UNESP - Universidade Estadual Paulista
"Júlio de Mesquita Filho"
Faculdade de Odontologia de Araraquara

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CIMENTO DE SILICATO DE CÁLCIO MODIFICADO COM NANOPARTÍCULAS DE VIDRO BIOATIVO PARA APLICAÇÃO COMO SUBSTITUTO DE DENTINA

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Cimento de silicato de cálcio modificado com nanopartículas de vidro bioativo para aplicação como substituto de dentina

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Orientador: Prof. Dr. Hernane da Silva Barud

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Prof. Dr. Osmir Batista de Oliveira Junior
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Corral Núñez CM. Cimento de Silicato de Cálcio Modificado com Nanopartículas de Vidro Bioativo para Aplicação como Substituto de Dentina. [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2017.

RESUMO

Os cimentos de silicato de cálcio têm se desenvolvido rapidamente durante as últimas décadas; com a aparição de novos cimentos para uso em odontologia restauradora. Estes cimentos apresentam diferentes composições químicas e por tanto provavelmente diferente radiopacidade. A incorporação de nanopartículas de vidro bioativo poderia melhorar a bioatividade dos cimentos de silicato de cálcio. O **objetivo** foi avaliar a composição química e radiopacidade dos cimentos a base de silicato de cálcio Biodentine (BD) e TheraCal LC e a contribuição do acréscimo de nanopartículas de vidro bioativo (nVB) no BD. **Método**: Foram realizados testes de radiopacidade de acordo com norma ISO 9917 e caracterização com microscopia eletrônica de varredura e análise elementar (MEV/EDX) dos novos cimentos de silicato de cálcio BD e TheraCal LC. Posteriormente, nVB foram sintetizadas com técnica sol-gel, e foram preparados nanocompósitos do BD com 1 e 2% em peso de nVB (1%nVB/BD e 2%nVB/BD). A bioatividade dos nanocompósitos foi avaliada in vitro e caracterizado com MEV/EDX, análise de espectroscopia no infravermelho por transformada de Fourier e difração de raios-X (DRX). Também os nanocompósitos foram aplicados em discos de dentina e a interface caracterizada com MEV-EDX. **Resultados:** BD apresentou zircônio como elemento radiopacificante e maiores valores de radiopacidade do que o TheraCal LC, que apresentou bário, estrôncio e zircônio como radiopacificadores. A incorporação de nVBs em BD melhorou a bioatividade in vitro do BD não modificado, acelerando a formação de uma camada de apatita cristalina em sua superfície. Comparado com BD não modificado, nVB/BD mostrou uma área interfacial maior com maior incorporação de Si e precipitação intratubular de depósitos quando em contato com dentina. **Conclusão:** Os cimentos de silicato de cálcio melhorados, BD e TheraCal LC apresentam diferente composição química com distintos agentes radiopacos, este é reflexado em diferencias em suas radiopacidades. A incorporação de nVB em BD aumenta as propriedades bioativas in vitro do BD, acelerando a formação de apatita cristalina na sua superfície após um curto período de imersão em solução.

Palavras-chave: Materiais Dentários. Cimentos Dentários. Capeamento da Polpa Dentária. Cimento de Silicato. Nanopartículas. Vidro. Permeabilidade da Dentina.

Corral Núñez CM. Calcium silicate based cement modified with bioactive glass nanoparticles for application as dentine substitute. [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2017

ABSTRACT

Calcium silicate cements have developed rapidly during the last decades; with the appearance of new cements for use in restorative dentistry. These cements have different chemical composition, therefore they probably present different radiopacity values. The incorporation of bioactive glass nanoparticles could improve the bioactivity of calcium silicate cements. The **objective** was to evaluate the chemical composition and radiopacity of calcium silicate cements, Biodentine (BD) and TheraCal LC and to evaluate the bioactive properties of nanocomposites, based on the incorporation of bioactive glass nanoparticles (nBG) in BD. **Method:** Radiopacity tests were performed according to ISO 9917 standard and characterization with scanning electron microscopy and elemental analysis (SEM/EDX) of new calcium silicate cements, BD and TheraCal LC. Subsequently, nBG were synthesized using the sol-gel technique, and nanocomposites of BD with 1 and 2 wt% nBG were prepared (1%nBG/BD and 2%nBG/BD). The bioactivity of the nanocomposites was evaluated in vitro and characterized with SEM/EDX, analysis of Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analysis (XRD). In addition, the nanocomposites were applied in dentin discs and maintained in SBF and the interface characterized with SEM-EDX. **Results:** BD presented zirconium as a radiopacifying element and higher values of radiopacity than TheraCal LC, which presented barium, strontium and zirconium as radiopacifiers. The incorporation of nBGs into BD improves the in vitro bioactivity of the unmodified BD, accelerating the formation of a layer of crystalline apatite on its surface after immersion in SBF. When compared with unmodified BD, nBG/BD showed a larger interfacial area with greater Si incorporation and intratubular formation of deposits when in contact with dentin. **Conclusions:** The new calcium silicate cements, BD e TheraCal LC present different chemical compositions with different radiopacifiers, this is expressed as differences in their radiopacity values. The incorporation of nBG in BD enhances the bioactive properties of BD, accelerating the formation of crystalline apatite after a short time of immersion in solution.

Keywords: Dental Materials. Dental Cements. Dental Pulp Capping. Silicate Cement. Nanoparticles. Glass. Dentin Permeability.

SUMÁRIO

1 INTRODUÇÃO

Os cimentos de silicato de cálcio (CSC, compostos principalmente por cimento Portland do tipo I) têm despertado alto interesse, por poderem ser utilizados em situações clínicas nas quais os outros cimentos odontológicos falham (Prati et al.[59], 2015). Pesquisadores tem estudado e proposto modificações em sua composição a fim de aprimorar ainda mais suas propriedades físico-mecânica e biológicas.

A alta compatibilidade com os tecidos biológicos e a capacidade de tomar presa em presença de umidade (Prati et al.[59], 2015), fazem com que o CSC seja indicado como material ideal para o capeamento pulpar direto (Nowicka et al.[54], 2015), reparação de perfuração radiculares e de furca (Aggarwal et al.[4], 2013; Bachoo et al.[7], 2013; Guneser et al.[33], 2013), apicificação e retrobturação, (Bachoo et al.[7], 2013) e como dentina artificial nos tratamentos restauradores (Torabinejad et al.[72], 1999; Septodont[65], 2012; Koubi et al.[47], 2013; Hashem et al.[36], 2015).

O Agregado Trióxido Mineral (MTA, ProRoot MTA, Dentsply) foi o primeiro CSC desenvolvido para fins odontológicos (Parirokh et al.[58], 2010). Rapidamente, outros produtos comerciais a base de CSC foram lançados no mercado, entre eles: MTA Angelus (Angelus Soluções Odontológicas do Brasil) e Endo CPM Sealer (Egeo, Argentina), devido a alta aceitação e recomendação da comunidade cientifica (Parirokh et al.[58], 2010).

No entanto, a falta da radiopacidade necessária para identificá-lo nas radiografias, (Camilleri et al.[14], 2010; Saghiri et al.[63], 2015; Bosso-Martelo et al.[11], 2016), fez com que a formulação básica (Cimento de Portland) fosse modificada pela incorporação de radiopacificadores que podem prejudicar seu desempenho biológico e suas propriedades físico mecânicas. Pesquisadores informam que em MTA, a incorporação de óxido de bismuto ao cimento Portland na proporção de 1:4 (% em peso) proporciona uma ótima radiopacidade (Torabinejad et al.[71], 1995; Islam et al.[40], 2006). Porém, destacam que a presença desse radiopacificador aumenta a citotoxicidade do CSC e afeta adversamente suas propriedades físicas diminuindo sua resistência à compressão, aumentando o seu tempo de cura e sua porosidade

(Camilleri et al.[17], 2004; Coomaraswamy et al.[19], 2007; Camilleri[12], 2008; Gomes Cornelio et al.[31], 2011; Antonijevic et al.[6], 2014).

Além disso, o MTA apresenta outros inconvenientes, como longo tempo de presa (165 +/- 5 min), o tom acinzentado (Torabinejad et al.[73], 1995) e as alterações de cor que tanto a formulação original como o MTA branco causam na estrutura dental, limitando seu uso clínico nos procedimentos restauradores e estéticos (Felman et al.[26], 2013; Keskin et al.[43], 2015).

Em 2011, a Septodont lançou uma nova formulação de CSC visando resolver estes problemas (Septodont[66], 2012; Watson et al.[78], 2014; Kaup et al.[42], 2015). Com um tempo de presa de 12 minutos, o Biodentine™ é composto por uma mistura de silicato tricálcico, silicato dicálcico, carbonato cálcio, óxido de ferro e óxido de zircônio, e o liquido contém cloreto de cálcio e um polímero solúvel em água (Septodont[66], 2012). Segundo o fabricante, a tecnologia utilizada para produzir o Biodentine™ (Active Biosilicate Technology™) resulta em um CSC mais puro e com menor nível de contaminantes, comumente encontrados nos materiais a base de cimento Portland (Septodont[66], 2012).

A redução do tempo de presa do Biodentine™ é atribuída a maior área de superfície devido ao uso de partículas de pó de tamanho menor e ao uso do cloreto de cálcio, um reconhecido acelerador de reações químicas (Kogan et al.[45], 2006; Wiltbank et al.[79], 2007). Quanto a radiopacidade do Biodentine™, a literatura é controversa. Alguns autores destacam que o óxido de zircônio, utilizado como radiopacificador, não oferece o contraste radiográfico necessário, sendo bastante difícil de identificar esse material nas radiografias (Bachoo et al.[7], 2013; Caron et al.[18], 2014). Estudos independentes têm relatado valores de radiopacidade que variam entre 4 e 5 mm de Al (Camilleri et al.[15], 2013; Grech et al.[32], 2013), enquanto outros relatam valores muito baixos de 2,8 a 1,5 mm Al (Tanalp et al.[70], 2013; Kaup et al.[42], 2015).

Mais recentemente foi desenvolvido o TheraCal LC (Bisco Inc, Schamburg, IL, EUA), um composto de CSC modificado por resina, que permite controlar o tempo de presa por fotoativação (Bisco[9], 2015). Esse material é composto por óxido de cálcio, partículas de silicato de cálcio, vidro de estrôncio, sílica, sulfato de bário,

zirconato de bário e resina (BisGMA e PEGDMA). De acordo com a sua patente, a radiopacidade desse novo material pode ser fornecida pelo fluoreto de itérbio, sulfato de bário e óxido de bismuto (Suh et al.[68], 2008). Porém, segundo Gandolfi et al. informam que o material não apresenta radiopacidade necessária (1,07 mm Al) para o adequado acompanhamento radiográfico do material (Gandolfi et al.[28], 2012).

Além das diferenças destacadas, existem inúmeras dúvidas sobre a efetiva contribuição destes novos cimentos em cada um dos diversos usos para os quais tem sido indicado e qual dos sistemas é o que apresenta os melhores desempenhos. Tanto o MTA, como o Biodentine™ e o Theracal LC podem ser classificados como materiais bioativos, ou seja, materiais que reagem ativamente com os tecidos circundantes favorecendo a deposição e formação de compostos químicos em sua superfície. Várias análises podem ser realizadas para caracterizar e classificar os materiais bioativos. Dentre estas avaliações podem-se destacar as caracterizações da morfologia superficial realizada por microscopia eletrônica de varredura (MEV), composição química elementar (EDX) e estrutural (DRX), composição molecular (FTIR) e bioatividade (formação de compostos químicos na superfície do material quando imerso em fluido corpóreo simulado (Camilleri et al.[15], 2013; Camilleri et al.[16], 2014; Kim et al.[44], 2014). Embora tenha sido observado que Biodentine apresenta melhores propriedades bioativas do que TheraCal, existem dúvidas sobre a estabilidade dos depósitos formados; só fosfatos de cálcio amorfo é observado e não apatita cristalina (Han et al.[35], 2013; Camilleri[13], 2014; Kim et al.[44], 2014).

Há um outro tipo de material com elevadas propriedades bioativas, conhecido como “vidro bioativo” (VB, composição 46,1% molar de SiO_2, 24,4% molar de Na_2O, 26,9% molar de CaO e 2,6% molar de P_2O_5, denominado 45S5 e Bioglass) (Jones[41], 2013). Ele é capaz de formar uma ligação com tecidos ósseos (Hench[37], 2006) por um mecanismo complexo baseado na lixiviação iônica, dissolução controlada de vidro e precipitação de uma camada de apatita na sua superfície (Jones[41], 2013).

Recentemente, uma revisão sistemática confirmou que o tratamento da dentina desmineralizada com vidro bioativo leva a formação de apatita (Fernando et al.[27], 2017). Além disso, um estudo avaliando o uso de vidro bioativo (de tamanho

micrométrico) como substituto da dentina confirmou a sua bioatividade sobre este tecido (Gjorgievska et al.[30], 2013). No entanto, a adaptação na cavidade mostrou-se pobre e foi sugerido a utilização de partículas de menor tamanho (Gjorgievska et al.[30], 2013).

Atualmente, com a técnica de "sol-gel", é possível sintetizar vidro bioativo de tamanho nanométrico (Zheng et al.[83], 2017). Estas nanopartículas de vidro bioativo são biomateriais atraentes, devido à sua grande área superficial específica, e sua elevada razão de superfície por volume. Devido a estas características, elas apresentam melhor bioatividade, porque podem acelerar o processo de formação de depósitos de apatita comparado com vidro bioativo micro-dimensionado (Hong et al.[39], 2009; Valenzuela et al.[74], 2012). Em particular, considera-se que seu uso é promissor em materiais compostos, devido a que suas características morfológicas facilitam a sua incorporação em outras matrizes (Zheng et al.[83], 2017).

Tem sido sugerido que a utilização de nanopartículas poderia melhorar as características específicas de certos materiais, como seria, neste caso, a bioatividade (Besinis et al.[8], 2015; Padovani et al.[56], 2015). Considerando o seu tamanho pequeno, é esperada que adições de nanopartículas em pequenas proporções são capazes de gerar efeito positivo desejado, sem modificar de forma significativa o material nas suas outras propriedades (Aguilar-Perez et al.[5], 2016). A incorporação de nanopartículas de vidro bioativo em CSC provavelmente aumentará sua bioatividade, melhorando a mineralização da dentina.

