

Evaluating the bioactivity of endodontic sealers with respect to their thermo-nanomechanical properties

Andreea Marica¹, Luminita Fritea¹, Florin Banica¹, Iosif Hulka², Gerlinde Rusu³, Cosmin Sinescu⁴, Traian Octavian Costea⁵, Simona Cavalu^{1,*}

¹Faculty of Medicine and Pharmacy, University of Oradea, P-ta 1 Decembrie 10, 410087 Oradea, Romania

²Research Institute for Renewable Energies, University Politehnica Timisoara, G. Muzicescu 138, 300501 Timisoara, Romania

³Faculty of Industrial Chemistry and Environmental Engineering, University Politehnica Timisoara, C. Telbisz 6, 300001 Timisoara, Romania

⁴Faculty of Dentistry, "Victor Babes" University of Medicine and Pharmacy, P-ta E. Murgu 2, 300041 Timisoara, Romania

⁵Advanced Materials Research Infrastructure—SMARTMAT, University of Oradea, 1 Universitatii Street, 410087 Oradea, Romania

Physico-chemical and mechanical features of endodontic sealers are essential functional properties involved in their sealing ability, osteoconductivity, and biocompatibility. Four different root canal sealers (Ceraseal, MTA Fillapex, AH Plus and Sealapex) were investigated in this study in order to evaluate the thermal and nanomechanical features in relation to their bioactivity potential. The nano-hardness values respected the descendent trend: $H_{\text{Ceraseal}} > H_{\text{Sealapex}} > H_{\text{MTA}} > H_{\text{AHPlus}}$, being influenced by the percent of the inorganic component in the samples, identified as residual mass in TGA/DTG (Thermogravimetric Analysis/ Derivative Thermogravimetry) thermograms. Based on electrochemical measurement in SBF (Simulated Body Fluid) and surface investigations by SEM/EDX, we found that the bioactivity potential decreased in the following order: Ceraseal > MTA Fillapex > AH Plus > Sealapex. The highest bioactivity in the case of Ceraseal was evidenced in terms of apatite-like spherulites observed to cover the surface after 28 days incubation in SBF, and Ca/P ratio 1.71, along with the dynamic release and consumption of Ca^{2+} and PO_4^{3-} in SBF. On the opposite side, lack of mineralization was noticed on the surface of Sealapex sample. Knowledge about the proper thermo-mechanical, biological and physico-chemical properties of the root canal materials is important in order to allow a correct material selection, either as premixed or two-component formulation, requiring good sealing or immediate therapeutic effect.

Keywords: root canal sealers, bioactivity, endodontic treatment, thermal analysis, nanoindentation

1. Introduction

According to the criteria suggested by Grossman [1] and revised in accordance with the actual standards [2], the properties of an ideal root canal sealer should fulfil the following requirements: (1) to provide a good adhesion and hermetic seal of the canal wall when set, (2) radiopaque, (3) very fine powder so can mix easily with liquid, (4) dimensionally and color stable after setting, (5) insoluble in tissue fluids, (6) bioactive, stimulating the formation of hydroxyapatite in contact with

body fluids, (7) biocompatible (non-mutagenic, non-sensitizing, and noncytotoxic) after setting.

However, when Grossman published his criteria for an ideal root canal sealer, the formulation consisted of two separate components (powder and liquid). Nowadays, both two components and premixed single formulations are currently available. The endodontic sealers constituted by two components (powder and liquid) require a manual mixing following the manufacturer's instructions. Any imbalance of the powder/liquid ratio may negatively influence the sealing properties of the sealer [3, 4]. Generally, the incorporation of inorganic particles is performed in order to obtain

* E-mail: simona.cavalu@gmail.com

a composite material with improved mechanical properties, especially nanosized filler particles was found to provide substantial improvements in the material hardness [5].

Although there are many available types of sealers, they are classified into five main groups according to their composition: zinc oxide eugenol (ZOE)-based, calcium hydroxide-based, glass ionomer-based, resin-based, and bioceramic sealers [3, 6–9]. Calcium silicate-based sealers are also classified as bioceramic sealers, as well as MTA (Mineral Trioxide Aggregate) – based. The continuous development of new and more efficient endodontic sealers has emphasized an evolution from the conventional ZOE to the contemporary ones like epoxy-based resin, calcium phosphate-based sealer and MTA or bioceramic sealer, with double benefits: creating a hermetic seal and inducing biomineralization through the formation of hydroxyapatite crystals [10]. On the other hand, eugenol was found to leak from conventional zinc oxide eugenol sealers, inducing toxic effect, inflammatory response, and impairing the transmission in nerve cells [11]. Whereas the cytotoxicity of conventional endodontic sealers is well described in literature, little is known about the long-term toxicity of newer endodontic sealers. They exhibit a variable degree of cytotoxicity depending on the conditions under which testing was performed, while the toxic effect is exerted preponderant when they are fresh or in short testing times [12]. These factors are critical in endodontic treatments and directly impact the success of dental procedures.

Setting time and solubility are also clinically important features: a slow setting-sealer will penetrate more profound in the complex morphological environment of the root canal, while a rapid setting one is suitable for quick closure indicated in sensitive situations [13]. Solubility depends on sealer matrix chemistry, being an adverse characteristic for a root canal sealer, while the bioactivity potential is related to their ability to provide a high alkaline environment, which promote the release of Ca ions, stimulates biomineralization and triggers the antibacterial activity [14–16]. Additionally, viscosity and surface tension are also important issues

affecting interfacial phenomena such as wettability, penetration and bonding.

Although the definition of bioactivity of dental materials differs in several studies [17–20], it can be defined as the periodontal cellular response induced by the release of ions from the bio-material and subsequent biomineralization. However, with the emergence of new and advanced instrumentation and sensitive analytical methods, the bioactivity potential exerted by different types of endodontic materials represent a continuous debate. Moreover, the incorporation of various inorganic particles, either as a radiopacifier or as reinforcement agent, may have a controversial effect in terms of bioactivity potential. It has been shown that nanosized filler particles offers significant improvements in the material hardness and elasticity, but also favorable optical properties and durability [5, 21]. However, to date, this mechanism is not clear, but theoretically, if the amount of filler increases, with decreasing particle sizes and, consequently, decreasing interparticle spacing, the elasticity modulus and hardness of the material should increase considerably. On the other hand, heat application during obturation of root canal and water loss can induce changes in the structural and mechanical properties of some sealers affecting their long-term success [22, 23]. This is mainly important in the case of two components endodontic materials requiring a rigorous powder/liquid ratio and manual mixing. Each particular endodontic sealer may have simultaneously benefits and drawbacks. While calcium silicate possesses a slow setting time, they are considered to be not suitable for build-up on the same day with endodontic obturation, while calcium-hydroxide-containing sealers are considered good choices for large apical radiolucency, in cases with poor evolution prognosis [7]. However, regardless of sealer choice, a practitioner must keep in mind that long-term clinical success depends not only on the properties of the sealer, but also on the obturation technique and proper preparation and irrigation of the root canal in order to promote periapical healing, regeneration and sealing by biomineralization through apatite deposition at the interface.

In the context of contradictory information presented in literature regarding the bioactivity potential of different types of endodontic sealers, which often influence the choice of the best material according to the priority of each patient, requiring good sealing or immediate therapeutic effect, and the lack of clinical data [24], the aim of this study was to evaluate and compare the bioactivity potential of four root canal sealers (Ceraseal, MTA Fillapex, AH Plus and Sealapex) in relation to their composition, thermal behavior and nanomechanical properties. The null hypothesis is that the bioactivity potential of the selected endodontic sealer is influenced by its thermal features and nanomechanical properties (nano-hardness).

