRACT

Introduction and objectives: *One of the essential phases of root canal treatment is root canal obturation. Solid or semi-solid materials are the most common obturating materials (paste or softened form). Sealer is a biomaterial that enables the sealing process to be carried. This study aimed to evaluate the inflammatory response to Adseal sealer and Sure-Seal Root sealer in rats.*

Materials and methods: *This experimental study was conducted on 28 Wistar rats that were divided into four groups of seven animals per group based on four time periods (7, 14, 30 and 60 days). Each rat received subcutaneous implants containing Adseal sealer (Meta Biomed, Cheongju, Korea) and Sure-Seal Root sealer (Sure Dent Corporation, Gyeonggi-do, South Korea) tubes as well as an empty tube as a control. After the insertion of the tubes, the first to fourth groups were sacrificed on days 7, 14, 30 and 60, respectively, by injecting a high dose of anesthetics. Subsequently, the histopathologic features of the samples were investigated. Data were analyzed in SPSS software (version 26) using Freidman, Wilcoxon, Kruskal-Wallis and Mann-Whitney U tests. A p-value less than 0.05 was considered statistically significant.*

Results: *On day 7, the severity of inflammation was higher in the Adseal sealer and Sure-Seal Root sealer groups compared to the control group. Moreover, on day 14, the level of inflammation was higher in the Sure-Seal Root sealer group than the Adseal sealer and control groups. In addition, on days 30 and 60, the severity of inflammation was similar in both the case and control groups and decreased in all samples. Formation of granulation tissue was observed in all samples on day 14. There was fibrosis tissue in the*

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Sure-Seal Root sealer samples (71.4%) on day 60; however, no fibrosis tissue was observed in the Adseal sealer and control groups.

Conclusion: *It is concluded that the Sure-Seal Root sealer might lead to a more inflammatory response compared to the Ad Seal sealer. However, due to decreasing inflammation in sealers over time, both sealers are biocompatible.*

Keywords: Adseal, sealer, sure seal root, tissue reaction.

INTRODUCTION

Root canal treatment seeks to remove the dental pulp to prevent potential infection and repair root periapical tissue. Root canal filling provides complete sealing in the whole root canal from crown tooth entry to the end of the apical. The primary root canal filling materials are usually solid or semi-solid (paste or softened form) and are used with or without a sealer (1). Endodontic treatment aims to prevent and treat apical periodontitis by using proper disinfection and three-dimensional root canal filling (2). Root canal obturation is one of the essential phases of root canal treatment. It affects various factors, including the technical quality of the root canal. Root canal filling prevents the dissemination of microbes and their byproducts, and it has been employed in various methods from solid material to gutta-percha cones with root canal sealers (3). The material used for tooth sealing remains permanently in the root system. Therefore, it might affect the surrounding tissue (4). Although the endodontic sealer is vital in root canal treatment, the differences in the methods of root canal filling, thickness and uniform distribution of sealers are significant (5).

The toxicity of an endodontic material is vital in the treatment procedure because most sealers have demonstrated cytotoxic responses (6). The direct contact of incompatible sealers with the surrounding tissue or tissue fluids can affect the subsequent repair of the tissue and make a favorable environment for bacterial invasion. It can also cause inflammation and decreased repair of apical periodontitis by increasing macrophage activity, adhesion, and phagocytosis (7, 8). Generally, before introducing a new biomaterial in clinical use, it must always be evaluated by histocompatibility tests.

Tissue compatibility is assessed through cell culture studies *in vitro*, and then, at higher levels, intraosseous or subcutaneous implantation *in vivo* (9). The biomaterials which are composed of ceramics are called hydraulic calcium silicate-based sealers and among them, Endoseqence BC Hiflow and Endoseqence BC are examples of biocompatible materials when exposed to human periodontal ligament stem cells (hPDLSCs) (10). These sealers contain calcium phosphate and/or calcium silicate. Bioactive bioceramics react with tissue components and may be bioresorbable, such as calcium phosphate materials-alternatively, non-bioresorbable, including calcium silicate or hydraulic cement used in endodontics (11). Bioactivity of materials refers to their capacity to form a hydroxyapatite layer when exposed to a calcium and phosphate-rich tissue fluid. This feature adds to the sealing ability of the material and makes it highly biocompatible, osteoinductive, and osteoconductive (12).

