



# Biom mineralization ability of an experimental bioceramic endodontic sealer based on nanoparticles of calcium silicates

Lucia Timis<sup>1</sup>, Maria Gorea<sup>2</sup>, Nicolae Har<sup>3</sup>, Sanda Cimpean<sup>1</sup>, Ada Delean<sup>1</sup>, Marius Gheorghe Bud<sup>1</sup>, Radu Septimiu Campian<sup>4</sup>

1) Department of Conservative Odontology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Chemical Engineering, Babes-Bolyai University, Cluj-Napoca, Romania

3) Department of Geology, Babes-Bolyai University, Cluj-Napoca, Romania

4) Department of Oral Rehabilitation, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

## Abstract

**Background and aims.** The ultimate goal of endodontic therapy is to prevent periradicular disease or to promote the healing of the periradicular lesions. The use of nontoxic, biocompatible, and bioactive materials designed for root canal obturation is preferred due to their increased potential to induce healing and bone regeneration, thereby restoring the functionality of the tooth and the adjacent tissues. The aim of this study was to analyze the biom mineralization ability of an experimental endodontic sealer based on synthesized nanoparticles of calcium silicates.

**Methods.** Six plastic moulds were filled with the freshly prepared experimental endodontic sealer and kept for 3 days at room temperature in a moist environment. After hardening, four samples were subsequently immersed in simulated body fluid (SBF) and introduced in incubator at 37° C and 100% relative humidity; two of them were kept for 7 days and the other two for 14 days. Two samples were not immersed in SBF and were used for comparison. The biom mineralization potential was assessed by XRPD, SEM and EDS analysis.

**Results.** Following immersion in SBF, XRPD analysis identified apatite crystals for experimental material both after 7 and 14 days. SEM images displayed the specific microstructure for bioceramic materials alongside with the presence of apatite crystals on their surface. EDS identified the presence of phosphorus and calcium elements, underlining the biom mineralization potential of the experimental material.

**Conclusion.** Interaction between experimental material and SBF succeeded in inducing precipitation of apatite on its surface, evidenced by XRDP, SEM and EDS analysis.

**Keywords:** biom mineralization, nano calcium silicate, bioceramic endodontic sealer, SBF

DOI: 10.15386/mpr-2660

Manuscript received: 02.08.2023

Accepted: 29.08.2023

Address for correspondence:

Nicolae Har  
nicolae.har@ubbcluj.ro

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License <https://creativecommons.org/licenses/by-nc-nd/4.0/>

## Background and aims

The aim of the endodontic treatment is to completely remove the content of the root canal and to perform a three-dimensional obturation, without voids, in order to obtain a proper seal of the endodontic space. Root canal obturation is achieved by combining a fluid material called endodontic sealer with a core material, using different obturation techniques [1]. Endodontic sealers have been extensively investigated over time

and up to date, no endodontic sealer fulfills all the requirements formulated by Grossman; nevertheless the new class of biomaterials based on calcium silicates is promising [2,3].

Biomaterials are biocompatible materials used in many fields of medicine, including dentistry. They are constituted of inorganic compounds, being efficient in replacing human tissues or stimulating their regeneration [4,5].

Endodontic sealers alongside

core materials are placed in vicinity of the periapical tissues, therefore their biological properties are of great importance. Bioceramic sealers have proven to be superior to conventional ones, especially regarding biocompatibility and bioactivity [2,6].

Bioactivity is the property of a biomaterial to interact with the living tissue and to induce or modulate a biological activity, leading to healing [7]. Calcium silicate materials, when in contact with body fluids generate a layer of apatite on their surface, inducing hard tissue formation when adjoining bone or dental pulp [8,9]. When contiguous with radicular dentin, a mineral layer of apatite was observed at dentin-sealer interface, described as “mineral infiltration zone” [10,11]. The presence of this interfacial layer has a positive influence upon the adhesion of endodontic sealers to radicular dentin, improving their resistance to dislocation [12,13]. This activity is based on ion exchange between the bioactive material and body fluids leading to a dissolution-precipitation reaction [12,14].

Kokubo et al. demonstrated that bioactivity is reproducible in vitro when utilizing a fluid similar to human plasma regarding its ion composition and pH, opening new ways in investigating the bioactivity potential of a material without using animal models [15].

In the present study we intended to determine the biomineralization potential of an experimental bioceramic endodontic sealer, as a first step in investigating its bioactivity, after aging in simulated body fluid (SBF) for 7 and 14 days.

