

## Biocompatibility of a novel hydroxyapatite-based endodontic sealer enriched with silicon and strontium ions in rats

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### Abstract

**Objective:** This study aimed to evaluate the biocompatibility of a novel bioceramic endodontic sealer formulation, combining tricalcium silicate/dicalcium silicate, silicon hydroxyapatite, and strontium hydroxyapatite.

**Methods:** Sixty polyethylene tubes were filled with the following four materials (n = 15): AH Plus sealer, Sure-Seal Root bioceramic sealer, an experimental bioceramic sealer, and empty control tubes. Fifteen adult Wistar rats were used, each receiving all four tube types implanted subcutaneously on the dorsal skin. The rats were divided into three groups, evaluated at 15, 30, and 60 days postoperation. Histological analysis assessed inflammation, fibrous capsule thickness, giant cell infiltration, and biomineralization. The Kruskal-Wallis test was used for statistical analysis, with the significance level set at  $P < 0.05$ .

**Results:** There were no statistically significant differences in tissue reaction measures among the groups ( $P > 0.05$ ). Intragroup comparisons revealed a significant reduction in inflammation in the AH Plus group ( $P = 0.04$ ). Fibrous capsule thickness and giant cell infiltration decreased significantly in the Sure-Seal Root and AH Plus groups over the experiment ( $P < 0.05$ ). Biomineralization increased in the experimental sealer group but without statistical significance ( $P > 0.05$ ).

**Conclusions:** Sure-Seal Root and AH Plus sealers exhibited significant decreases in some tissue reactions over time, but the experimental sealer did not. Despite the lack of statistical significance in between-group comparisons, the biocompatibility of commercially available sealers appears to be better than the experimental sealer, based on the results of this study.

**Keywords:** AH Plus, Biocompatibility, Endodontic treatment, Hydroxyapatites, Root canal sealer, Tricalcium silicate

### Introduction

The primary goal of endodontic treatment is to eliminate microorganisms, followed by three-dimensional obturation of the root canal to reduce the risk of microbial recolonizations (1). Root filling typically consists of a core material and a sealer (2). Since gutta-percha, the most commonly used core material does not bond directly to the dentinal walls, a sealer is essential to create an impenetrable seal and fill any accessory canals (3). Due to its potential extrusion from the apical foramen and direct contact with apical tissues, the

biocompatibility of filling materials becomes critical (4). In the presence of toxic materials, a prolonged inflammatory reaction in periradicular tissues may occur, potentially leading to delayed healing or treatment failure. (5). An ideal sealer must possess several essential characteristics, including favorable physical, chemical, and bonding properties, optimal antibacterial activity, acceptable biocompatibility, and excellent sealing ability (6-8).

Endodontic sealers can be classified into seven major groups, including zinc oxide eugenol (ZOE)-based, epoxy resin-based, silicon-based, mineral trioxide aggregate (MTA)-based, bioceramic-based, methacrylate resin-based, and calcium phosphate-based sealers (9). Several studies have evaluated the inflammatory potential, foreign body reactions, effects on the mineralization of dental pulp cells, and overall biocompatibility of endodontic sealers (10-13). However, the currently available sealers met only some ideal criteria, each presenting specific limitations. For instance, ZOE-based

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sealers exhibit apical leakage over time (14). AH-26, an epoxy resin-based sealer, releases toxic levels of formaldehyde (15). Because of their high viscosity, silicon-based sealers can be extruded into the periapical tissue under pressure (16). When extruded from the apical foramen, MTA-based sealers may cause severe pain (17). The properties of bioceramic sealers, such as setting time and microhardness, are easily affected by environmental moisture (18). Given the critical role of sealers in achieving successful obturation and preventing reinfection, there is a clear need for continued investigation into alternative materials.