2. Materials and methods

Four different root canal sealers were tested in this study, listed in Table 1, along with their manufacturer, composition, and setting time.

Six specimens of each material were prepared in accordance with the manufacturers' instructions, using polyethylene molds (20 mm diameter and 2 mm high) and kept for 3 days at room temperature, except CERASEAL specimens, which were kept for 3 days in a dark container at 37°C and 95% humidity. After hardening, the specimens were separated from the molds by cutting the walls of the molds and submitted to analysis.

2.1. Thermogravimetric analysis (TGA/DTG)

TGA thermograms were recorded using a TG 209 F1 Libra (NETZSCH-Gerätebau GmbH, Selb, Germany) thermogravimetric analyzer. The measurements were carried out in a nitrogen atmosphere, in the temperature range of 20–850°C, with a heating rate of 10°C/min, the data being processed with the Netzsch Proteus-Thermal Analysis program version 6.1.0. (NETZSCH- Gerätebau GmbH, Selb, Germany). Although the clinically relevant temperature range is considered to be up to 200°C (which is the maximum temperature of thermoplasticized gutta-percha), the application of

higher temperature during thermogravimetric analysis may offer additional information in terms of long-term stability.

2.2. Nanoindentation measurements

The specimens were subjected to nanomechanical tests using a Nanoindenter G200 device (Agilent Technologies, Santa Clara, CA, USA), at room temperature and normal humidity (45–52%), by applying a diamond Berkovich pyramidal-shaped tip. Each indentation test consisted of a loading and unloading phase repeated 10 times, while the microscopic image of the selected area on the surface allowed precise control of the selected area and indenter position. The indentation tests were carried out in quasi-static depth-control mode, in which the maximum displacement depth was fixed at 2400 nm while the load force was continuously measured. The loading rate was modulated by the machine and hold at a constant value of 0.05/s. The surface detection sensitivity parameter was set to 100 N/m, while the surface approach velocity was set to 10 nm/s with a starting point of 1000 nm. Before starting the test, the instrument was set to wait for the drift rate to reach and to remain below 0.05 nm/s. Both hardness and Young's modulus were obtained from the load-displacement curves generated by the measurements, using the Oliver–Pharr method [25], taking into account the tip shape function and considering that the value of the displacement may include contributions from both the specimen and the indenter.

2.3. Evaluation of bioactivity potential

Simulated body fluid (SBF) was prepared and manipulated according to the methodology described by Kokubo and Takadama [26] using the following reagents purchased from Sigma Aldrich (Steinheim, Germany): NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, Na₂SO₄ and (HOCH₂)₃CNH₂. All these components were dissolved at 37°C and pH = 7.4 to avoid spontaneous precipitation. The final pH was adjusted to 7.4 by adding 1 M HCl solution. The specimens from each material were immersed in 5 mL SBF and incubated for 28 days at 37°C and 95–100%

Table 1. The tested root canal sealers: composition, manufacturers and setting time (according the manufacturer description)

Sealer	Composition	Setting time (min)	Manufacturer
CERASEAL	Calcium silicates, zirconium oxide, thickening agent	210	Meta Biomed Co., Cheongju, Korea
MTA Fillapex	<u>Base paste</u> : Salicylate resin, natural resin, calcium tungstate, nanoparticulated silica, pigments <u>Catalyst paste</u> : Diluting resin, tricalcium silicate, di-calcium silicate, calcium oxide, tricalcium aluminate, nanoparticulated silica, pigments.	130	Angelus Dental Solutions, Londrina, PR, Brazil
SEALAPEX	<u>Base paste</u> : sulphonamide resin, N-ethyl toluene, silicon dioxide, zinc oxide, calcium oxide; <u>Catalyst paste</u> : isobutyl salicylate resin, silicon dioxide, bismuth trioxide, titanium dioxide, pigments	1400	SybronEndo - Sybron Dental Specialties, Glendona, CA, USA
AH Plus	<u>Paste A</u> : bisphenol A epoxy resin, bisphenol F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments; <u>Paste B</u> : dibenzylamine, aminoadamantane, tricyclodecane-diamide, calcium tungstate, zirconium oxide, silica, silicone oil	480	Dentsply Sirona, York, PA, USA

humidity. At different time intervals (24 hours, 7, 14, 21 and 28 days), the SBF solution was changed, while the pH, calcium and phosphate ions measurements were performed. The samples were carefully rinsed with deionized water, and dried, and fresh SBF fluid was added. Calcium ions determination and pH analysis were performed by using an ion-selective electrode (Calcium Combination Electrode – Consort) and a pH electrode connected to a CiberScan PCD 6500 (pH/Ion/Conductivity/DO Meter). The square wave voltammetry method was used for the phosphate determination. The electrochemical experiments were recorded on screen-printed electrodes (SPEs – Metrohm Dropsens, Belgium) having carbon as working and counter electrodes and silver as reference electrodes, being connected to Autolab PGSTAT 128N potentiostat (Metrohm, Belgium). The square wave voltammetry method employed the following parameters: -0.01 V step, 0.05 V modulation amplitude, 25 Hz frequency for a range scan from 1.0 V to -1.5 V.

In order to evaluate the mineralization process, the surface of the specimens was investigated after

28 days incubation period, by SEM/EDX using Quanta FEG 250 SEM instrument (FEI, Breda, The Netherlands) operating at 15 kV, $\text{HFW} = 82.9$ μm , the images being captured with back scattered electron detector at different magnifications. The instrument was equipped with energy dispersive X-ray spectroscopy (EDX) unit and Apollo SSD detector (EDAX Inc., Mahwah, NJ, USA). The microstructure investigations and EDX analysis were performed at about 10 mm working distance, in low vacuum mode (using a pressure limiting aperture) and 15 kV accelerating voltage, while the probe current was 10 μA , in order to avoid surface charging and damage to the analyzed samples. Serial SEM photomicrographs were taken at different magnifications (5 k and 20 k). In order to assess the atomic Ca/P ratio, the spectrometer was calibrated with a standard reference material used to evaluate the physical and chemical properties of apatites of biological, geological or synthetic origins. According to the standard, the calcium to phosphorus (Ca/P) molar ratio is consistent with the theoretical value $\text{Ca/P} = 1.67$ for stoichiometric hydroxyapatite.

2.4. Statistical analysis

Data were analysed with the SPSS 22.0 software (IBM, New York, NY, USA). The results of the physical, chemical and mechanical properties were subjected to statistical analysis by one-way ANOVA test followed by a post hoc Tukey test in order to provide statistical significance. P values below 0.01 were considered significant.

3. Results

3.1. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) and first derivative curves (DTG) of the endodontic sealer specimens are presented in Figures 1–4, showing a very different behavior according to their composition. In the thermogram corresponding to CERASEAL (Fig. 1), it can be noticed the presence of water, either in the free form, unbound (2.21%), which is visible in the TGA curve as an initial mass loss in the temperature interval 0–100°C, or bound water (6.07%) in the temperature interval 100–160°C. Between 200–400°C, a decomposition process occurred, the associated mass loss being 6.12%. The composition of CERASEAL is based mainly on premixed inorganic compounds (calcium silicates and zirconium oxide), with thermal stability, and hence, the final residual mass at 850°C was 84.26%.

