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BIOCOMPATIBILITY OF DIFFERENT ROOT CANAL SEALERS (IN VIVO STUDY)

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ABSTRACT

Aim: This study evaluated the biocompatibility of three different root canal sealers (CeraSeal, NeoSEALER Flo, and AH-Plus) by implanting them into the subcutaneous tissues of rats and analyzing the tissue response through histopathological and immunohistochemical methods.

Materials and Methods: Sixty male *Calomys callosus* (Rodentia, Cricetidae) rats were used. The sealers were placed in polyethylene tubes that were inserted into certain dorsal subdermal locations. After 3, 7, and 30 days, the implants were removed, fixed, and processed for microscopic inspection using a glycol methacrylate-embedding procedure.The descriptive analysis focused on fibrous capsule thickness, severity of the inflammatory response, presence of giant cells, and evidence of biomineralization.

Results: CeraSeal and the control group exhibited a milder inflammatory response compared to NeoSEALER Flo and AH-Plus. Additionally, the control group showed the thinnest fibrous capsule, followed by CeraSeal, while AH-Plus demonstrated the thickest capsule.

Conclusions: Within the limitations of this study, CeraSeal demonstrated superior biocompatibility compared to NeoSEALER Flo and AH-Plus sealers.

KEYWORDS: CeraSeal, Neosealer Flo, AH plus, biocompatibility, inflammatory reaction

INTRODUCTION

The intent of endodontic therapy is to effectively treat root canal infections by thoroughly cleaning and shaping the canals, followed by filling the canal space to prevent the infiltration of microorganisms and fluids from both the coronal and apical directions. [1,2]

Most root canals are filled using an appropriate sealer, which serves as primary component of root canal obturation to create a fluid-tight seal.[3,4]

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Endodontic root canal sealers are widely used to seal dentinal tubules and form a uniform interface between the dentinal walls and the obturation material[5], thereby enhancing the sealing of the canal space.[6,7] Root canal sealers' main purposes are to fill in the canal's gaps and imperfections, eliminate residual bacteria remaining after cleaning and shaping, and exert a germicidal effect to further disinfect the root canal system.[4,8]

Biological interactions are characterized by factors such as biocompatibility, cytotoxicity, differentiation potential, cell plasticity and bioactive properties.[9] Biocompatibility refers to a material's capacity to display qualities that are compatible with and non-harmful to living tissues.[10]

Biocompatible materials produce persistent and positive host responses throughout application and do not cause immunological or toxic reactions when in contact with tissues or tissue fluids.[10,11] In contrast, bioactive materials possess a structure it allows for a direct bond between the substance and the tissue. [12,13] Research into biological interactions is essential for assessing the clinical performance and impact of these materials. [12,14,15] Tissue responses to endodontic materials are typically evaluated through histological studies after implanting the materials into animal tissues.[16,17]

A wide variety of root canal sealers have been utilized alongside solid or semisolid fillers. These sealers are formulated in different types, including epoxy resin, zinc oxide–eugenol, calcium hydroxide, silicone, bioceramic, and glass ionomer-based materials, each with distinct setting mechanisms.[5,18]

Despite the availability of numerous formulations, research continues to seek the most suitable root canal sealers.[19] Recently, several new sealers have been introduced to the market under various commercial names.[12,20]

The integration of substances into dental products to enhance tissue remineralisation and antibacterial activity is of great interest. [21]Additives that improve the biocompatibility and bioactivity of sealants are a prominent emphasis in clinical applications. [18,22] These advancements show great promise in improving root canal treatment outcomes and may play a key role in the prevention and management of endodontic pathologies.[21]

Numerous in vitro (cell culture) investigations have shown that calcium silicate-based materials, primarily made up of CaSi particles, are highly biocompatible and have good biological characteristics [23,24] and ex vivo (animal model) investigations. [25,26] These favorable interactions with biological tissues are largely attributed to the release of biologically active ions, such as calcium[27,28] and the nucleation of an apatite layer on the material's surface[29], a process that begins immediately following material hydration.[30] Due to these properties, calcium silicate-based materials have become essential in addressing challenging endodontic cases, including as perforation repairs and the placing of apical plugs in teeth with open apices. [31]

The initial version of calcium silicate-based materials had some disadvantages, notably long setting times, low radiopacity, handling challenges, and grayish discoloration. These issues limited their use as root canal sealers.[32]

Modifications to these endodontic materials have been made to overcome many of their limitations, and calcium silicate-based root canal sealers have been introduced in the past 10–15 years in powder-liquid or paste-to-paste formulations .[31]

Recently, already mixed flowable sealers were produced for root canal therapy. In contrast to other formulations, these materials are readily available to use and don't need mixing, as their setting reaction occurs in the presence of moisture. Calcium silicate-based materials have more recently been collectively referred to as "bioceramics." It is vital to remember that the term "ceramic" refers to any

inorganic substance that contains both metallic and nonmetallic components.

The expression "bioceramics," which was developed to highlight its favorable biological function, encompasses ceramic materials utilized for healing or replacement damaged bone structures. Bioceramics may react alongside surrounding tissue to either assist tissue growth or promote new tissue regeneration.[33] As such, the word "bioceramic" is wide and does not exclusively relate to calcium silicate-based materials. Materials based on calcium silicate have been shown to interact favorably with the surrounding periapical tissues without triggering inflammation or foreign body reactions.[25,26]

These new bioceramics were created by adding variable amounts of calcium silicate (CaSi) and radiopacifiers into their formulation. In this setting, it is critical to distinguish between calcium silicate-based sealers (which largely include CaSi particles) and calcium silicate-containing sealers (which contain trace amounts of CaSi).