Adseal sealer (Meta Biomed, Cheongju, Korea) is a resin-based endodontic sealer which is insoluble in tissue fluids (13); cytotoxicity assays for Adseal sealer show increased mesenchymal cells and good biocompatibility in human periodontal ligament cells (14). Sure-Seal Root sealer (Sure Dent Corporation, Gyeonggi-do, South Korea) is a new root canal hydraulic sealer premixed to use injectable bioactive calcium silicate-based material for root canal filling; it has a biocompatible, osteogenic, antibacterial, radiopaque, hydrophilic and hydroxyapatite formation; moreover, it has a proper shelf time and ideal hardening. It does not compress during set-up and has useful physical features (15).

Calcium silicate cement has shown good biocompatibility, bioactivity and osteoconductivity (16). These sealers also can stimulate osteoblastic and osteocementogenic genes (17). To our knowledge, there is no published study investigating

the Sure-Seal Root sealer compatibility. Accordingly, the present study aimed to evaluate the biocompatibility of a new hydraulic calcium silicate-based root canal sealer (Sure-Seal Root sealer) compared to Adseal sealer through subcutaneous implantation in an experimental rat model. The null hypothesis was that there would be no difference among groups regarding the histological reactions on days 7 and 14.

MATERIALS AND METHODS

Preparation of samples

The present experimental study was conducted at Mazandaran University of Medical Sciences, Mazandaran, Iran. All experimental procedures were evaluated and approved by the Ethics Committee of Mazandaran University of Medical Sciences, Mazandaran, Iran (IR.MAZUMS.REC.1397.2718).

The Wistar rats (n=28), weighing 250-300 g, were obtained from the Mazandaran University of Medical Sciences, Mazandaran, Iran. They were housed in standard conditions, fed with a standard diet and kept under standard conditions (12-h light: dark cycle, temperature 20-25°C, and 25%-35% humidity). Experimental protocols were conducted according to the Guide for the Care and Use of Laboratory Animals (18).

Toxicity evaluation

The rats (n=28) were randomly divided into four groups of seven animals per group based on four intervals (7, 14, 30, and 60 days), and each rat received three tubes, including two tubes of Adseal sealer (Meta Biomed, Cheongju, Korea) and Sure-Seal Root sealer (Sure Dent Corporation, Gyeonggi-do, South Korea). Moreover, and an empty tube was regarded as the control in their subcutaneous tissues. The animals were intraperitoneally anesthetized using 75 mg/kg ketamine 10% (Dopaser, Caliers A, Barcelona, Spain) and 10 mg/kg xylazine (Dopaser, Caliers A, Barcelona, Spain). Following the shaving of the dorsal skin and disinfection with 5% povidone-iodine solution (Behvarzan, Rasht, Iran), three 15-mm longitudinal incisions were made through the skin with a # 15 scalpel, equidistant from the spine with an orientation from head to tail, Then, the separated subcutaneous pockets were prepared by blunt dissection at each side of the incision. The premixed and injectable Sure-Seal Root sealer was used in this study. The Adseal sealer in aseptic condition and the base of the manufacturing kit brochure was mixed and placed in sterile polyethylene tube (10 mm height and 3 mm in inner diameter) (Figure 1).

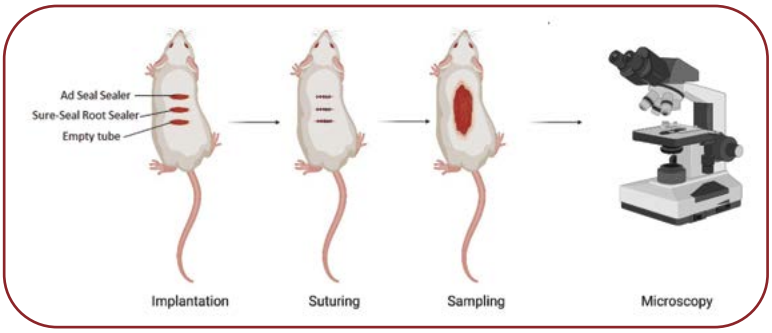

FIGURE 1. Rat models receiving three tubes, including two tubes of Adseal sealer and Sure-Seal Root sealer and an empty tube as the control in their subcutaneous tissues