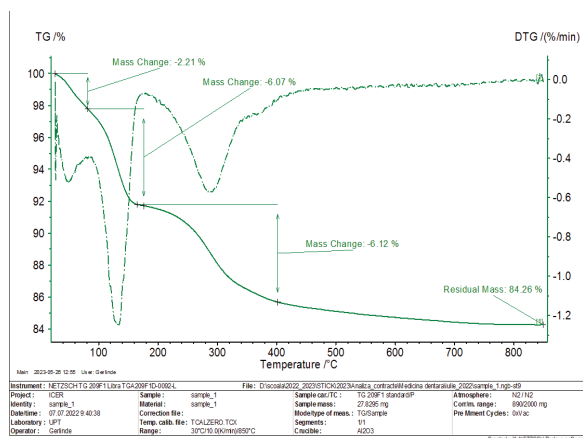


Fig. 1. TGA and DTG thermograms of CERASEAL root sealer

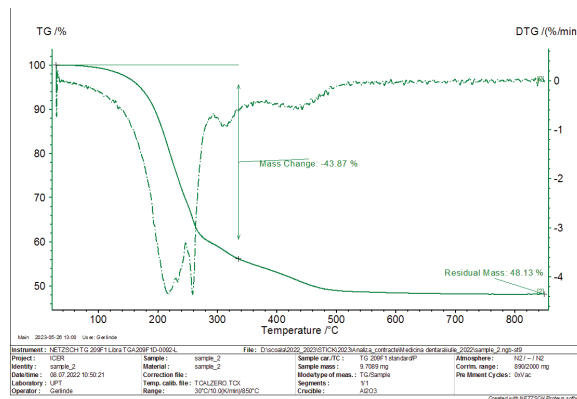
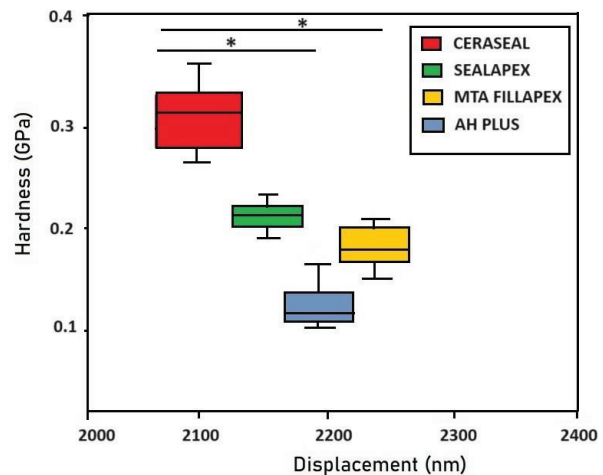
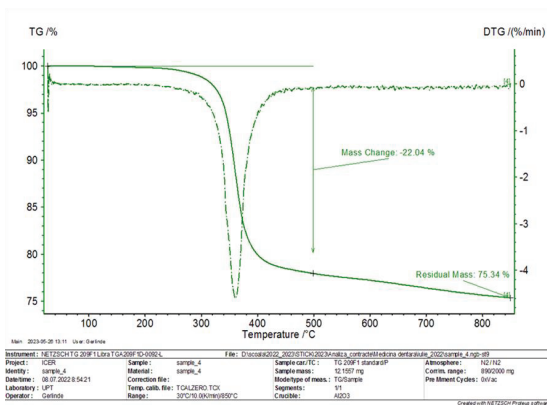
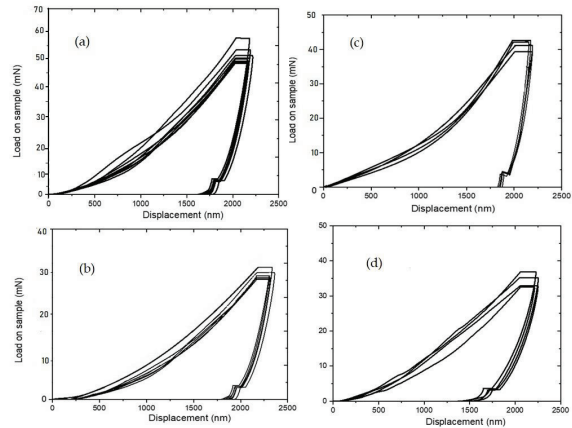
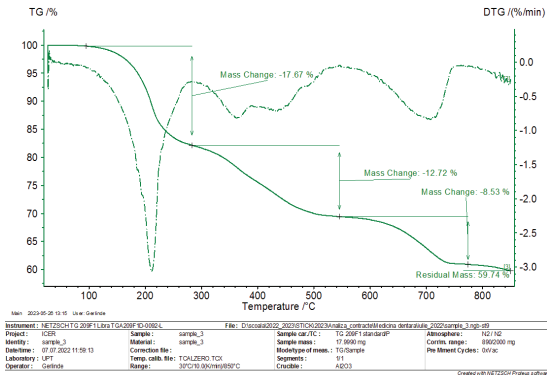


Fig. 2. TGA and DTG thermograms of MTA FILLAPEX

The thermogravimetric pattern of MTA FILLAPEX (Fig. 2) is different compared to the previous one, since its composition is a mixture of natural resins and a considerable amount of mineral trioxide aggregate (tricalcium oxide, tricalcium silicate, tricalcium aluminate), in a two pastes formulation (base and catalyst). As the sample contains no water, the decomposition occurred in one step, in the temperature interval between 180–320°C, with a mass loss of 43.87%, the final residual mass at 850°C being 48.13%, associated with the inorganic compounds. However, similar to CERASEAL, we can notice a decomposition temperature below the maximum temperature of thermoplasticized gutta-percha (which is approximate 200°C).

SEALAPEX is a calcium hydroxide-based sealer containing a mixture of different resins (sulphonamide resin and isobutyl salicylate resin) and inorganic fillers in a two pastes formulation. As a consequence, this sample presented 3 steps of decomposition (Fig. 3). The first one, between 180–230°C accompanied by 17.67% mass loss, is associated with the decomposition of the organic compounds, the 2nd one between 325–470°C with 12.72% mass loss, and the final one between 640–730°C with 8.53% mass loss. The final residual mass at 850°C was 59.74%, corresponding to all the inorganic compounds in the sample.

AH PLUS is an epoxy resin-based root canal sealer which is also dispensed in 2 pastes: Paste A is composed of bisphenol epoxy resins mixed with



mineral compounds, while Paste B contains amino adamantane, aromatic amine hardeners along with calcium tungstate and zirconium oxide fillers. Within the clinically relevant temperature interval (up to 200°C) we noticed a stable behavior and no mass loss, as this sealer contains no water. In this case, the decomposition process (Fig. 4) occurred at higher temperature (between 330–380°C) compared to previous samples and presented one single step, associated with the decomposition of the organic compounds and a mass loss of 22.04%, the final residual mass at 850°C being 75.34%, which correspond to the inorganic components.

3.2. Nanoindentation measurements

After setting and hardening, load-displacement curves were recorded for each specimen, as

Fig. 5. Load-displacement curves recorded on the surface of the specimens: a) CERASEAL; b) MTA FILLAPEX; c) SEALAPEX and d) AH PLUS

Fig. 6. Box plot graphical representation of hardness values obtained from nanoindentation tests as a function of contact depth for each specimen (*p values < 0.01)

presented in Figure 5(a–d), while the hardness values as a function of contact depth are displayed in Figure 6.

