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Obtaining bioceramic cements for dental repair procedures based on hydroxyapatite and bismuth oxide

Obtenção de cimentos biocerâmicos para procedimentos de reparo dentário à base de hidroxiapatita e óxido de bismuto

Obtención de cementos biocerámicos para procedimientos de reparación dental a base de hidroxiapatita y óxido de bismuto

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Abstract

Introduction: The use of hydroxyapatite-based cements in dental and bone tissue regeneration procedures has shown favorable results. However, structural fragility and lower levels of radiopacity at clinical evaluation make it difficult to use in direct clinical procedures. Objective: This study aimed to synthesize a new cement with properties to be considered for use in dental repair procedures using hydroxyapatite and a hydrogel, with the addition of bismuth oxide as a radiopacifying agent. Methodology: The materials were obtained by a mixture of hydroxyapatite produced by the precipitation method, a hydrogel with carboxymethylcellulose and calcium silicate and bismuth oxide. The products were characterized by X-ray diffraction, Fourrier transform X-ray fluorescence, scanning electron microscopy, setting time, pH and radiographic appearance. Data were analyzed using Jamovi® software version 1.6 to calculate absolute frequencies, as well as measures of central tendency and variability. Results: The proposed cements obtained presented phase compositions without alterations in the composites, with a nanometric porous structure. Basic pH contributes to its bioactivity and antimicrobial action. The drying time of the proposed cements was prolonged. From the radiographs, the cement containing bismuth oxide was radiopaque when compared to the cement without this component. Conclusion: A new dental cement based on hydroxyapatite and bismuth oxide was obtained, homogeneous, with satisfactory radiopacity property, enabling its analysis through radiographic examinations.

Keywords: Hydroxyapatite; Bismuth; Regeneration; Dental materials.

Resumo

Introdução: O uso de cimentos à base de hidroxiapatita em procedimentos de regeneração de tecidos dentários e ósseos tem demonstrado resultados favoráveis. No entanto, a fragilidade estrutural e os níveis mais baixos de radiopacidade à avaliação clínica dificultam o uso em procedimentos clínicos diretos. Objetivo: Este estudo teve como objetivo sintetizar um novo cimento com propriedades a serem consideradas para uso em procedimentos de reparo dentário utilizando hidroxiapatita e um hidrogel, com adição de óxido de bismuto como agente radiopacificador.

Metodologia: Os materiais foram obtidos por uma mistura de hidroxiapatita produzida pelo método de precipitação, um hidrogel com carboximetilcelulose e silicato de cálcio e óxido de bismuto. Os produtos foram caracterizados por difração de raios X, fluorescência de raios X com transformada de Fourrier, microscopia eletrônica de varredura, tempo de presa, pH e aspecto radiográfico. Os dados foram analisados no software Jamovi® versão 1.6 para cálculo de frequências absolutas, bem como medidas de tendência central e variabilidade. Resultados: Os cimentos propostos obtidos apresentaram composições de fases sem alterações nos compósitos, com estrutura porosa nanométrica. O pH básico contribui para sua bioatividade e ação antimicrobiana. O tempo de secagem dos cimentos propostos foi prolongado. Pelas radiografias, o cimento contendo óxido de bismuto mostrou-se radiopaco quando comparado ao cimento sem este componente. Conclusão: Foi obtido um novo cimento odontológico a base de hidroxiapatita e óxido de bismuto, homogêneo, com propriedade satisfatória de radiopacidade viabilizando sua análise por meio de exames radiográficos.

Palavras-chave: Hidroxiapatita; Bismuto; Regeneração; Materiais odontológicos.

Resumen

Introducción: El uso de cementos a base de hidroxiapatita en procedimientos de regeneración de tejidos dentales y óseos ha mostrado resultados favorables. Sin embargo, la fragilidad estructural y los niveles más bajos de radiopacidad en la evaluación clínica dificultan su uso en procedimientos clínicos directos. Objetivo: Este estudio tuvo como objetivo sintetizar un nuevo cemento con propiedades a considerar para su uso en procedimientos de reparación dental utilizando hidroxiapatita e hidrogel, con la adición de óxido de bismuto como agente radiopacificador. Metodología: Los materiales fueron obtenidos por una mezcla de hidroxiapatita producida por el método de precipitación, un hidrogel con carboximetilcelulosa y silicato de calcio y óxido de bismuto. Los productos se caracterizaron por difracción de rayos X, fluorescencia de rayos X por transformada de Fourrier, microscopía electrónica de barrido, tiempo de fraguado, pH y aspecto radiográfico. Los datos fueron analizados utilizando el software Jamovi® versión 1.6 para calcular frecuencias absolutas, así como medidas de tendencia central y variabilidad. Resultados: Los cementos propuestos obtenidos presentaron composiciones de fase sin alteraciones en los compuestos, con una estructura porosa nanométrica. El pH básico contribuye a su bioactividad y acción antimicrobiana. El tiempo de secado de los cementos propuestos fue prolongado. A partir de las radiografías, el cemento que contenía óxido de bismuto resultó radiopaco en comparación con el cemento sin este componente. Conclusión: Se obtuvo un nuevo cemento dental a base de hidroxiapatita y óxido de bismuto, homogéneo, con propiedad de radiopacidad satisfactoria, que permite su análisis mediante exámenes radiográficos.