Table 1 summarizes the chemical components of the sealers and their producer. The sealers were carefully placed into the pockets to a depth of 20 mm to prevent smearing of the test material on the outer tube areas. An empty tube was used as the negative control. After implantation, the incisions were closed using 4-0 silk sutures (Silkam HR26, B. Braun Surgical, Rubí, Spain). All animals had free access to a standard diet and water during the entire study period. The first to fourth groups were sacrificed by injecting a high dose of anesthetics on days 7, 14, 30, and 60, respectively. The samples were prepared with a

TABLE 1. Sealer names, producers and chemical components

Sealer	Producer	Components
Adseal sealer	Meta Biomed, Cheongju, Korea	**BASE** <20% epoxy resin NS calcium phosphate NS zirconium dioxide NS calcium oxide Ns ethylene glycol salicylate **Catalyst** 2.5%–10% N, n-dibenzyl-5-oxanonandiamin-1,9 2.5%–10% amantadine
Sure seal root sealer	Sure Dent Corporation, Gyeonggi-do, South Korea	Calcium silicate Calcium sodium phosphosilicate Zirconium oxide

thickness of 5 µm. The specimens were fixed in 10% formalin (PH=7) (Merck, Darmstadt, Germany) from 24 to 28 hours at 4°C before the histopathological study. The full thickness of the fragmentations was embedded in paraffin and stained with hematoxylin and eosin (H&E). Afterward, histopathological changes were microscopically analyzed with a light microscope (Nikon-Eclipse-E100, JaPan) and documented by a pathologist. The inflammation reactions were categorized and each score was examined in three 7.5 mm^2 fields under a 100× and 400× microscope (19). The scores of reactions are listed below:

- Score 1 (no reaction or a few acute inflammatory cells);
- Score 2 (mild reaction): the presence of less than 25 chronic inflammatory cells;
- Score 3 (moderate reaction): 25-125 chronic inflammatory cells;
- Score 4 (severe reaction): 125 or more chronic inflammatory cells.

The presence or absence of hemorrhagic and fibroblast cells in each group was recorded (20), followed by reporting the presence of fibrosis tissues and granulation (21).

Statistical analysis

The results were analyzed in SPSS software (version 26). For comparing the severity of inflammation, the Kruskal-Wallis test was used in both the case and control groups for each interval (7, 14, 30, and 60). If results were significant, the Mann-Whitney U test was employed to compare the pairwise groups. Furthermore, the severity of inflammation in every group for each interval was compared utilizing the Freidman test. Significance Wilcoxon test was used to compare two-to-two times in each group. A p-value less than 0.05 was considered statistically significant.

RESULTS

This study investigated 28 rats that were divided into four groups of seven animals *per* group based on four time periods (7, 14, 30, and 60 days). According to the results of Kruskal-Wallis test, there were significant differences among the Sure-Seal Root sealer, Adseal sealer, and control groups in terms of inflammation severity after seven (P=0.002) and 14 (P=0.002) days; however, no difference was observed on days 30 (P=1) and 60 (P=1). In addition, the results of Mann-Whitney U test revealed significant differences between the Sure-Seal Root sealer and control groups in the severity of inflammation after seven (P=0.008) and 14 (P=0.011) days, so that the severity of inflammation was more severe in the Sure-Seal Root sealer than the control group. Furthermore, there was a significant difference between the two groups of Sure-Seal Root sealer and Adseal sealer only on day 14, and the severity of the Sure-Seal Root sealer inflammation was more severe than that in the Adseal sealer group (P=0.014; Table 2).

The Freidman test results indicated that the severity of inflammatory response decreased in the Sure-Seal Root sealer (P=0.001), Adseal sealer (P=0.001) and control (P=0.001) groups on days 7-60, and it was also significant based on the Wilcoxon test results. Wilcoxon test results showed significant differences among all the samples in terms of the severity of inflammatory response on days 7 and 14 compared to the findings on days 30 and 60 (P=0.001). It is worth mentioning that the severity of inflammation has decreased on days 30 and 60 (Table 3).