Considerando-se o rápido desenvolvimento dos CSC, com novos materiais tentando suprir as carências dos já disponíveis, torna-se premente a realização da revisão da literatura sobre as propriedades e uso dos novos CSC. Além disso, também é importante determinar a composição dos novos materiais de CSC melhorados, como Biodentine o TheraCal, além de avaliar a sua radiopacidad, uma vez que há antecedentes controversos a este respeito. Finalmente, considerando que a bioatividade é uma das características mais relevantes deste cimento, o presente trabalho visa avaliar propriedades bioativas do CSC com a incorporação de

nanopartículas de vidro bioativo, buscando desenvolver um novo cimento de CSC com melhores propriedades bioativas.

2 OBJETIVOS

OBJETIVO GERAL

Avaliar a composição química e radiopacidad dos cimentos a base de silicato de cálcio Biodentine e TheraCal e a contribuição do acréscimo de nanopartículas de vidro bioativo no Biodentine.

OBJETIVOS ESPECÍFICOS

1. Revisão da literatura sobre as propriedades e uso dos cimentos de silicato de cálcio na odontologia;
2. Avaliar a composição química e radiopacidade de cimentos de silicato de cálcio melhorados.
3. Avaliar o efeito da incorporação de nanopartículas de vidro bioativo sobre a bioatividade de cimentos de silicato de cálcio.

3 PUBLICAÇÕES

3.1 Publicação 1*

The current state of calcium silicate cements in restorative dentistry: A review

Revista: Revista de Facultad de Odontología Universidad de Antioquía

Revista abreviada: Rev. Fac. Odontol. Univ. Antioq.

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* ANEXO 1. O artigo segue as normas do periódico ao qual foi publicado.

REVISIÓN DEL ESTADO ACTUAL DE CEMENTOS DE SILICATO DE CALCIO EN ODONTOLOGÍA RESTAURADORA

THE CURRENT STATE OF CALCIUM SILICATE CEMENTS IN RESTORATIVE DENTISTRY: A REVIEW

CAMILA CORRAL NÚÑEZ[1], EDUARDO FERNÁNDEZ GODOY[2], JAVIER MARTÍN CASIELLES[3], JUAN ESTAY[4], CRISTIAN BERSEZIO MIRANDA[4], PATRICIA CISTERNAS PINTO[5], OSMIR BATISTA DE OLIVEIRA Jr.[6]

RESUMEN. *Los cementos de silicato de calcio se han aplicado como materiales dentales desde hace más de veinte años; sin embargo, su uso en el área de la odontología restauradora es más reciente. Mejores propiedades mecánicas y menores tiempos de endurecimiento le permiten ser indicados para una variedad de aplicaciones en las que este material se utiliza como sustituto dentinario, entre ellas el recubrimiento pulpar directo/indirecto y como base/liner cavitario. A su vez, también se podría utilizar como material para restaurar esmalte de manera temporal. El presente artículo busca revisar la evidencia científica disponible, enfocándola a sus aplicaciones en odontología restauradora. La información se obtuvo a partir de artículos originales de investigación científica y revisiones de literatura, publicados en revistas disponibles en bases de datos como Medline/Pubmed y Scielo, junto a la información técnica otorgada por los fabricantes de estos cementos. El presente trabajo describe la composición, el modo de empleo, la reacción de fraguado y la evidencia científica sobre las aplicaciones de los cementos de silicato de calcio en odontología restauradora.*

Palabras clave: *cementos de silicato, cementos dentales, materiales dentales, recubrimiento de la pulpa dental.*

Corral-Núñez C, Fernández-Godoy E, Martin-Casielles J, Estay J, Bersezio-Miranda C, Cisternas-Pinto P et al Revisión del estado actual de cementos de silicato de calcio en odontología restauradora. Rev Fac Odontol Univ Antioq 2016; 27(2): 425-441. DOI: http://dx.doi.org/10.17533/udea.rfo.v27n2a10

Abstract. *Calcium silicate cements have been used as dental materials for more than twenty years; however, their use in restorative dentistry is more recent. Better mechanical properties and shorter curing times make them suitable for a variety of applications in which they are used as a substitute of dentin, including direct/indirect pulp capping and as cavity base/liner. These materials may also be used to restore enamel temporarily. This article seeks to review the available scientific evidence with a focus on their applications in restorative dentistry. The information was gathered by reviewing original scientific research articles and literature reviews published in journals available in databases such as Medline/Pubmed and Scielo, along with technical information provided by the manufacturers of these cements. This article describes the composition, instructions for use, and curing reaction of calcium silicate cements, as well as the scientific evidence on their applications in restorative dentistry.*

Key words: *silicate cements, dental cements, dental materials, dental pulp capping.*

Corral-Núñez C, Fernández-Godoy E, Martin-Casielles J, Estay J, Bersezio-Miranda C, Cisternas-Pinto P et al. O. The current state of calcium silicate cements in restorative dentistry: A review. Rev Fac Odontol Univ Antioq 2016; 27 (2): 425-441. DOI: http://dx.doi.org/10.17533/udea.rfo.v27n2a10

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THE CURRENT STATE OF CALCIUM SILICATE CEMENTS IN RESTORATIVE DENTISTRY: A REVIEW *

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Abstract. Calcium silicate cements have been used as dental materials for more than twenty years; however, their use in restorative dentistry is more recent. Better mechanical properties and shorter curing times make them suitable for a variety of applications in which they are used as a substitute of dentin, including direct/indirect pulp capping and as cavity base/liner. These materials may also be used to restore enamel temporarily. This article seeks to review the available scientific evidence with a focus on their applications in restorative dentistry. The information was gathered by reviewing original scientific research articles and literature reviews published in journals available in databases such as Medline/Pubmed and Scielo, along with technical information provided by the manufacturers of these cements. This article describes the composition, instructions for use, and curing reaction of calcium silicate cements, as well as the scientific evidence on their applications in restorative dentistry.

Key words: silicate cements, dental cements, dental materials, dental pulp capping.

INTRODUCTION

Calcium silicate cements are gradually making their way through the various materials used in restorative dentistry. While it is true that they have long been used in endodontics, their introduction in restorative dentistry is more recent. Mineral Trioxide Aggregate (MTA) was the first of this type of materials to be developed (patented in 1995). As a result of the favorable properties of biocompatibility and bioactivity of this first material, many manufacturers developed other MTA-like products, such as MTA Angelus (Angelus Soluções Odontológicas, Brazil) and Endo COM Sealer (Egeo, Argentina).[1] These materials are largely used in endodontic treatments; however, they can also be used in restorative dentistry, including direct pulp capping.[1, 2]

Later, in 2011, a new material appeared in the market: Biodentine™ (Septodont, Saint Maur des Fossés, France), which is indicated as a replacement for both coronal and root dentin.3 The quick hardening of this cement, in comparison with previous calcium silicates, and its improved mechanical properties made it suitable for definitive restorations in replacing dentin and as a temporary cement to restore enamel.[3] Other materials, such as TheraCal LC (Bisco Inc., Schamburg, IL, USA), have been developed more recently suggesting the use of calcium silicates mixed with composite resins, which can control hardening times since they are light-curing materials.

One of the greatest advantages of calcium silicates is their so-called bioactive property. Bioactive materials are defined as those that "trigger a biological response in the tissue-material interface, resulting in the formation of bonding between material and tissue".[4, 5] This is evident in the favorable responses observed when the material is in contact with soft tissues such as pulp and periodontal tissues, or with hard tissues such as dentin.[6-8]

Research shows that these cements can produce strong bonding with dentin through an area of mineral infiltration, with formation of mineral tags and diffusion of calcium and silicon to dentin.[9, 10] In addition, in contact with pulp tissue, the material can stimulate dentin bridge formation.[11] This is why the study of these materials is of particular interest to restorative dentistry, due to their potential use as restorative materials in case of deep dentin cavities, as well as in direct and indirect pulp capping therapies.

Since calcium silicate cements have expanded their range of indications, including some for restorative dentistry, and due to the emergence of new silicate calcium-based materials with important variations in their compositions, it is necessary to review the available scientific literature that assess their use in these applications, due to the lack of reviews focusing on this particular topic. Therefore, this review article aims to evaluate the available information on calcium silicate cements, focusing on their possible applications in restorative dentistry. Thus, it seeks to update the clinicians' knowledge about calcium silicate cements, helping them make more informed clinical decisions.

We conducted a topic review by searching on the Pubmed/Medline and Scielo databases using the following key words: calcium silicate cement, tricalcium silicate cement, Mineral Trioxide Aggregate, BiodentineTM, TheraCal LC, and bioactive

cements. In addition, technical information provided by manufacturers of these cements was collected. Data analysis involved reviewing the abstracts of available articles, and only those that were considered relevant to the subject matter were included.

MINERAL TRIOXIDE AGGREGATE

Mineral Trioxide Aggregate (MTA) was the first calcium silicate developed for dental use; it was developed and patented by Torabinejad and White in 1995.[12] Its main component is Portland cement type I (calcium silicate), known as regular Portland cement used in construction, which is added bismuth oxide (Bi_2O_3) to provide it with radiopacity.[12]

Composition and instructions for use

The original MTA formula was developed at the University of Loma Linda, United States, and was manufactured by Dentsply International (ProRoot MTA and Tooth-Colored MTA; Dentsply-Tulsa Dental, Tulsa-USA; Dentsply-Johnson City- USA). However, various similar products have been manufactured by other companies.1 Several studies have provided detailed information on the components of the main types of MTA, ProRoot MTA (Grey MTA or GMTA) and Tooth-Colored MTA (White MTA or WMTA).[13] The main components of GMTA are described in table 1, while the components of the white version, WMTA, are tricalcium silicate and oxide bismuth.[13] Studies comparing their composition have concluded that the difference in color between these materials is due to the lack of iron compounds in the WMTA formula. The observations have also found smaller particles in WMTA compared with GMTA, suggesting that this may be connected to the easier handling of WMTA.[13-15]

Table 1. Components of ProRoot MTA (Grey MTA or GMTA)

Powder	Liquid
Tricalcium silicate	Sterile water
Dicalcium silicate	
Bismuth oxide	

These cements are prepared by mixing MTA powder with sterile water in a 3:1 ratio.[16] A plastic or metal spatula is used to mix the cement in a glass lab, and the mix can be applied with an instrument such as a plastic or metal amalgam carrier to bring the material to the application site.[16]

Curing reaction

Mixing the powder with sterile water produces a colloidal gel which soon solidifies.[1] During this mixing, a hydration reaction occurs with the components, leading to the formation of calcium silicate hydrate (C-S-H) and calcium hydroxide as by-products.[17] Once the mixture starts, its pH value increases sharply, reaching to pH 12 after 20 m, which remains for three hours.[18, 19] Camilleri has studied the chemical changes that occur when the cement hydrates. It has been observed that a high proportion of calcium ions is released quickly, due to the dissolution of calcium hydroxide to a progressive decalcification of C-S-H. This occurs more rapidly than the release of silica and bismuth. It is thought that high levels of calcium released are connected to the biocompatibility of the material, since the elution of calcium hydroxide induces cell proliferation in vitro.[17] The curing time of the original version of MTA, GMTA, is 165 (+/– 5) m; [18] while WMTA takes 70 (+/– 8.5) m, with a working time of 5 (+/– 0.79) m.[20] This extended curing time is one of the biggest disadvantages of this type of material, and is one of the reasons why it cannot be used in single-session procedures.[2] Generally, clinicians should confirm the material's curing time in a second session before moving to the next step.

Scientific evidence supporting its applications in restorative dentistry

Direct pulp capping

Several review articles on the clinical applications of MTA have been published.[2, 21-24] In 2010, Parirokh and Torabinejad conducted a review suggesting that MTA is a promising material to preserve pulp vitality when used in direct pulp capping.[2] The authors state that this seems to be the material of choice for direct capping therapies, compared with other available materials for the treatment of permanent teeth.[2] In 2011, Aguilar and Linsuwanont published a systematic review on pulp therapy in permanent teeth with pulp exposure due to caries and treated with MTA and calcium hydroxide.[22] They found out that both materials can provide satisfactory results in pulp therapies, such as direct pulp capping and total or partial pulpotomy. Success

rates after 3 years were high: 72.9% for direct pulp capping (in patients aged 6 to 10 years), 99.4% for partial pulpotomy (in patients aged 6 to 27 years), and 99.3% for total pulpotomy (in patients aged 6 to 70 years).[22] However, the authors also stated that the evidence available at the time provided inconclusive information and they highlighted the need for more high-quality studies.[22]

These revisions were followed by four publications of trials comparing MTA and calcium hydroxide (a material generally used in vital pulp therapies in permanent teeth), most of which found better results for MTA.[25-28] Mente et al assessed 149 patients (with an average of 27 months follow-up) who were treated with direct capping following pulp exposure, using calcium hydroxide and MTA.[27] They observed a higher rate of success with MTA (78%) compared with calcium hydroxide (60%), concluding that MTA seems to be more effective in maintaining pulp vitality after direct capping.[27] Similar results were obtained by Hilton et al in their randomized clinical study, finding out a lower probability of failure in teeth treated with MTA (19.7%) compared with calcium hydroxide (31.5%).[25] Their study included a large sample of 376 patients who were monitored for up to 2 years.[25] On the other hand, Chailertvanitkul et al found no difference in terms of success rate when performing direct capping following pulp exposure with MTA and calcium hydroxide, but they did find a tendency to a higher probability of failure in pulp exposure greater than 5 mm^2, with a 2-year follow-up.[26] Leye et al found no significant differences in survival rates with MTA and calcium hydroxide at 6 months, but they did find differences at 3 months, with more favorable results for MTA.[28] A clinical study has also been published evaluating the preservation of the vitality of teeth treated with MTA in direct capping.[29] The success rate (conservation of vitality) after 3.6 (+/–1.1) years was 91.3%.[29]

The scientific evidence of the use of MTA in direct pulp capping therapies has been growing slowly. However, despite the favorable results for MTA, the amount of high-quality clinical studies is still low in this area, with follow-ups in the short and medium term.