Palabras clave: Hidroxiapatita; Bismuto; Regeneración; Materiales dentales.

1. Introduction

The research for new biomaterials obtained from a polymeric organic matrix reinforced by a nanoparticulate inorganic matrix such as ceramics is constant (Cucuruz et al., 2016). The development of new biocements to be used as bone substitute or in dental repair procedures is largely researched, especially the calcium phosphate cements where efforts have been made to improve properties such as biodegradability, macroporosity, mechanical stability and osteoconduction (Lodoso-Torrecilla et al., 2021).

Synthetic hydroxyapatite (HAp) has been used as a bone replacement because of its natural-like composition found in human bones and teeth. It has important properties such as a good resistance, an adequate color, a low thermal and electrical conductivity, osteoconduction, favorable cellular and vascular response, without changes in inflammation patterns, easy manufacturing process and low cost (Costa et al., 2009; Oliveira et al., 2016; Sa et al., 2015).

However, its low mechanical resistance, low ductility and chemical instability in the implanted area limit its use in areas that require a high resistance against mechanical stresses (Uskokovic et al., 2016). It also does not have a considerable radiopaque value that allows its evaluation *in vivo* (Barros, 2012).

Thus, it is necessary to add a radiopacifying agent to its formulation that does not interfere with its physical and biological properties. It must also allow an evaluation of the material when implanted *in vivo* by imaging studies (Barros, 2012; Viapina et al., 2014).

Bismuth oxide (Bi_2O_3) is a non-toxic semi-conductive inorganic compound with excellent optical and electrical properties used commercially as a radiopacifying agent in dental products such as composite resins and in Mineral Trioxide

Aggregate (MTA) (Maria-Hormigos et al., 2015). Its radiopacification property is provided by its high atomic number (Z = 83) and atomic mass (A = 208.9u), and the high nuclear density of its atoms makes it difficult for radiation to pass through (Cutajar et al., 2011).

In order to improve the mechanical properties and the setting time of ceramic materials for dental use, carboxymethylcellulose (CMC) in calcium silicate-based bone cements has been evaluated. Hydrogels based on CMC have been shown to direct accumulation, differentiation and organization of human mesenchymal stem cells in order to elaborate native extracellular chondrogenic matrices (Chen & Liu, 2016).

Thus, this work aimed to obtain a bioceramic cement based on HAp, calcium silicate and bismuth oxide as a radiopacifying agent to be used for dental repair procedures.

2. Methodology

For the synthesis of HAp, solutions of phosphoric acid (H_3PO_4 - 85% P.A. Vetec, Rio de Janeiro, RJ, Brazil) and calcium hydroxide ($Ca(OH)_2$ - 95% P.A. Vetec, Rio de Janeiro, RJ, Brazil) were used. For the synthesis of the hydrogel, carboxymethylcellulose (100% P.A. Sigma-Aldrich, São Paulo, SP, Brazil) and Glycerin (99.5% P.A. Sigma-Aldrich, São Paulo, SP, Brazil) diluted in deionized water were used. For the formulation of the cement, calcium silicate ($CaSiO_3$ - 87% P.A. Sigma-Aldrich, São Paulo, SP, Brazil) and bismuth oxide (Bi_2O_3 -100% P.A. Vetec, Rio de Janeiro, RJ, Brazil) were used.

Synthesis and characterization of HAp

The HAp was synthesized by wet precipitation using 2M dilute phosphoric acid and 2M calcium hydroxide solutions (Saeri et al., 2003).

60 ml of H_3PO_4 solution were added dropwise to the solution of $Ca(OH)_2$, remaining under magnetic stirring for 50 minutes until a visual consistency of a dry paste was obtained. The dried material was then oven-dried at 110ºC for 24 hours.

The resulting product was triturated in an agate mortar and sieved in a 100 mesh aperture mesh (149 μm), thereby obtaining the HAp designated as HAPI. Subsequently, the HAPI was calcined in a furnace at 900°C for a period of 2 hours, then receiving the nomenclature HAPC. The resulting products were subjected to characterizations.

Cements preparation

The cements were prepared from the mixture of a ratio of 30% HAp and 70% hydrogel based on a methodology to produce a bone scaffold substitute (Dutta et al., 2019). For this work, two different compositions of dental cement were prepared. The amounts of each component material of the evaluated cements are described in Table 1.

Table 1. Compositions of each element for the formulation of dental cements.

Experimental Cements	Deionized Water (g)	Glycerin (g)	CMC (g)	HAp (g)	$CaSiO_3$ (g)	Bi_2O_3 (g)	Total weight (g)
C3	20.72 (56%)	4.48 (12%)	0.74 (2%)	6.66 (18%)	4.44 (12%)	--	37 (100%)
C4	20.72 (56%)	4.48 (12%)	0.74 (2%)	5.55 (15%)	3.33 (9%)	2.22 (6%)	37 (100%)

Source: Research data.

