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Original Research Article

Characterization of chemical elements of calcium silicate-based cements

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Abstract

Objective: The aim of this study was to characterize the chemical elements and surface of the cements: Biodentine®, Bio-C Sealer®, Endosequence® BC Sealer, MTA Angelus® and Portland Cement (PC). **Material and methods:** The cements were manipulated according to manufacturer's recommendations, inserted in polyethylene tubes and kept in a 95% humidity incubator for 72 hours. The analysis of the chemical elements was performed by energy dispersive X-ray spectroscopy (EDS) coupled to a low vacuum Scanning Electron Microscope (SEM). The surface analysis was performed according to the distribution pattern, shape and particle size in images obtained using Field Emission Gun SEM (SEM-FEG). Data were analyzed descriptively for EDS chemical composition spectra and qualitatively for SEM-FEG images. **Results:** EDS analysis showed that all evaluated cements contained calcium and silicon rich particles. MTA Angelus® presented bismuth-rich particles, while Biodentine®, Bio-C Sealer® and Endosequence® presented zirconium-rich particles, the latter having tantalum in its composition. All materials showed a uniform distribution pattern of particles on the surface. Biodentine® and Endosequence® showed similarly shaped particles. **Conclusion:** The cements present particles with uniform distribution pattern and similar chemical composition, differentiating the presence of bismuth or zirconium.

Introduction

In last decade calcium silicate-based cements, also known as bioceramics, have been introduced into endodontics, consisting of ceramic materials developed especially for biomedical applications, resulting from the combination of calcium silicates and calcium phosphates, which may include alumina particles, zirconia, bioactive glasses, glass ceramics and hydroxyapatite [15, 29, 40].

The properties of calcium silicate-based cements have been widely evaluated, including good sealing ability, tissue tolerance, setting expansion, high pH, easy manipulation and chemical stability in biological environment [1, 13, 29, 33-35, 44, 47]. These materials have been considered bioactive due to their ability to interact with surrounding tissues and promote osteogenesis [24]. Also, from the hydration of the material during the setting process, the formation of hydroxyapatite crystals between material surface and dentin wall has been reported, which allow adequate sealing and better marginal adaptation [34, 41]. Calcium silicate-based endodontic cements are presented with indication for both root canal filling and as repair material [41].

MTA (mineral trioxide aggregate) consists of hydrophilic particles based on tricalcium silicate, dicalcium silicate, tricalcium aluminate and calcium oxide [9]. It is a repair endodontic cement in the form of white or gray powder and considered bioactive due to its sealing ability, tissue tolerance and the ability to stimulate mineralization [38]. Currently, it has been indicated in several clinical situations, such as sealing of perforations and resorption, retrograde filling in apical surgeries, pulp capping, pulpotomy, apexification and in pulp revascularization processes [4, 7, 10, 33]. However, MTA has limitations such as possibility of dental discoloration [30], long setting time and difficulty in handling [7, 43].

Chemical composition of MTA presented similarity of composition with Portland cement (PC), having as difference the presence of bismuth in MTA, which confers radiopacity to the material [21]. MTA and PC are composed of tricalcium and dicalcium silicate, and when hydrated produce hydrous calcium silicate gel and calcium hydroxide [9].

Biodentine® (Septodont, Saint-Maur-des-Fosses, France) is presented as a powder in a capsule that must be added to the liquid supplied by the manufacturer and is indicated as a substitute for dentin and endodontic clinical applications similar to MTA [11, 46]. This material has ability to induce pulp cell differentiation and *in vitro* biomineralization [25]. Endosequence® (Brasseler, Savannah, GA, USA) and Bio-C Sealer® (Angelus, Londrina, PR, Brazil) are indicated for root canal obturation and presented in syringes in form of ready-to-use premixed pastes. A systematic review [41] compared the physicochemical and biological properties of premixed bioceramic endodontic cements with conventional endodontic cements. From *in vitro* and *in vivo* studies in animals included, it may be concluded that bioceramic cements had good physicochemical and biological properties and were similar or better than conventional cements. However, they highlighted the lack of long-term and well-conducted clinical trials evaluating these materials.