CeraSeal, developed by Meta-BioMed in South Korea, is a pre-prepared bioceramic sealer including bioactive components tricalcium silicate (20-30%) and dicalcium silicate (1-10%), as well as radiopacifiers tricalcium aluminate (1-10%) and zirconium dioxide (45-50%). The manufacturer also mentions the existence of minor amounts of thickening agents.

NeoSealer Flo, produced by Avalon BioMed, is a premixed bioceramic sealer with bioactive components tricalcium silicate (<25%) and dicalcium silicate (<10%). Radiopacifiers include calcium aluminate (<25%), calcium aluminum oxide (<6%), tricalcium aluminate (<5%), and tantalite (50%). The manufacturer reports trace amounts of calcium sulfate (<1%).

AH Plus was chosen as the reference because to its well-established biological features; it is an epoxy resin-based root canal sealant with a number of advantages. It has been extensively evaluated, exhibiting low microleakage, the capacity to attach to dentin, antibacterial activity against E. faecalis, and excellent dimensional stability with negligible polymerization shrinkage when placed in the root canal.[34]

In this study, we will compare the biocompatibility of two bioceramic sealers with an epoxy resin-based sealer.

MATERIALS AND METHODS

The ethics committee of the faculty of dental medicine, Al-Azhar University, Assiut (AUAREC20250004-3) is constituted and operates according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern IRB operation.

Sample Size Calculation:

In animal studies, power analysis is considered the most scientifically appropriate method for determining sample size, often utilizing ANOVA for analysis. Using the "G*Power 3.1" software and based on the following assumptions — a 95% two-sided confidence interval, an effect size of 0.8, a statistical power (1-β) of 0.95, and an alpha error (α) of 0.05 — the minimum required sample size was calculated. A total of 60 mice were deemed sufficient to detect a true effect in the experiment. These animals were divided into four equal groups of 15 mice each: three experimental groups (each testing a different root canal sealer) and one control group. The groups were monitored over a one-month period, with consideration given to ethical standards regarding animal use and minimizing resource waste.

Sixty male *Calomys callosus* (Rodentia, Cricetidae) rats, each weighing between 150 and 200 grams, were used in the study. The animals were randomly divided into four groups of 15 specimens each, corresponding to the CeraSeal, NeoSEALER Flo, AH-Plus sealers, and a control group.

Polyethylene tubes measuring 1.5 mm in inner diameter, 2.0 mm in outer diameter, and 10.0 mm in length were sterilized in an autoclave before use. The root canal sealers tested were: Group 1 — CeraSeal (Meta Biomed Co., Cheongju, Korea); Group 2 — NeoSEALER Flo (Avalon Biomed™, Houston, Texas, USA); and Group 3 — AH Plus (Dentsply De Trey GmbH, Konstanz, Germany). Each material was prepared according to the manufacturers' instructions. Newly mixed sealers were inserted in sterile polyethylene tubes and then implanted into the rats' subcutaneous tissue.Empty tubes served as the control (Group 4). For Groups 1 to 3, each tube was filled with its respective material using a lentulo spiral and properly labeled.

The mice were anesthetized intraperitoneally with 0.2 mL of a 1:1 ketamine/acepromazine mixture. The dorsal region was shaved (Figure 1) and disinfected with 5% iodine tincture. Small incisions measuring about 15 mm in length were made on both sides of the dorsum (Figure 2). Blunt dissection to a depth of 15 mm established two independent subcutaneous spaces for tube placement. The tubes with the freshly mixed sealers were then gently put into the right and left compartments of each animal (Figure 3), ensuring no material spilled into the surrounding tissue. Following implantation, the incisions were closed with sutures (Figure 4).

The animals were euthanized in groups of five at 3, 7, and 30 days post-implantation using cervical dislocation, following the guidelines of the Brazilian College of Animal Experimentation. Whenever possible, sedation or light anesthesia was administered prior to euthanasia, as recommended.

The tubes, together with the surrounding skin and connective tissue, were carefully removed. The specimens were then submerged in 10% formalin produced in a 0.1 mol/L phosphate-buffered solution for 24 hours before being dehydrated at room temperature using a graded ethanol series. Next, the samples were embedded in glycol methacrylate.(Historesin; Leica Microsystems, Nussloch GmbH, Germany).

For cross-sectioning, the blocks were aligned with the tube's long axis. Sections were 3 μm thick and stained with 1% toluidine blue.

The histological sections were examined under a light microscope at various magnifications, with particular attention to tissue reactions at the sealer–connective tissue interface near the open ends of the tubes.

The interface between the material and the surrounding tissue at the tube's open end was studied and evaluated for fibrous capsule thickness, inflammatory response severity, the presence of giant cells, and biomineralization evidence.