A novel hydroxyapatite-based bioceramic sealer, which contains tricalcium silicate/dicalcium silicate, silicon hydroxyapatite, and strontium hydroxyapatite, has been developed in the Dental Materials Research Center of Mashhad University of Medical Sciences. A previous study (19) assessed the physical properties of this sealer, but research on its biocompatibility remains limited.

This study aimed to evaluate the tissue reaction of rat subcutaneous tissue to this novel sealer formulation and compare its biocompatibility with two commonly used sealers: an epoxy resin-based sealer (AH Plus) and a bioceramic sealer (Sure-Seal Root).

## Materials and methods

This study was conducted in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (20) and approved by the ethics committee of Mashhad University of Medical Sciences with the approval number IR.MUMS.DENTISTRY.REC.1398.115.

### Study design

A power analysis was conducted to determine the appropriate sample size for the study. Based on a prior study (21), the sample size was calculated using an alpha level of 0.05 and a beta level of 0.20. Consequently, 15 adult male Wistar rats were selected. These rats were approximately two months old and weighed around 2.2 kg. Based on postoperative intervals (15, 30, and 60 days), they were randomly assigned to three experimental groups (n=5 per group).

All animals were housed under standard laboratory conditions, including controlled temperature, humidity, and a 12-hour light/dark cycle, and were provided with a consistent diet and water. Each rat was observed daily to ensure their well-being throughout the study.

### Material preparation

Sixty sterile polyethylene tubes (10 mm in length, 1.2 mm in diameter) were prepared as carriers for the root canal sealers. These tubes were divided into four groups of 15 tubes and filled with the following sealers:

Group 1 (AH Plus): The AH Plus sealer (Dentsply Sirona, Konstanz, Germany), an epoxy resin-based formulation, was prepared following the manufacturer's instructions.

Group 2 (Sure-Seal Root): The Sure-Seal Root sealer (Sure-endo, Sure Dent Corporation, Seoul, South Korea), a bioceramic-based formulation, was prepared according to the manufacturer's instructions.

Group 3 (Experimental sealer): The experimental bioceramic sealer was formulated as a novel mixture consisting of 50 wt% tricalcium silicate (TCS) and dicalcium silicate (DCS), along with 25 wt% silicon hydroxyapatite and 25 wt% strontium hydroxyapatite. This mixture was combined with distilled water in a 1:1 weight ratio. Detailed preparation of the sealer is described in a previous study (19).

Group 4 (Control): This group consisted of empty tubes that served as controls in the study.

In groups 1 to 3, each tube was filled with its respective material using a lentulo spiral and carefully labeled.

### Surgical procedure

The rats were anesthetized with an intramuscular injection of ketamine hydrochloride 10% (47.5 mg/kg; Alfasan International B.V., Woerden, Netherlands) and xylazine 2% (10 mg/kg; Bayer HealthCare, Shawnee Mission, KS, USA). The dorsal areas of the rats (upper right and left shoulders, lower right and left flanks) were shaved and disinfected with a 10% povidone-iodine solution (Betadine; Avrio Health L.P., Stamford, CT, USA). A 20-mm incision and a subcutaneous pocket were created to insert the tubes. The AH Plus sealer was implanted in the upper left shoulder, the Sure-Seal Root bioceramic sealer in the upper right shoulder, the experimental bioceramic sealer in the lower right flank, and the empty control tube in the lower left flank.

The incisions were sutured using 3-0 silk sutures (Ethicon Inc., Somerville, NJ, USA), and all rats received postoperative care, including subcutaneous injections of ketoprofen (5 mg/kg; Mylan, Canonsburg, PA, USA) for analgesia and enrofloxacin (10 mg/kg) for three days to prevent infection. At the designated intervals (15, 30, and 60 days), the rats were euthanized by CO<sub>2</sub> inhalation. The implant sites and 1 cm of surrounding tissue were carefully excised for histological examination.