To obtain the hydrogel, 25 mL of water and glycerin were placed in a beaker and kept under magnetic stirring on a BIOSTAR heating plate until reaching 50°C. Then, the CMC was added under stirring for 1 hour to prevent the formation of lumps, resulting in a translucent hydrogel.

Then, HAp, calcium silicate and bismuth oxide (only in the C4 cement) were added, keeping under constant mechanical stirring for 1 hour at 10 RPM for total blending of the powder with the hydrogel. The dental produced cements were named C3 and C4. Each group analyzed were produced with 5 samples; the samples were submitted to physicochemical characterizations described below.

The results obtained to the variables ph and setting time were statistically analyzed by the software Jamovi® version 1.6 to calculate absolute frequencies as well as measures of central tendency and variability.

X-ray diffraction (XRD)

The raw materials used on cements production as well as the cements produced were analyzed on X-ray diffraction using a Bruker model D2 Phaser X-ray diffractometer, (Cu Kα radiation), voltage of 40kV, 30mA current, with scanning $5° < 2\Theta < 95°$ with an increment of 0.016. to determine the crystal phases.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra analysis of the raw materials and cements obtained were realized on a Vertex 70 Bruker model spectrometer operated between 4000 e 400cm^{-1}. This analysis was used to obtain an infrared spectrum of absorption scattering, objecting to identify molecular compounds and their chemical composition by the spectral groups present on the composites structures.

Scanning electron microscopy (SEM)

The microstructural arrangement and morphology of the biocements were analysed by TESCAN VEGA3 scanning electron microscopy.

pH analysis

The pH analyzer was performed using a digital pH meter AK90, Akso, with replaceable electrode. A solution of potassium hydroxide was mixed drop by drop in deionized water with constant magnetic stirring in order to calibrate the pH of the equipment. Then, 0.1g of each cement was diluted in 10 ml of deionized water; with neutral pH, the values were measured in the solution of the diluted cements.

Setting time

The initial and final setting time of the cements were assessed using Gillmore needles (ISO, 2012). Five proof bodies measuring 10 mm diameter per 2 mm of height were analyzed with a inox needle weighing approximately 113g (initial setting time) and 453 g (final setting time), which was lowered on the discs of the evaluated material every minute, considering the final prey time when the material was no longer marked by the tip of the needle.

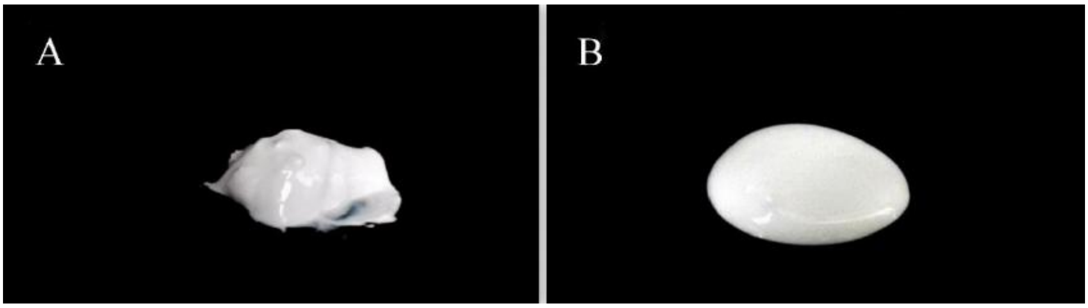
Radiographic analysis

The radiographic analysis aims to evaluate the radiopacity power of the material in question. For this purpose, following the protocol recommended by ISO 6876/2012, specimens measuring 10 mm in diameter and 1 mm in thickness were made in steel rings, placed on an occlusal radiographic film and radiographed (X-ray Gnatus®, Timex 70E mobile column, Campina Grande, Brazil) with 90 ° angulation and exposure time of 0.32 milliseconds (ISO, 2012). Also was analyzed the radiopacity of the cements when inserted in very deep Class I cavities of Black classification, measuring 4 mm of height by 5 mm of length and 3.5 mm of thickness, realized with diamond bit 1093 (Fava®).

3. Results and Discussion

The cements produced can be seen on Figure 1, C3 (a) and C4 (b). All cements had a homogeneous consistency, with white coloring.