Experimental endodontic sealer (EES) was developed as a powder/liquid system. The powder consisted of calcium silicates, forsterite (magnesium silicates) and zirconium dioxide. The liquid used for the material preparation was zinc chloride solution, which also had the role of an accelerator, reducing the setting time.

Tricalcium silicate, the main component of EES was synthesized by a sol-gel method as described previously [16]. Forsterite was added as a filler and also to increase the material's mechanical resistance. Up to date, to our knowledge, there is no endodontic sealer containing forsterite. Its bioactive potential was described previously by Gorea et al [17]. Zirconium dioxide was used as a radio-opacifier.

## Methods

The samples for the biomineralization assessment were obtained using 10 mm diameter and 2 mm thickness plastic moulds which were filled with the freshly prepared endodontic sealers and kept for 3 days at room temperature in a moist environment. Six samples were prepared for the investigated material.

After hardening, the specimens were removed from the plastic moulds. Two samples were stored for comparison

while the other four were subsequently immersed in 10 ml of SBF, in individual plastic containers and introduced in an incubator at 37° C and 100% relative humidity. Two samples were removed from SBF solution after 7 days, and the remaining ones after 14 days. The superficial layer of all investigated samples was collected with a sharp blade, mashed into a fine powder and used for further analysis, in order to illustrate its mineralogical and punctual chemical composition.

### X-ray Powder Diffraction (XRPD)

In order to identify the mineral phases of each sample, XRPD investigations were performed using Bruker D8 Advance diffractometer (Bruker, Karlsruhe, Germany) with Cu-K $\alpha$  radiation ( $\lambda=1.541874$  Å), Fe 0,01 mm filter and a LynxEye one-dimensional detector. The working parameters were 40 kV and 40 mA. The data was collected in a 2 $\theta$  range, between 5° and 63° with a step interval of 0.02° and 0.2 s/step. The PDF-2 database was used to identify the mineral phases.

### Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

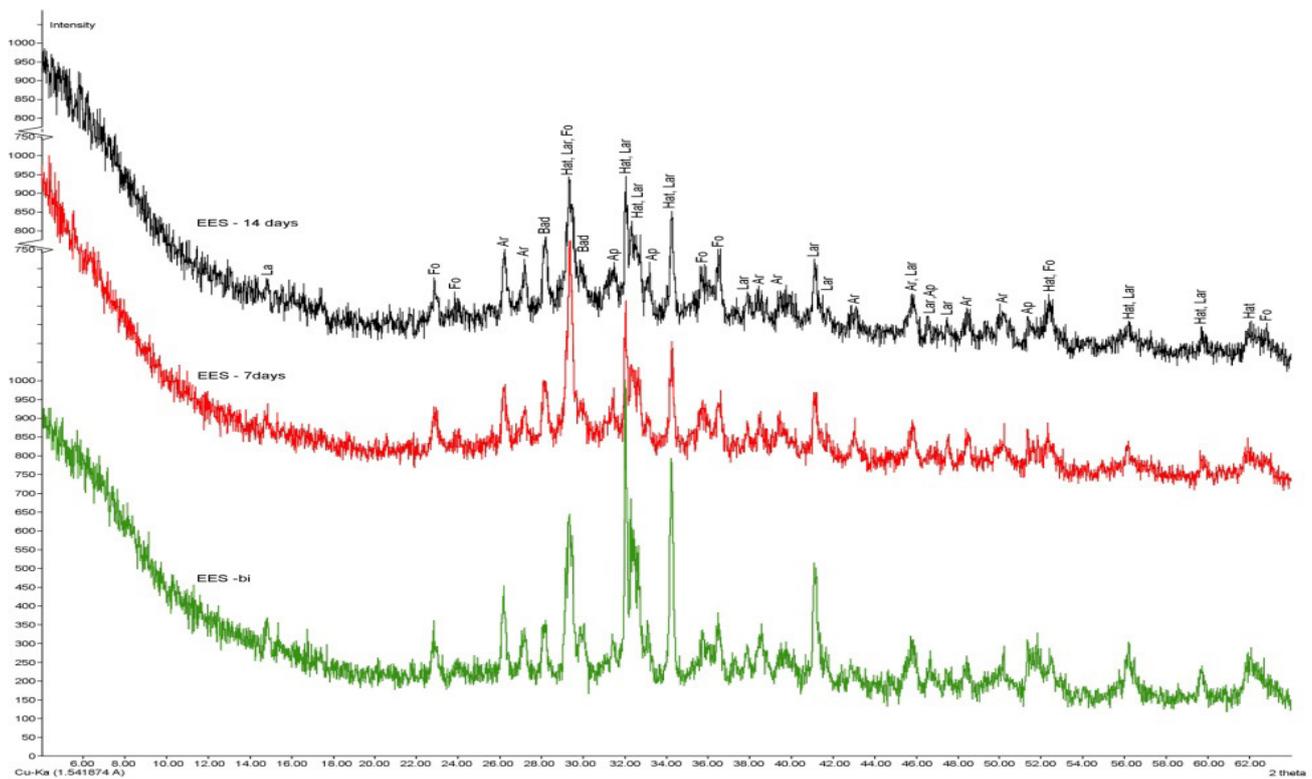
The powder samples were sprinkled on adhesive carbon tape mounted on aluminium stubs, and afterwards sputter-coated with platinum in a 10 nm coat using Agar Sputter Coater (Agar Scientific, Stansted, UK). High Resolution Scanning Electron Microscope (Hitachi – SU8230) equipped with EDS detector (AZtech, Oxford Instruments, High Wycombe, UK) with a resolution of 0.8 nm @ 15KV / 1.1 nm @ 1KV was used to investigate the specimens for (semi)quantitative chemical composition of the mineralogical phases.