### Histopathological examination

The excised tissue samples were fixed in 10% formalin (Sigma-Aldrich, St. Louis, MO, USA) for 48 hours, then embedded in paraffin blocks. Sections of 3-4 µm were cut using a microtome and stained with hematoxylin and eosin (H&E) in a 2 × 2 mm field. A blind pathologist examined the slides under 400x magnification using a light microscope (Leica Microsystems, Wetzlar, Germany). Tissue reaction was graded based on the following four parameters:

1. Intensity of inflammatory reaction: This criterion was evaluated by counting polymorphonuclear cells (PMNs) and scored as follows: no or minimal PMN infiltration (0), fewer than 25 PMNs indicating a low reaction (1), between 25 and 125 PMNs representing a moderate reaction (2), and over 125 PMNs indicating a severe reaction (3).

2. Fibrous capsule thickness: This was classified as thin (thickness less than 150 µm) or thick (thickness greater than 150 µm).

3. Giant cell infiltration: The presence or absence of giant cell infiltration, indicating necrotic tissue, was recorded.

4. Biomineralization: The presence or absence of calcified areas was evaluated.

### Statistical analysis

The Kruskal-Wallis test was used to compare the tissue reactions among the different groups, with the level of statistical significance set at  $p < 0.05$ . statistical analysis

was performed using SPSS software (version 23; SPSS Inc, Chicago, IL, USA).

### Results

Table 1 presents intergroup and comparisons of tissue reactions to the different materials used in this study. ANOVA revealed no significant differences between the groups at different intervals concerning the inflammatory response, fibrous capsule thickness, giant cell infiltration, or biomineralization ( $P > 0.05$ ; Table 1).

Table 2 presents intragroup comparisons of different variables throughout the study period. Histological analysis showed a reduced inflammatory reaction over time in all groups. However, this reduction was statistically significant only in the AH Plus group ( $P = 0.04$ ). The thickness of the fibrous capsule decreased in the control, AH Plus, and Sure-Seal Root groups, with the decrease being significant in the Sure-Seal Root ( $P = 0.04$ ) and AH Plus ( $P = 0.04$ ) groups. In the experimental bioceramic sealer group, a slight increase in fibrous capsule thickness was noted after 60 days, though this change was not statistically significant. Figure 1 represents tissue reactions observed in the study groups.

Giant cell infiltration decreased over time in all groups except for the experimental bioceramic sealer group, where a slight increase was observed after 60 days. However, this change was not statistically significant. The reduction in giant cell infiltration was significant in the Sure-Seal Root ( $P = 0.02$ ) and AH Plus ( $P = 0.02$ ) groups.

**Table 1.** Intergroup comparison of study groups at each time interval, presenting the frequency of observed tissue reactions across the different sealing materials

| Assessment Time | Group               | Inflammatory reaction |          |        | Fibrous capsule thickness |       | Giant cell infiltration |         | Biomineralization |         |
|-----------------|---------------------|-----------------------|----------|--------|---------------------------|-------|-------------------------|---------|-------------------|---------|
|                 |                     | mild                  | moderate | severe | thin                      | thick | absent                  | present | absent            | present |
| Day 15          | AH Plus             | 0                     | 1        | 4      | 1                         | 4     | 2                       | 3       | 5                 | 0       |
|                 | Sure-Seal Root      | 1                     | 1        | 3      | 1                         | 4     | 2                       | 3       | 4                 | 1       |
|                 | Experimental sealer | 2                     | 0        | 3      | 2                         | 3     | 3                       | 2       | 4                 | 1       |
|                 | Control             | 3                     | 1        | 1      | 4                         | 1     | 5                       | 0       | 5                 | 0       |
|                 | <i>P-value</i>      | 0.22                  |          |        | 0.17                      |       | 0.17                    |         | 0.52              |         |
| Day 30          | AH plus             | 4                     | 1        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                 | Sure-Seal Root      | 4                     | 0        | 1      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                 | Experimental sealer | 4                     | 1        | 0      | 4                         | 1     | 4                       | 1       | 5                 | 0       |
|                 | Control             | 4                     | 1        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                 | <i>P-value</i>      | 0.99                  |          |        | 0.36                      |       | 0.36                    |         | 1.00              |         |
| Day 60          | AH plus             | 4                     | 1        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                 | Sure-Seal Root      | 3                     | 2        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                 | Experimental sealer | 3                     | 1        | 1      | 3                         | 2     | 3                       | 2       | 3                 | 2       |
|                 | Control             | 5                     | 0        | 0      | 4                         | 1     | 5                       | 0       | 5                 | 0       |
|                 | <i>P-value</i>      | 0.41                  |          |        | 0.23                      |       | 0.08                    |         | 0.08              |         |