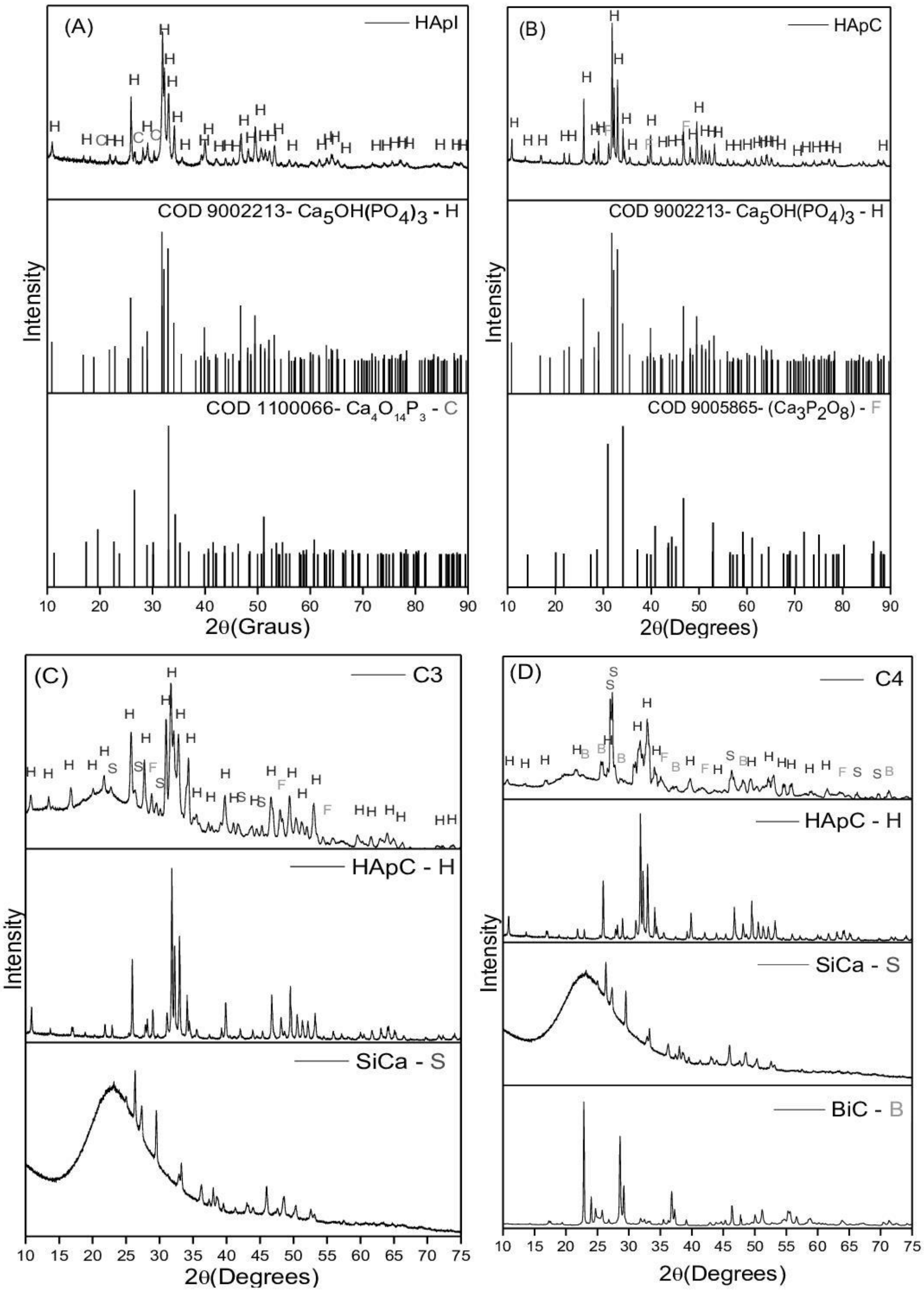
Figure 1. Visual aspect of synthesized cements (A) C3 and (B) C4.



Source: Research data.

The X-ray diffractograms obtained for the samples under study are shown on Figure 2. Figure 2(A) shows the result of pure HAp obtained by wet precipitation method (HApI), after calcination evidenced by the presence of peaks corresponding to the hydroxyapatite phase $Ca_5OH(PO_4)_3$ identified by the letter (H) according to the standard COD 9002213. The presence of traces of the second phase of calcium phosphate ($Ca_4O_{14}P_3$), identified by the letter (C) according to the standard COD 1100066, were also obtained. Its presence caused a small displacement of the peaks related to HAp.

Figure 2. XRD results (A) initial HAp. (B) calcined HAp. (C) endodontic cement C3 (D) endodontic cement C4.



Source: Research data.

Figure 2(B) shows the result of XRD evaluation of HAp after calcination process at 900 °C (HApC). In addition to the characteristic peaks of the phase $Ca_5OH(PO_4)_3$ identified by the letter (H) according to the standard COD 9002213, it also resulted in the presence of the second phase of tricalcium phosphate $_3(PO_4)_2$, identified by the letter (F) according to the standard COD 9005865.

The XRD of the samples C3 and C4 of the dental cements obtained are presented in Figure 2(C) and Figure 2(D). Peaks corresponding to the phases of hydroxyapatite (H), tricalcium phosphate (F) and calcium silicate (S) were found in all diffractograms. These are the materials used for forming the cements. The organic reagents CMC and glycerin, in addition to water, which were used to obtain the cements, were not identified in the XRDs because they are amorphous.

For the calcium silicate (SCA) sample (Figure 2C), there was an initial characteristic within the range 17-29° of an amorphous band related to the hydration of calcium silicate. The other peaks observed in the diffractogram indicate the presence of the calcium silicate wollastonite according to the standard JCPDF 9011913.

For the sample of commercial bismuth oxide (BiC) (Figure 2D), the material is presented in crystalline and monophasic form, according to the standard JCPDF 71-465.