BIODENTINE

Biodentine is a cement-based calcium silicate that has been advertised as “the first all-in-one material” to be used whenever dentin has been damaged.[30] This material

has been developed in an effort to produce a calcium silicate with better mechanical properties [31] and hardening times.[32]

Composition and instructions for use

Biodentine comes as a capsule containing powder and a liquid contained in a vial. According to the mixing instructions, the contents of the vial should be squeezed into the capsule and then mixed in an amalgamator for 30 s. Depending on preference, the contents of the capsule is applied with a porta amalgam, a spatula, or a device such as the Root Canal Messing Gun.[33] Table 2 shows the components as stated by the manufacturer.[32]

Table 2. Components of Biodentine, modified from Septodont [32].

Powder	Liquid
Tricalcium silicate	Calcium chloride
Dicalcium silicate	Water-soluble polymer
Calcium carbonate and oxides	
Iron oxide	
Zirconium oxide	

According to the manufacturer, the Active Biosilicate Technology™ used to produce Biodentine™ ensures the purity of calcium silicate, as opposed to other calcium silicate cements based on Portland cement which contain non-purified mixtures with low concentrations of metal impurities.[32] However, recent studies have found remains of arsenic, lead, and chromium in Biodentine™.[34] Moreover, the found levels of arsenic are higher than those allowed by ISO 9917. Nevertheless, the same components have been reported for MTA, but since the release in the physiological solution is minimal, they have been considered safe.[34]

The manufacturer has suggested that this material's reduced curing time (12 m) compared to traditional calcium silicates such as MTA (70 ±8,5 m)[20] is due to the smaller size of the powder particles, thus allowing a greater reaction area. In addition, the calcium chloride added to the liquid has proven to be a powerful accelerator of reaction in these materials.[35, 36] The manufacturer also states that the material's best mechanical properties are due to the lack of impurities, along with the addition of

calcium carbonate powder and the optimal density of the powder obtained in the mix.[32] The water soluble polymer probably plays an important role in achieving better powder density, since an easy to-handle mix is obtained with a smaller amount of water.[32] Finally, it has been supposed that zirconium oxide is added in order to provide it with radiopacity, since it is has been used in other materials for the same purpose.[37] This is another important difference with MTA, where radiopacity is provided by means of

oxide bismuth—a compound that according to some authors has an unwanted effect on the material—.[38]

Curing reaction

The curing reaction of Biodentine™ is similar to that of MTA, with production of hydrated calcium silicates and calcium hydroxide as by-products,[39] but the speed of reaction is greater in Biodentine™.[40, 41]

The initial curing reaction takes about 12 m.[41] However, impedance spectroscopy has shown that the reaction continues for up to 14 days.[42] The study of Villat et al suggests that the complete hydration reaction of this silicate is much slower than that observed in the acid-base reaction of glass ionomer cements, concluding that this reaction could continue for months, extending ion exchange, decreasing porosity, and increasing the material's mechanical properties.[42]

Applications in restorative dentistry

Biodentine™ is indicated as a substitute for dentin in both the coronal portion and the root.[32] Indications for restorative dentistry include:

- Temporary restoration of enamel
- Final restoration of dentin
- Restoration of lesions of large and/or deep cavities (sandwich technique)
- Restoration of deep cervical or root lesions
- Direct and indirect pulp capping

The manufacturer indicates that applying the product does not require any prior treatment and that, once hardened, the cement should be treated as if it were healthy dentin. In the case of a sandwich technique using this material, it has been

recommended to fully restore the cavity in the first session, remove the outer part after one week to six months and cover it with composite resin.[33]

Scientific evidence supporting its applications in restorative dentistry

Direct pulp capping

Only one clinical study assessing Biodentine™ as a restorative material in direct pulp capping has been published to date. The study by Nowicka et al involved drilling pulp premolars extracted for orthodontic purposes capping with BiodentineTM (n = 11) and MTA (n = 11). After 6 weeks, most premolars showed formation of full dentin bridge, with absence of pulp inflammatory response; no significant differences were found between Biodentine™ and MTA during the observation period.[11]

Other articles have evaluated this material in animal models and in extracted molars. Tran et al conducted a study in rats also showing the consistent formation of dentin bridge in pulp cappings made with Biodentine™ and MTA.[43]

In these cases, the formed bridge is located in the affected area, with an ortodentine type of organization, in contrast to what was observed in treatments performed with calcium hydroxide, which showed cell inclusions similar to osteodentine.[43]

In their study, Laurent et al used healthy premolars recently extracted, which were kept in a culture and subjected to direct capping procedures with Biodentine™.[8] In all the evaluated premolars (n = 15), they noted the formation of mineralization foci, which increased in size until day 28—date of the last observation—. They also noticed the expression of markers of mineralization, suggesting that the material is capable of inducing the differentiation of odontoblast cells, involved in the formation of dentin tissue.[8]

However, the level of evidence in studies in animals or in ex vivo models is smaller than that achieved in clinical trials. Therefore, it is necessary to conduct additional clinical trials to provide more evidence on the use of this material in direct pulp capping.

Indirect pulp capping

A randomized clinical study recently evaluated the use of Biodentine™ in indirect pulp capping. The study analyzed 72 restorations (36 made with BiodentineTM and 36 with glass ionomer), with a follow-up of up to one year, finding out no differences

between the materials when measuring the clinical efficacy of pulp vitality conservation.[44] However, the authors noted that most teeth with apical radiotransparency (which was not detected at baseline with periapical x-rays but later with computed tomography) that decreased in size or were eliminated were treated with BiodentineTM,[44] while most recent lesions or their progression were found in teeth treated with glass ionomer.[44] These results were attributed to the bioactive characteristics of BiodentineTM, which have been reported from in vitro studies.[6-8, 45]

Permanent restoration of dentin and temporary restoration of enamel

Only one clinical study using Biodentine™ as a restorative material (of enamel and dentine) has been published to date.[46] This clinical, multicentered, randomized study with a three-year follow-up has only published the results obtained during the first year.[46] Class I and Class II restorations (n = 397) were performed with BiodentineTM and composite resin.[46] The initial assessment of the product shows very satisfactory results in terms of anatomical shape, marginal adaptation, and proximal contacts; however, the composite resin restorations showed better clinical behavior in these parameters after six months. This is why this study recommends that after 6 months it is necessary to remove the outermost layer of Biodentine™ and to restore with composite resin, leaving it only as permanent replacement of dentin and temporary replacement of enamel.[46]

THERACAL LC

TheraCal LC is a resin-modified calcium silicate cement developed by Bisco Inc. to be used as a barrier and protection of the pulp-dentin complex.[47] It comes in a syringe containing a photo-curable paste composed of calcium oxide, particles of calcium silicate, glass of strontium, barium sulfate, silica, barium zirconate, and resin (BisGMA and PEGDMA). According to the manufacturer, it is indicated for direct and indirect pulp capping applied as a cavity liner.[47]

In vitro studies have examined its physical and chemical properties.[48-50] Camilleri noted that, just as Biodentine™, TheraCal LC allows calcium phosphates to deposit on its surface when in contact with a saline solution;[50] however, the release of calcium ions is significantly lower that than of BiodentineTM.[49, 50] Gandolfi has demonstrated that TheraCal LC solubility is less than that of MTA and calcium

hydroxide; in addition, it has a weak radiopacity (less than required by standard ISO 6976) and can be light-cured in thickness of 1.7 mm.[48]

Since this material has been recently released, there are no clinical studies evaluating its behavior, and so far, there is only one published study in animals. Cannon et al conducted a study in primates performing direct pulp capping with TheraCal LC. The authors noted that teeth treated with this material had way more frequent dentin bridge formation, compared with calcium hydroxide and glass ionomer.[51]

DISCUSSION

Calcium silicates have long been part of the variety of dental materials available in the market; however, their use in restorative dentistry used to be limited to a few applications. Mineral Trioxide Aggregate (MTA), due to its excellent biocompatibility and bioactivity properties and low mechanical properties, is indicated for direct capping.[1, 2] In comparing it with alternative materials for these therapies, the scientific evidence shows favorable results when using it for these indications. Both systematic reviews and randomized clinical trials agree that this material is effective in maintaining vital teeth, with consistent formation of dentin bridge.[2, 22, 26, 27] The success rates of therapies using this material are comparable (and in some studies even higher) to conventional materials, such as calcium hydroxide (tempered in the studies by Hilton et al, Chailertvanitkul et al, and Leye Benoist et al, and non-tempered in the study by Mente et al).[25-28] However, it is necessary to conduct more long-term clinical studies in order to provide further evidence.

The development of Biodentine™ expanded the indications of calcium silicates in restorative dentistry. The composition of this material is similar to that of MTA but with significant variations that imply changes in its physical properties.[32] The alleged best mechanical properties of Biodentine™, as well as its reduced curing time, allows it to be used in a wide range of indications. It has been suggested as a material for dentin replacement in Class I, II and V cavities, and as replacement of enamel on a temporary basis (up to 6 months). These applications of Biodentine™ are brand new within calcium silicates, so further assessment is needed.

The results of clinical studies are promising. Biodentine™, in addition to having faster curing times compared to other calcium silicates, is easy to handling as it comes in capsules, allowing its clean and accurate application on teeth. This material makes a

very good alternative for the treatment of deep dental caries, including cases with reversible pulpal inflammation already occurring. Due to its bioactive properties, Biodentine™ may provide appropriate pulp-dentin sealing, favoring pulp response and changing the conditions of tissues affected by tooth decay.

TheraCal LC is a cement of recent availability in the market; as an advantage, it can be photo-curable.[47] The effects of this incorporation of resin to a calcium silicate cement have been explored in some in vitro studies;[48-50] however, no clinical studies have been reported to date.

The scientific evidence on calcium silicate cements is in general focused on materials that have been available for a longer time, such as MTA.[11, 44, 46] There are no many clinical studies on newer calcium silicate cements.[11, 44] This prevents from having more consistent information to determine their clinical efficacy. This level of evidence is certainly needed in order to make conclusions on these materials; it is therefore necessary to conduct evaluations through randomized clinical trials, in order to provide clinicians with accurate information for decision making.

CONCLUSIONS

Calcium silicates are alternative dental materials that can be used in direct and indirect capping, cavitary liner, dentin replacement in class I, II and V cavities, and as semi-permanent restorations of enamel.

Indications for direct and indirect capping are supported by clinical studies, especially in the case of MTA for direct capping. New applications proposed for these materials, such as replacement of dentin in class I, II and V cavities have still insufficient clinical evidence; however, in vitro studies show promising results.

The biocompatibility and bioactivity properties make of calcium silicates one of the restorative materials that offer a more favorable response by pulp tissue.

CONFLICTS OF INTEREST

The authors state that they have no conflict of interest.

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3.2 Publicação 2*

Radiopacity and chemical assessment of calcium silicate-based cements for direct pulp capping

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RADIOPACITY AND CHEMICAL ASSESSMENT OF CALCIUM SILICATE-BASED CEMENTS FOR DIRECT PULP CAPPING

Abstract New calcium-silicate-based cements with superior mechanical and handling characteristics have been commercialized for pulpar protection. However, differences in their composition affect their radiopacity and compromises their visualization on radiographs. The aim of this study was to evaluate their chemical composition and radiopacity. Methods: Discs of 10 mm x 1 ± 0.1mm were prepared of Biodentine, TheraCal LC, Dycal and GC Fuji IX (n=5). The samples were radiographed directly on an photostimulable phosphor (PSP) occlusal plate adjacent to an aluminium step wedge. The radiopacity of each specimen was determined according to ISO 9917/2007. Statistical analyses were carried out using ANOVA and Tukey test at a significance level of 5%. The chemical constitution of materials was determined by scanning electron microscopy and energy dispersive X-ray element mapping. In addition, quantitative chemical analysis was carried out. Results: The mean radiopacities of Biodentine, TheraCal LC, Dycal and GC Fuji IX were 2.79 ± 0.22, 2.17 ± 0.17, 3.18 ± 0.17 and 3.45 ± 0.16 mm of Al, respectively. TheraCal LC showed the lowest radiopacity compared to the other materials, followed by Biodentine. Dycal and GC Fuji IX radiopacity values did not present significant statistical differences. Scanning electron microscopy and energy dispersive X-ray analysis revealed the main constituents of the cements, which were different for all of them. The majority of the constituent elements were uniformly distributed, with the exception of zirconium in Biodentine, and tungsten in Dycal. Conclusions: The differences in chemical composition of new calcium silicate cements are reflected in differences in their radiopacity values.

Key Words

Biodentine, calcium silicate-based cement, chemical composition, TheraCal LC, radiopacity.

Introduction

Direct pulp capping consists of covering the vital pulp exposed with a dental material (1). The aim is to maintain pulpal health, allowing patients to retain teeth longer and at lower costs than alternative interventions (2). The dental material used should promote formation of new reparative dentin (3) and to present sufficient radiopacity to allow its identification in radiographic examinations.

Conventionally, calcium hydroxide-based materials have been used due to their ability to stimulate pulp repair (3). However, they have some disadvantages such as high solubility and low mechanical properties (4). More recently, calcium-silicate-based cements have demonstrated promising clinical results (5, 6). Mineral Trioxide Aggregate (MTA) was the first of these cements, introduced in 1993 (7). Nevertheless, it presents some disadvantages that discourage its use for pulp capping, such as long setting time and discoloration (6). New calcium-silicate-based cements are commercialized that overcome some of these drawbacks, such as Biodentine, with faster setting time (8) and better colour stability (9, 10). In addition, TheraCal was developed, which is a light-cured, resin-modified material.

The changes in composition of the new calcium-silicate-based cements include changes in the radiopacifier incorporated. Bismuth oxide is added to MTA in a 1:4 (wt.%) ratio (11). However, several studies have shown that bismuth oxide affects negatively its biocompatibility (12, 13) and physical properties (14, 15). In addition, bismuth oxide leaches out from the material with time (16). Consequently, alternative radiopacifiers have been used in Biodentine and TheraCal. Biodentine uses zirconium oxide as a radiopacifier (17), however, independent research conducted have reported a wide range of radiopacity values (ranging from 1.5 to 4.1 mm Al) (18-21). According to TheraCal's patent, ytterbium fluoride, barium sulphate or bismuth oxide could be incorporated as radiopacifiers (22), and to the best of our knowledge only one study has evaluated its radiopacity (23).