**Table 2.** Intragroup comparisons within each study group across the assessment time points, presenting the frequency of tissue reactions observed at various intervals

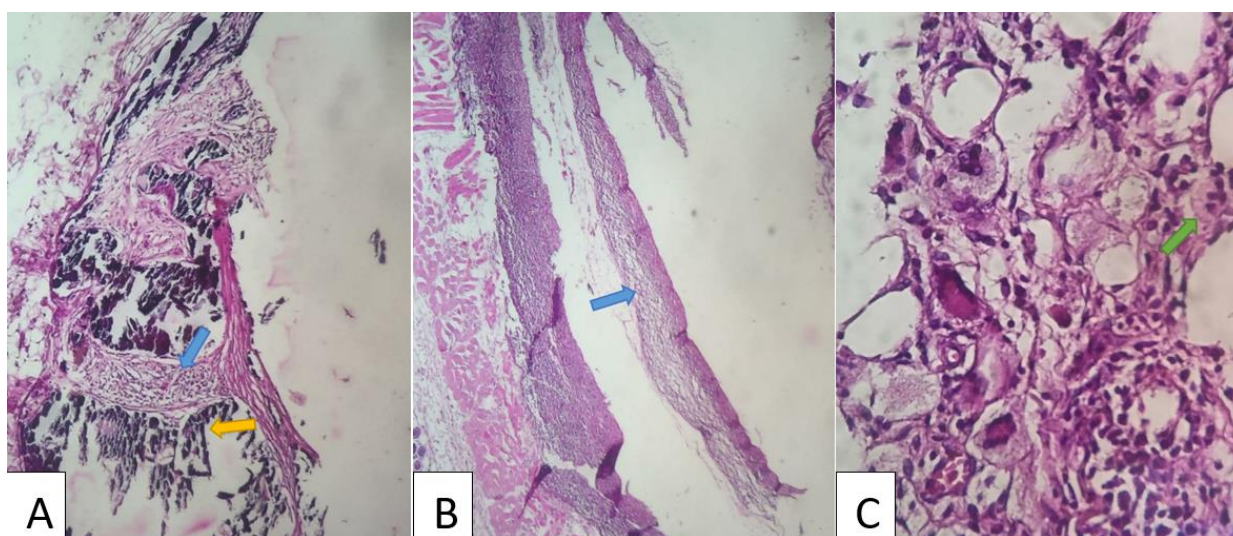
| Group               | Assessment Time | Inflammatory reaction |          |        | Fibrous capsule thickness |       | Giant cell infiltration |         | Biomineralization |         |
|---------------------|-----------------|-----------------------|----------|--------|---------------------------|-------|-------------------------|---------|-------------------|---------|
|                     |                 | mild                  | moderate | severe | thin                      | thick | absent                  | present | absent            | present |
| AH Plus             | Day 15          | 0                     | 1        | 4      | 1                         | 4     | 2                       | 3       | 5                 | 0       |
|                     | Day 30          | 4                     | 1        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                     | Day 60          | 4                     | 1        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                     | <i>P-value</i>  | 0.04                  |          |        | 0.04                      |       | 0.02                    |         | 1.00              |         |
| Sure-Seal Root      | Day 15          | 1                     | 1        | 3      | 1                         | 4     | 2                       | 3       | 4                 | 1       |
|                     | Day 30          | 4                     | 0        | 1      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                     | Day 60          | 3                     | 2        | 0      | 5                         | 0     | 5                       | 0       | 5                 | 0       |
|                     | <i>P-value</i>  | 0.12                  |          |        | 0.04                      |       | 0.02                    |         | 0.34              |         |
| Experimental sealer | Day 15          | 2                     | 0        | 3      | 2                         | 3     | 3                       | 2       | 4                 | 1       |
|                     | Day 30          | 4                     | 1        | 0      | 4                         | 1     | 4                       | 1       | 5                 | 0       |
|                     | Day 60          | 3                     | 1        | 1      | 3                         | 2     | 3                       | 2       | 3                 | 2       |
|                     | <i>P-value</i>  | 0.26                  |          |        | 0.43                      |       | 0.74                    |         | 0.28              |         |
| Control             | Day 15          | 3                     | 1        | 1      | 4                         | 1     |                         |         | 5                 | 0       |
|                     | Day 30          | 4                     | 1        | 0      | 5                         | 0     |                         |         | 5                 | 0       |
|                     | Day 60          | 5                     | 0        | 0      | 4                         | 1     |                         |         | 5                 | 0       |
|                     | <i>P-value</i>  | 0.29                  |          |        | 0.56                      |       |                         |         | 1.00              |         |