For the FTIR spectra results showed in Figure 3, the hydroxyapatites synthesized (Figure 3A) and after calcination (Figure 3B), we observed vibrational bands and a wave number typical of the hydroxyapatite by the PO_4^{3-},OH and CO_3^{2-} groups at approximately 1,027 and 611 cm^{-1}, 2,345 cm^{-1} and 1,426 cm^{-1}, respectively. The phosphate bands (PO_4^{3-}) are present in the HAps, and were observed in all spectra. The observed vibrations of CO_3^{2-} may come from the air, since the samples were produced in an open atmosphere and in an aqueous solution.

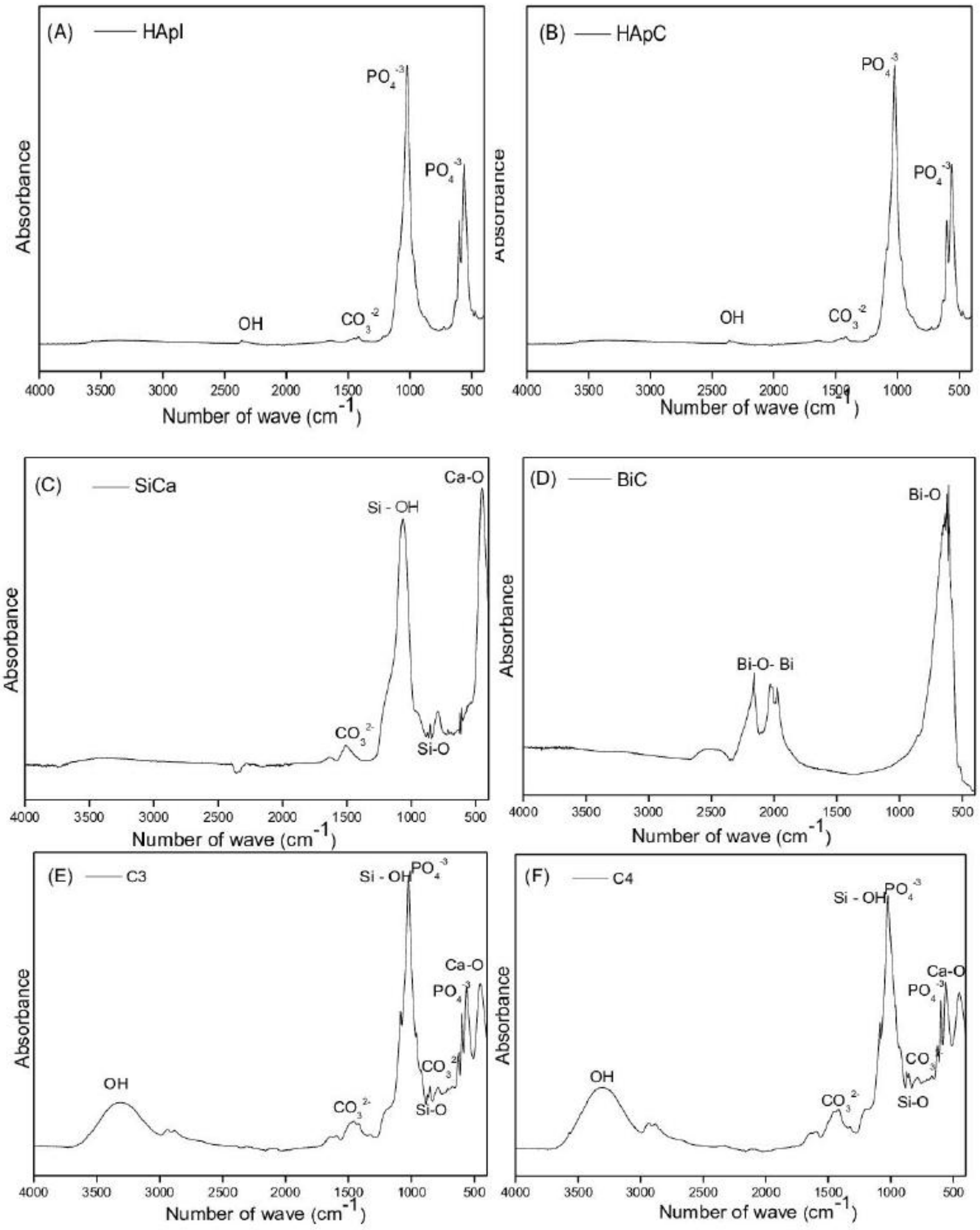
According to the FTIR results for the SiCa sample (Figure 3C), there were bands at the frequency range between 831 cm^{-1} and 1,066 cm^{-1}, possibly resulting from the vibrations between the silicon and the oxygen. The band at 1,000 cm^{-1} and at 900 $cm^{-1}$is also related to the Si-O stretch vibration (Ślósarczyk et al., 2005). The intensities at approximately 445 cm^{-1} and 355 cm^{-1} result from the stretching vibrations of calcium and oxygen. For the BiC sample (Figure 3D), there were functional groups corresponding to bismuth oxide at approximately 2,161 - 2,000 cm^{-1}, and characteristic Bi-O-Bi vibrations at approximately 650 - 590 cm^{-1}.