The characterization of chemical elements is extremely relevant since they can be directly related to the cytotoxicity of the material and affect the biocompatibility, as well as the physical structure and surface, which can influence cell adhesion and propagation. The size and shape of the cement particles influence the surface area, the potential of the reactivity of the particles with human tissues and, consequently, the biological quality of the material [28, 31]. The similarity of particle size and shape may further influence the mechanical strength of the material [16].

New calcium silicate-based cements have been developed, and although *in vitro* and *in vivo* animal studies have reported good properties of these materials, there is a lack of research to evaluate their chemical elements and surface characteristics, particularly comparing them to precursor cements, such as PC and MTA. Given the above, the aim of the present study was to evaluate the chemical elements and surface characteristics of calcium silicate-based cements and compare them to MTA and Portland Cement.

Material and methods

For sample preparation, 10 standardized polyethylene tubes (Levine Probe 12, Embramed, Jurubatuba, SP, Brazil) with 3 mm internal diameter and 3 mm height were prepared using a 0.01 mm resolution digital caliper (Mitutoyo MTI Corporation, Tokyo, Japan) and a n.11 scalpel blade (Swann Morton, Sheffield, United Kingdom).

In this study the following cements were applied: Biodentine® (Septodont, Saint-Maur-des-Fosses, France); Bio-C Sealer® (Angelus, Londrina, PR, Brazil); Endosequence® BC Sealer (Brasseler, Savannah, GA, USA); MTA Angelus® (Angelus, Londrina, PR, Brazil) and Portland Cement (Cia. Itaú Cement, Itaú de Minas, MG, Brazil).

The materials were manipulated according to manufacturer's recommendations, inserted inside the polyethylene tubes (n=2 for each material) placed on a glass plate and transferred to an incubator (ECB 1.3, OdontoBrás, Ribeirão Preto, SP, Brazil) at a temperature of 37°C with a relative humidity of 95%, where it remains for 72 hours.

Chemical element analyzes were performed by Energy Dispersive Spectroscopy (EDS) with an X-ray detector (Oxford Instruments X-MaxN) coupled with a Scanning Electron Microscope (SEM) (JEOL JSM – IT300, Tokyo, Japan). One sample of each cement was fixed in an aluminum sample holder and the upper surface metallized with carbon. The SEM was operated at low vacuum and electron acceleration voltage of 20 kV, where the back scattered electrons were analyzed. Cement surface images were evaluated at 500x magnification, and then, according to the particle size of each cement, a specific magnification for EDS analysis was selected, ranging from 500x to 5000x. In the central surface area of each sample were selected points with differences in contrast and surface appearance between the particles, representative of distinct chemical compositions. Then the points were numbered, and their spectra presented as graphs.

Analysis of the surface characteristics of the cements was performed using images obtained with a Field Emission Gun Scanning Electron Microscope SEM-FEG (JEOL JSM-7100F, Tokyo, Japan). One sample of each cement was fixed in an aluminum sample holder, gold-plated on the upper surface and transferred under a microscope.

Each sample was evaluated in the central area by images with increasing magnifications of 25x, 500x and 5000x, in high vacuum and secondary electrons detection with a maximum voltage of 3 kV. The surface characteristics of the samples were evaluated by an endodontist examiner according to the following criteria: uniformity of particle distribution (characterization or not of a distribution pattern); shape (similarity or otherwise between particle shapes) and size (similarity or otherwise between particle size). Images with 5000x magnifications were analyzed.

Results

The images obtained by SEM in EDS analysis of each cement with the representative spectra of selected points are shown in figure 1. MTA Angelus®, Biodentine®, Endosequence® and Bio-C Sealer® showed particles with different contrasts and appearance in the low vacuum SEM images. Portland Cement had particles with different surface aspects and similar contrasts. MTA Angelus® showed bismuth rich particles dispersed between calcium and silicon rich particles. In addition to these elements, the presence of oxygen, aluminum and magnesium was detected (figure 1A). PC presented particles with different superficial aspects, in which a predominant presence of calcium, silicon and oxygen were identified. Other elements identified in PC were sulfur, potassium, aluminum, magnesium, sodium and iron (figure 1B). Biodentine® and Bio-C Sealer® showed calcium and zirconium rich particles dispersed between calcium, silicon and oxygen rich particles. In addition to these elements, the presence of aluminum was detected in both cements (figures 1C and 1E). Endosequence® showed calcium, tantalum and silicon rich particles dispersed among calcium, silicon and zirconium rich particles. Oxygen and sulfur were also identified (figure 1D).