## Results

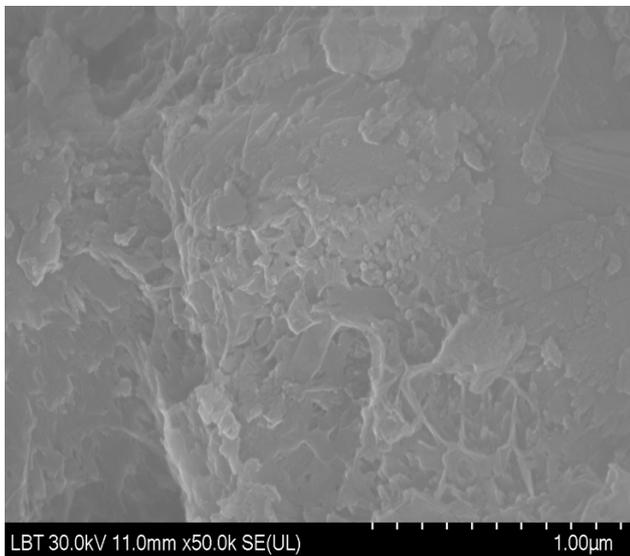
X-ray Powder Diffraction (XRPD) performed on the experimental endodontic sealer (EES) before immersion in SBF pointed out the presence of tricalcium silicate (hatrurite- Ca<sub>3</sub>SiO<sub>5</sub>), dicalcium silicate (larnite- Ca<sub>2</sub>SiO<sub>4</sub>), forsterite (Mg<sub>2</sub>SiO<sub>4</sub>), zirconium oxide (baddeleyite-ZrO<sub>2</sub>) as initial components, in addition to which calcium carbonate (aragonite- CaCO<sub>3</sub>) was present. After immersion in SBF, apatite (Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub> (CO<sub>3</sub>)<sub>0.75</sub> (OH)<sub>0.5</sub>), was also identified beside the enumerated crystals, both for 7 and 14 days samples (Figure 1).

Scanning Electron Microscopy (SEM) performed on EES before immersion in SBF revealed a homogeneous material corresponding to bioceramic hydraulic materials. It consisted of an amorphous matrix of calcium silicate hydrate gel (CSH), in which crystals in different stages of development, with a lamellar, round or irregular shape were observed (Figure 2).

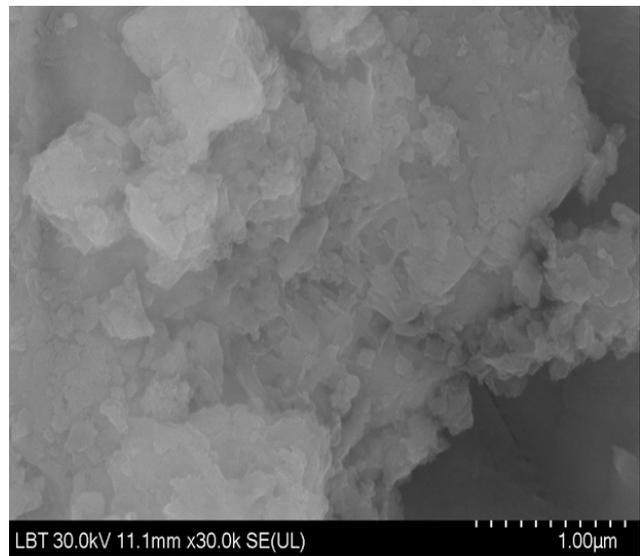
After 7 and 14 days of immersion in SBF, newly formed crystals with dimensions of up to 0.2 micrometres covered the material's surface (Figure 3).