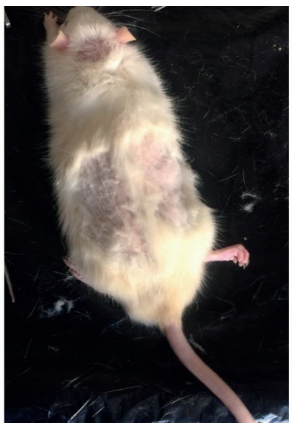

Fig. (1) The dorsal skin of rat was shaved

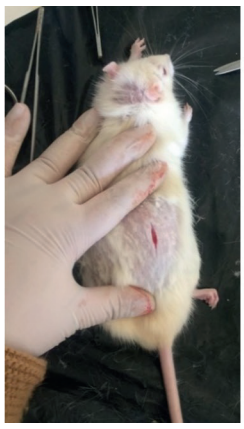

Fig. (2) Small incisions with a blade

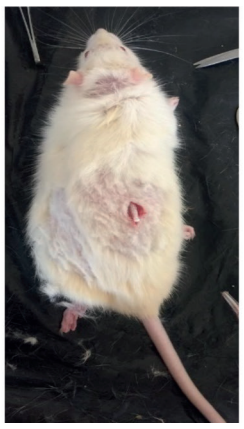

Fig. (3) Tubes containing freshly mixed sealers carried to the subcutaneous tissue

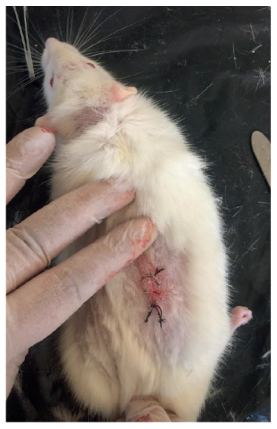

Fig. (4) Suturing of the wounds

A previously trained, blinded pathologist performed the histopathological analyses at three separate time points.

Histopathological examination

Tissue reactions were assessed using four parameters:

1. **Intensity of Inflammatory Reaction**: This was determined by counting polymorphonuclear cells (PMNs) and scored as follows: no or minimal PMN infiltration (0), fewer than 25 PMNs indicating a mild reaction (1), between 25 and 125 PMNs representing a moderate reaction (2), and more than 125 PMNs indicating a severe reaction (3).
2. **Fibrous Capsule Thickness**: This was categorized as thin (less than 150 μm) or thick (greater than 150 μm).
3. **Giant Cell Infiltration**: The presence or absence of giant cell infiltration, a sign of necrotic tissue, was recorded.
4. **Biomineralization**: The presence or absence of calcified areas was evaluated.

Statistical analysis

All data were collected, calculated, tabulated, and analyzed statistically using the following tests. A normality test (Kolmogorov-Smirnov) was conducted to assess the distribution of the samples. Descriptive statistics were reported as mean ± standard deviation (SD). One-way ANOVA was used to compare the groups and time intervals within each group. Post hoc pairwise comparisons were performed using Tukey's test. The Chi-square test was applied to evaluate qualitative data between groups. A p-value of ≤ 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS software for Windows, version 26.0 (IBM Corp, Armonk, NY), with a significance level of $p < 0.05$.

RESULTS

Histological Examination

The histopathological examination of the subcutaneous tissues revealed varying degrees of inflammatory reactions and an increase in the thickness of the fibrous capsule across all groups at 3, 7, and 30 days (Figure 5).

After 3 days, both the control and CeraSeal groups exhibited moderate inflammatory reactions, characterized by infiltration of neutrophils, lymphocytes, and macrophages. In contrast, the NeoSEALER Flo and AH Plus groups displayed severe inflammatory cell infiltration, primarily consisting of lymphocytes and macrophages. The NeoSEALER Flo group exhibited the thickest fibrous capsule, followed by the AH Plus and CeraSeal groups. The control group showed the thinnest fibrous capsul

After 7 days, the control group exhibited a mild inflammatory reaction, with infiltration by neutrophils, macrophages, and lymphocytes; the fibrous capsule was thinner, and no giant cells or areas of necrosis were observed. In the CeraSeal and NeoSEALER Flo groups, the intensity of inflammation had decreased. However, in the AH Plus group, a severe inflammatory response persisted. The thickness and organization of the fibrous capsule remained greatest in the NeoSEALER Flo group, followed by the AH Plus and CeraSeal groups.

After 30 days, the control group exhibited a thinner fibrous capsule and a reduced inflammatory reaction compared to the observations at 3 and 7 days. The CeraSeal group showed a significant decrease in inflammatory cell infiltration, followed by the NeoSEALER Flo and AH Plus groups. Both the CeraSeal and NeoSEALER Flo groups demonstrated thinning of the fibrous capsule relative to earlier time points. The AH Plus group maintained the greatest mean fibrous capsule thickness compared to the other groups.