A research gap had been identified regarding the radiopacity of Biodentine and the scarce number of studies investigating TheraCal´s radiopacity and composition.

Therefore, this work aims to close this gap by evaluating the chemical composition and radiopacity of new commercial calcium-silicate-based cements

Materials and methods

The calcium-silicate-based cements used in this study were BiodentineTM (Septodont, Saint- Maur-des-Fossés, France) and TheraCal (Bisco Inc., Illinois, USA). Dycal (Dentsply, Connecticut, USA) and GC Fuji IX Capsule (GC America Inc., Illinois, USA) were used as reference.

Radiopacity evaluation

The radiopacity test was performed according to the methods described by the ISO 9917:1 and 2 for water-based cements (24, 25). The dental materials were mixed following manufacturer instructions and placed into moulds measuring 1 mm in thickness and 10 mm in diameter. The specimens were covered with glass coverslips, and assembled with a clamp to ensure the correct thickness. TheraCal was supplied by the manufacturer in pre-mixed syringes, it was dispensed into the mould, then covered with glass coverslip, assembled with a clamp and polymerized with a light-curing unit for 20 secs (EliparTM LED, 3M ESPE, Seefeld, Germany), through upper and lower coverslips. Specimens with notorious clefts, voids, discontinuities or air bubbles were discarded. Thickness was checked with a digital calliper, and only specimens whose thickness fell in the range of 1.0 $\pm$ 0.1 mm were used.

Five specimens of each material were placed directly on a PSP occlusal plate (48x54 mm, FireCR Dental, 3DISC Corp., Daejeon, Korea) adjacent to an aluminium (99% pure) step wedge with step height ranging from 1-10 mm (Odeme, Santa Catarina, Brazil, **Fig. 1a**). Radiographs were taken with an X-ray appliance model Myray RXAC (Imolia, Italia), at tube voltage of 70 Kv, current of 8 mA, exposure time of 0.4 s, and target-film distance of 40 cm. A custom 3D printed device was used to ensure standardization of focal distance and angulation of the central ray.

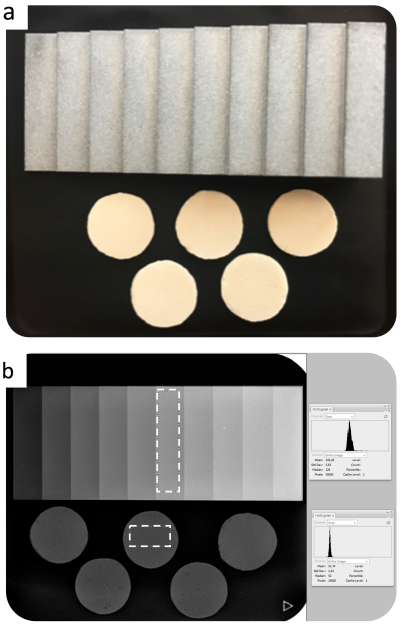

Figure 1. Cement samples with aluminium step wedge placed on a PSP occlusal plate (a). Digital image with average grey value reading in software (b).

The radiographs were processed (FireCR Dental Reader, 3DISC Imaging. Virginia, USA) and a digital image was obtained. The digital image file was exported to a greyscale analysis software Adobe Photoshop CS6 (Adobe, California, USA). The average grey value (between 0 and 255, with 0 representing pure black and 255 pure white) for each material sample and each step of the wedge were measured (Fig. 1b). A graph of aluminium thickness (in mm) vs. grey value of each aluminium step was plotted and the logarithmic trend line was drawn. The radiopacity of each specimen, expressed in mm Al, was then determined using the equation of the trend line.

Elemental analysis of cements

For each material, one of the specimens was dehydrated, mounted on aluminium stubs and gold coated. Specimens were examined using a scanning electron microscope (Jeol JSM-IT300LV, JEOL USA Inc., USA) coupled to an energy dispersive x-ray detector for elemental analysis with computer-controlled software Aztec EDS system (Oxford Instruments, Abingdon, UK). Micrographs of the material

surface at 1000x magnifications with element EDX mapping were captured and EDX quantitative chemical analysis was carried out.

Statistical analysis

The data of radiopacity test was evaluated using SPSS software (SPSS Inc., Chicago, IL, USA). The results obtained for all materials were submitted to normality test Shapiro-Wilk. After proving the normality of the sample data distribution, the data were submitted to ANOVA test and post hoc Tukey test at a 5% level of significance.

Results

Radiopacity measurements

The results for radiopacity evaluation are shown in Table 1. TheraCal showed the lowest radiopacity values, followed by Biodentine. Dycal and GC Fuji IX Capsule showed the highest radiopacity value, and presented statistically similar radiopacity values ($p > 0.05$).

Table 1. Radiopacity values of dental cements in equivalent mm of aluminium.

Materials	Means (±standard deviation)
TheraCal LC	2.17 ± 0.17^{a}
Biodentine	2.79 ± 0.22^{b}
Dycal	3.18 ± 0.17^{c}
GC Fuji IX GP	3.45 ± 0.16^{c}

Different letters indicate a statistically significant differences (analysis of variance and post hoc Tukey, $p < 0.05$).

Compositional analysis

Major elements (<10 wt.%) of Biodentine are oxygen, carbon and calcium; its minor element components (1-10 wt.%) are silicon, zirconium and chlorine. The constituent elements display homogeneous distribution, with the exception of zirconium which is

observed as accumulations (**Fig. 2 a-c**). TheraCal is composed mainly by carbon and oxygen, with silicon, calcium, strontium, barium and aluminium as minor element components. The constituent elements display homogeneous distribution (**Fig. 2 d-f**). Dycal and GC Fuji IX EDX analysis are shown in **Fig. 3**.

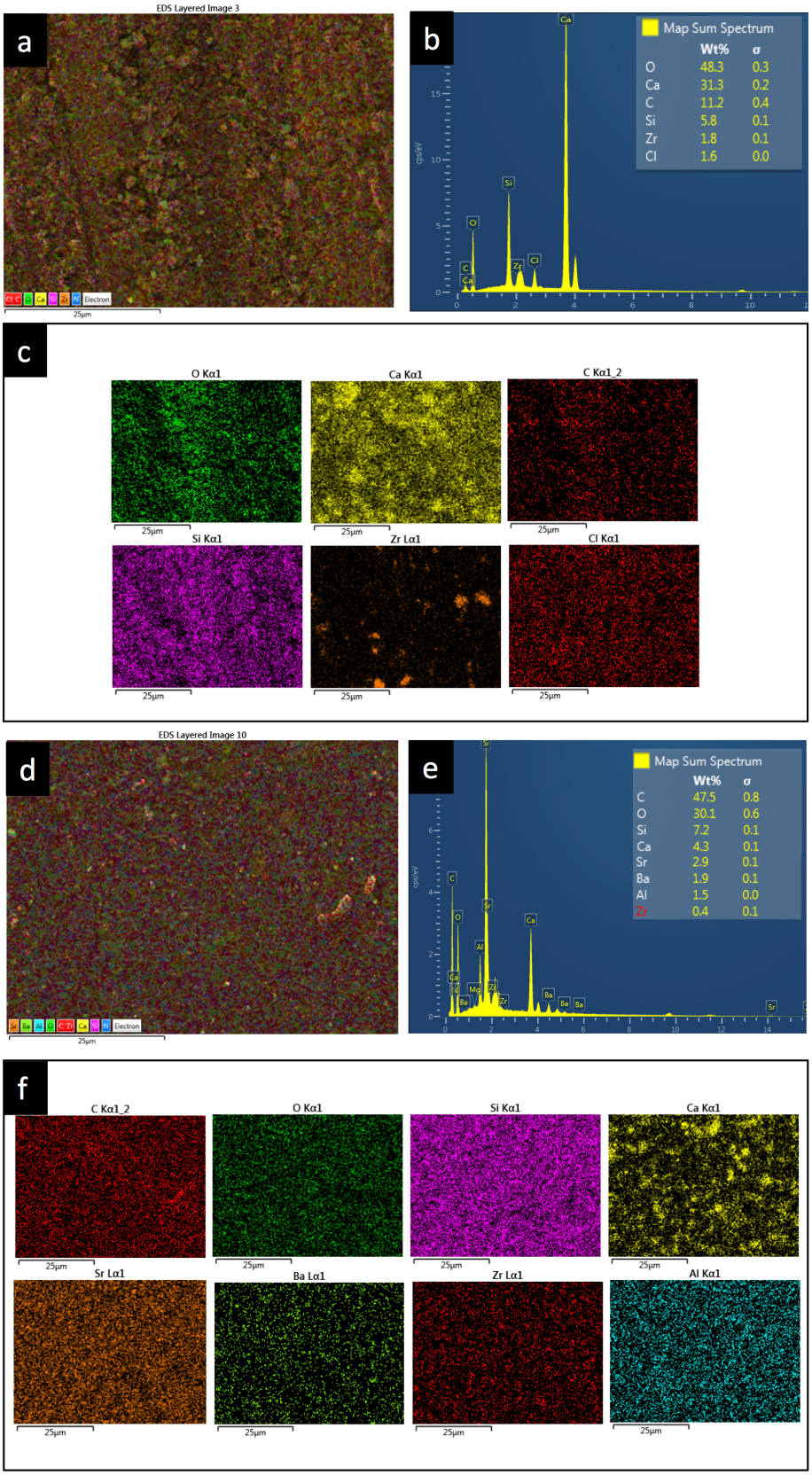

Figure 2. Representative SEM elemental distribution maps at 1000 x magnification (a, d) with EDX bulk analysis (b, e) and elemental distribution maps of radiopaque elements (c, f) of Biodentine (a-c) and TheraCal (d-f).

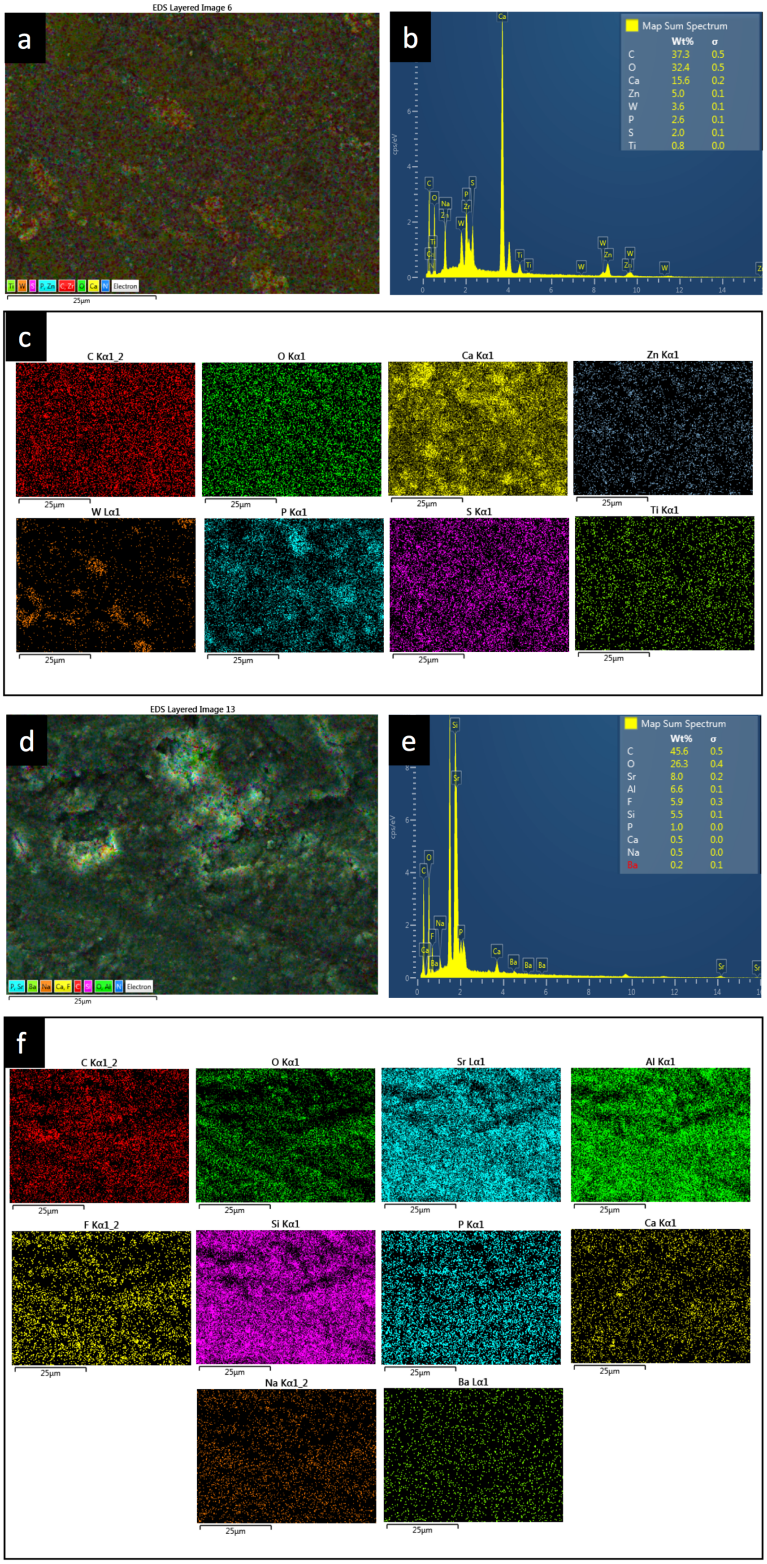

Figure 3. Representative SEM elemental distribution maps at 1000 x magnification (a, d) with EDX bulk analysis (b, e) and elemental distribution maps of radiopaque elements (c, f) of Dycal (a-c) and GC Fuji IX (d-f).