**Figure 1.** X-ray pattern for experimental endodontic sealer (EES) before immersion in SBF (EES-bi) and after immersion in SBF for 7 and 14 days (EES– 7 days; EES – 14 days), with the specific peaks for tricalcium silicate (Hat), dicalcium silicate (Lar), forsterite (Fo), zirconia (Bad), calcium carbonate (Ar), and apatite (Ap).



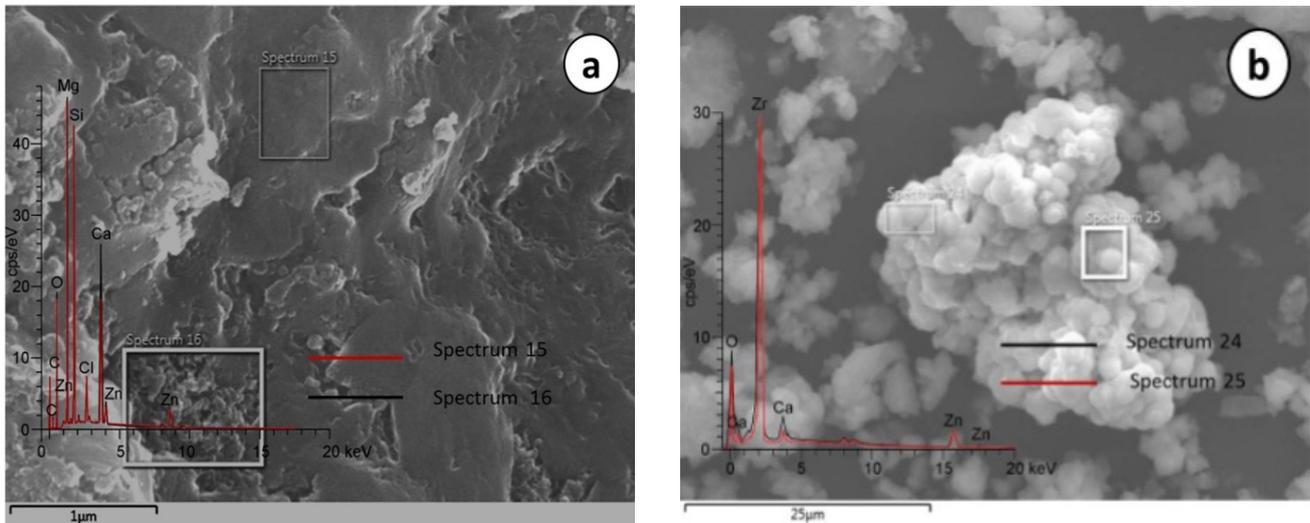
**Figure 2.** SEM image of experimental endodontic sealer (EES) before immersion in SBF.



**Figure 3.** SEM image of experimental endodontic sealer (EES) after immersion in SBF.

The (semi)quantitative **Energy Dispersive Spectroscopy (EDS)** spectra before immersion in SBF (Figure 4a), revealed the constitutional elements of the material: calcium (Ca=5.3-7.2 wt.%), silicon (Si=10.7–11.7 wt.%), magnesium (Mg=18.2-18.8 wt.%) and oxygen (O=42.4-43.5 wt.%) as main elements, suggesting that