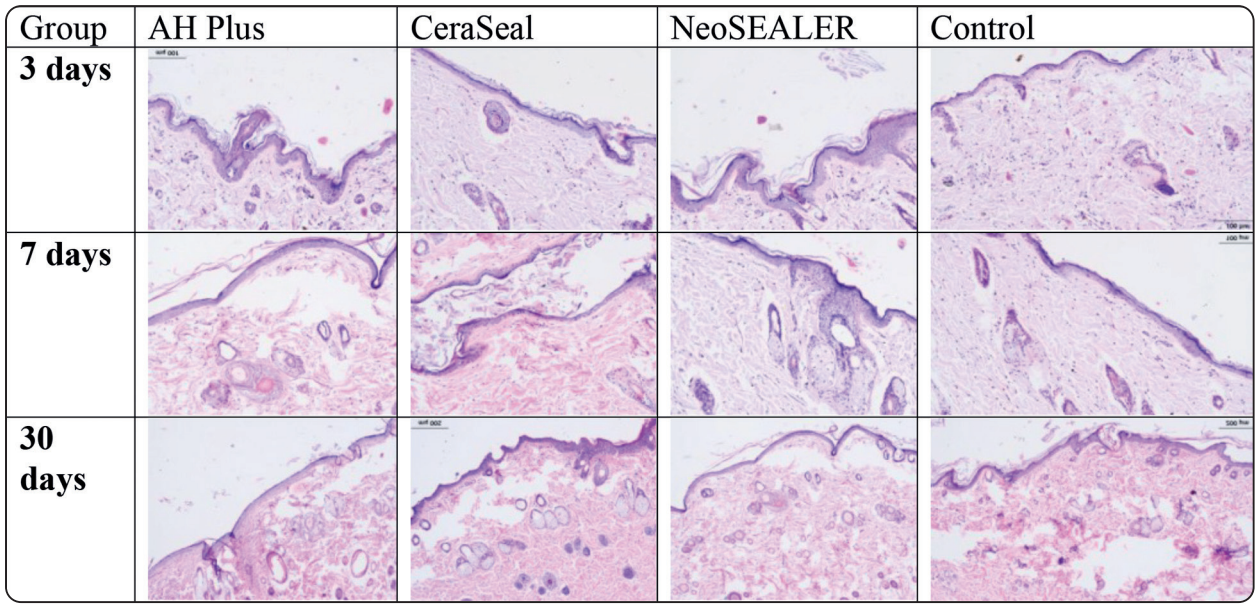

Fig. (5) Photomicrograph of subcutaneous tissues representing inflammation intensity and thickness of fibrous capsule of study groups after 3, 7 and 30 days at 10X, and 20X magnification.

1. Number of Neutrophils:

Figure 6, showed the comparison of intra-group and inter-group differences for the number of neutrophils across different materials (CeraSeal, NeoSEALER Flo, AH Plus, and a control groups) at 3, 7, and 30 days:

Inter-group Comparisons

Statistical analysis showed significant difference G1, G2, G3 with control at 3, 7 and 30 days with $P<0.05$.

3 Days: AH Plus shows the highest mean number of neutrophils (42.6), followed by NeoSEALER Flo (37.4) and CeraSeal (34.0). The control group has a significantly lower mean (13.4).

7 Days: AH Plus again has the highest mean (19.4), followed by CeraSeal (17.4) and NeoSEALER Flo (15.0). The control group remains significantly lower (4.6).

30 Days: AH Plus has a mean of 4.4, NeoSEALER Flo has a mean of 2.6, CeraSeal has a mean of 2.2, and the control group has the lowest mean (1.6).

Intra-group Comparisons

CeraSeal: The mean number of neutrophils decreases over time (34.0a at 3 days to 2.2a at 30 days). **for NeoSEALER Flo,** Similar to CeraSeal, the mean decreases over time (37.4 at 3 days to 2.6 at 30 days). Also, **the AH Plus** decreases over time, but it starts higher and remains comparatively higher at each time point (42.6 at 3 days to 4.4a at 30 days). **Control Group,** the mean number of neutrophils also decreases significantly over time (13.4 at 3 days to 1.6 at 30 days). Statistical analysis showed significant difference between groups at 3 and 7 days while at 30 days, the P-value is not significant (NS)

2. Number of Macrophages:

Figure 7 showed the comparison of inter-group and intra-group differences for the number of macrophages across different materials (CeraSeal, NeoSEALER Flo, AH Plus, and a control group) at 3, 7, and 30 days:

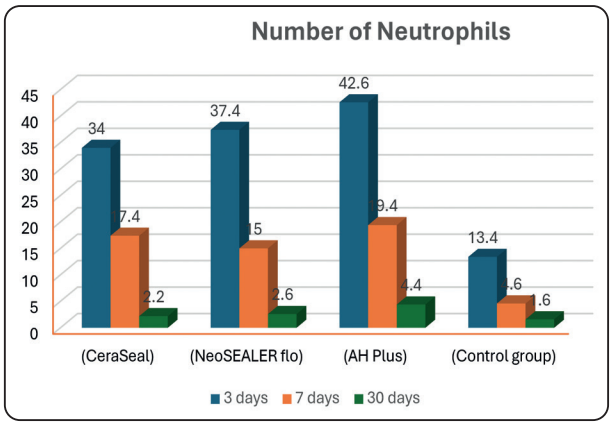

Fig (6) Bar chart representing ccomparison between Different Groups for Number of Neutrophils

Inter-group Comparisons

3 Days: AH Plus shows the highest mean number of macrophages (28.8), followed by NeoSEALER Flo (19.0) and CeraSeal (17.4). The control group has the lowest mean (11.4). Statistical analysis showed significant difference between **AH Plus with other groups.**

7 Days: AH Plus again has a high mean (18.8), similar to NeoSEALER Flo (16.0). CeraSeal is slightly lower (10.8), and the control group remains the lowest (6.4). pair wise comparison showed significant difference between **NeoSEALER Flo** and **AH Plus** with **CeraSeal** and control groups.

30 Days: AH Plus has a mean of 7.2a, NeoSEALER Flo has a mean of 5.6, CeraSeal has a mean of 2.8, and the control group has the lowest mean (1.4). statistical analysis showed the same found at 7 days.

Intra-group Comparisons

At all-time points (3, 7, and 30 days), the P-values are highly significant more than 0.05, indicating significant differences between the groups.