Biomineralization increased in the experimental sealer group, decreased in the Sure-Seal Root sealer group, and remained unchanged in the AH Plus and control groups. However, none of these changes were statistically significant ( $P > 0.05$ ; Table 2).

## Discussion

The study used 60 tubes, 45 filled with different sealing materials, including AH Plus sealer, Sure-Seal Root sealer, and the experimental bioceramic sealer. Additionally, 15 empty tubes served as control samples.

Each rat had four sites where the sealers or controls were implanted, and the tissue reactions at 15, 30, and 60 days post-implantation were recorded. The time intervals of 15, 30, and 60 days were selected based on a previous study (21) to assess short- and long-term tissue reactions. While some studies include a 7-day interval (11, 22), we excluded this to minimize the impact of surgical trauma on early inflammatory responses. On day 15, surgical trauma begins to subside, and on day 30, tissue repair processes are underway. By



**Figure 1.** A) Tissue reaction to the experimental sealer on day 60, shown at 400 x magnification, with a thick fibrous capsule (blue arrow) and evidence of dystrophic calcification (yellow arrow). B) Tissue reaction to AH Plus sealer on day 15, shown at 100 x magnification, highlighting a thick fibrotic capsule (blue arrow). C) Tissue reaction to Sure-Seal Root sealer on day 15, shown at 400 x magnification, displaying the presence of giant cells (green arrow).

60 days, a more complete healing response is expected (23).

The novel bioceramic sealer formulated in this study is a calcium silicate-based sealer. This new generation of sealers is known for its improved sealing ability, antibacterial properties, biocompatibility, and superior bonding to dentine (8). Several studies have demonstrated the advantages of incorporating silicon and strontium into endodontic materials (12, 24, 25). In 2015, Vahabzadeh et al. (26) explored the use of silicon in brushite (calcium phosphate-based) cement, showing that silicon enhanced osteogenesis, vascularization, and new bone formation. Similarly, Bakhit et al. (12) found that strontium promoted odontoblast and osteoblast differentiation and mineralization, stimulating the formation of mineralized osteodentin-like tissue *in vivo*. Barbosa et al. (25) also supported using calcium and strontium in endodontic sealers and observed improved setting time, strength, and workability.

In the present study, the experimental sealer showed an initially severe inflammatory reaction, which decreased over time but did not reach statistical significance. One sample showed a severe reaction at 60 days, which may be attributed to the sealer's longer setting time, consistent with the findings of other studies (22, 27).