Discussion

In the present study, the radiopacity and chemical composition of new commercial calcium silicate-based cements was investigated. Pulp capping treatment involves the direct cement application on the pulp. This material should promote dental pulpal complex reparation (3) and have enough radiopacity to allow its identification. However, since neat calcium-silicate cements have low intrinsic radiopacity, it is necessary to incorporate different radiopacifier to increase it (26).

Biodentine™ presented an equivalent radiopacity of 2.79 ± 0.22 mm Al. Other studies have reported a wide range of radiopacity values for this cement. Camilleri et al. reported a radiopacity between 4 and 5 of mm Al (18), Grech et al reported 4.1 mm Al (19), Tanalp et al. reported 2.8 mm Al (20) and Kaup et al reported radiopacity value of 1.5 mm Al (21). The variation in the results obtained in different studies could be due to a poor standardization of the manufacturing of the material, as it has been previously suggested (21), or due to methodological variations with other studies such as film to focus distance (21), step wedge with different mm increments (18, 19) and different conditions to store samples (18, 19). In the present study a 3D printed device was used to standardize the film to focus distance and to assure the central ray is perpendicular to the film. Digital radiography was used rather than conventional radiographs, in contrast with other studies (21), avoiding the use of optical densitometer and possible errors due to film processing (27). In addition, the aluminium step wedge used in these tests had 1 mm increment as it has been suggested by ISO standards (24, 28), to carry out a more accurate analysis.

According to ISO 6876:2002 “Dental root canal sealing materials”, the radiopacity should be equivalent to not less than 3 mm Al (28), and according to ISO 9917:2007 “Water based cements” should be at least 1 mm Al. Therefore, according to the results of this study Biodentine does not comply with ISO 6876 requirements of radiopacity, but it does for ISO 9917:2007. However, regarding ISO 6876 for root canal sealing material, when Biodentine is used in pulp capping treatments it is not used for permanent obturation of the root canal, therefore if it is consider strictly, the material for this application is out of the scope of this standard (28). Similarly, ISO 9917 is only for cements that set by acid-base reaction (24), which is not the case for

calcium-silicate-based cement that set by hydration reaction. Nevertheless, several authors have reported the difficulty to differentiate Biodentine when assessed with radiographies, which has been mentioned as a disadvantage of the material (29). Since Biodentine is indicated for a wide range of indications, including pulp capping, pulpotomy, repair of root/furcation perforation, apexification, among others, (30) the thickness of the applied material for these different indications varies, but it is still relevant for all of them to be able to easily distinguish the cement from anatomical structures on a radiograph. The need for ISO standards requirements specific for calcium silicate-based cements (conventional and resin-modified) in relationship to their specific clinical applications has been already mentioned (23).

The radiopacity is related to the atomic number of the elements that constitute the material and its physical density. Tooth structures are mainly made up of calcium and phosphorus, with 20 and 15 atomic number respectively, therefore materials with higher atomic number will be easier to detect in radiographs. In this study it was demonstrated the presence of zirconium as a minor component (1.8 wt.%) of Biodentine, which has an atomic number of 40. Interestingly, zirconium distribution in contrast with other element constituent of the cement is uneven, which is probably the resulting distribution of zirconium oxide particles present in Biodentine powder. Zirconium oxide particles have been detected in set Biodentine, and it has been suggested that these particles do not take part of the setting reaction of the cement (18). Previous studies have shown that Biodentine powder presents a 5.1 wt.% of zirconium oxide (18), however, higher incorporations of zirconium oxide (30%) have shown to increase radiopacity values to more than 6 mm Al maintaining adequate physical properties (27).

The present study found oxygen, carbon, calcium, silicon, zirconium and chlorine as constituent elements of set Biodentine. This elemental composition correlates well with the components of the cement reported by the manufacturer (17), with powder composed of tricalcium and dicalcium silicate, calcium carbonate and oxide, zirconium oxide and liquid composed of calcium chloride and hydrosoluble polymer (17). Camilleri et al. have previously described the presence of these elements with SEM/EDX analysis, with the exception of carbon and chlorine (18). However, in the same study it was described the presence of calcium carbonate particles in the set

cement, engulfed in the calcium silicate hydrate (18), therefore carbon should be present. Chlorine has been added in the form of calcium chloride to the liquid to accelerate the reaction (17).

TheraCal LC presented an equivalent radiopacity of 2.17 ± 0.17 mm of Al, which was lower than the radiopacity of Biodentine. To the best of our knowledge and probably due to the novelty of this cement, only Gandolfi et al. have previously evaluated its radiopacity, reporting an equivalent radiopacity of 1.07 mm Al (23). Similar to Biodentine, TheraCal LC is not cover by the scope of ISO 9917, part 2 for resin-modified cements nor for 6876:2002, due to the same reasons. TheraCal LC is indicated as a material for direct and indirect pulp capping. The manufacturer suggests the application of the material in layers of maximum 1 mm thickness, which for pulp capping procedures should be just enough to seal the communication between the pulp and the oral cavity (31). Consequently, it is relevant that the material is sufficiently radiopaque to be able to distinguish it, even when the material is used as a thin layer.

According to the TheraCal LC's patent, the radiopaque material incorporated in the cement could be ytterbium fluoride, barium sulphate or bismuth oxide (22). In this study, the presence of strontium (2.9 wt.%), barium (1.9 wt.%) and zirconium (0.4 wt.%) was demonstrated, which have high atomic numbers (38, 56 and 40 respectively). The addition of barium sulphate and strontium zirconate to calcium silicate cements, as radiopacifiers, has been tested before (26, 32). Cement replaced by 25- 30% barium sulphate showed radiopacity values greater than 3 mm Al (26). However, it has been reported the leaching of barium and strontium in calcium silicate-based cements (32), therefore it would be interesting to assess if the leaching also occurs in a resin-modified calcium silicate, such as TheraCal LC.

According to EDX analysis, in addition to the radiopaque elements TheraCal LC presents carbon, oxygen, silicon, calcium, and aluminium. Other study have reported similar composition, however in contrast with the present study the presence of zirconium has been previously reported (33). In agreement with other studies (33, 34), it was also demonstrated the presence of aluminium in the cement. Aluminium has been associated with several adverse health effects, including neurotoxicity,

genotoxicity, Alzheimer´s disease, dementia, hyperactivity, and learning disorders in children (34). However, it was not seen a significate increase of Al plasma levels in liver of rats when TheraCal LC was used implanted in tooth socket, in contrast with other calcium silicate cements, such as MTA, that showed increased plasma Al levels (34).

Dycal and GC Fuji IX were included in this study as reference materials, both presented radiopacity values higher than 3 mm Al. Dycal is a self-setting calcium hydroxide-based cement used for pulp capping treatments (4). The presence of tungsten was detected, which possess a high atomic number of 74 that probably provides the radiopacity for this cement. Similar to Biodentine with zirconium as radiopacifier, tungsten was also observed distributed in accumulations in the cement. GC Fuji IX is a glass ionomer cement used as dentine replacement, strontium and barium as radiopacifiers were detected in its composition.

Conclusions

The differences in chemical composition of new commercial calcium silicate cements are reflected in differences in their radiopacity values. Biodentine presented zirconium as radiopacifiying element and higher radiopacity values than TheraCal LC, which presented barium and strontium as radiopacifiers.

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3.2 Publicação 3*

Enhanced bioactive properties of Biodentine™ modified with bioactive glass nanoparticles

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Original Article
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Enhanced bioactive properties of Biodentine™ modified with bioactive glass nanoparticles

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Abstract

Objective: To prepare nanocomposite cements based on the incorporation of bioactive glass nanoparticles (nBGs) into Biodentine™ (BD, Septodent, Saint-Maur-des-Fosses Cedex, France) and to assess their bioactive properties. Material and Methods: nBGs were synthesised by the sol-gel method. BD nanocomposites (nBG/BD) were prepared with 1 and 2% nBGs by weight; unmodified BD and GC Fuji IX (GIC, GC Corporation, Tokyo, Japan) were used as references. The *in vitro* ability of the materials to induce apatite formation was assessed in SBF by X-ray diffraction (XRD), attenuated total reflectance with Fourier transform infrared spectroscopy (ATR-FTIR), and scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis. BD and nBG/BD were also applied to dentine discs for seven days; the morphology and elemental composition of the dentine-cement interface were analysed using SEM-EDX. Results: One and two percent nBG/BD composites accelerated apatite formation on the disc surface after short-term immersion in SBF. Apatite was detected on the nBG/BD nanocomposites after three days, compared with seven days for unmodified BD. No apatite formation was detected on the GIC surface. nBG/BD formed a wider interfacial area with dentine than BD, showing blockage of dentine tubules and Si incorporation, suggesting intratubular precipitation. Conclusions: The incorporation of nBGs into BD improves its *in vitro* bioactivity, accelerating the formation of a crystalline apatite layer on its surface after immersion in SBF. Compared with unmodified BD, nBG/BD showed a wider interfacial area with greater Si incorporation and intratubular precipitation of deposits when immersed in SBF.

Keywords: Apatite-forming ability. Bioactive glass. Bioactivity. Biodentine. Nanocomposites.

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ENHANCED BIOACTIVE PROPERTIES OF BIODENTINE™ MODIFIED WITH BIOACTIVE GLASS NANOPARTICLES

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ABSTRACT

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Key words: Apatite-forming ability. Bioactive glass. Bioactivity. Biodentine. Nanocomposites.

INTRODUCTION

Biodentine™ (BD), a tricalcium silicate-based cement, was developed as a dentine substitute with clinical applications, including direct and indirect pulp capping[9] and the restoration of coronal dentine[16]. For some of these applications, the material may come into direct contact with pulpal tissues or with deeply carious dentine, making its biocompatibility and ability to seal in moist environments relevant clinical properties. It is well established that the placement of a permanent, properly sealed restoration is crucial to clinical success in indirect and direct pulp therapies[11], a property that closely relates to the bioactivity of the applied restorative material.

Bioactivity is defined as the capacity of a material to "elicit a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material"[2]. BD has been shown *in vitro* to induce the formation of calcium and phosphorous surface precipitates after immersion in biological fluids[7] and allows the formation of an interfacial layer with dentine[8, 13]. The mechanism of action proposed for BD effect on dentin is that first occurs a degradation of collagenous components due to an alkaline caustic effect, which forms a porous structure that facilitates the permeation of Ca^{2+}, OH^-, and CO_3^{2-} ions, mineralising this substrate[26]. *In vivo* studies demonstrated the formation of reparative dentine after BD pulp capping, which is evidence for its bioactivity, which results in a bond with the tissue[19]; however, there are concerns about the stability of this interfacial layer, since only amorphous-calcium-phosphate has been identified, not dentine-like hydroxyapatite[13].

Bioactive glass (BG) is a well-known bioactive ceramic material that has gained attention due to its ability to chemically bond with hard tissues through the formation of an apatite layer on its surface[12]. This apatite layer forms following solution-mediated dissolution of the glass[12]. It has been proposed that when BG is in contact with physiological fluids a series of reactions occurs, including: a rapid ionic exchange, creation of silanol bonds on the glass surface, increase of pH with formation of silica-rich region, migration of Ca^{+2} and PO_4^{3-} groups from the solution, which leads to the formation of an apatite layer[12]. For this ability, it has been incorporated into a range of products, including synthetic bone grafts to induce hard-tissue regeneration and toothpastes that treat hypersensitivity[12]. Recently, a study evaluated the use of BG as a dentine substitute; however, the BG particle size was

ca. 700 μm, resulting in poor cavity adaptation with empty spaces between the BG particles, and therefore the use of smaller particles was recommended[6].

Contemporary manufacturing processes allow the synthesis of nanometre-size BG, and BG nanoparticles (nBGs) have superior bioactivity compared to traditional micrometre-size BG, accelerating the formation of hydroxyapatite when it is incorporated into different biomaterials[23]. Brushing exposed dentin tubules with nBGs forms occlusing and tightly bonded hydroxyapatite rods that extend deep into the dentinal tubules[4]. Hydroxyapatite is less susceptible to degradation than other amorphous calcium phosphate phases with different Ca/P ratio than the stoichiometric crystalline hydroxyapatite (1.67)[25]. Therefore, it is expected that a hydroxyapatite based interface provides a more stable seal than an amorphous-calcium-phosphate-based interfase, as seen in BD/dentine interfase[13]. To the best of our knowledge, nBGs have not been utilised in the formulation or modification of calcium silicate-based cements. The incorporation of nBGs into BD could enhance BD's bioactive properties to stimulate the formation of crystalline hydroxyapatite, equivalent to that of dentine hard tissue.

The aim of this study was to prepare BD modified with nBGs and assess the *in vitro* and *ex vivo* bioactivity of these novel nanocomposite cements (nBG/BDs). The hypothesis is that the incorporation of nBG into BD improves its bioactivity, inducing a higher degree of mineralisation in dentin-cement interfase.

MATERIALS AND METHODS

Materials

This study included two commercially available cements, Biodentine (BD, Septodent, Saint-Maur-des-Fosses Cedex, France; Lot No. B08571) and GC Fuji IX Capsule (GIC, GC Corporation, Tokyo, Japan; Lot No. 1208061), and two experimental nanocomposite cements (nBG/BD), 1%nBG/BD and 2%nBG/BD, which were composed of BD with 1% nBGs and 2% nBGs by weight respectively.

Preparation of nanocomposite cements.

nBG particles (size ca. 40-70 nm) were synthesised by the sol-gel method, using the following previously-described molar composition: $58SiO_2$:40CaO:$5P_2O_5$[23]. 1%nBG/BD and 2%nBG/BD were prepared by adding 7 and 14 mg of nBG powder to

the BD capsule, respectively. The resulting nBG/BD nanocomposites powder was then mixed dry within the BD capsule in an amalgamator (Ultramat 2, SDI, Australia) for 30 seconds. Five drops of BD liquid were then added to the capsule before mixing, according to the BD manufacturer's instructions.

***In vitro* bioactivity assay**

The ability of the cement materials to induce the formation of apatite was assessed in acellular SBF, which was prepared as described by Kokubo et al.[15] using the standard ion composition (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM) and buffered at pH 7.4 at 37 °C. For this purpose, discs of material, measuring 7 mm in diameter and 2.5 mm thick, were prepared (for BD and GIC, the manufacturers' mixing instructions were followed) and allowed to fully set during incubation at 37°C and 100% humidity for 24 hours. Specimens were individually immersed in 50 mL of SBF, using polyethylene containers, for 3 or 7 days at 37°C in a thermostatic bath.