Images obtained from each cement in the SEM-FEG, used for surface characterization, are shown in figure 2. All evaluated cements showed a uniform distribution pattern of the particles on the surface. MTA Angelus® and PC exhibited surface containing particles of similar shapes and sizes. Biodentine® presented surface containing round shaped particles of varying sizes, while Endosequence® showed particles of similar size and shape dispersed in a dense matrix. Bio-C Sealer® presented particles with varying shapes, some being rounded and others in needles with varying sizes.

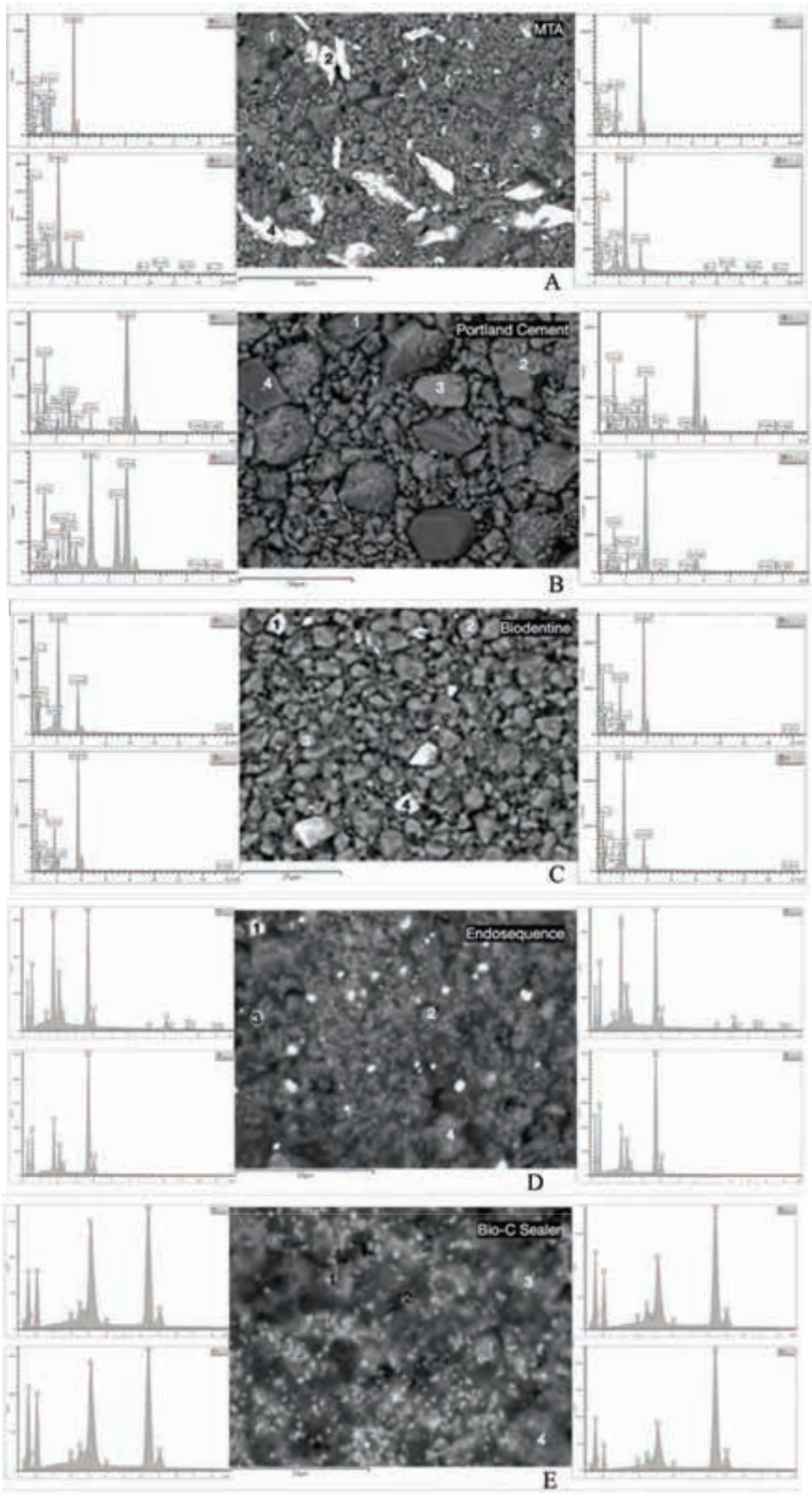

Figure 1 – Backscattered scanning electron micrographs and EDS analysis from MTA Angelus (A), Portland Cement (B), Biodentine (C), Endosequence (D) and Bio-C Sealer (E) with the identified points and their respective spectra