CeraSeal: The mean number of macrophages decreases over time (17.4 at 3 days to 2.8 at 30 days). **NeoSEALER Flo:** The mean also decreases over time (19.0 at 3 days to 5.6 at 30 days). For **AH Plus:** The mean decreases over time (28.8 at 3 days to 7.2 at 30 days). **Control Group:** The mean number of macrophages decreases significantly over time (11.4 at 3 days to 1.4 at 30 days).

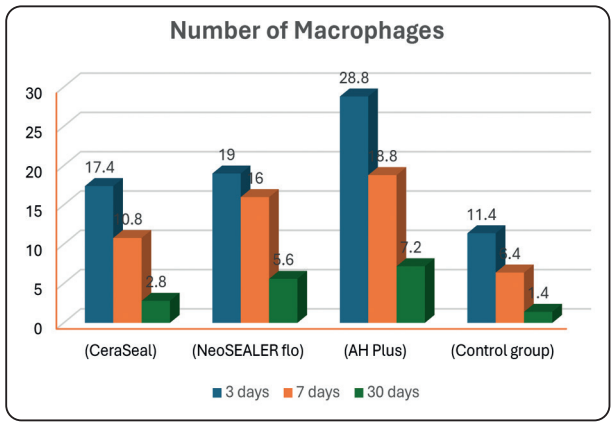

Fig (7) Bar chart representing comparison between Different Groups for Number of Macrophages

3. Number of Lymphocytes:

Figure 8 showed the comparison of inter-group and intra-group differences for the number of lymphocytes across different materials (CeraSeal, NeoSEALER Flo, AH Plus, and a control group) at 3, 7, and 30 days:

Inter-group Comparisons

3 Days: AH Plus shows the highest mean number of lymphocytes (47.0), followed by NeoSEALER Flo (32.2) and CeraSeal (25.0). The control group has the lowest mean (18.6). there is no significant between CeraSeal with NeoSEALER **Flo**.

7 Days: AH Plus again has the highest mean (28.4), followed by NeoSEALER Flo (23.0). CeraSeal (17.4) is next, and the control group remains the lowest (11.8c) and there is no significant between **NeoSEALER Flo** with AH Plus.

30 Days: AH Plus has the highest mean (17.8), followed by NeoSEALER Flo (10.6). CeraSeal (5.8) and the control group (3.4) are the lowest, and there is no significant between CeraSeal with control.

Intra-group Comparisons

CeraSeal: The mean number of lymphocytes decreases over time (25.0 at 3 days to 5.8 at 30 days). **NeoSEALER Flo:** The mean also decreases over time (32.2 at 3 days to 10.6 at 30 days). **AH Plus:** The mean decreases over time (47.0 at 3 days to 17.8 at 30 days). **Control Group:** The mean number of lymphocytes decreases significantly over time (18.6at 3 days to 3.4 at 30 days).

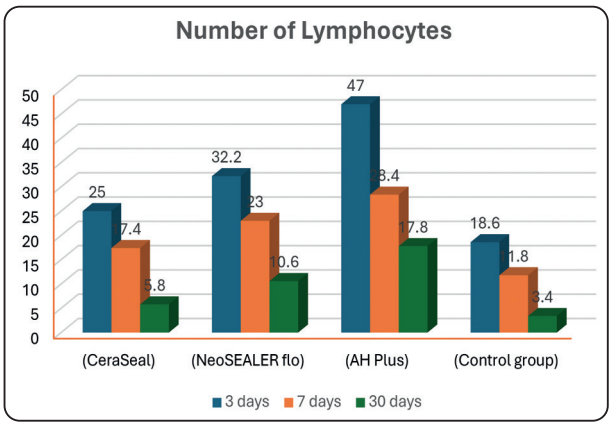

Fig. (8) Bar chart representing comparison between Different Groups for Number of Lymphocytes

4. Intensity of Inflammatory Reaction:

This criterion was evaluated by counting inflammatory cells infiltration (neutrophils, macrophages, and lymphocytes) and scored as follows: no or minimal infiltration (0), more than 5 fewer than 25 indicating a low reaction (1), between 25 and 125 representing a moderate reaction (2), and over 125 indicating a severe reaction (3) (Figure 9,10).

Inter-group Comparisons

3 Days: AH Plus shows the highest mean **Intensity of inflammatory reaction** (118.4), followed by NeoSEALER Flo (88.6) and CeraSeal (76.4). The control group has the lowest mean (40.2). there is no significant between CeraSeal with NeoSEALER **Flo**.

7 Days: AH Plus again has the highest mean (66.6), followed by NeoSEALER Flo (56.0). CeraSeal (45.6) is next, and the control group remains the lowest (20.6) and there is a significant between **NeoSEALER Flo** with AH Plus.

30 Days: AH Plus has the highest mean, followed by NeoSEALER Flo. CeraSeal and the control group are the lowest, and there are significant between all groups with control.

Intra-group Comparisons

CeraSeal: The mean of **Intensity of inflammatory reaction** decreases over time (76.4 at 3 days to 10.8 at 30 days). **NeoSEALER Flo:** The mean also decreases over time (88.6 at 3 days to 20.2 at 30 days). **AH Plus:** The mean decreases over time (118.4 at 3 days to 27.6 at 30 days). **Control Group:** The mean **of Intensity of inflammatory reaction** decreases significantly over time (40.2 at 3 days to 5.8 at 30 days).