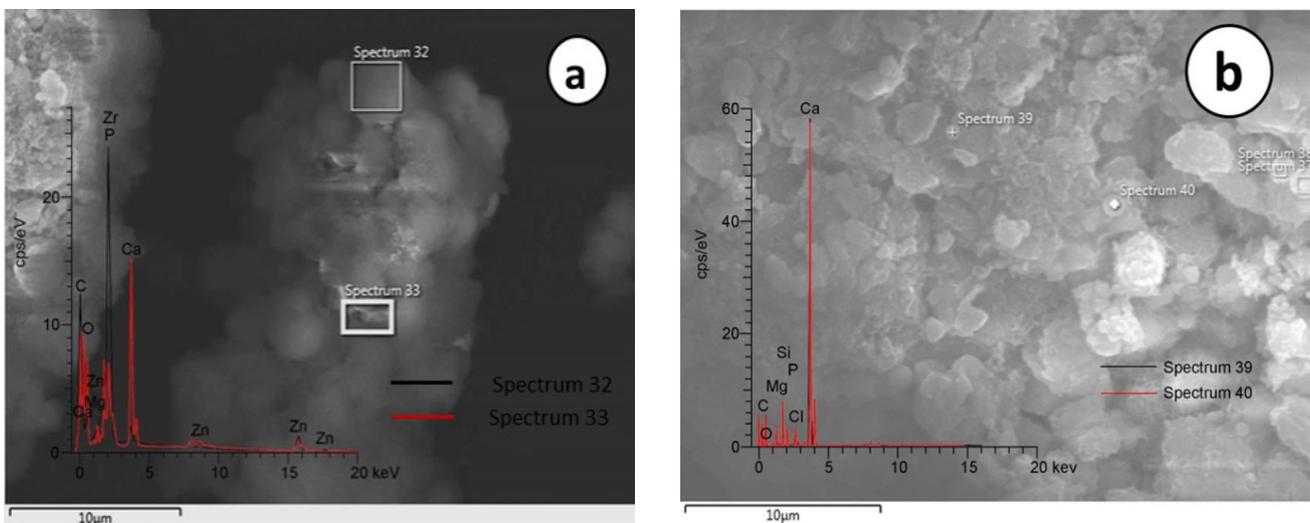
material consisted mainly of calcium silicate and forsterite. Zinc (Zn=2.3-2.8 wt.%) and chlorine (Cl =1.7 -2.0 wt.%) were subordinate elements, originated from 2% zinc chloride solution. Zirconia  $ZrO_2$  was observed as globular aggregates with diameters under  $1\ \mu\text{m}$ , with the participation of zirconium (Zr =56.1-56.3 wt.%) and oxygen (O=41.6-43.9 wt.%) (Figure 4b).



**Figure 4 a, b.** SEM images and EDS spectra of experimental endodontic sealer (EES) before immersion in SBF.

The EDS spectra collected on the experimental endodontic sealer after immersion in SBF for 7 and 14 days, indicated, besides the initial elements of the material calcium (Ca=4.7-28.3 wt.%), silicon (Si=1.8-2.8 wt.%), magnesium (Mg=1.5-2.6 wt.%), oxygen (O=41.2-52.6 wt.%), zinc (Zn=2.3-2.4 wt.%), chlorine (Cl=1.1-

1.0), zirconium (Zr=0.0-93.0 wt.%), also the presence of phosphorus (P) in different concentrations depending on the investigated areas (Figure 5 a, b). After 7 days phosphorus (P) was present in 0.6 wt.% (spectrum 33), while after 14 days it increased up to 0.8 wt.% and 0.9 wt.% (spectrum 39 and 40).



**Figure 5.** SEM images and EDS spectra of experimental endodontic sealer (EES) after immersion in SBF for 7 days (a) and 14 days (b).

## Discussion

Various methods are available for investigating the biomineralization and bioactivity potential of endodontic sealers, both *in vivo* and *in vitro* [18]. Biomineralization potential of a material can be evidenced *in vitro*, by the apatite crystals formed after immersion in simulated body fluid(s). Kokubo et al. demonstrated a strong quantitative correlation between *in vitro* apatite formation and *in vivo* bone bioactivity [19]. Consequently, it was speculated that materials that are able to induce apatite on their surface would manage to bond to bone tissue [20].

Calcium silicates were proven to be superior to other materials in generating apatite, due to calcium ( $\text{Ca}^{2+}$ ) and silicon ( $\text{Si}^{4+}$ ) ions released by the material.  $\text{Si}^{4+}$  ions are able to enhance the proliferation and osteogenic differentiation of different stem cells, including periodontal ligament cells and dental pulp cells [21], while  $\text{Ca}^{2+}$  ions are known to act as extracellular signals for mineralizing cells [22], being able to convert the pluripotent-mesenchymal cells into osteoblast or chondroblasts [23].

Instead of using MTA or Portland cement as a source for calcium silicates, synthesized nano tricalcium silicate ( $\text{Ca}_3\text{SiO}_5$ ) is preferred, since this method allows obtaining a powder with increased purity and smaller sized particles [24]. Having this in mind, we have chosen to synthesize the nano-powder of  $\text{Ca}_3\text{SiO}_5$ , by a sol-gel method especially for developing the experimental endodontic sealer.