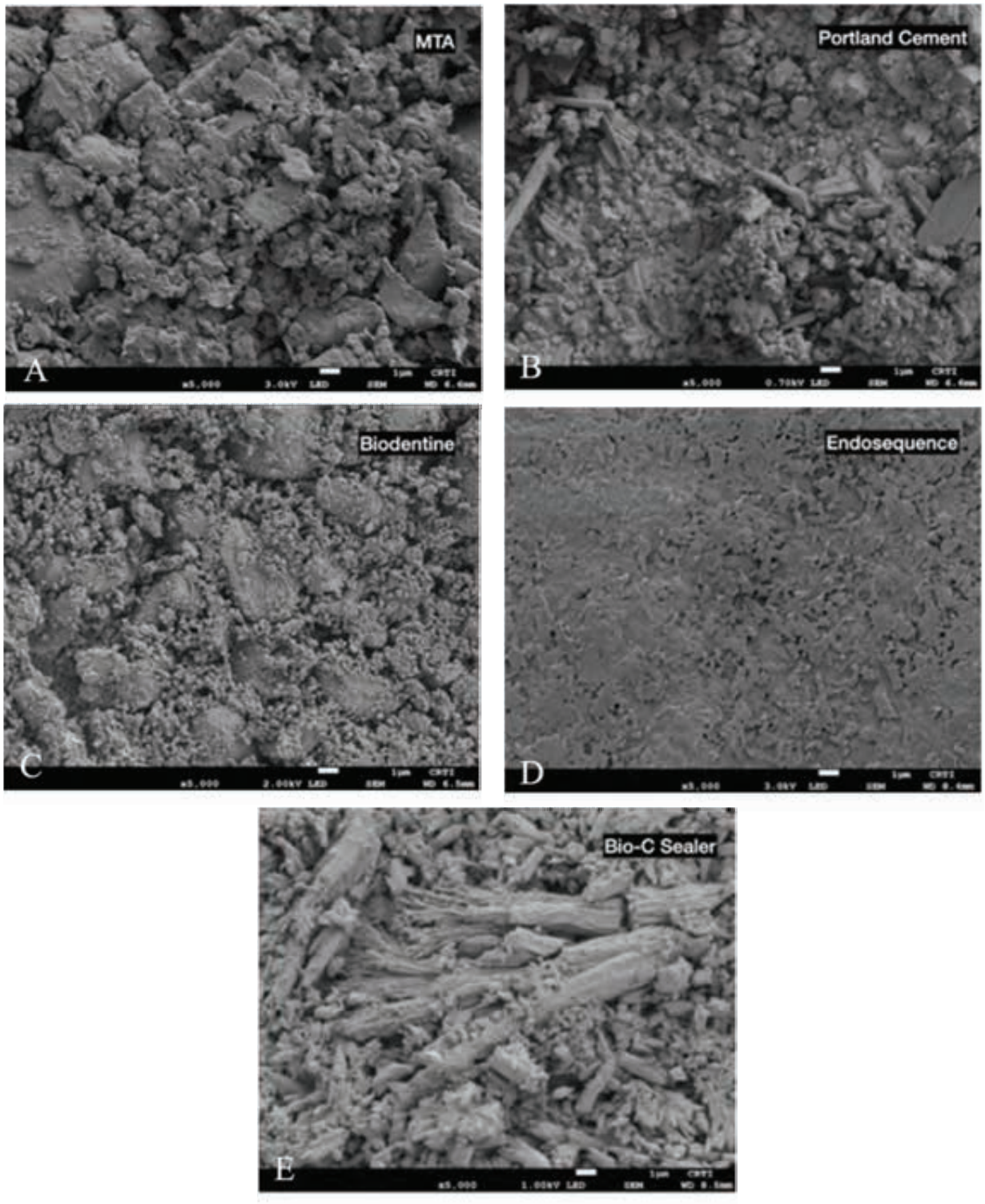

Figure 2 – SEM-FEG secondary electron capture images of each material. MTA Angelus (A); Portland Cement (B); Biodentine (C); Endosequence (D); Bio-C Sealer (E)

Discussion

Knowledge of the chemical elements and surface characteristics of cements that maintain close contact with the periapical tissue is a predictive factor of the physicochemical and biological properties [16]. Characterization of the chemical elements of endodontic materials has been performed by X-ray energy dispersive spectroscopy (EDS) [9, 11, 28]. This method allows chemical information to be obtained in micrometer order areas from a microanalysis equipment coupled to the SEM. Information about the elements present is obtained by capturing the characteristic x-rays resulting from the interaction of the primary electron beam with the sample. This technique can detect the presence of chemical elements in solid materials, especially those of high molecular weight [37].

The main differences observed in the chemical composition of the evaluated cements were in the presence of bismuth in MTA Angelus® and zirconium in Biodentine®, Bio-C Sealer® and Endosequence®. Elements with high atomic numbers, such as zirconium and bismuth, identify radiopaque characteristics, while elements with low atomic numbers, such as silicon, result in radiolucent materials, and there is therefore a correlation between the atomic number of the element and its radiopacity [39]. Bismuth oxide and zirconium oxide have been incorporated into the materials as radiopacifying agents [8, 11, 12, 20, 41]. Camilleri *et al.* [11] reported that zirconia (ZrO_2) has advantages over bismuth because it is inert and does not affect the physical properties of the material.

The chemical elements identified in Biodentine® in the present study were similar to those of Camilleri *et al.* [11] through EDS. However, in this same study, by X-ray fluorescence spectroscopy the authors also detected the presence of iron in this material. This difference can be explained by the method, since among the limitations of EDS are the impossibility of distinguishing between the phases in which the elements are present, as well as the detection of low atomic number elements [37].