The control group in this study exhibited a mild to moderate inflammatory reaction. This reaction was likely due to the neutrality of the polyethylene tubes and the faster healing of surgical inflammation without any additional substances (11, 27, 28). A severe reaction in one sample was likely due to surgical trauma or delayed healing caused by tissue manipulation.

In the AH Plus sealer group, a severe inflammatory reaction was observed on the 15<sup>th</sup> day, likely exacerbated by an allergic reaction to the sealer's components. However, inflammation and foreign body reactions decreased significantly by 30 and 60 days. This reduction is attributed to the increased vascular activity and tissue repair observed during this period (28, 29). Similar decreases in inflammation have also been reported in studies by Santos et al. (28) and Zhang and Peng (27).

The inflammatory reaction in the Sure-Seal Root bioceramic sealer group decreased after 30 days. However, it showed a slight increase after 60 days, likely due to animal-induced irritation of the surgical area. Regarding fibrous capsule thickness, the results indicated a decrease over time for all three sealers. This finding contrasts with studies that have reported increased capsule thickness over time (30). The findings

of this study align with those of Zhang et al. (27), who observed a thin capsule formation around bioceramic sealers after 60 days, possibly reflecting lower irritation levels of these materials. Delfino et al. (31) also found that fibrous capsule thickness decreased over time due to the rearrangement of collagen fibers and reduced pro-inflammatory factors like IL-6. The concentration of endodontic materials also plays a crucial role in cytotoxicity. Sheela et al. (32) showed that lower concentrations of bioceramic sealers and AH Plus were non-toxic, while higher concentrations exhibited cytotoxic effects.

The presence of giant cells indicates the organism's effort to eliminate foreign materials through phagocytosis and to clear necrotic tissues resulting from prior injuries (30, 33, 34). This study noted a significant reduction in giant cell infiltration in the Sure-Seal Root and AH Plus groups. However, the experimental sealer group exhibited an initial decrease followed by a slight increase in giant cell presence, although these changes were not statistically significant. The sustained presence of giant cells in the experimental sealer group may be linked to its higher solubility, which could promote the release of substances and the formation of calcific precipitates (35, 36).

Regarding biomineralization, the experimental sealer showed a higher degree of biomineralization compared to the other sealers. However, the differences in biomineralization among the study groups were not statistically significant. Biomineralization was noted in three samples with the experimental sealer, indicating its potential for hard tissue formation, but further research is necessary to investigate its occurrence at different intervals.

Intergroup comparisons revealed no significant differences in tissue reaction measures between the study groups, suggesting that the experimental sealer has biocompatibility comparable to commercially available sealers. However, this lack of significance may be caused by the small sample size. Further research with larger sample sizes and advanced techniques, such as von Kossa staining and immunohistochemical analysis, is essential to evaluate the long-term performance of the experimental sealer.

## Conclusions

The novel formulation of tricalcium silicate/dicalcium silicate (50 wt%), silicon hydroxyapatite (25 wt%), and strontium hydroxyapatite (25 wt%) showed comparable biocompatibility to commercially available sealers. However, Sure-Seal Root and AH Plus sealers exhibited

significant decreases in some tissue reactions over time, whereas the experimental sealer did not. Overall, the biocompatibility of commercially available sealers appears to be better than the experimental sealer, based on the results of this study.

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## Conflict of interests

The authors have no conflict of interest to declare

## Authors' contributions

M.Z. contributed to the conceptualization of the study. M.J. was responsible for the methodology. N.B. contributed to the editing and reviewing of the manuscript. M.B. conducted the investigation, and M.P. contributed to writing and reviewing the manuscript.

## Ethical approval

Ethical approval was obtained from the Ethics Committee of Mashhad University of Medical Sciences with the approval number IR.MUMS.DENTISTRY.REC.1398.115.

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