The novelty of EES was the incorporation of forsterite ( $\text{Mg}_2\text{SiO}_4$ ) into its formula. Its biomineralization potential has been proven previously; when in contact with simulated body fluid, Mg can be replaced by Ca and Si replaced by P respectively, generating apatite over time [17]. Moreover, *in vitro* studies concluded that  $\text{Mg}^{2+}$  ions enhance the proliferation and differentiation of human dental pulp stem cells (hDPSCs) [25].

In the present study, the biomineralization potential of EES was evaluated. Besides SEM and EDS analysis, XRPD was used as an additional method of investigation, for a more accurate characterization of the mineralogical composition [26].

EES's mineral phase evidenced by X-ray powder diffraction (XRPD) was composed of tricalcium silicate ( $\text{Ca}_3\text{SiO}_5$ ), dicalcium silicate ( $\text{Ca}_2\text{SiO}_4$ ), forsterite ( $\text{Mg}_2\text{SiO}_4$ ), and zirconium dioxide ( $\text{ZrO}_2$ ) (Figure 1). Beside these crystals, which were part of the material's initial composition, calcium carbonate ( $\text{CaCO}_3$ ) was also identified, both before and after immersion in SBF, being formed as a result of the chemical reaction between calcium hydroxide and carbon dioxide from the air or water. Its presence is favorable, as it was previously proven to exhibit a good biocompatibility [27].

SEM analysis of hydrated material, before immersion in SBF (Figure 2) revealed a homogeneous surface containing an amorphous phase of calcium silicate hydrate (CSH) and multiple crystals, in different stages of

development.

Setting reaction of calcium silicates is a complex process that occurs when the powder is in contact with water. Hydrolysis of  $\text{SiO}_4^{4-}$  groups from calcium silicate generates amorphous nano-porous calcium silicate hydrate (CSH) gel, which in time will harden by crystallization [28]. The hydration process also leads to formation of calcium hydroxide (portlandite  $\text{Ca}(\text{OH})_2$ ), as a result of the reaction between calcium ions ( $\text{Ca}^{2+}$ ) released by the material and hydroxyl ions ( $\text{OH}^-$ ) derived from water. Newly formed  $\text{Ca}(\text{OH})_2$  creates an alkaline environment which promotes the subsequent reactions [29].

As expected, after EES's immersion in SBF, apatite was evidenced by XRPD as a crystalline compound, starting with day 7 (Figure 1). Apatite was generated due to interaction between  $\text{Ca}^{2+}$  present on the surface of hardened material and phosphate ions ( $\text{HPO}_4^{2-}$ ) from SBF solution [28].

This chemical reaction continues as long as  $\text{Ca}^{2+}$  and  $\text{HPO}_4^{2-}$  ions are available. Release of  $\text{Ca}^{2+}$  ions has been demonstrated to be increased and prolonged for calcium silicate materials [30] also playing an important role in mineralization and hard tissue formation [31].

The presence of  $\text{Ca}_3\text{SiO}_5$  and  $\text{Ca}_2\text{SiO}_4$  in EES samples, both before and after immersion in SBF indicated that calcium silicates were only partially hydrated, probably due to the reduced amount of time available for this process. Their hydration had not occurred completely when investigated, being known that a complete hydration of calcium silicate based endodontic sealers may require a longer time [32].

SEM analysis performed on EES after immersion in SBF for 7 and 14 days revealed the specific surface aspect of apatite (Figure 3). EDS analysis (Figure 4 a,b), illustrated the participation of phosphorus (P) alongside calcium (Ca), with an increased concentration after 14 days ( $\text{P}=0.9$  wt.%), compared to 7 days ( $\text{P}=0.6$  wt.%). Due to the affinity of  $\text{Ca}^{2+}$  ions for  $\text{HPO}_4^{2-}$  ions, the presence of phosphorus in the analyzed powder was an indicator that apatite were formed and precipitated on material's surface after immersion in SBF for both 7 and 14 days (Figure 5 a,b). *In vivo*, this biomineralization activity increases the chemical adhesion of bioceramic materials to root canal dentine, improving their resistance to dislocation [33].

Apatite forming ability and intratubular biomineralization was previously reported for calcium silicate based endodontic sealers [34,35]. Siboni et al investigated the release of calcium ions for different materials soaked in simulated body fluid for 28 days and observed that leakage of calcium ions was significantly higher for calcium silicate based endodontic sealers compared to epoxy resin based endodontic sealers [36]. Similar results were observed by Candeiro et al [37]. Release of calcium ions play an important role in hard tissue deposition and mineralization, thus the increased

bioactivity and biocompatibility for calcium silicate endodontic sealers [38].