Seven-day period

The Kruskal-Wallis test showed significant differences among the groups (P=0.002). The severity of

TABLE 2. Determination and comparison of inflammation in experimental and control groups

Day	Sure-Seal Root	Adseal sealer	Control	Overall p-value*	p-value for SSR *vs* ASS**	p-value for SSR *vs* control**	p-value for ASS *vs* control**
7	4	3.57 ± .53	2	0.002	0.083	0.008	0.015
14	3.14 ± .37	2.28 ± .48	2	0.002	0.014	0.011	0.157
30	1	1	1	1	1	1	1
60	1	1	1	1	1	1	1

*Kruskal-Wallis test; **Mann-Whitney test; SSR: Sure-Seal Root; ASS: Adseal sealer

Data are expressed as mean± standard deviation

TABLE 3. The significant level of inflammation severity in different groups over time

GROUP	Overall p-value*	p-value for 7 *vs* 14**	p-value for 7 *vs* 30**	p-value for 7 *vs* 60**	p-value for 14 *vs* 30**	p-value for 14 *vs* 60**	p-value for 30 *vs* 60**
Sure-Seal Root	0.001	0.356	0.001	0.001	0.001	0.001	1
Adseal sealer	0.001	0.089	0.001	0.001	0.001	0.001	1
Control	0.001	1	0.001	.0010	0.001	0.001	1

*Friedman test; **Wilcoxon test

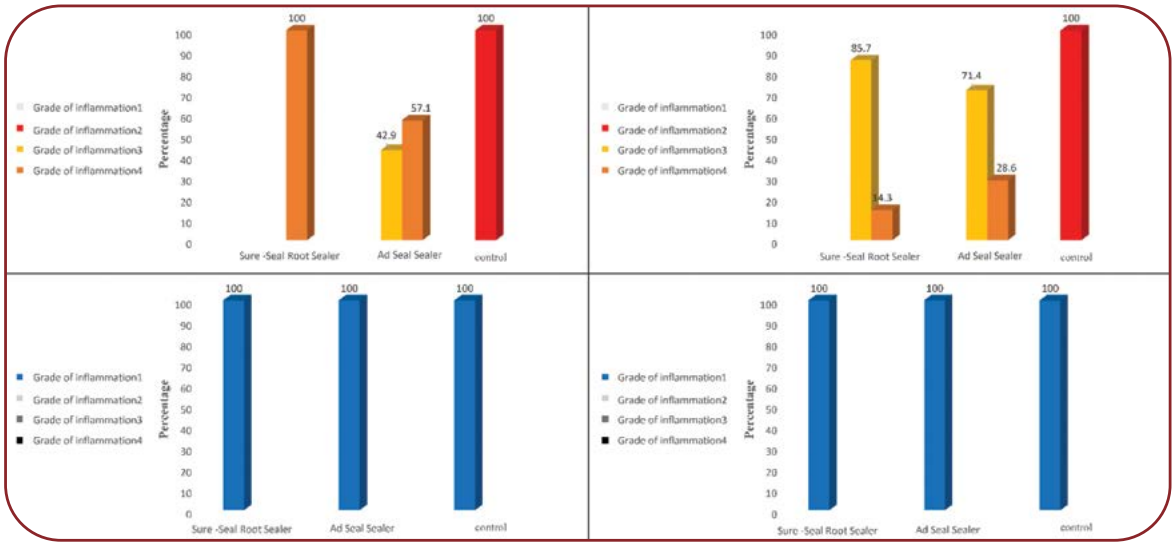

FIGURE 2. A) Distribution of inflammation grades, in percentages, for seven days; B) distribution of inflammation grades, in percentages, for 14 days; C) distribution of inflammation grades, in percentages, for 30 days; D) distribution of inflammation grades, in percentages, for 60 days

inflammation was higher in the Adseal sealer and Sure-Seal Root sealer groups compared to the control group. The differences were also significant according to the Mann-Whitney U test results (P<0.05; Table 2, Figure2). On the other hand, the severity of inflammation in the control group, which received empty tubes, was mild (100%). However, the severity of inflammation in rats with Sure-Seal Root sealer tubes was severe (100%) due to acute inflammation, and most cells were neutrophils. In the Adseal sealer group, the rate of inflammation was moderate (42.9%) to severe (57.1%), and inflammation was acute; moreover, most of the cells were neutrophils. Hemorrhage and fibroblast cells were observed in most of the samples (Figures 3A-C).