In terms of nanomechanical features, we can notice similar patterns of the load-displacement curves showing a well-composed and uniform composition of the materials after mixing the pastes, but different values of the peak force were measured in order to penetrate the same depth in

Table 2. Measurement of pH, Ca^{2+} and PO_4^{3-} ions in SBF solution, at different time point

Sample	1 day	7 days	14 days	21 days	28 days
pH					
CERASEAL	10.2 ± 0.9^a	11.7 ± 0.9^a	9.7 ± 0.1^a	9.2 ± 0.2^a	8.7 ± 0.5^a
MTA FILLAPEX	9.2 ± 0.1^b	9.8 ± 0.2^b	8.9 ± 0.1^b	8.2 ± 0.2^b	8.2 ± 0.2^a
AH PLUS	7.4 ± 0.2^c	7.9 ± 0.2^c	7.2 ± 0.3^c	7.2 ± 0.3^c	7.0 ± 0.2^b
SEALAPEX	8.2 ± 0.2^d	8.4 ± 0.2^d	8.4 ± 0.1^b	8.2 ± 0.1^b	8.4 ± 0.1^a
Ca^{2+} (mg/L)					
CERASEAL	180.2 ± 20.2^a	90.7 ± 10.5^a	80.5 ± 10.2^a	51.7 ± 10.1^a	31.4 ± 3.9^a
MTA FILLAPEX	90.4 ± 10.5^b	130.5 ± 11.6^b	110.8 ± 10.9^b	60.5 ± 7.5^b	42.2 ± 5.5^b
AH PLUS	110.2 ± 15.5^c	92.5 ± 10.2^a	88.7 ± 9.2^c	90.3 ± 9.8^c	90.7 ± 10.2^c
SEALAPEX	97.5 ± 10.0^d	105.5 ± 11.5^b	98.2 ± 10.2^d	96.1 ± 10.5^d	94.2 ± 10.8^d
PO_4^{3-} (mg/L)					
CERASEAL	48.3 ± 5.0^a	37.2 ± 4.0^a	25.1 ± 2.0^a	19.2 ± 1.5^a	15.5 ± 2.0^a
MTA FILLAPEX	42.8 ± 5.0^b	30.5 ± 3.5^b	22.1 ± 2.0^b	16.8 ± 2.0^b	14.0 ± 2.0^a
AH PLUS	40.5 ± 5.0^c	39.2 ± 4.0^c	32.9 ± 3.0^c	30.5 ± 3.5^c	29.2 ± 3.0^b
SEALAPEX	42.1 ± 5.0^b	38.5 ± 4.0^c	35.0 ± 3.0^d	34.0 ± 4.0^d	35.0 ± 3.0^c

Legend: Different letters represent significant differences of the values in the same column (at the same point time) with $p < 0.01^*$.

each specimen. A load of 59 mN was recorded on the surface of CERASEAL in order to obtain 2100 nm displacement, while only 45 mN was necessary to penetrate the same depth in SEALAPEX. Much lower values were recorded for MTA FILLAPEX peak force (29 mN) and AH PLUS (35 mN). The average hardness values obtained from the fitting parameters range from $H = 0.12 \pm 0.05$ GPa in the case of AH PLUS, to $H = 0.31 \pm 0.07$ GPa for CERASEAL, demonstrating that the type and quantity of the filler (inorganic component), as well as the matrix type influence the material hardness.

3.3. Evaluation of bioactivity potential

3.3.1. Electrochemical measurements in SBF

The monitorization of pH, Ca^{2+} and PO_4^{3-} ions in SBF solution was performed at different time intervals (1, 7, 14, 21 and 28 days) and the results are presented in Table 2.

It can be observed that, except AH PLUS, all the materials investigated in this study exhibited an alkaline pH and the highest mean of pH value at each time point was observed in CERASEAL. It was also noticed that the maximum pH values were reached by each material after 7 days. In terms of Ca measurements, we noticed an initial burst

released from CERASEAL sample, after the first day, followed by sustained calcium consumption for the next period, until day 28, demonstrating long term activity. A similar behavior in terms of Ca consumption can be observed for MTA FILLAPEX, but with a slower rate, while the burst release was achieved after 7 days. Very poor activity in terms of Ca exchange was noticed for AH PLUS and SEALAPEX during the investigated period, as no significant modifications of Ca concentrations were found. On the other hand, a sustained consumption of phosphate ions was also detected in the SBF related to CERASEAL and MTA FILLAPEX samples, while very small variations of PO_4^{3-} concentration was observed in AH PLUS and SEALAPEX. The dynamic release and consumption of Ca^{2+} concomitant with PO_4^{3-} reflects the apatite growth, as in physiological solutions, the bioactive materials consume calcium and hydroxyl ions for apatite formation.

3.3.2. Surface characterization and biomineralization capacity: SEM/EDX analysis

SEM-EDX analysis showed different surface morphologies of the samples after 28 days incubation in SBF. Figures 7–10 display the SEM micrographs recorded on the surfaces of each sample

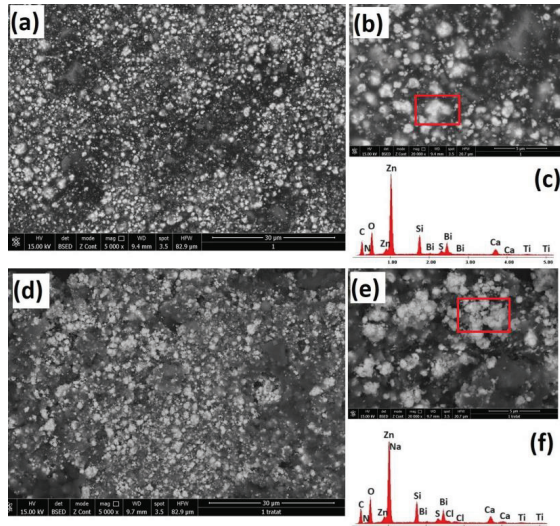


Fig. 7. SEM micrograph recorded on the surface of SEALAPEX sample before (a–c) and after immersion in SBF (d–f) along with the EDX spectrum

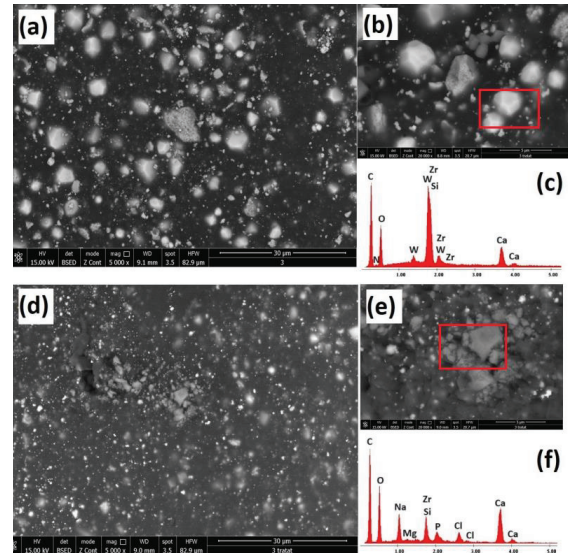


Fig. 9. SEM micrograph recorded on the surface of AH PLUS sample before (a–c) and after immersion in SBF (d–f) along with the EDX spectrum