The elements detected in Endosequence® were similar to those found by Xuereb *et al.* [45] using the same method, which also identified particles rich in zirconium, calcium and silicon. However, there was a divergence regarding the presence of tantalum detected in the present study, which was not identified in the previous investigation. This element is used in the medical and dental area for its biocompatibility, because its chemical inertia allows it to not corrode in the body fluids and not cause tissue damage [5, 42].

The chemical elements identified in MTA Angelus® in the present study are in agreement with findings from previous investigations that used EDS [6, 11, 28, 36]. Estrela *et al.* [21] used X-ray fluorescence spectroscopy as chemical characterization method and also identified the presence of iron and strontium in MTA (ProRoot®, Dentsply) and strontium, titanium and manganese in PC.

In the present study, presence of magnesium in MTA cement was identified. Camilleri *et al.* [11] report that the presence of magnesium in the MTA detected by EDS indicates the use of natural limestone as a source of calcium carbonate for clinker manufacture, instead of pure calcium carbonate obtained by precipitation.

MTA and PC, as well as all calcium silicate-based cements, are considered hydraulic cements as they require moisture for setting reaction. PC is made from the grinding of a product called clinker, obtained by burning a mixture of limestone and clay and composed mainly of calcium oxide, silicon oxide, aluminum oxide, iron oxide and magnesium oxide [28, 19]. After grinding, calcium sulfate is added to accelerate the setting reaction of the material. The presence of sodium and potassium in PC may be related to common clinker impurities of this material from the raw materials used in its manufacturing process [11].