Fourteen-day period

The Kruskal-Wallis test results showed significant differences among groups (P=0.002). The severity of inflammation was higher in the Sure-Seal Root sealer group compared to the Adseal sealer and control groups. The differences were also significant according to the Mann-Whitney U test results (P<0.05; Table 2, Figure 2). The severity of inflammation in the control group, which received empty tubes, was mild (100%) on day 14, and the severity of inflammation in the rats with the Sure-Seal Root sealer tubes decreased from severe (14.3%) to moderate (85.7%). The inflammation of rats in the Adseal sealer group was reduced to moderate (28.6%) and mild (71.4%). The formation of the granulation tissue was observed in all samples (Figures 3D-G).

Thirty-day period

The Kruskal-Wallis test results showed no significant differences among the groups (P=1), and the severity of inflammation was at the same level 1 in both the case and control groups (P>0.05;

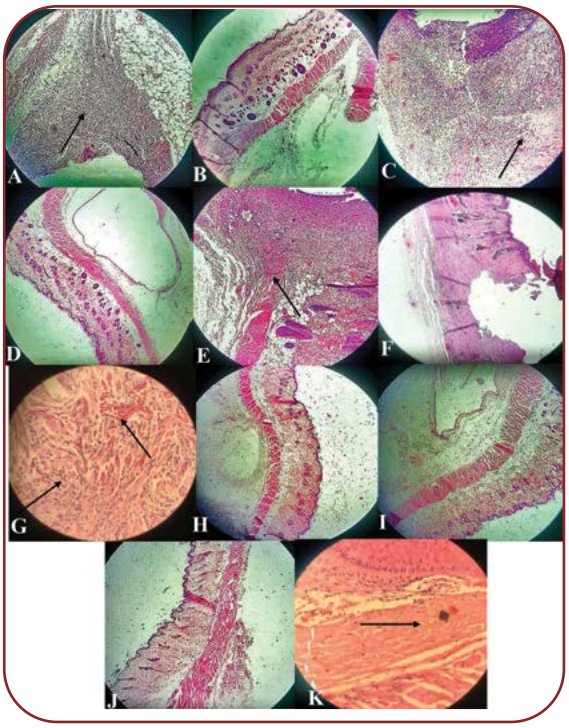

FIGURE 3. Images from the rat subcutaneous tissue reaction in the experimental tubes on the 7th day (H&E staining): A) severe to moderate inflammation and reaction tissue in Adseal tubes (400×) (the black spike shows the tissue reaction to the sealer); B) empty tube as the control group (100×); C) severe to moderate inflammation and reaction tissue in the Sure-Seal tubes (400×) (the black spike shows the tissue reaction to the sealer)

Images from the rat subcutaneous tissue reaction in the experimental tubes on the 14th day (H&E staining): D) empty tube as the control group; E) moderate to severe inflammation in the Sure-Seal Root tubes with lymphocyte and granulation cells; few neutrophils, macrophage, and severe inflammation in the Sure-Seal Root (400×); F) Moderate to mild inflammation in the Adseal tubes; Severe inflammation in the Sure Seal (400×); G) Inflammatory infiltration in reaction to the Sure-Seal Root on the 30th day (the black spike shows the tissue reaction to the sealer)

Images from the rat subcutaneous tissue reaction in the experimental tubes on the 30th and 60th days (H&E staining): H) Absence of inflammation reaction in the Adseal tubes on the 30th day; I & J) absence of inflammation reaction in the Adseal and Sure-Seal Root tubes on the 60th day; K) fibrous tissue in the Sure-Seal Root tubes on the 60th day (the black spike shows the tissue reaction to the sealer)

Table 2, Figure 2). No inflammation was noted among the groups, and no specific tissue changes were observed (Figure 3H).