Results of the current study indicated the presence of apatite after 7 and 14 days of immersion in SBF for EES. As expected, there was a correlation between the presence of apatite crystals identified by XRPD analysis and the presence of phosphorus evidenced at EDS analysis.

Phosphorus was not part of the material's initial composition for EES, but it was present in the SBF used as the immersion solution. Therefore, the presence of phosphorus on the material's surface occurred due to its participation in the generation of apatite.

### Conclusion

Within the limitations of the current study, we were able to determine the biomineralization potential of EES, as a first step in investigating its bioactivity. The preliminary results obtained in the present study by XRPD, SEM and EDS analysis demonstrated that interaction between experimental material and SBF succeeded to induce precipitation of apatite on its surface. Incorporation of forsterite in the EES' formula did not alter its biomineralization activity and can be considered a suitable filler for increasing its mechanical resistance.

Calcium silicate based endodontic sealers are a good alternative to conventional sealers in terms of biomineralization and bioactivity, taking into consideration their potential interaction with viable human cells in/from the periapical area.

### Acknowledgments

This work was supported by PhD Research Project no. PCD 1033/66/13.01.2021, granted by Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania.

### References

- Ørstavik DA. Materials used for root canal obturation: technical, biological and clinical testing. *Endodontic Topics*. 2005;12:25-38. Doi: 10.1111/j.1601-1546.2005.00197.x
- Silva Almeida LH, Moraes RR, Morgental RD, Pappen FG. Are Premixed Calcium Silicate-based Endodontic Sealers Comparable to Conventional Materials? A Systematic Review of In Vitro Studies. *J Endod*. 2017;43:527-535.
- Lee JK, Kwak SW, Ha JH, Lee W, Kim HC. Physicochemical Properties of Epoxy Resin-Based and Bioceramic-Based Root Canal Sealers. *Bioinorg Chem Appl*. 2017;2017:2582849.
- Williams DF. On the mechanisms of biocompatibility. *Biomaterials*. 2008;29:2941-2953.
- Raghavendra SS, Jadhav GR, Gathani KM, Kotadia P. Bioceramics in endodontics - a review. *J Istanbul Univ Fac Dent*. 2017;51(3 Suppl 1):S128-S137.
- Jitaru S, Hodisan I, Timis L, Lucian A, Bud M. The use of bioceramics in endodontics - literature review. *Clujul Med*. 2016;89:470-473.
- Williams DF. Biocompatibility pathways and mechanisms for bioactive materials: The bioactivity zone. *Bioact Mater*. 2021;10:306-322.
- 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.
- Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. *Int Endod J*. 2018;51:177-205.
- 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.
- Al-Haddad A, Che Ab Aziz ZA. Bioceramic-Based Root Canal Sealers: A Review. *Int J Biomater*. 2016;2016:9753210.
- Sfeir G, Zogheib C, Patel S, Giraud T, Nagendrababu V, Bukiet F. Calcium Silicate-Based Root Canal Sealers: A Narrative Review and Clinical Perspectives. *Materials (Basel)*. 2021;14:3965.
- Lin GSS, Ghani NRNA, Noorani TY, Ismail NH, Mamat N. Dislodgement resistance and adhesive pattern of different endodontic sealers to dentine wall after artificial ageing: an in-vitro study. *Odontology*. 2021;109:149-156.
- Watson TF, Atmeh AR, Sajini S, Cook RJ, Festy F. Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: biophotonics-based interfacial analyses in health and disease. *Dent Mater*. 2014;30:50-61.
- Kokubo T, Kushitani H, Sakka S, Kitsugi T, Yamamuro T. Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W. *J Biomed Mater Res*. 1990;24:721-734.
- Timiș L, Avram A, Gorea M, Bizo L, Cîmpean S, Câmpian RS. Synthesis and characterization of nano biotri-calcium silicate, as a component of an endodontic sealer. *Studia UBB Chemia*. 2020; LXV:157-169.
- Gorea M, Naghiu MA, Avram A, Petean I, Mocanu A, Tomoaia-Cotisel M. Novel Porous Forsterite Ceramics Biocompatibility and Bioactivity Evaluation. *Rev Chim*. 2020;71:343-351.
- Camilleri J, Atmeh A, Li X, Meschi N. Present status and future directions: Hydraulic materials for endodontic use. *Int Endod J*. 2022;55 Suppl 3(Suppl 3):710-777. [Erratum in: *Int Endod J*. 2023;56:402].
- Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials*. 2006;27:2907-2915.
- Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N, Nakamura T, et al. Apatite formation induced by Silica Gel in a simulated body fluid. *J Am Ceram Soc*. 1992;75:2094-2097. Doi: 10.1111/j.1151-2916.1992.tb04470.x
- Han P, Wu C, Xiao Y. The effect of silicate ions on proliferation, osteogenic differentiation and cell signalling pathways (WNT and SHH) of bone marrow stromal cells. *Biomater Sci*. 2013;1:379-392.
- Zamparini F, Prati C, Taddei P, Spinelli A, Di Foggia M,