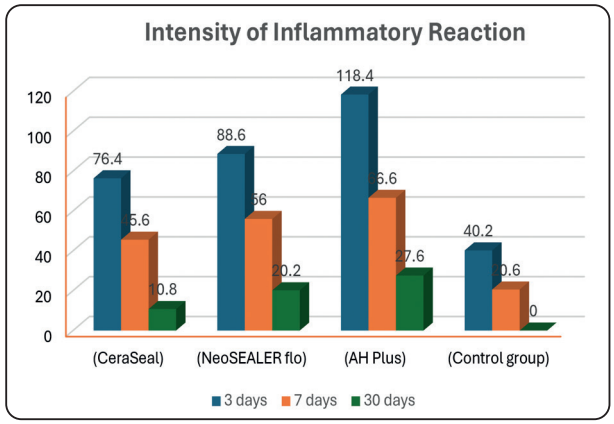

Fig. (9) Bar chart representing comparison between Different Groups for Intensity of Inflammatory Reaction

Thickness of the Fibrous Capsule

Figure 11 showed the comparison of inter-group and intra-group differences in the thickness of the fibrous capsule across different materials (CeraSeal, NeoSEALER Flo, AH Plus, and a control group) at 3, 7, and 30 days:

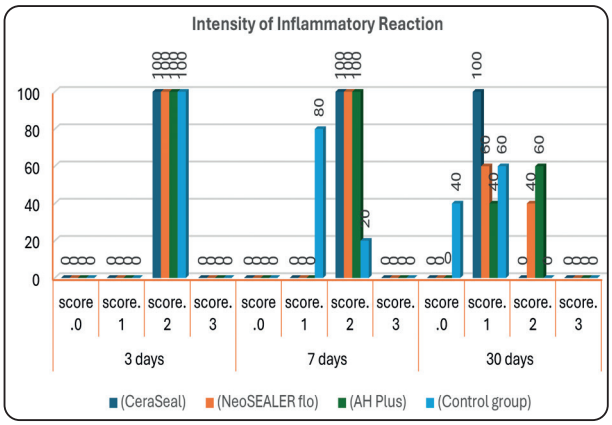

Fig (10) Bar chart representing intensity of inflammatory reaction

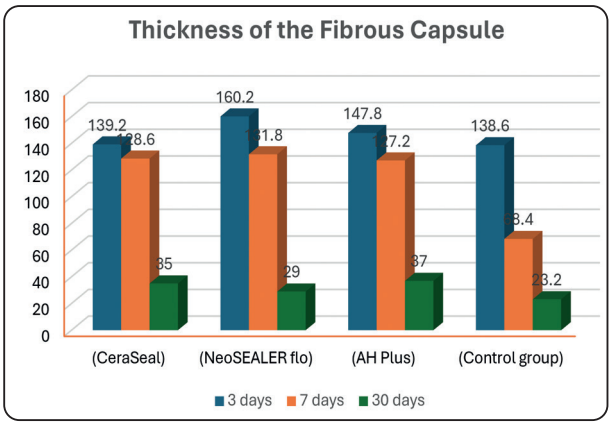

Fig. (11) Bar chart representing difference in the thickness of fibrous capsule

Inter-group Comparisons

3 Days: NeoSEALER Flo shows the highest mean thickness of the fibrous capsule, followed by AH Plus and CeraSeal. The control group has the lowest mean, and there is no significant between different groups.

7 Days: NeoSEALER Flo shows the highest mean thickness of the fibrous capsule, followed by AH Plus and CeraSeal. The control group has the lowest mean. and there are significant between different groups with control (P=0.003).

30 Days: AH Plus shows the highest mean thickness of the fibrous capsule, followed by CeraSeal and NeoSEALER Flo. The control group has the lowest mean. and there is no significant between different groups.

Intra-group Comparisons

CeraSeal: thickness of the fibrous capsule decreases over time (139.2 at 3 days to 35.0 at 30 days). **NeoSEALER Flo:** The mean also decreases over time (160.2 at 3 days to 29.0 at 30 days). **AH Plus:** The mean decreases over time (147.8 at 3 days to 37.0 at 30 days). **Control Group:** The mean of thickness of the fibrous capsule decreases significantly over time (138.6 at 3 days to 23.2 at 30 days).

At 3 Days: CeraSeal: 60% Thin, 40% Thick, **NeoSEALER Flo:** 40% Thin, 60% Thick, **AH Plus:** 40% Thin, 60% Thick and **Control Group:** 60% Thin, 40% Thick. **At 7 Days: CeraSeal:** 80% Thin, 20% Thick, **NeoSEALER Flo:** 80% Thin, 20% Thick, **AH Plus:** 60% Thin, 40% Thick and **Control Group:** 100% Thin, 0% Thick. **At 30 Days: CeraSeal:** 100% Thin, 0% Thick, **NeoSEALER Flo:** 100% Thin, 0% Thick, **AH Plus:** 100% Thin, 0% Thick and **Control Group:** 100% Thin, 0% Thick. **Generally,** at both 3 days and 7 days, the P-values indicate that there isn't a statistically significant difference in the proportions of "thin" and "thick" results *between* the different sealer groups. By 30 days, all groups show 100% "thin" results. No P-value was calculated to make any comments about statistical significance.