Sixty-day period

The Kruskal-Wallis test indicated no significant differences among the groups (P=1), and the severity of inflammation was at the same level 1 in both the case and control groups (P>0.05) (Table 2, Figure 2). No inflammation was observed among the groups. There were fibrosis tissues in the Sure Seal Root Sealer samples (71.4%); however, no fibrosis tissue was observed in the Adseal sealer and control groups (P=0.001; Figure 3 I-K). ❑

DISCUSSION

The biocompatibility of a material is defined as its ability to be compatible with the host tissue (22). Materials must possess the ability to have direct contact with the living tissues. Therefore, being aware of the host tissue response is vital and can be influenced by endodontic treatment (6). The use of new material for clinical methods must be evaluated by histocompatibility tests, cell culture and implantation in living tissues (23). Bioceramic sealers have been used for endodontic purposes for the past 30 years. The utilization of the bioceramic materials as root canal sealers has two advantages, including biocompatibility, which prevents the rejection by the surrounding tissues, and calcium phosphate, which enhances the setting properties of the bioceramics. It results in a chemical structure which is similar to that of the teeth and improves the bonding of the sealer to dentin (24).

Based on the results of the present study, the null hypothesis is rejected since the inflammatory response in the resin sealer was less than that in the bioceramic sealer on days 7 and 14. Almost all endodontic sealants are toxic when freshly prepared and should undergo clinical trials (25, 26). In some studies, due to sealer extrusion and tissue reaction, the set conditions of materials are preferred. In other studies, freshly mixed are used because of their similarity to clinical conditions. In our study, all tubes were filled with fresh sealers (27). There was no previous study on the tissue response of the sealers; therefore, our findings not be compared with those of previous studies. The present study showed that calcium silicate-based sealer had a more significant inflammatory response than resin sealer and control groups on days 7 and 14; however, over time more precisely on days 30 and 60, the inflammatory response of

both sealers was similar and decreased. The primary inflammatory response in the case and control groups may be due to the trauma caused by surgery and placement of tubes. In this respect, the results obtained by us are in line with those reported by El-Mansy (26); however, they are not consistent with the results of a study conducted by Azevedo LU (28), in which no inflammatory response was observed. The initial inflammatory reaction of calcium silicate-based sealer due to the release of calcium ions and the formation of alkaline pH can be a normal process.

Alkaline pH damages periapical tissues, releases inflammatory cytokines from inflammatory cells and reduces cell life (29). Heat due to the material setting reaction and high flow hesitated setting time lead to the long-term dissolution of toxic materials in the tissue (17, 30, 31). If the inflammatory reaction decreases over time, it can indicate the biocompatibility of a substance (13). In the present study, the inflammatory response decreased over time in both calcium silicate-based sealer and resin based sealer. Studies have also shown that zirconium oxide is similar to radioopacifir in the composition of both sealers, which is non-toxic and biocompatible. It also accelerates fibroblast proliferation and regression of the inflammatory response (17, 26, 32). Adseal is a resin based sealer which contains a catalyst component that accelerates the setting time process (33). Moreover, the low cytotoxicity of the Adseal sealer may be due to its calcium phosphate content, which can increase its compatibility and may have a bone-inducing effect (13). In the present study, granulation tissue formation was observed on day 14 in all case and control samples; however, fibrosis tissue formation was noted only in the Sure-Seal Root sealer on day 60. The formation of the fibrous tissue appears to be an attempt to limit the toxicity of an external substance (27). In some studies, the formation of fibrosis tissue around the sealer is inversely related to adaptation and is a sign of inflammation (17). Other studies have shown an association between fibrosis tissue and material compatibility (17, 26, 34). In the present study, the formation of fibrosis tissue in calcium silicate sealer over time is probably related to the compatibility of these materials.

Although this study has made an excellent effort to compare the inflammatory response to Adseal sealer and Sure-Seal Root sealer in rats, more clinical and preclinical investigations are needed to confirm the results of this experiment. It is also recommended that further studies compare the biocompatibility of the Sure-Seal Root sealer with more than one sealer to approve the accuracy of results. ❑

CONCLUSION

It can be concluded that Sure-Seal Root sealer might lead to a more inflammatory response than Adseal sealer. However, due to decreasing inflammation in sealers over time, both sealers are biocompatible, but it is recommended that they should be evaluated with more sealers and more tests. ❑

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