- Gandolfi MG. Chemical-Physical Properties and Bioactivity of New Premixed Calcium Silicate-Bioceramic Root Canal Sealers. *Int J Mol Sci.* 2022;23:13914.
23. Matsumoto S, Hayashi M, Suzuki Y, Suzuki N, Maeno M, Ogiso B. Calcium ions released from mineral trioxide aggregate convert the differentiation pathway of C2C12 cells into osteoblast lineage. *J Endod.* 2013;39:68-75.
  24. Camilleri J. Is Mineral Trioxide Aggregate a Bioceramic? *Odovtos – Int J Dent Sci.* 2016;18:13-17.
  25. Mommer A, Tabatabaei F, Tayebi L, Vahabzadeh S. Role of magnesium on phase composition of tricalcium phosphate and its interaction with human dental pulp stem cells. *J Mater Res.* 2023;38:228–36. Doi:10.1557/s43578-022-00851-4
  26. Badawy RE, Mohamed DA. Evaluation of new bioceramic endodontic sealers: An in vitro study. *Dent Med Probl.* 2022;59:85-92.
  27. Zhao P, Tian Y, You J, Hu X, Liu Y. Recent Advances of Calcium Carbonate Nanoparticles for Biomedical Applications. *Bioengineering (Basel).* 2022;9:691.
  28. Niu LN, Jiao K, Wang TD, Zhang W, Camilleri J, Bergeron BE, et al. A review of the bioactivity of hydraulic calcium silicate cements. *J Dent.* 2014;42:517-533.
  29. Chaudhari PS, Chandak MG, Jaiswal AA, Mankar NP, Paul P. A Breakthrough in the Era of Calcium Silicate-Based Cements: A Critical Review. *Cureus.* 2022;14:e28562.
  30. Zamparini F, Siboni F, Prati C, Taddei P, Gandolfi MG. Properties of calcium silicate-monobasic calcium phosphate materials for endodontics containing tantalum pentoxide and zirconium oxide. *Clin Oral Investig.* 2019;23:445-457.
  31. Seo DG, Lee D, Kim YM, Song D, Kim SY. Biocompatibility and Mineralization Activity of Three Calcium Silicate-Based Root Canal Sealers Compared to Conventional Resin-Based Sealer in Human Dental Pulp Stem Cells. *Materials (Basel).* 2019;12:2482.
  32. Donnermeyer D, Bürklein S, Dammaschke T, Schäfer E. Endodontic sealers based on calcium silicates: a systematic review. *Odontology.* 2019;107:421-436.
  33. Kakoura F, Pantelidou O. retreatment efficacy of endodontic bioceramic sealers: a review of the literature. *Odovtos - Int J Dent Sci.* 2018;20:39-50.
  34. Kim Y, Kim BS, Kim YM, Lee D, Kim SY. The Penetration Ability of Calcium Silicate Root Canal Sealers into Dentinal Tubules Compared to Conventional Resin-Based Sealer: A Confocal Laser Scanning Microscopy Study. *Materials (Basel).* 2019;12:531.
  35. Chisnoiu R, Moldovan M, Chisnoiu A, Hrab D, Rotaru D, Păstrav O, et al. Comparative apical sealing evaluation of two bioceramic endodontic sealers. *Med Pharm Rep.* 2019;92(Suppl No 3):S55-S60.
  36. Siboni F, Taddei P, Zamparini F, Prati C, Gandolfi MG. Properties of BioRoot RCS, a tricalcium silicate endodontic sealer modified with povidone and polycarboxylate. *Int Endod J.* 2017;50 Suppl 2:e120-e136.
  37. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. *J Endod.* 2012;38:842-845.
  38. Viapiana R, Guerreiro-Tanomaru JM, Hungaro-Duarte MA, Tanomaru-Filho M, Camilleri J. Chemical characterization and bioactivity of epoxy resin and Portland cement-based sealers with niobium and zirconium oxide radiopacifiers. *Dent Mater.* 2014;30:1005-1020.