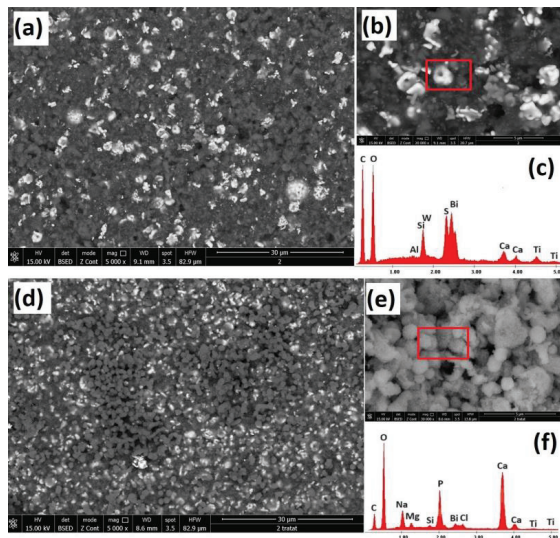


Fig. 8. SEM micrograph recorded on the surface of MTA FILLAPEX sample before (a–c) and after immersion in SBF (d–f) along with the EDX spectrum

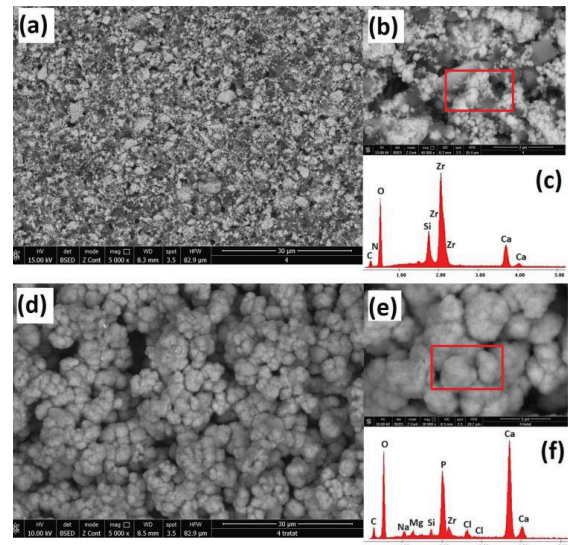


Fig. 10. SEM micrograph recorded on the surface of CERASEAL sample before (a–c) and after immersion in SBF (d–f) along with the EDX spectrum

before and after immersion in SBF, with different details and magnifications, along with the EDX microanalysis and Ca/P ratio calculations.

It can be observed that the surface of CERASEAL and MTA FILLAPEX samples was

covered by small spherulites and granules, detected as mineral deposits rich in Ca and P, with intercalated voids. Larger size of the granulated minerals was observed in the case of CERASEAL (approximative $3\ \mu\text{m}$ diameter) compared to MTA

FILAPEX (1.2 μm diameter). According to EDX measurements, Ca/P atomic ratio was 1.70 ± 0.09 and 1.65 ± 0.05 , respectively, which is an indicative of apatite type deposits.

A very poor mineral deposition was detected on the surface of AH PLUS, with only few spots detected as calcium phosphate, while the ratio Ca/P indicated 1.40 ± 0.05 , which is a non-apatite type mineral. No mineralization was detected on the surface of SEALAPEX sample, indicating a completely lack of bioactivity after 28 days.

4. Discussion

Root canals are special structures with complex and unique anatomy, and no two are alike. Successful root canal treatment, to achieve a three-dimensional obturation with complete coronal, lateral and apical sealing capability for long-term, is of crucial importance in preventing sealer leakage and infection [27, 28]. The chemical composition and thermo-mechanical features of endodontic sealers can influence their adhesion to root dentine and, consequently, the effectiveness of root canal obturation. Moreover, heat application during obturation has triggered attention about its compatibility with different sealers, especially in the case of epoxy-resin and calcium silicate-based, affecting their long-term success. TGA/DTG measurements allows evaluation of the effect of heat on the mass of tested materials, which can reflect chemical changes in its composition, whether due to reactions or material loss [22]. Hence, the knowledge of physico-chemical properties of sealing materials is important in order to allow the selection of an appropriate material for each particular situation in clinical practice [29].

Particularly, the proper evaluation of the bioactivity of different types of sealers is of crucial importance, being related to their functional properties, such as the sealing ability, osteoconductivity and biocompatibility. When compacted against dentine, following the specific root canal treatment, the formation of the interfacial layer occurs in the presence of phosphate ions contained in biological fluids, contributing to the prevention of bacterial leakage through the interface [30, 31]. The

mineral layer of apatite observed at dentin-sealer interface, is often described as “mineral infiltration zone” [32], promoting the adhesion of endodontic sealers to radicular dentin, and hence, improving their resistance to dislocation.

Our study evaluated the bioactivity potential of different types of commercial endodontic sealers in relation to their composition, thermal decomposition behavior, and nanomechanical features. The investigations performed in SBF and SEM/EDX analysis revealed a bioactivity potential in the following order: CERASEAL > MTA FILLAPEX > AH PLUS > SEALAPEX. CERASEAL is a pre-mixed Calcium Silicate-Based Sealer, and since then, little information has been available in the literature related to its biological and physico-chemical properties and performance *in vitro* and *in vivo* [3, 33].

A study conducted by Naji Kharouf *et al.* [3] aimed to compare the physico-chemical properties, filling ability, and antibacterial activity of Ceraseal and BioRoot RCS (a powder-liquid bioceramic sealer, based on tricalcium silicate, that required manual mixing procedures) and concluded that CERASEAL possesses superior filling ability and lower solubility compared to BioRoot RCS, suggesting that this behavior is due to its specific chemical composition and pre-mixed condition, as its main advantage consists in having a homogeneous mixture with no concern related to the alteration of powder/liquid ratio. However, the authors pointed out that both sealers exhibited high mineralization activity, as evidenced by SEM/EDX. In our study, the higher bioactivity of CERASEAL, compared to other sealers, was evidenced in terms of apatite-like spherulites observed to cover the surface after 28 days incubation in SBF, while Ca/P ratio was 1.70 ± 0.09 . At the same time, CERASEAL raised the pH of the immersion medium, reaching a maximum of 11.7 after 7 days, accompanied by a burst of Ca released, which triggered apatite nucleation. The prolonged exchange mechanism and consumption of Ca and phosphate ions in SBF is a key factor in promoting periodontal tissue regeneration [34]. It was previously reported that the high bioactivity potential of hydraulic calcium-silicate materials is associated

with osteogenic differentiation and mineralization capacity of these materials, according to alkaline phosphatase assays [34]. Calcium hydroxide is a by-product of the reaction of calcium silicate cement with water; dispersion and consequent release of hydroxyl and calcium ions in the environment may contribute to tissue healing and bacterial elimination [35, 36]. On the other hand, phosphate ions are able to collate with the readily available calcium ions to form calcium phosphate species, which can be the building blocks for hydroxycarbonate apatite (HCA). A sustained decreasing of phosphate ion concentration in the SBF solution is indicative of a phosphate-rich precipitate forming [37]. Moreover, in the case of CERASEAL, we noticed good thermal stability, as the decomposition process occurred in the temperature interval 200–400°C with an associated mass loss of only 6.12%.