Materials and surface deposit characterisation

The structures of the set cement materials and surface deposits formed in the SBF assays were examined by X-ray diffraction analysis **(**XRD) on a D 5000 X-Ray Diffractometer (Siemens, Karlsruhe, Germany), using CuKα radiation within a 2θ range of 5 – 40 ° at a scanning speed of 1.2 °min^{-1}. In addition, attenuated total reflectance with Fourier transform infrared spectroscopy (ATR-FTIR) analysis was performed using a Cary 630 Agilent Technologies FTIR-ATR (Agilent Technologies Inc., Santa Clara, CA, USA) spectrometer in the 400-4000 cm^{-1} wavenumber range.
Discs, before and after 7 days of SBF immersion, were dehydrated, mounted on aluminium stubs, and coated with gold. Specimens were examined using a scanning electron microscope (Jeol JSM-IT300LV, JEOL USA Inc., USA) connected to an energy dispersive x-ray detector for elemental analysis with computer-controlled software, the Aztec EDS system (Oxford Instruments, Abingdon, UK). Micrographs of the material surface at 500x and 2000x magnifications were captured, and EDX quantitative chemical analyses were performed of BD, 1%nBG/BD and 2%nBG/BD samples after 7 days of SBF immersion at 2000x augmentation, representative areas for each material were analysed, and Ca/P ratio was calculated.

Morphological and elemental analysis of the dentine-cement interface

The use of human extracted molars was approved by the Ethics Committee of the Faculty of Dentistry, University of Chile (PRI-ODO 14/011). Two-mm-thick dentine discs were obtained from human third molars via a water-cooled, low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA). The smear layer of the dentine discs was removed with 37% phosphoric acid for 20 seconds, and the discs were rinsed and dried with absorbent paper.

BD, 1%nBG/BD and 2%nBG/BD were applied to one of the surfaces of the disc and allowed to fully set during incubation at 37°C and 100% humidity for 24 hours. An acid-etched dentin disc without cement application was used as control (CT). These specimens were then individually immersed in 50 mL of SBF, using polyethylene containers, for 7 days at 37°C in a thermostatic bath**.**

Discs immersed in SBF were then dehydrated and fractured perpendicular to the dentine-cement interface. Specimens were mounted on aluminium stubs using carbon-coated, double-sided adhesive tape and then coated with gold. The discs were analysed using a scanning electron microscope with energy dispersive X-ray analysis (Jeol JSM-IT300LV, JEOL USA Inc). For morphological observations, dentine close to the dentine-cement interface was analysed. Representative micrographs of the interface were captured at 1000x and 2000x. EDX quantitative chemical analysis and element mapping was carried out with 2000x augmentation.

Statistical analysis

The Ca/P molar ratio of EDX quantitative chemical analyses was calculated for BD, 1%nBG/BD and 2%nBG/BD after 7 days of SBF immersion (n=5) and statistical analyses were performed using SPSS 23.0 (SPSS Inc, Chicago, IL, USA) considering $p<0.05$ statistically significant. Normality was tested using Shapiro Wilk test, since data were not normally distributed for all groups, nonparametric Kruskal–Wallis and Mann–Whitney tests were performed for comparison of the groups.

RESULTS

Characterisation and *in vitro* bioactivity of cements

The XRD patterns of the set cement materials are shown in **Figure 1a.** The BD diffractogram exhibits the characteristic peaks that correspond to the crystalline

phases (baddeleyite, calcite, calcium silicate, and portlandite) that constitute the BD cement[1]. The nanocomposite samples showed similar XRD peaks, although they were less intense in the nanocomposite with greater nBG content (2%nBG/BD). The XRD patterns of the materials after immersion in SBF are shown in **Figure 1b-c.** After 3 days of incubation, nBG/BD nanocomposite diffractograms presented one of the most characteristic apatite reflections (ICDD® PDF No: 9-432) at 25.9° (**Figure 1a**). In the case of BD, the appearance of this apatite peak was only detected after 7 days of incubation (**Figure 1c**). On the other hand, no XRD peaks were detected for the GIC before or after SBF incubation.

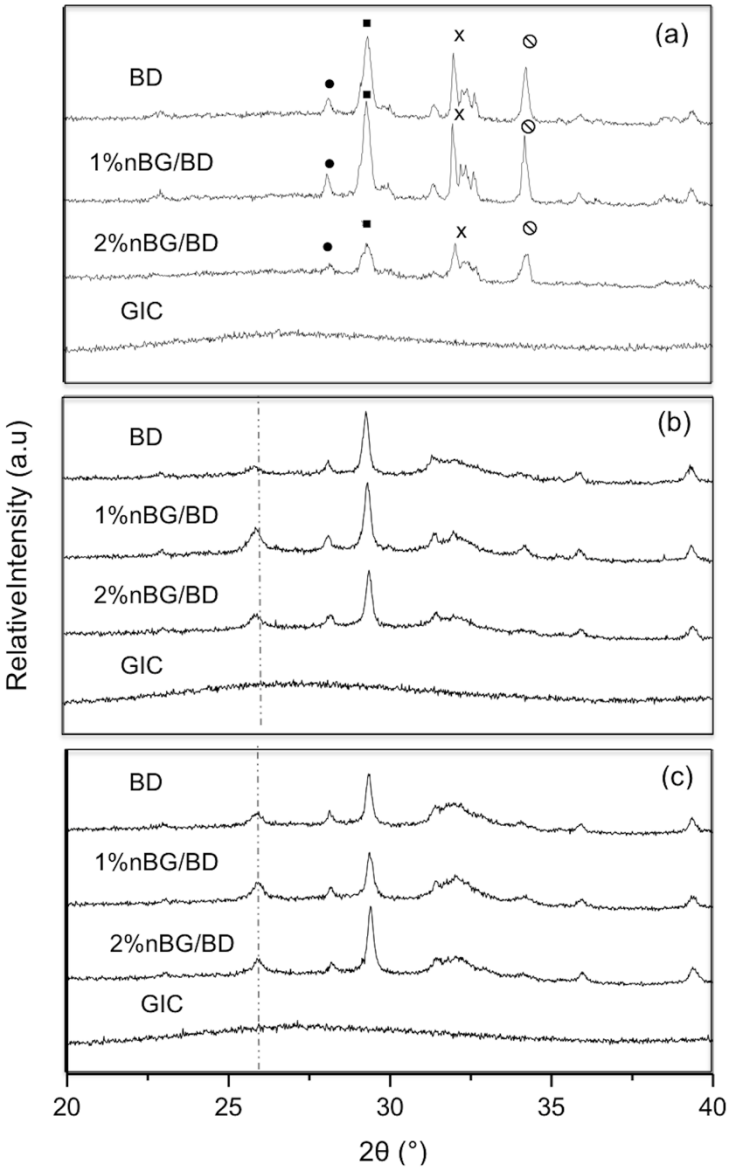

Figure 1. X-ray diffraction analysis of set cements (a) showing the main phases present (•: ZrO_2, Baddeleyite; ■ Calcite, $CaCO_3$; x: Calcium Silicate, Ca_3SiO_5; ⦸ Portlandite, $Ca(OH)_2$) and after 3 days (b) and 7 days (c) of immersion in SBF.

The ATR-FTIR analysis of the cement materials in the 1600-450 cm^{-1} region is shown in **Figure 2a.** The BD and nBG/BD spectra presented bands at 520 cm^{-1} that corresponded to silica vibration modes and at 1400, 870, and 710 cm^{-1} that were atributed to the vibrations of carbonate bonds[29] . GIC presents a wide and weak band around 1000 cm^{-1} caused by bridging (Si-O-Si) and non-bridging (terminal Si-O group) oxygen[1]. After 3 days of SBF immersion, the bands of the cement matrix components progressively disappeared in all of the materials (**Figure 2b**). The appearance of new bands at 560, 600, and 1040 cm^{-1} in the nBG/BF spectra is attributed to P-O vibrations of the apatite structure[17]; in contrast, these bands were hardly detected in the BD spectrum. After 7 days of immersion (**Figure 2c**), apatite bands became more intense for all the cement materials, but particularly for the nBG/BD nanocomposites.

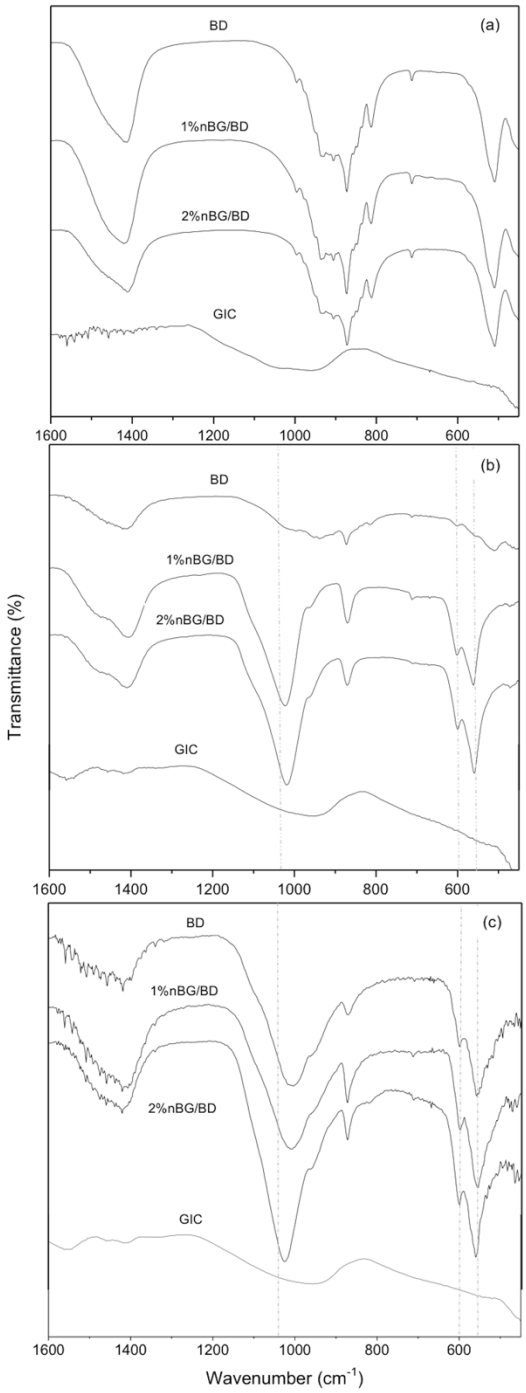

Figure 2. ATR-FTIR spectrum of set cements (a) and cements immersed in SBF for 3 days (b) and 7 days (c).

SEM/EDX images of samples before and after SBF soaking for 7 days are presented in **Figure 3.** The presence of mineral deposits can be observed on the cement surfaces after immersion in SBF. The BD surface was not significantly modified after a period of SBF immersion. In contrast, the formation of a new mineral phase was

clearly observed on the nBG/BD surfaces. Characteristic spherical apatite deposits covered the entire surface of the nanocomposites, and a denser and well-developed apatite layer was produced on 2%nBG/BD. EDX analysis revealed that the mineral deposits that formed on BD were mainly composed of carbon, oxygen, calcium, and phosphorous, with increasing phosphorous content on the nBG-modified cements. The median (minimum-maximum) Ca/P molar ratio values of BD, 1%nBG/BD, and 2%nBG/BD surfaces were 15.32 (11.05 - 21.40), 4.08 (2.67 - 4.35), and 1.88 (1.70 - 3.16), and the differences were statistically significant between all groups ($p<0.05$). On the other hand, no mineral deposit formation was detected on the GIC surface, and oxygen, aluminium, fluorine, silicon, and carbon appeared to be the main elemental components.

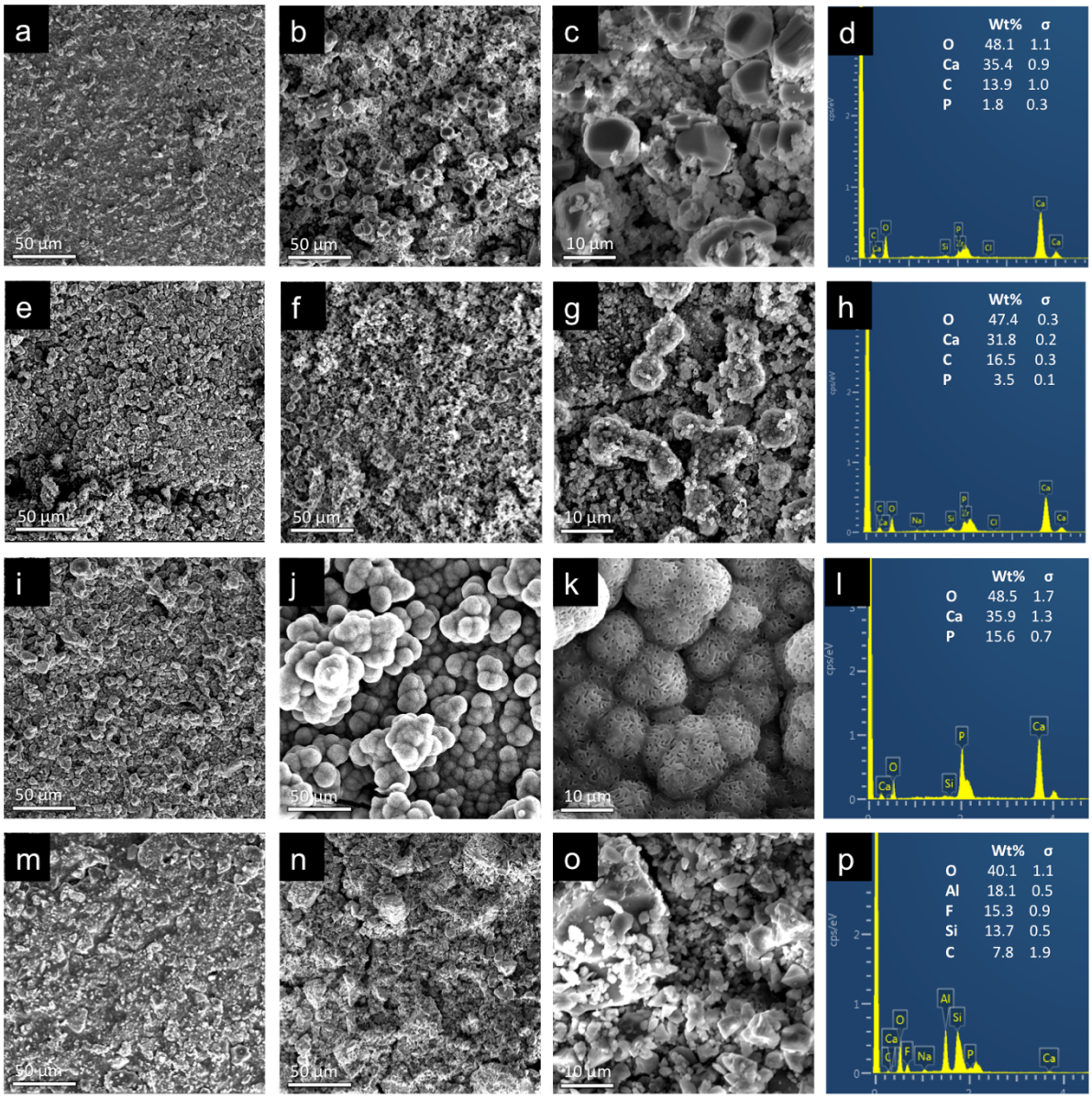

Figure 3. SEM micrographs and EDX analysis of set cements before (a, e, l, m) and after 7 days of SBF immersion (b-d, f-h, j-l, n-p) of BD (a-d), 1%nBG/BD (e-h), 2%nBG/BD (i-l), and GIC (m-p). Representative images at 500x (a-b, e-f, i-j, m-n) and 2000x (c, g, k, o) magnifications with EDX elemental analysis (d, h, l, p).