Despite the evidence that arsenic is a common limestone impurity used as a raw material in the manufacture of PC [18], Duarte *et al.* [19] observed that the release of this substance by PC is minimal, and state that there is no contraindication to the clinical use of PC due to the release of this element. These data were later confirmed by De-Deus *et al.* [17].

In this study the surface evaluation of the materials was performed, and it was observed that all cements showed uniform distribution of the particles on the surface. Biodentine® presented results similar to those of Camilleri *et al.* [11] and Han and Okiji [25], who also observed rounded particles of different sizes. Endosequence® presented small particles dispersed in a dense matrix, a result similar to Xuereb *et al.* [45], who did not observe porosity on the material surface.

MTA Angelus® and PC presented particles with varied shapes and sizes, which are in agreement with Borges *et al.* [6] and Camilleri *et al.* [11], who after analyzing the surface of the MTA by SEM, observed particles with round shapes and different dimensions, as well as needle-shaped crystals. However, these results are inconsistent with the study by Hwang *et al.* [28] regarding the

MTA, which showed uniform particle size, and concordant regarding the PC, which presented particles with a wide range of sizes. These different results regarding the MTA may be justified by the difference of the trademark, because the authors evaluated Pro Root® MTA (Dentsply, Tulsa, OK) and also by subjectivity during image analysis.

Surface characterization of dental materials has been carried out by SEM [23], whose principle is the emission of an electron beam by a tungsten filament which focuses on the sample, causing a series of signal emissions. These signals are emitted as secondary electrons, backscattered electrons and X-rays, which are captured by appropriate detectors, amplified and processed in a specific analyzer system for each type of signal. It is therefore possible, from obtaining a magnified and three-dimensional image of the sample surface, to perform surface analysis [37].

Uniform particle distribution and similarity of size and shape influence surface area and contribute to the biological response of the material [28, 31]. Currently, there is an understanding that being only an inert material when placed in contact with living tissue is not enough, it is expected that it will be able to induce processes that culminate in tissue regeneration or replacement of the injured tooth structure [21].

The hydration of tricalcium silicate, a basic component of PC and its derivatives, including bioceramic cements, produces hydrous calcium silicate gel and calcium hydroxide [11, 45]. Calcium hydroxide produced in the reaction is responsible for the bioactivity of the material [45]. The interaction of tricalcium silicate-based cements with dentin was observed Han and Okiji [25], which demonstrated the intertubular incorporation of calcium and silicon, apatite deposition and formation of a structure similar to mineral tags. Such ability may explain the dentin sealing and adhesion ability of these materials. The reaction of calcium ions with tissue phosphates results in the formation of calcium phosphate (apatite), responsible for the bioactivity of the material. The active participation of calcium in the repair process is demonstrated by several studies [22, 27]. Estrela *et al.* [22] described the mechanism by which calcium ions in the material participate directly in the formation of the mineralized barrier, with formation of calcite crystals.

According to Xuereb *et al.* [45], cements presented as premixed paste in syringes are easy to handle, however they need hydration for setting reaction. Due to the need of presence of moisture, in clinical conditions these materials would depend on the dentinal fluid inside the root canal to take hold, since the channels are dried after the sanitation and modeling process.

Bioceramic cements have shown good sealing ability and tissue tolerance in *in vitro* studies [2, 13-15, 32] but there is a lack of controlled clinical studies that prove the long-term efficacy and safety of these materials [3, 33]. Another limitation presented by these cements is related to the ability of these materials to be removed from the root canal system in case of retreatment. Hess *et al.* [26] showed that conventional retreatment techniques were unable to completely remove bioceramic cement from the root canal.

Despite the great advances achieved by the technological development in endodontic materials, there is no material that contemplates all the characteristics considered as ideal. Additionally, clinical studies are still needed to analyze the behavior of new calcium silicate-based cements.

Conclusion

The evaluated cements presented particles with uniform distribution pattern and similar chemical composition, with predominance of calcium and silicon, differing mainly the presence of bismuth or zirconium.

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