Giant Cell Infiltration:

Figure 13. The comparison between the different groups at each time point, focusing on the presence or absence of Giant Cell Infiltration, based on the information in your table.

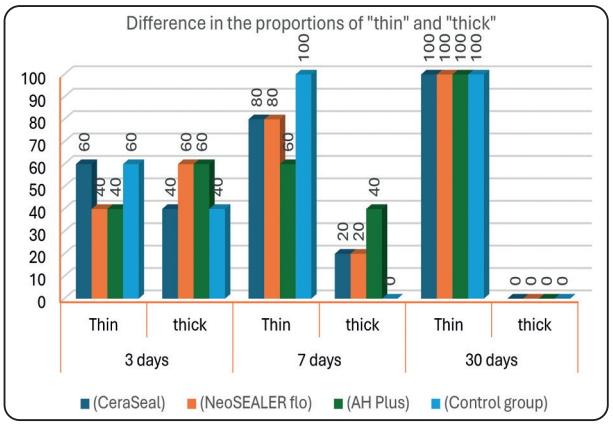

Fig. (12) Bar chart representing difference in the proportions of "thin" and "thick"

3 Days:

CeraSeal: Absent in 4 samples (80%), Present in 1 sample (20%), **NeoSEALER Flo:** Absent in 4 samples (80%), Present in 1 sample (20%). For **AH Plus:** Absent in 4 samples (80%), Present in 2 samples (40%) while the **Control Group was** Absent in 4 samples (80%), Present in 1 sample (20%) **and** there is no statistically significant difference between the groups regarding the presence or absence with P>0.05.

7 Days:

CeraSeal: Absent in 4 samples (80%), Present in 1 sample (20%), **NeoSEALER Flo:** Absent in 4 samples (80%), Present in 1 sample (20%). **AH Plus:** Absent in 4 samples (80%), Present in 1 sample (20%). For **Control Group:** Absent in 5 samples (100%), Present in 0 samples (0%) and, there is no statistically significant difference between the groups regarding the presence or absence (P=0.758)

30 Days:

CeraSeal: Absent in 5 samples (100%), Present in 0 samples (0%), **NeoSEALER Flo:** Absent in 5 samples (100%), Present in 0 samples (0%). For **AH Plus:** Absent in 5 samples (100%), Present in 0 samples (0%) on the other side, **the Control Group:** Absent in 5 samples (100%), Present in 0 samples (0%). Generally, at 3 and 7 days, the groups show similar distributions of Giant Cell Infiltration presence, and statistical analysis confirms no significant difference. By 30 days, Giant Cell Infiltration is absent in all samples across all groups.

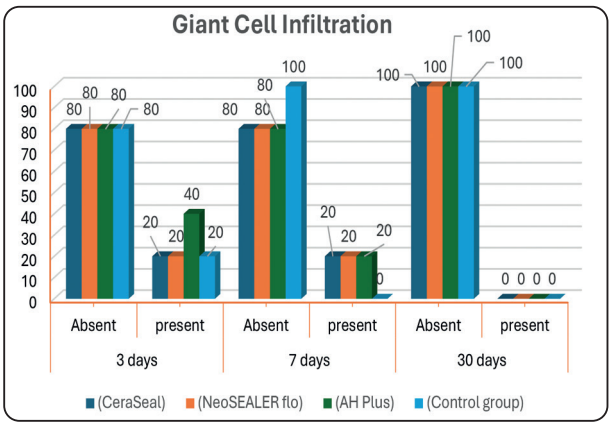

Fig. (13) Bar chart representing difference in giant cell infiltration

Biomineralization

Figure 14, the results showed that at **3, 7 and 30 Days,** All groups (CeraSeal, NeoSEALER Flo, AH Plus, Control) show 100% "Absent" and 0% "Present **and** There are no differences between the groups at any of the time points. All groups show the same result: whatever is being measured is "Absent" in all samples at all time points. No P-values are computed so it is not possible to comment on statistical significance.

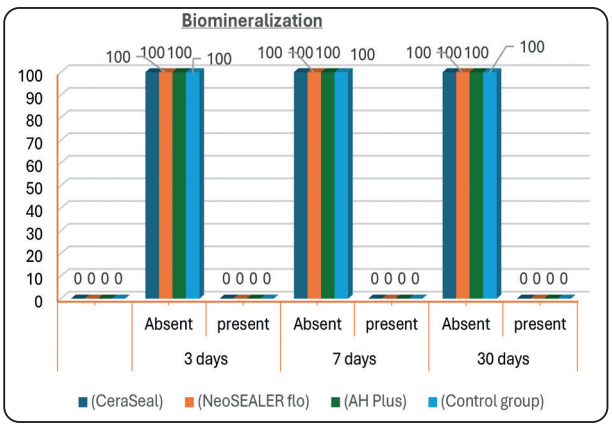

Fig. (14) Bar chart representing biomineralization in the different groups

DISCUSSION

Biocompatibility refers to a material's ability to carry out its intended function when implanted in living tissue without causing harm to that tissue. For dental materials, ensuring biocompatibility is crucial, as toxic elements can lead to irritation or even damage to nearby tissues—particularly if the material is unintentionally pushed into the periradicular area. [35,36] Nearly all endodontic sealers exhibit toxicity in their freshly mixed state; therefore, they must be evaluated under conditions that accurately reflect their clinical use to determine their safety profile.[36,37]