In vitro bioactivity of the dentine-cement interface

Cross-sectional SEM images of the cement-treated dentine interfaces after 7 days of immersion in SBF are shown in **Figure 4**. The presence of an interfacial mineralised layer in the dentine treated with both BD and nBG/BD nanocomposites can be observed, which can be identified as an area of morphologically altered dentine where dentine tubules appeared blocked by the new mineral phase. This

area was largely developed in the cement modified with nBGs; it appeared to be approximately four times thicker in the 2%nBG/BD nanocomposite than in BD. In contrast, in CT sample open and funnelled dentine tubules towards the surface are observed.

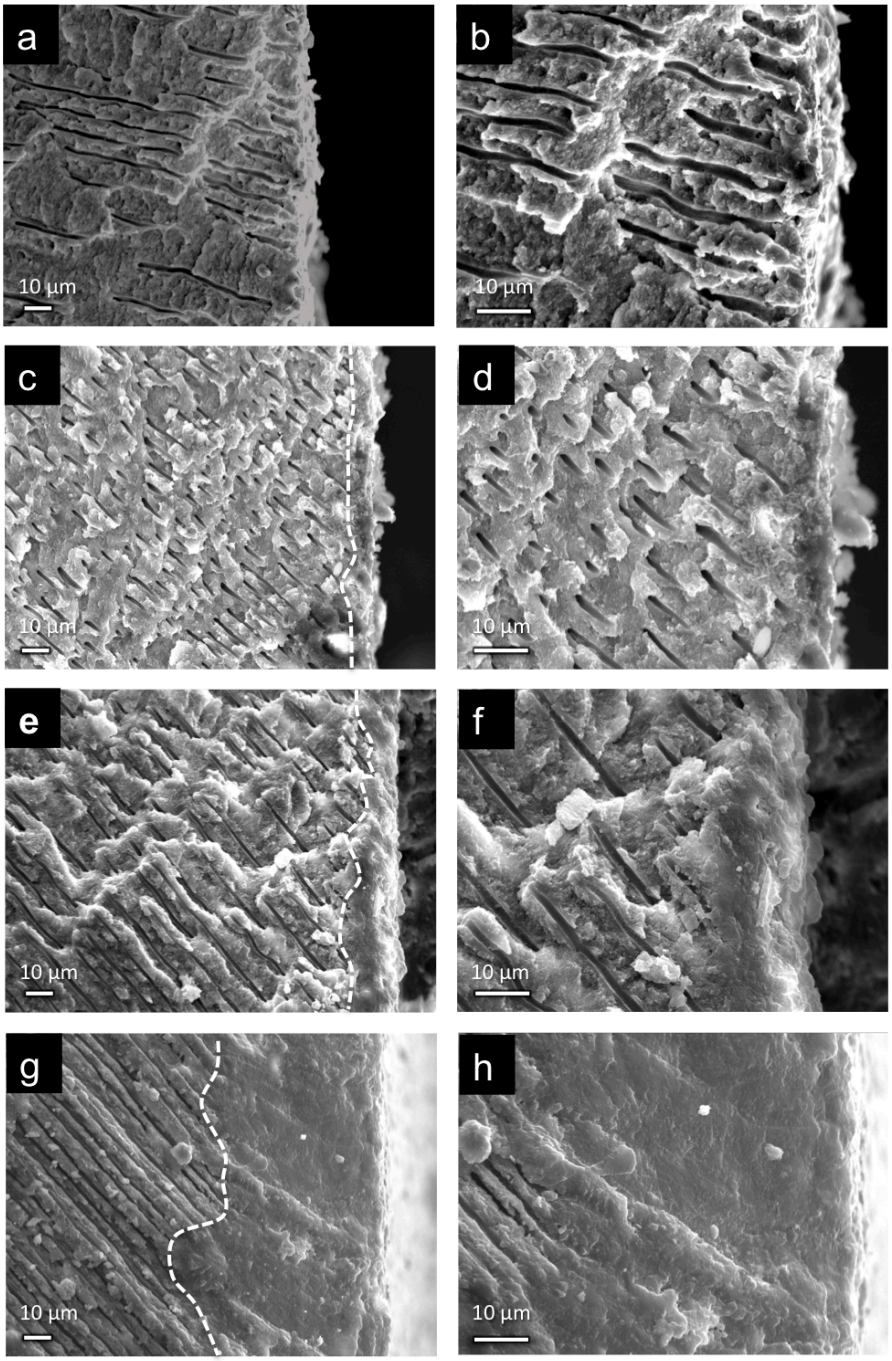

Figure 4. SEM micrographs of dentine-material interface of CT (a-b), BD (c-d), 1%nBG/BD (e-f), and 2%nBG/BD (g-h) samples, where material was on the left side before fracture. Dotted lines show the approximate position of the interfacial area.

Element mapping of BD and 2%nBG/BD samples (**Figure 5**) revealed the distribution of calcium, phosphorous, and silica within the dentine treated with the cements. BD- and 2%nBG/BD-treated dentine presented equivalent calcium and phosphorous distributions, whereas the 2%nBG/BD- treated dentinal tissue had a higher silicon density, which decreased with dentinal depth.

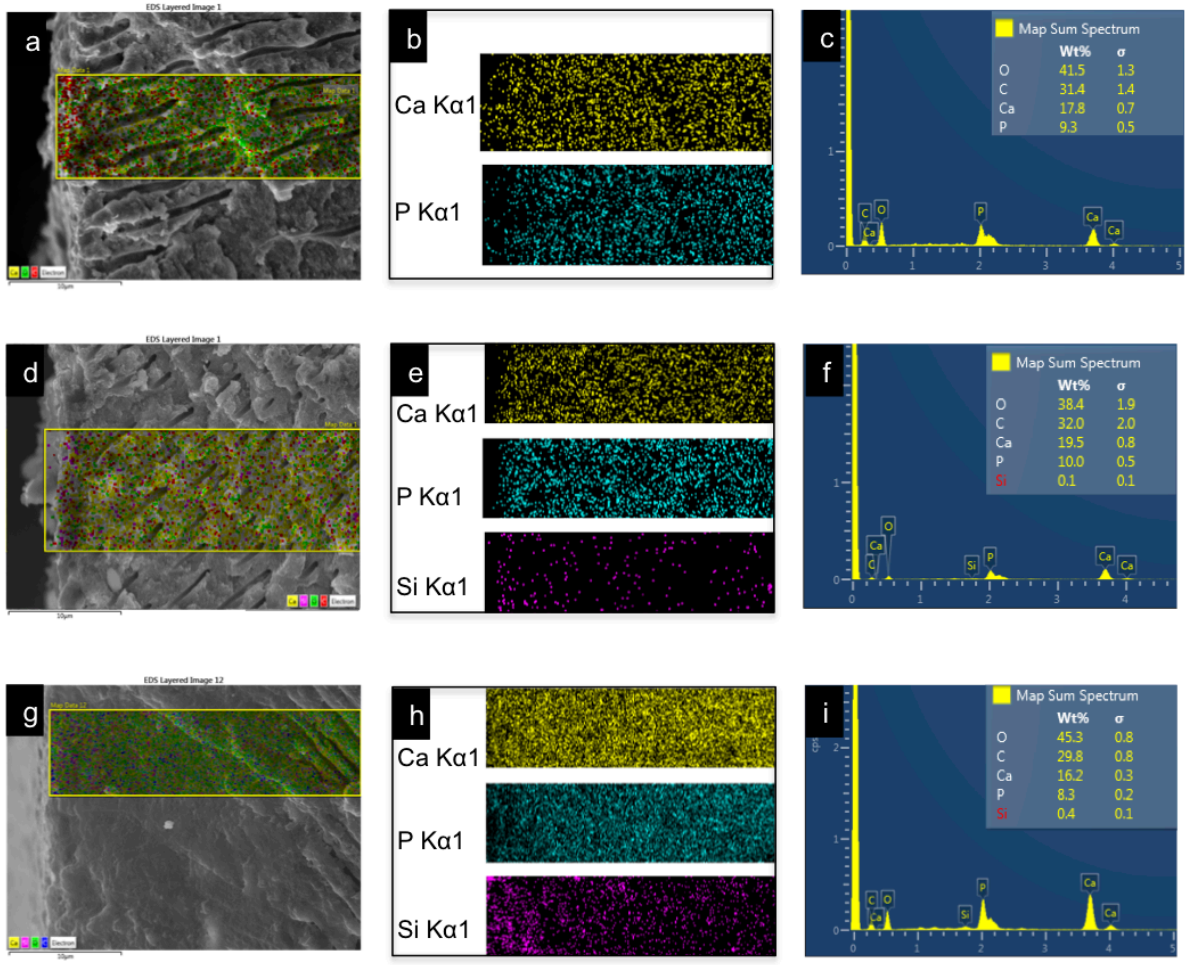

Figure 5. Micrographs and mapping images obtained by SEM-EDX of the CT (a-c), BD-dentine (d-f) and 2%nBG/BD (g-i) interfacial areas. SEM micrographs at 2000x with a selected area (a, d, g) for EDX mapping (b, e, h) and EDX bulk analysis (c, f, i).

DISCUSSION

This study demonstrated the feasibility of preparing bioactive cement nanocomposites, based on the incorporation of nBGs into the BD matrix. The assessment of nBG/BD nanocomposite bioactivity revealed accelerated apatite-phase formation, compared to unmodified BD. XRD analysis showed earlier apatite

crystallisation (after 3 days), as judged by the appearance of one of the most characteristic apatite reflections at 25.90°, corresponding to the (002) plane of the apatite crystal structure (ICDD® PDF No: 9-432). ATR-FTIR similarly confirmed the earlier presence of apatite deposits on the nanocomposites by the appearance of characteristic PO_4 vibrations of the crystalline apatite structure[17]. Consistently, SEM images showed surface precipitates with globular morphology on the nBD composites, which had the distinctive appearance of apatite deposits. Similar cauliflower-like clusters have been observed by others when nBGs were immersed in SBF[18]. In addition Ca/P value of nBG/BDs deposits was closer to the stoichiometric value of the hydroxyapatite structure (1.67)[25] than BD deposits; Taken together, the combined structural, chemical, morphologic, and elemental analyses showed that the nanocomposites possess enhanced bioactivity, expressed by an accelerated formation of the crystalline apatite-phase on their surface when immersed in SBF, compared to BD.

The improved bioactivity exhibited by the nanocomposites can be directly attributed to the incorporation of nBGs into the BD matrix. BG is a bioactive material well known for its ability to form an apatite layer on its surface through solution-mediated dissolution of the glass structure[10, 12]. Therefore, the results in this study suggest that when nBG was incorporated into BD and immersed in SBF, the bioactive ability of the nanoparticles was expressed. The dissolution of BG silicate network into ionic components forms a supersaturated solution, resulting in the nucleation of an amorphous calcium-phosphate layer, which transforms into apatite[14]. This phenomenon was observed in the nanocomposites after shorter periods of immersion compared to BD, which is due to the rapid dissolution of the nBGs that can form an apatite layer in periods of 24 hours[3]. The bioactivity of BG has been also confirmed in *in vivo* and *in vitro* studies, demonstrating the formation of a bone-like apatite layer on its surface in the living body, and bonding to bone through this layer[10, 12]. Other attempts to incorporate BG in restorative dental materials, such as resin adhesive [20] and glass ionomer cements, have been reported[27-28]; however, to date there are no published studies assessing the effects of its incorporation into calcium-silicate-based materials. Moreover, BG particles have been incorporated into resin adhesive and glass ionomer cements by using traditional micrometre-size particles[20, 27, 28], in contrast to the nanosized BG used in the current work.

nBGs present high surface area, with greater available surface for interaction with the physiological medium, which accelerates the ionic dissolution process of BG structure and, consequently, apatite formation and crystallisation[24]. This could explain why the incorporation of low levels of nBGs into the cement matrix (1% wt.) can improve the cement's bioactivity, accelerating the formation of crystalline apatite.