By comparison, the high bioactivity of MTA FILLAPEX is due to its complex inorganic composition derived from Portland cement: mineral trioxide aggregate (MTA) consists of tricalcium silicate, di-calcium silicate, calcium oxide, tri-calcium aluminate and nanoparticulated silica. MTA-based composites are known to interact with dentine and incorporate intertubular Ca and Si accompanied by apatite deposition in the presence of biological fluids [38], and such a biomineralization ability is responsible for the sealing capacity. Although there are controversial results in the literature, several studies are in agreement with our findings in terms of bioactivity potential. For example, Tamomaru-Filho et al. evaluated the physico-chemical properties and bioactive potential of MTA Fillapex compared to SEALAPEX and AH Plus and found that MTA Fillapex was the only one endodontic material demonstrating a bioactive potential [39], while other studies evidenced that MTA Fillapex sealer showed lower and delayed apatite formation ability, compared to a premixed calcium silicate-based sealer (TotalFill BC), and this may be attributed to its high resin content and lower calcium silicate content [40]. Similarly, our study indicated variable response between CERASEAL and MTA Fillapex upon incubation in SBF, as the first one contains higher

amount of Ca and lower level of silica, compared to MTA-based. We suggest a possible complementary effect between Ca (as an accelerator of apatite precipitation) and Si (as a nucleator). In our experiment, the Ca/P ratio recorded for MTA Fillapex was 1.65 ± 0.05 with a smaller size of mineral spherulites compared to CERASEAL. It is well known that the Ca/P ratio acts as an indicator of mineralization potential, and the formation of carbonated apatite correlates with increased Ca/P ratios. According to the literature [41, 42], the development of calcium carbonate occurs when the Ca/P ratio exceeds 1.67.

However, it is important to mention the influence of variations in environmental pH on the solubility and water sorption of root canal sealers. The different environments have been shown to affect the material chemistry, as the alkaline capacity is related to the dissolution and ionic releasing of the sealers, resulting in different values for the solubility of the same material when tested using different storage media [43, 44]. Additionally, the alkaline pH ensures the bacteriostatic effect, promoting apical healing and tissue mineralization. In terms of thermal behavior, the decomposition process occurred at a lower temperature (180–320°C) compared to CERASEAL, accompanied by a higher mass loss (12.72%).

AH Plus was considered a gold standard epoxy resin-based sealer used in endodontic treatments due its excellent physico-chemical properties, but recently, the resinous component has been associated with the increased cytotoxicity [45]. Some studies evidenced that AH sealer was unable to promote bioactivity or hydroxyapatite precipitation when immersed in PBS or simulated body fluid [46, 47]. Our study indicated only poor bioactivity potential, which is in concordance with several previous findings [48]. This is not surprising, being an epoxy resin-based material, with low calcium content in a form of calcium tungstate (as a radiopacifier), which is visible in EDX spectrum [Fig. 9c]. The lower ionic exchange (both Ca and phosphate) recorded by electrochemical measurements may be associated with the cross-linking mechanism in epoxy resins, and consequently a low solubility. Obviously, calcium and hydroxyl ion release after

hydration is associated with a favorable bioactivity of calcium silicate-based and MTA-based endodontic biomaterials, in opposition to epoxy resin matrix. The same arguments could be invoked for the lack of bioactivity in the case of SILAPEX, which is a non-eugenol, calcium hydroxide-based sealer, also containing two types of resins. Sealers containing calcium hydroxide were primarily intended to promote osteogenesis concomitant with an antimicrobial environment, but unfortunately, they have not achieved the desired clinical outcomes [7]. In terms of thermal features, within the clinically relevant temperature interval (up to 200°C), SEALAPEX presented the lowest initial decomposition temperature and moderate mass loss, while AH Plus showed a stable behavior and no mass loss. A good thermal stability of AH Plus was previously evidenced by Atmeh *et al.* [22], showing that heat application on the epoxy resin-based sealer induced very minimal changes on the chemical structure at low temperature, but higher temperatures (over 250°C) and/or longer duration was accompanied by earlier polymerization and consequently, changes in the chemical structure of epoxy monomers, amine hardeners, and calcium tungstate filler. Our findings suggest that the bioactivity potential of the selected endodontic sealers is not related to their thermal behavior, but the differences in terms of decomposition temperature and mass loss might have significant influence on long term evolution of the endodontic treatment. The results are clinically relevant in the context of necessity to assess the thermal stability and to determine if they can tolerate the heating temperatures generated during thermal-based obturation techniques without decomposition. The knowledge about thermal stability of endodontic sealers would give clinicians insights for better clinical decisions for higher treatment outcomes [44].

In terms of nanomechanical features, the hardness values respect the following descendent trend: $H_{\text{Ceraseal}} > H_{\text{Sealapex}} > H_{\text{MTA}} > H_{\text{AHPlus}}$, demonstrating that the composition in terms of the inorganic component and the matrix type influence the material hardness. According to the literature, the mean hardness of the root dentine nearest the pulp was 0.52 ± 0.24 GPa in a study performed by

Radoslav Halgas *et al.* [49], while values between 0.4 GPa and 0.64 GPa, depending on the longitudinal and transversal positions, were found by Michael Kucher *et al.* [50]. Although there is no requirement for the hardness of endodontic sealers, we noticed that CERASEAL possesses the closest hardness value ($H = 0.31 \pm 0.07$ GPa) to that of root canal dentine. Hardness refers to a material's resistance to permanent deformation and, from a clinical point of view, is important for understanding the clinical behavior of many biomaterials, in the field of restorative dentistry and to predict the wear resistance of a material against applied forces such as occlusal loading. The results are also relevant as sealers should show some elasticity to accommodate root flexures when subjected to masticatory or other stresses [50–52].

Our results did not reveal any influence of the mechanical or thermal properties on the bioactivity potential of the selected sealers. Therefore, the null hypothesis was rejected.

We are aware of the fact that the major problem with the *in vitro* experimental test is that the results cannot be extrapolated to clinical conditions [13]. The testing environment of *in vitro* studies only mimics the physiological conditions of humidity and temperature and cannot replicate all the uncontrolled clinical variables that potentially influence the bioactivity of an endodontic sealer. It is well known that exposure to moisture is different under *in vitro* versus *in vivo* conditions. Hence, *in vivo* experimental animal models are further necessary to attempt to reproduce the clinical conditions as closely as possible.

However, within the limitations of our study, by corroborating the results, we suggest that CERASEAL meets the criteria for the best bioactivity and nano-hardness, along with good thermal stability, which makes it a preferred material to promote an impermeable root canal sealing for long term. To the best of our knowledge, this is the first paper dealing with the bioactivity potential and thermo-nanomechanical properties of root canal sealers. Our findings are important in the context of a great variety of new-generation sealers available on the market, each of them claiming their antimicrobial efficacy of a broader spectrum.

The development of new sealers and obturation techniques will advance significantly concomitant with the advancement of technology. A combination of sealing ability and biocompatibility, accompanied by the clinician's knowledge and adequate techniques, can ensure the success of endodontic treatment. However, continuous research is required in order to assess the long-term antibacterial effect of endodontic sealers.

5. Conclusions

In this work, a comparison of the bioactivity potential and thermal and nanomechanical properties of different types of endodontic sealers was performed. Based on electrochemical measurement in SBF and surface investigations by SEM/EDX, we found that the bioactivity potential decreased in the following order: Ceraseal > MTA Fillapex > AH Plus > Sealapex, while the nano-hardness values respected the descendent trend: $H_{\text{Ceraseal}} > H_{\text{Sealapex}} > H_{\text{MTA}} > H_{\text{AHPlus}}$. Our results did not reveal any influence of the mechanical or thermal properties on the bioactivity potential of the selected sealers. Further research and laboratory studies simulating the natural environment to assess the biological interaction between different types of endodontic sealers and root canal dentine are still necessary.

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Conflicts of Interest

The authors declare no conflict of interest.