For the dental cements obtained (Figures 2E and 2F), FTIR results show spectra corresponding to the O-H bond for the hydroxyl group, to the group CO_3^{2-} of carbonates, and to the group $PO_4^{3-}$of phosphates. The band observed in the frequency range between 891 cm^{-1} and 1,230 cm^{-1} of the simulated spectrum suggests a result of vibrations between the silicon and the oxygen. There were no molecular vibrations corresponding to the bismuth oxide in the C4 endodontic cement spectrum, thus confirming that it did not enter into the structure of the hydroxyapatite.

The results of SEM analysis for C3 cement (Figure 4A) showed an intense particle aggregation morphology and less porosity, which gives this cement a higher alloy characteristic. C4 cement (Figure 4B) presented a finer morphology of smaller agglomerates, but with greater porosity present between the particles and the amorphous region, resulting from the presence of bismuth oxide nanoparticles, forming a more homogeneous matrix.

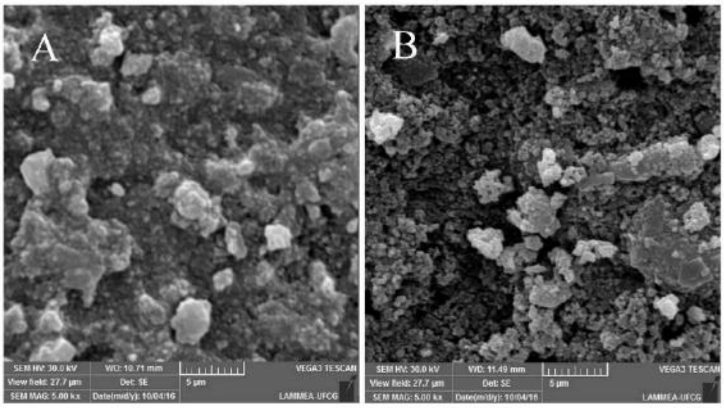
The results of the pH analysis presented on Table 2 showed that the pH of the cements were within an alkaline range, with median values of 10.7 and 10.5 for the C3 and C4 cements, respectively. Table 3 shows the setting times of the cements obtained verified according to the ISO 6876. By analyzing the data, the cement C3 presented a shorter mean time to reach the final prey (5.5h) when compared to the C4 cement (10h).

Figure 3. Results of the initial FTIR (A) initial HAp. (B) calcined HAp. (C) SiCa. (D) BiC. (E) C3 cement. (F) C4 cement.



Source: Research data.

Figure 4. SEM micrographs of cement samples obtained on a 5µm scale (A) C3. (B) C4.



Source: Research data.

Table 2. Descriptive analysis of pH values for the cements samples.

	pH C3		pH C4
N	5		5
Missing	0		0
Mean	10.7		10.5
Median	10.7		10.5
Mode	10.7	a	10.5
Standard deviation	0.0837		0.0707
Minimum	10.6		10.4
Maximum	10.8		10.6

[a] More than one mode exists, only the first is reported

Source: Jamovi®, Research data.

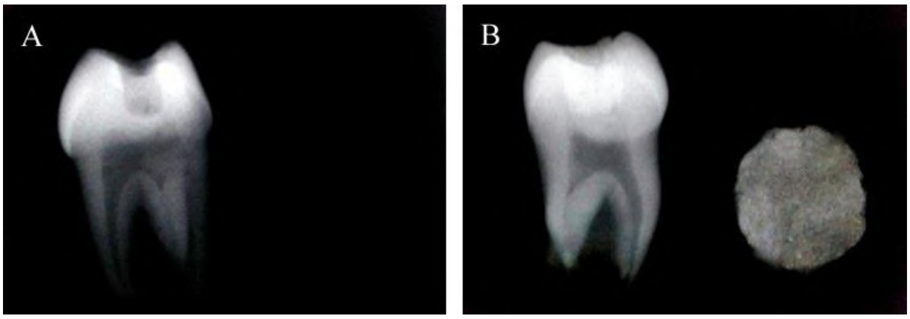
Table 3. Cement setting time means of the evaluated cements C3 and C4

	ST C3 (hours)	ST C4 (hours)
N	5	5
Missing	0	0
Mean	5.50	10.0
Median	5.50	10.0
Standard deviation	0.122	1.58
Minimum	5.40	8.00
Maximum	5.70	12.0

Source: Jamovi®, Research data.

The radiographs results are shown on Figure 5. For the cement C3 (Figure 5A), it is observed by radiography result that it does not present any radiopacity of the cement pellet, as well as of the material inserted in the tooth. This evidences a radiolucency in the tooth by the appearance that there is an empty space which can be easily confused with a carious process or the absence of material and the cement disc positioned next to the tooth cannot even be seen. However, the radiographed pellet of the cement C4 (Figure 5B) is clearly observed inside the tooth and on the cement disc next to it. The implanted material of the enamel and dentin structure of the tooth can also be easily distinguished because the C4 cement proved to be more radiopaque than these dental structures.