The biocompatibility of biomaterials is evaluated through assessing the severity and duration of the inflammatory reaction they cause. Various methods have been employed to assess the biocompatibility of endodontic materials, with subcutaneous implantation tests being among the most common. In this study, rats were chosen as the experimental model due to their low susceptibility to postoperative infections, ready availability, and their established use in biocompatibility research.[38,39]

Rat models are advantageous because their periapical anatomy closely resembles that of humans, and they are cost-effective to acquire and breed.[40]

To maintain likeness to clinical circumstances and standardization, polyethylene tubes were used in this study.The implantation of polyethylene tubes into the subcutaneous tissue of rats is recommended by the International Organization for Standardization (ISO 10993-6)[41] to evaluate the biocompatibility and bioactivity of calcium silicate-based endodontic materials. [42] Polyethylene tubes are inert and useful for testing compounds in direct touch with surrounding tissues. [43]

An extra empty tube was implanted as a control to reduce variables, prevent selection bias, and eliminate any confounders that could influence the results. [44]

Therefore, histopathological evaluation of tissue responses to the materials should assess the duration of the reaction.[(39)] In this study, both early (3 days) and late (30 days) responses were considered. The initial modest reaction detected in the control samples was most likely caused by surgical trauma, as sterile polyethylene tubes are inert and do not cause an inflammatory response. [43] By day 30, the reactions around the control tubes had faded, leaving a healthy connective tissue capsule around the implants.

Two bioceramic root canal sealers (RCSs), CeraSeal and NeoSEALER Flo, were selected for this study due to their popularity among endodontists for their biocompatible properties. In contrast, AH Plus, which has been extensively studied in cytotoxicity evaluations, was used as the reference sealer in accordance with recent reports. [45,46]

AH Plus, selected as the reference material due to its well-documented biological properties [47], exhibited the highest inflammatory reaction scores. Its pronounced initial toxicity may be attributed to its high amine content, which serves to accelerate the setting time. [48] Additionally, the release of bisphenol A diglycidyl ether, a mutagenic component present in resin-based materials, may also contribute to cytotoxicity [49] and could be responsible for the stronger early inflammatory response.

In contrast, both silicone-based sealers showed a faster recovery from the initial inflammatory response. These findings align with previous observations, highlighting that the chronic inflammatory reaction to epoxy resin-based materials is typically more prolonged and intense compared to other endodontic sealers.[47]

Three inspection time points were used to determine the severity of the inflammatory reaction to the tested elements. Initially, histological research revealed inflammatory cell infiltration in all tested sealers. These findings were most likely caused by surgical trauma from the incision and

the physical presence of the tubes, which may have provoked the initial inflammatory response shown in the control group. [50] Both calcium silicate-based sealers produced a moderate to high inflammatory response. Calcium silicate compounds are known to release calcium ions when in contact with tissue fluids.

Thus, the increase in alkaline pH following the setting of the materials may account for the initially pronounced inflammatory response. Moreover, the heat generated during the setting process can promote the recruitment of inflammatory cells and the subsequent release of cytokines. [51] However, the inflammation associated with the calcium silicate-based sealers diminished rapidly, whereas AH Plus continued to exhibit a higher level of inflammation compared to the controls.

Thus, the biocompatibility of CeraSeal may be attributed to its release of Ca^{2+} ions, which contribute to a more alkaline environment. [52] Furthermore, due to its low cytotoxicity as demonstrated in other study[8], the inflammatory response to CeraSeal diminishes rapidly. The release of calcium ions also promotes cell growth, further supporting its biocompatibility.[50]

The results of this study demonstrated that CeraSeal was the most biocompatible material, followed by NeoSEALER Flo and then AH Plus. This finding is consistent with previous research showing that AH Plus induces a greater inflammatory response compared to CeraSeal.[53]

We found that both bioceramic sealers exhibited a time-dependent cytotoxic effect, with the cytotoxicity of NeoSEALER Flo being slightly higher than that of CeraSeal. In contrast, AH Plus displayed the highest level of cytotoxicity.

The lower inflammatory potential of CeraSeal may make it the preferred choice in settings with a higher risk of sealer extrusion or in patients with greater sensitivity. [54] However, although NeoSEALER Flo's superior sealing properties distinguish it from other sealers, its higher cytotoxicity limits its use, particularly in cases where sealer extrusion is a concern.[55]

Bioceramic materials, like most bioceramic sealers, are likely to elicit an inflammatory response through a variety of mechanisms. pH, ion release, and barrier breakdown byproducts all have an effect on cellular stress and cytokine production [56] proposed that the formulation of NeoSEALER Flo may release more calcium ions or other reactive species, thus producing a larger inflammatory response. In contrast,CeraSeal's formulation may promote tissue regeneration by balanced ion release, reducing inflammation. [57]

CONCLUSIONS

Based on the results of this investigation, CeraSeal is the most biocompatible endodontic sealer when compared to NeoSEALER Flo and AH Plus. Both NeoSEALER Flo and CeraSeal exhibited a time-dependent cytotoxic impact, with NeoSEALER Flo being somewhat more toxic than CeraSeal. These findings indicate that, while both bioceramic sealers provide desired sealing qualities, CeraSeal may have a better biocompatibility profile and hence be more appropriate for clinical scenarios requiring minimum tissue irritation. However, the inherent cytotoxic and inflammatory activity of bioceramic sealers emphasizes the significance of careful material selection and application techniques in order to obtain the best clinical outcomes and protect periapical tissue integrity.

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