References

- [1] Grossman, L. Obturation of root canal. In *Endodontic Practice*, 10th ed. Lea and Febiger, Philadelphia, 1982; pp. 297.
- [2] ISO, 6876. Dentistry-Root canal sealing materials. Geneva: International Organization for Standardization; 2012.
- [3] Kharouf N, Arntz Y, Eid A, Zghal J, Sauro S, Haikel Y, Mancino, D. Physicochemical and Antibacterial Properties of Novel, Premixed Calcium Silicate-Based Sealer Compared to Powder-Liquid Bioceramic Sealer. *J Clin Med*. 2020;9:3096. doi: 10.3390/jcm9103096
- [4] Muedra P, Forner L, Lozano A, Sanz JL, Rodríguez-Lozano FJ, Guerrero-Gironés J, Riccitiello F, Spagnuolo G, Llena C. Could the Calcium Silicate-Based Sealer Presentation Form Influence Dentinal Sealing? An In Vitro Confocal Laser Study on Tubular Penetration. *Mater*. 2021;14:659. doi: 10.3390/ma14030659
- [5] Peskersoy C, Culha O. Comparative Evaluation of Mechanical Properties of Dental Nanomaterials. *Hindawi J of Nanomater*. 2017;2017:8. doi: 10.1155/2017/6171578
- [6] Estivalet MS, de Araújo LP, Immich F, da Silva AF, Ferreira NdS, da Rosa WLdO, Piva E. Bioactivity Potential of Bioceramic-Based Root Canal Sealers: A Scoping Review. *Life*. 2022;12:1853. doi: 10.3390/life12111853
- [7] Komabayashi T, Colmenar D, Cvach N, Bhat A, Carolyn Primus C, Imai Y. Comprehensive Review of Current Endodontic Sealers. *Dent Mater J*. 2020;39:703–720. doi: 10.4012/dmj.2019-288
- [8] Marica A, Sipos L, Iurcov R, Stefanescu T, Ciavoi G, Lucan A-I, Miere (Groza) F. Current Use of Nanoparticles in Endodontics: A Systematic Review. *Rom J Oral Rehabil*. 2022;14:115–126.
- [9] Joseph T, Joy M, Joy J, Hari K, Joy B. Bioceramics as Root Canal Sealers: A Review. *Int J Sci Res*. 2020;9:494–498. doi: 10.21275/SR201101174003
- [10] Tyagi S, Mishra P, Tyagi P. Evolution of Root Canal Sealers: An Insight Story. *Eur J Gen Dent*. 2013;2:199–218. doi: 10.4103/2278-9626.115976
- [11] Kaur A, Shah N, Logani A, Mishra N. Biototoxicity of Commonly Used Root Canal Sealers: A Meta-Analysis. *J Conserv Dent*. 2015;18:83–88. doi: 10.4103/0972-0707.153054
- [12] Silva, E.J.N.L.; Santos, C.C.; Zaia, A.A. Long-Term Cytotoxic Effects of Contemporary Root Canal Sealers. *J Appl Oral Sci*. 2013;21:43–47. doi: 10.1590/1678-7757201302304
- [13] Silva EJNL, Ehrhardt IC, Sampaio GC, Cardoso ML, da Silva Oliveira D, Uzeda MJ, Calasans-Maia MD, Cavalcante DM, Zuolo ML, De-Deus G. Determining the Setting of Root Canal Sealers Using an In Vivo Animal Experimental Model. *Clin Oral Invest*. 2021;25:1899–1906. doi: 10.1007/s00784-020-03496-x
- [14] Gandolfi MG, Taddei P, Tinti A, Dorigo ES, Rossi PL, Prat C. Kinetics of Apatite Formation on a Calcium-Silicate Cement for Root-End Filling During Ageing in Physiological-Like Phosphate Solutions. *Clin Oral Invest*. 2010;14:659–668. doi: 10.1007/s00784-009-0356-3
- [15] Shekhar S, Jaiswal S, Nikhil V, Gupta S, Mishra P, Raj S. Comparative pH and Calcium Ion Release in Newer Calcium Silicate-Based Root Canal Sealers. *Endod*. 2019;31:29–33. doi: 10.4103/endo.endo_53_18
- [16] Simon V, Cavalu S, Simon S, Mocuta H, Vanea E, Prinz M, Neumann M. Surface Functionalisation of Sol-Gel Derived Aluminosilicates in Simulated