Figure 5. Radiographic analysis of cements in human teeth and tablets following ISO 6876/2012 (A) C3. (B) C4



Source: Research data.

4. Discussion

The obtention of calcium phosphate cements with good mechanical properties, coesion and a consistency that allow the material to be injected is appealing, once this material can be used with minimally invasive procedures and can be fitted in irregular spaces (Lodoso-Torrecilla et al., 2021). The use of such materials has been widely researched in the repair of bone and even cartilaginous tissues (Bi et al., 2020). In this research it was possible to obtain a biocement from the use of hydroxyapatite and the polymer CMC.

For the synthesis of this cements, the HAp produced in laboratory by method of precipitation was calcined. This process did not change the main phase (HAp), but changed the secondary phases. This change in the transformation from the secondary phase of $Ca_4O_{14}P_3$ into $Ca_3(PO_4)_2$ is justified in a function of the calcination temperature at 900°C (Rojas, 2015). Futhermore, these results also corroborate with others studies that report the presence of HAp associated with other components identified as calcium phosphate and tricalcium phosphate (Oliveira et al., 2016; Rojas, 2015).

The SCA XRD showed an amorphous band related to the hydration of calcium silicate analyzed to the adhesion to CMC (Zhang et al., 2015). All these materials were found on the XRD of the cements produced C3 and C4 without other phases.

The FTIR results showed symmetrical axial vibration of the group CO_3^{2-}, which was also observed at 1,340 cm^{-1}. This indicates that the CO_3^{2-} group can be adsorbed into the surface of the particles or may act as a replacement for the PO_4^{3-} group in the HAp network (Ślósarczyk et al., 2005). In general, the bands characteristic of co-precipitates and hydrothermal HAp are consistent with the literature (Bi et al., 2020; Oliveira et al., 2016).

Prodana et al. reported that upon obtaining a new ceramic coating with carbon nanotubes, HAp and TiO_2 for biomedical applications, they observed the elongation, flexion and vibration modes of OH^- identified at 3,270 cm^{-1} and 634 cm^{-1}, respectively. Modes of elongation and flexion were observed at 560 cm^{-1}, 3,960 cm^{-1}, 1,099.33 cm^{-1} and 1,042 cm^{-1} referring to the group PO_4^{-3}, and confirmed the formation of well-crystallized apatite (Prodana et al., 2015). The carbonate band was observed at 1,640 cm^{-1}, and the observed peak at 2,354 cm^{-1} was attributed to the adsorbed carbon dioxide.

The bands of Bi-O-Bi at 846 cm^{-1} and characteristic vibrations are still observed at approximately 548-460 cm^{-1} of the binding of the Bi-O metal oxide. These results corroborate with the conclusions also observed on other studies (Viapina et al., 2014; Maria-Hormigos et al., 2015). Bismuth oxide has a vibration at 3,448 cm^{-1} for -OH, 1,652 for H_2O, 1,383 for NO_3, 825 for Bi-O-Bi, and 430 for Bi-O. These results indicate that Bi_2O_3 may contain significant amounts of the groups OH^- and NO_3^- on its surface (Maria-Hormigos et al., 2015).

According to the FTIR results, the biocements in development presented vibrational frequencies only corresponding to the chemical groups of the compounds, which were evaluated isolated. The CMC broadbands is presented between 3800-3000 cm^{-1} were correspond to the hydrogen ligation of free hydroxyl groups, the peak 2967 cm^{-1} refer to C-H stretching, in accordance to previous study (Bi et al., 2020).

The SEM results showed that the cements presented a morphological characteristic of clusters of small particles surrounded by an amorphous matrix, since they are all composed of the same components. The polymeric matrix must allow a good distribution of the HAp particles in its structure, since they tend to agglomerate, and Hap prepared via precipitation method is presented like an aggregate particles with no stabilizing factor (Bi et al., 2020), so the hydrogel confers microstructural stability in the distribution of these particles. HAp contributes to form the porous surface structure of cements, which acts as a passive support for angiogenesis and as an additional substrate for cell proliferation, allowing the migration and phenotypic expression of cells bones, resulting in the formation of new bone tissue (Cucuruz et al., 2016).

CMC provides a porous structure, which influences the physical properties of the material, and HAp particles increase the bioactive potential of cement for mineralization and bone growth, without decreasing its mechanical properties. HAp-loaded cements make their surface more favourable for the apatite nucleation and, therefore, for Ca-P precipitation due to the CMC and HAp carboxyl functional groups inducing the formation of this precipitate, which provide more nucleation sites for the formation of precipitates as a chain reaction, resulting in denser and thicker deposits over time, thus improving the mineralization capacity of cements (Sa et al., 2005).

All the cements presented an alkaline pH. This allows osteogenic differentiation and bone formation, anti-inflammatory effects and antimicrobial activity, because a high pH can damage the cell membrane and the DNA of microorganisms (Song et al., 2017). The evaluation of pH is important for the analysis of dissolution degree of the CMC network of cements, since hydrogels are dissolved through changes in the environmental conditions such as pH, temperature and the ionic strength of the solution (Caló; Khutoryanskiy, 2015). At a physiological pH, the carboxylic acid of the carboxymethyl group of the CMC is deprotonated, resulting in a negatively charged polymer network similar to the extracellular matrix network formed by glycosaminoglycan’s of cartilaginous tissues (Raza et al., 2015).

Obtaining a bone and dental graft material with effective regenerative properties is still a challenge, but there are three properties that historically used materials have shown to be important: high pH, antimicrobial activity and calcium ion release (Song et al., 2017).

As most of the materials that compose the cement have a neutral pH, this value was attributed to the presence of calcium silicate, which, when hydrated, becomes very basic. A research evaluated the antimicrobial properties of a calcium silicate cement with endodontic application against some common endodontic pathogens such as *Enterococcus faecalis*, and concluded with positive results for this antimicrobial action only because of the high pH (Prati; Gandolfi, 2015).

This result indicates a possible antimicrobial potential of the cements studied, similar to the antimicrobial potential of MTA that is attributed to its high pH (12.5) (Poggio et al., 2014). The antimicrobial property of materials for bone grafts is important for decreasing the risk of bacterial infections that may be possible threats after surgeries (Tommasi et al., 2016). The possible antimicrobial property of these non-antibiotic-related materials is an important point for the effort against the growing concern of increasing the resistance of pathogens against antibiotics.

The antimicrobial activity of bone grafts is also primordial to avoid re-absorption of natural bone hydroxyapatite or synthetic graft. Biofilms associated with bone infection may directly reabsorb or destroy the hydroxyapatite in the absence of host inflammation or osteoclastogenesis, similar to what occurs with chronic bone infections such as osteomyelitis, osteonecrosis or periodontitis (Tommasi et al., 2016).

HAp may decrease osteoclast cell activity by decreasing osteoclast gene expression and bone resorption capacity by mature osteoclasts (Uskokovic; Wu, 2016). Considering these results, it can be inferred that the analyzed material proved to be propitious to present important properties that act in an organism as a graft in order to accelerate the regeneration of mineralized tissues.

Setting time is one of the most clinically important properties for material operability and should be adequate to insert the material, maintain its structural shape and withstand stress. The cement C4 presented a reduction in the initial and final setting time, which can be attributed to the increase in the ratio of calcium silicate in relation to HAp. These results are related to the reduction in the proportion of hygroscopic materials (calcium silicate and hydroxyapatite) in relation to the addition of a water insoluble material. All materials that make up the evaluated cement directly influence this characteristic. Calcium silicate and CMC, when present in increasing proportions in the cements, decrease this time due to the fast calcium hydration reaction and the water absorption capacity of the CMC as a viscous agent (Sa et al., 2015; Zhang et al., 2016).

The HAp is a hydrophilic particle which provides the reduction of setting time with adsorption capacity, influencing the drying time of the material. However, this adsorption capacity is related to the pore structure and the physical-chemical nature of the solid surface (Costa et al., 2009).

The initial drying time of the cements evaluated is not within the average drying time of calcium silicate endodontic cements, such as MTA (20 min), Bio MM (32 min), BioRoot RCS (27 min) and AH plus (1,154 min). The clinical application requires an initial drying time between 3 and 10 minutes. The setting time must occur within a time interval that allows the manipulation and implantation of the material (Khalil et al., 2016). It should not be too long, since it may move from the indicated location; the temperature of this reaction cannot be high and it should not cause tissue damage and degradation of the cement.

The CMC gel may act as a heat sink, avoiding the increase in temperature during the hardening reaction of the cements, and the reaction temperature may influence the material setting time. The drying of cements can be attributed not only to calcium silicate, but also to HAp, which decreases the drying time of calcium silicate cements (Barros, 2012).

The increase in the drying time of the cement can also be attributed to the presence of glycerin whose addition into calcium phosphate cements improves their viscosity and applicability by means of edges, but also increases the hardening time of the material, decreasing its physical properties (Tommasi et al., 2016).

The radiographic analyses showed the importance on addition of some radiopaque material in order to confer clinical distinction of the tooth structure and the biomaterial implanted. The cement C4 containing bismuth oxide showed a radiopacity relevant for its clinical follow-up after *in vivo* implantation by radiographs results. Bismuth oxide is a material widely used to confer radiofrequency to dental products as composite resins for restorative procedures and endodontic cements such as MTA (Cetenovic et al., 2017).

A similar research used Yttribium Trifluoride as a radiopacifying agent in a calcium silicate cement with HAp, and the results demonstrated that a 20% radiopacifier addition was sufficient to impart good radiopacity to this cement, a quantity similar to bismuth oxide in the MTA (Barros, 2012; Viapina et al., 2014).

5. Conclusion

The development of new hydroxyapatite-type bioceramics cements must take in consideration the biological and physicochemical properties for use in dental repair procedures. Among the evaluated properties, the cement C4 showed the best results to be an alternative on the development of bioactive agents. However, the reduction in the setting time of the cements obtained is important for a better clinical and surgical application. After, the material obtained must be submitted to cytotoxicity tests and in vivo tests before its clinical analysis.

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