- Body Fluids. *Solid St Ionics*. 2009;180:764–769. doi: 10.1016/j.ssi.2009.02.011
- [17] Vallittu PK, Boccaccini AR, Hupa L, Watts DC. Bioactive Dental Materials—Do They Exist and What Does Bioactivity Mean? *Dent Mater*. 2018;34:693–694. doi: 10.1016/j.dental.2018.03.001
- [18] Han L, Okiji T. Bioactivity Evaluation of Three Calcium Silicate-Based Endodontic Materials. *Int Endod J*. 2013;46:808–814. doi: 10.1111/iej.12062
- [19] Hoppe A, Guldal NS, Boccaccini AR. A Review of the Biological Response to Ionic Dissolution Products from Bioactive Glasses and Glass–Ceramics. *Biomater*. 2011;32:2757–2774. doi: 10.1016/j.biomaterials.2011.01.004
- [20] Forsenkova AA, Fadeeva IV, Deyneko DV, Gosteva AN, Mamin GV, Shurtakova DV, Davydova GA, Yankova VG, Antoniac IV, Rau JV. Polyvinylpyrrolidone—Alginate—Carbonate Hydroxyapatite Porous Composites for Dental Applications. *Mater*. 2023;16:4478. doi: 10.3390/ma16124478
- [21] Sarosi C, Biris AR, Antoniac A, Boboia S, Alb C, Antoniac I, Moldovan M. The Nanofiller Effect On Properties Of Experimental Graphene Dental Nanocomposites. *J Adhes Sci Tech*. 2016;30:1779–1794. doi: 10.1080/01694243.2016.1161969
- [22] Atmeh, A.R., AlShwaimi, E. The Effect of Heating Time and Temperature on Epoxy Resin and Calcium Silicate-based Endodontic Sealers. *J Endod* 2017;43:2112–2118. doi: 10.1016/j.joen.2017.08.008
- [23] Antunes TBM, Janini ACP, Pelepenko LE, Abuna GF, Paiva EM, Sinhoretic MAC, Raimundo IM Jr, Gomes BPFA, de-Jesus-Soares A, Marciano MA. Heating Stability, Physical and Chemical Analysis of Calcium Silicate-Based Endodontic Sealers. *Int Endod J*. 2021;54:1175–1188. doi: 10.1111/iej.13496
- [24] Mustafa M. The Use of Bioceramic Root Canal Sealers for Obturation of The Root Canal System: A Review. *IJUM J Orofacial Health Sci*. 2021;2:14–25. doi: 10.31436/ijohs.v2i1.55
- [25] Oliver WC, Pharr GM. An Improved Technique for Determining Hardness and Elastic Modulus Using Load and Displacement Sensing Indentation Experiments. *J Mater Res*. 1992;7:1564–1583. doi: 10.1557/JMR.1992.1564
- [26] Kokubo T, Takadama H. How Useful is SBF in Predicting In Vivo Bone Bioactivity? *Biomater*. 2006;27:2907–15. doi: 10.1016/j.biomaterials.2006.01.017
- [27] Santos-Junior AO, De Castro Pinto L, Mateo-Castillo JF, Pinheiro CR. Success or Failure of Endodontic Treatments: A Retrospective Study. *J Conserv Dent*. 2019;22:129–132. doi: 10.4103/JCD.JCD_507_18
- [28] Gasner NS, Brizuela M. Endodontic Materials Used To Fill Root Canals. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Feb 1]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK587367/>
- [29] Cimpean SI, Burtea ALC, Chiorean RS, Dudescu MC, Antoniac A, Robu A, Campian RS, Timis LI. Evaluation of Bond Strength of Four Different Root Canal Sealers. *Mater*. 2022;15:4966. doi: 10.3390/ma15144966
- [30] Kulkarni D, Damiri F, Rojekar S, Zehravi M, Ramproshad S, Dhoke D, Musale S, Mulani AA, Modak P, Paradhi R, et al. Recent Advancements in Microneedle Technology for Multifaceted Biomedical Applications. *Pharm*. 2022;14:1097. doi: 10.3390/pharmaceutics14051097
- [31] Edanami N, Ibn Belal RS, Takenaka S, Yoshiba K, Yoshiba N, Ohkura N, et al. Apatite-Forming Ability of Flowable vs. Putty Formulations of Newly Developed Bioactive Glass-Containing Endodontic Cement. *App Sci*. 2021;11(19):8969.
- [32] Timis L, Gorea M, Har N, Cimpean S, Delean A, Bud MG, Campian RS. Biomaterialization Ability of an Experimental Bioceramic Endodontic Sealer Based on Nanoparticles of Calcium Silicates. *Med Pharm Rep*. 2023;96:434–440. doi: 10.15386/mpr-2660
- [33] López-García S, Myong-Hyun B, Lozano A, García-Bernal D, Forner L, Llena C, Guerrero-Gironés J, Murcia L, Rodríguez-Lozano FJ. Cytocompatibility, Bioactivity Potential, and Ion Release of Three Premixed Calcium Silicate-Based Sealers. *Clin Oral Investig*. 2020;24:1749–1759. doi: 10.1007/s00784-019-03036-2
- [34] Lim M, Jung C, Shin DH, Cho YB, Song M. Calcium Silicate-Based Root Canal Sealers: A Literature Review. *Restor Dent Endod* 2020;45:e35. doi: 10.5395/rde.2020.45.e35
- [35] Koutroulis A, Valen H, Ørstavik D, Kapralos V, Camilleri J, Sunde PT. Effect of Exposure Conditions on Chemical Properties of Materials for Surgical Endodontic Procedures. *Eur J Oral Sci*. 2023;131:e12943. doi: 10.1111/eos.12943
- [36] Cavalu S, Damian G. Rotational correlation times of 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-yloxy spin label with respect to heme and nonheme proteins. *Biomacromol*. 2003;4(6):1630–1635. doi: 10.1021/bm034093z
- [37] Nommeots-Nomm A, Hupa L, Rohanová D, Brauer DS. A Review of Acellular Immersion Tests on Bioactive Glasses-Influence of Medium on Ion Release and Apatite Formation. *Int J App Glass Sci*. 2020;11:537–551. doi: 10.1111/ijag.15006
- [38] Han L, Okiji T, Okawa S. Morphological and Chemical Analysis of Different Precipitates on Mineral Trioxide Aggregate Immersed in Different Fluids. *Dent Mater J*. 2010;29:512–7. doi: 10.4012/dmj.2009-133.
- [39] Tanomaru-Filho M, Prado MC, Torres FFE, Viapiana R, Pivoto-João MMB, Guerreiro-Tanomaru JM. Physicochemical Properties and Bioactive Potential of a New Epoxy Resin-Based Root Canal Sealer. *Braz Dent J*. 2019;30:563–568. doi: 10.1590/0103-6440201802861
- [40] Elsayed MA, Hassanien EE, Elgendy AAE. Ageing of TotalFill BC Sealer and MTA Fillapex in Simulated Body Fluid. *Eur Endod J*. 2021;6:183–188. doi: 10.14744/ej.2020.43043
- [41] Lu X, Leng Y. Theoretical Analysis of Calcium Phosphate Precipitation in Simulated Body Fluid. *Biomater*.

- 2005;26:1097–1108. doi: 10.1016/j.biomaterials.2004.05.034
- [42] Fadeeva IV, Goldberg MA, Preobrazhensky II, Mamin GV, Davidova GA, Agafonova NV, Fosca M, Russo F, Barinov SM, Cavalu S, Rau JV. Improved cytocompatibility and antibacterial properties of zinc-substituted brushite bone cement based on β -tricalcium phosphate. *J Mater Sci Mater Med*. 2021;32:99. doi: 10.1007/s10856-021-06575-x
- [43] De Moraes TG, de Menezes AS, Grazziotin-Soares R, Moraes RUME, Ferreira PVC, Carvalho CN, Bauer J, Carvalho EM. Impact of Immersion Media on Physical Properties and Bioactivity of Epoxy Resin-Based and Bioceramic Endodontic Sealers. *Polymers*. 2022;14:729. doi: 10.3390/polym14040729
- [44] Saber S, Galal MM, Ismail AG, Hamdy TM. Thermal, Chemical and Physical Analysis of VDW.1Seal, Fill Root ST, and ADseal Root Canal Sealers. *Sci Rep*. 2023;13:14829. doi: 10.1038/s41598-023-41798-8
- [45] Bakir EP, Yildirim ZS, Bakir Ş, Ketani A. Are Resin-Containing Pulp Capping Materials as Reliable as Traditional Ones in Terms of Local and Systemic Biological Effects? *Dent Mater J*. 2022;41:78–86. doi: 10.4012/dmj.2021-065
- [46] Abu Zeid ST, Alnoury A. Characterisation of the Bioactivity and the Solubility of a New Root Canal Sealer. *Int Dent J*. 2023. doi: 10.1016/j.identj.2023.04.003
- [47] Sanz JL, López-García S, Rodríguez-Lozano FJ, Melo M, Lozano A, Llena C, Forner L. Cytocompatibility and Bioactive Potential of AH Plus Bioceramic Sealer: An In Vitro Study. *Int Endod J*. 2022;55:1066–1080. doi: 10.1111/iej.13805
- [48] Bolanos DC, Komabayashi T, Shen I, Vega-Baudrit J, Gandolfi MG, Prati C, Montero-Aguilar M. Effects of heat on seven endodontic sealers. *J Oral Sci*. 2022;64(1):33–39. doi: 10.2334/josnurd.21-0178
- [49] Halgaš R, Dusza J, Kaiferová J, Kováčsová L, Markovská N. Nanoindentation Testing of Human Enamel and Dentin. *Ceramics – Silikáty*. 2013;57:92–99.
- [50] Kucher M, Dannemann M, Modler N, Bernhard MR, Hannig C, Weber M-T. Mapping of the Micro-Mechanical Properties of Human Root Dentin by Means of Microindentation. *Mater*. 2021;14:505. doi: 10.3390/ma14030505
- [51] Mokeem-Saleh A, Hammad M, Silikas N, Qualtrough A, Watts DC. A Laboratory Evaluation of the Physical and Mechanical Properties of Selected Root Canal Sealers. *Int Endod J*. 2010;43:882–888. doi: 10.1111/j.1365-2591.2010.01762.x
- [52] Cavalu S, Simon V, Goller G, Akin I. Bioactivity and Antimicrobial Properties of PMMA/Ag₂O acrylic bone cement collagen coated. *Dig J Nanomater Biostructures* 2011;61(2):779–790.

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