In addition, there was a distinctive mineral-rich interfacial layer within the dentine in contact with BD and the nanocomposite cements, which was thicker and had greater Si uptake in the dentine treated with nBG-modified cement. This may indicate that the nanocomposites have more prominent mineralisation ability than unmodified BD. The ability of calcium silicate cements to mineralise dentine when immersed in fluids has been previously reported[8, 21]. It is believed that the formation of this interfacial layer could be related to the good marginal seal of calcium silicate cements[8], supported by reports where immersion in fluids decreased the marginal leakage of apical plugs[19] and increased push-out strengths[22]. For BD, this interfacial layer has also been named the "mineral infiltration zone", which has been attributed to the dual effects of an alkaline caustic etching followed by mineral exchange[13]. This leads to the belief that the mineral deposits could reduce leakage by filling spaces along the interface and via interactions with dentine, such as intrafibrilar apatite deposition[7]. The precise role of Si uptake remains unclear, but it is thought that silica uptake in dentine may increase its acid resistance and physical strength[8].

In the present study, this interfacial layer was considerably thicker when BD was loaded with nBGs, possibly due to the additional effect of the bioactive ionic species produced by the nBG dissolution, which may diffuse along dentin tubules and induce the formation of apatite deposits. This new remineralisation layer could be formed by the penetration of the biomaterial into the open dentine tubules, and consequent transformation into an apatite phase by contact with the physiological medium. Silica is a key component of nBG and, following dissolution, could act as a nucleation site for the precipitation of dissolved calcium and phosphate ions to form hydroxyapatite[4]. This correlates well with the EDX mapping, where a higher Si content is observed in the mineral phase formed by nBG/BD. The presence of this morphologically different layer occluding previously open tubules suggested that the cement with nBG become lodge within the tubules, where apatite formation took place. Curtis et al. have reported similar effect by brushing micro and nano-BG on dentine with exposed tubules. It was found that when nano-BG is applied, a rod-like

apatite structure is formed within the tubules[4], whereas only a surface layer apatite onto the tubule opening was detected with micro-BG. Therefore, the use of BG with nanometric dimensions strongly favours the BG diffusion into the tubules and its consequent transformation into apatite phase. In addition, demineralised dentine can be faster remineralised by nBGs than with micron-sized BG as consequence of the substantially higher rate of dissolution of nBGs[3, 24].

To study the long term stability of this interfacial layer would be interesting in conditions that mimic the dynamics of its possible clinical applications. When BG has been added to other carriers, such as toothpastes, it has been demonstrated that the dentin tubule occlusion layer formed is resistant to acid challenge[5], and reduces significantly dentine permeability under simulated oral environment[30]. In addition, it has been suggested that the apatite rods formed into dentine tubules after brushing with nBGs slurries would have excellent retention[4]. This based on the observation that the observed continuous occluding apatite rods are tightly bonded to the dentine tubules, therefore the mechanical retention of these rods may be ensured as the angling and contours of the dentin tubule will avoid dislodgement of this interface[4]. Nevertheless, it would be of interest the study of the stability of the mineralized layer observed when nBG/BD were applied onto dentine.

The outstanding ability of BD cement modified with nBGs to accelerate the formation of dentine-like crystalline apatite inside of dentine tubules could have favourable clinical consequences. nBG-modified cement could generate a strongly mineralised seal when moisture control is difficult or could remineralise dentinal tissue in restorative therapies.

CONCLUSIONS

The incorporation of nBGs into BD enhances BD's *in vitro* bioactive properties, accelerating the formation of a crystalline apatite layer on its surface after a short period of immersion in SBF and greatly enhances the formation of a mineral-rich interfacial layer when in contact with dentine.

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4 DISCUSSÃO

Desde há muito tempo, os CSC são parte da variedade de materiais dentais disponíveis, sim embargo, seu uso em odontologia restauradora foi mais limitado a algumas aplicações (Prati et al.[59], 2015). MTA foi o primeiro cimento deste tipo, mas o desenvolvimento de Biodentine ampliou as indicações dos cimentos de silicatos de cálcio em odontologia restauradora (Koubi et al.[47], 2013; Kaup et al.[42], 2015).

As aclamadas melhores propriedades mecânicas e o reduzido tempo de presa de Biodentine permite que ele seja utilizado em uma ampla variedade de indicações, como material de substituição em dentina, e como substituição temporária de esmalte (Watson et al.[78], 2014; Kaup et al.[42], 2015). Estas aplicações de Biodentine são completamente novas entre os cimentos de silicatos de cálcio, assim sua avaliação é necessária. Mais recentemente, TheraCal LC tem sido comercializado, com a vantagem de ser fotopolimerizavel (Bisco[9], 2015). Os efeitos desta incorporação de resina a cimentos de silicato de cálcio têm sido investigados em alguns estudos in vitro, mas são necessários mais estudos in vivo (Camilleri[13], 2014; Camilleri et al.[16], 2014; Bortoluzzi et al.[10], 2015; Demirkaya et al.[23], 2016).

As alterações na composição dos novos cimentos com base de silicato de cálcio, incluem também alterações no agente radiopacificador incorporado. Devido aos cimentos de silicato de cálcio terem baixo valor de radiopacidade intrínseca precisa ser adicionado um radiopacificador (Camilleri et al.[14], 2010; Saghiri et al.[63], 2015; Bosso-Martelo et al.[11], 2016). Neste estudo Biodentine apresentado uma radiopacidade de 2,79 $\pm$ 0,22 mm Al e TheraCal LC de 2,17 $\pm$ 0,17 mm Al. Óxido de zircônio é usado como radiopacificador em Biodentine (Septodont[66], 2012), no entanto investigações independentes avaliando radiopacidade relataram grande variabilidade (de 1,5 a 4,1 mm Al) (Camilleri et al.[15], 2013; Grech et al.[32], 2013; Tanalp et al.[70], 2013; Kaup et al.[42], 2015). A variação nos resultados obtidos em diferentes estudos pode ser devido a uma pobre padronização da fabricação do material como tem sido sugerido anteriormente (Kaup et al.[42], 2015), ou devido a variações metodológicas com outros estudos (Camilleri et al.[15], 2013; Grech et al.[32], 2013; Kaup et al.[42], 2015). Uma inovação deste estudo foi usar um dispositivo impresso 3D para padronizar a distância de filme a foco e também para assegurar

que o raio central é perpendicular a amostra. Além disso, a radiografia digital foi utilizada em vez de radiografias convencionais, em contraste com outros estudos (Kaup et al.[42], 2015), evitando o uso de densitômetro óptico e possíveis erros devido ao processamento do filme (Cutajar et al.[22], 2011).

Embora não existam normas específicas de radiopacidade para os cimentos de silicato de cálcio, são normalmente utilizados dois padrões ISO para avaliar estes materiais. De acordo com a ISO 6876: 2002 “Dental root canal sealing materials”, a radiopacidade deve ser equivalente a pelo menos 3 mm Al (6876 ISO[1], 2002), e de acordo com a norma ISO 9917: 2007 “Water based cements” deve ser de pelo menos 1 mm Al (9917-1 ISO[2], 2007; 9917-2 ISO[3], 2010). Portanto, de acordo com os resultados deste estudo Biodentine e TheraCal LC não cumprem com os requisitos de ISO 6876 de radiopacidade, mas faz para a ISO 9917: 2007. No entanto, no que diz respeito ao ISO-6876 “Dental root canal sealing materials”, quando a Biodentine é utilizada em tratamentos de capeamento pulpar, não é utilizada para a obturação permanente do canal radicular, portanto, sob rígida consideração, o material para esta aplicação não está coberto por este padrão. Da mesma forma, o ISO-9917 é para cimentos de endurecimento por reação ácido-base, o que não é o caso do cimento à base de silicato de cálcio que endurecem por reação de hidratação.

É interessante também mencionar que alguns clínicos relataram a dificuldade de diferenciar a Biodentine quando avaliada com radiografias, que foi descrita como uma desvantagem do material (Bachoo et al.[7], 2013). Considerando-o acima e que a Biodentine é indicada para uma ampla gama de indicações, incluindo capeamento pulpar, pulpotomia, reparação da perfuração radiculares e de furca, apecificação, entre outros (Septodont[65], 2012). Espessura do material aplicado para estas diferentes indicações varia, mas ainda é relevante para todas as indicações distinguir o cimento das estruturas anatômicas em uma radiografia. A necessidade de requisitos de padrões específicos para cimentos à base de silicato de cálcio (convencional e modificado por resina) em relação às suas aplicações clínicas específicas já foi mencionada (Gandolfi et al.[28], 2012). Seria por tanto interessante avaliar diferentes maneiras de aumentar a radiopacidade destes cimentos. Assim, poder distingui-los em radiografias mesmo quando aplicado em camadas finas.

A radiopacidade está relacionada ao número atômico dos elementos que constituem o material e sua densidade física. As estruturas dentárias são constituídas principalmente por cálcio e fósforo, com números atômicos de 20 e 15 respectivamente, portanto, materiais com maior número atômico serão mais fáceis de detectar em radiografias. Neste estudo, demonstrou-se a presença de zircônio (o que possui um número atômico de 40) como componente menor (1,8% em peso) de Biodentine. De maneira interessante, o zircônio, em contraste com os outros elementos constituintes do cimento, não está uniformemente distribuído e é provavelmente a distribuição resultante das partículas de óxido de zircônio presentes no pó. As partículas de óxido de zircônio foram detectadas na Biodentine, e sugeriu-se que essas partículas não participam da reação do cimento (Camilleri et al.[15], 2013). Estudos anteriores mostraram que o pó de Biodentine contém 5,1% em peso de óxido de zircônio, no entanto, maiores incorporações de óxido de zircônio (30%) mostraram aumentar os valores de radiopacidade para mais de 6 mm, mantendo propriedades físicas adequadas (Cutajar et al.[22], 2011).

O presente estudo encontrou oxigênio, carbono, cálcio, silício, zircônio e cloro como elementos constituintes da Biodentine. Esta composição elementar correlaciona-se bem com os componentes do cimento relatados pelo fabricante (Septodont[66], 2012), com pó composto de silicato tricálcico e dicálcico, carbonato e óxido de cálcio, óxido de zircônio e líquido composto de cloreto de cálcio e polímero hidrossolúvel. Camilleri et al. descreveram anteriormente a presença desses elementos com análise SEM / EDX, com exceção de carbono e cloro (Camilleri et al.[15], 2013). No entanto, o mesmo estudo descreveu a presença de partículas de carbono como carbonato de cálcio (Camilleri et al.[15], 2013) e o cloro é adicionado na forma de cloreto de cálcio ao líquido para acelerar a reação (Septodont[66], 2012).

A TheraCal apresentou uma radiopacidade equivalente de 2,17 ± 0,17 mm de Al, que foi inferior à radiopacidade da Biodentine. Até o nosso conhecimento e provavelmente devido à novidade deste cimento, apenas Gandolfi et al. avaliaram previamente sua radiopacidade, relatando uma radiopacidade equivalente de 1,07 mm Al (Gandolfi et al.[28], 2012). Semelhante à Biodentine, a TheraCal não é coberta

pelo ISO 9917, parte 2, para cimentos modificados com resina (9917-1 ISO[2], 2007) e para 6876: 2002, pelas mesmas razões (6876 ISO[1], 2002). A TheraCal é indicada como um material para capeamento direta e indireta da polpa. O fabricante sugere a aplicação do material em camadas com uma espessura máxima de 1 mm, que, para os procedimentos de cobertura da polpa, deve ser suficiente para selar a comunicação com a polpa (Bisco[9], 2015). Consequentemente, é relevante que o material seja suficientemente radiopaco para poder distingui-lo como uma camada fina.

De acordo com a patente da TheraCal, o material radiopaco incorporado no cimento poderia ser fluoreto de itérbio, sulfato de bário ou óxido de bismuto (Suh et al.[69], 2008). Neste estudo, demonstrou-se a presença de estrôncio (2,9% em peso), bário (1,9% em peso) e zircónio (0,4% em peso), que possuem altos números atômicos (38, 56 e 40, respectivamente). A adição de sulfato de bário e zirconato de estrôncio a cimentos de silicato de cálcio, como radiopacificadores, foi testada por outros autores (Camilleri et al.[14], 2010; Xuereb et al.[80], 2016). O cimento substituído por 25 a 30% de sulfato de bário apresentou valores de radiopacidade superiores a 3 mm (Camilleri et al.[14], 2010). No entanto, a lixiviação de bário e estrôncio em cimentos à base de silicato de cálcio foi reportada (Xuereb et al.[80], 2016), portanto, seria interessante avaliar se a lixiviação também ocorre em um silicato de cálcio modificado com resina, como TheraCal.

De acordo com a análise EDX, além dos elementos radiopacos, a TheraCal apresenta carbono, oxigênio, silício, cálcio e alumínio. Outros estudos relataram composição similar (Camilleri[13], 2014). De acordo com outros estudos a presença de alumínio no cimento também foi demonstrada (Camilleri[13], 2014; Demirkaya et al.[23], 2016). O alumínio tem sido associado a vários efeitos adversos para a saúde, incluindo neurotoxicidade, genotoxicidade, doença de Alzheimer, demência, hiperatividade e transtornos de aprendizagem em crianças. No entanto, nenhum aumento significativo dos níveis plasmáticos de Al no fígado de ratos foi observado quando a TheraCal foi avaliada como implante, em contraste com outros cimentos de silicato de cálcio, como o MTA, que apresentaram níveis aumentados de Al de plasma (Demirkaya et al.[23], 2016).

Dycal e GC Fuji IX foram incluídos no estudo de analise químico e de radiopacidade como materiais de referência, ambos apresentaram valores de radiopacidade superiores a 3 mm Al. Dycal é um cimento à base de hidróxido de cálcio usado para tratamentos de cobertura de polpa (Desai et al.[24], 2009). A presença de tungstênio foi detectada, que possui um alto número atômico de 74. GC Fuji IX é um cimento de ionômero de vidro usado como substituição da dentina, foram detectados em sua composição estrôncio e bário, como seus radiopacificadores.

As aplicações de Biodentine e TheraCal LC são muito variadas, incluindo barreira de proteção dentinária, capeamento pulpar direto, reparação de perfuração radiculares e de furca, apicificação, retrobturação e em outros tratamentos endodônticos e restauradores para substituto da dentina. No caso de materiais com essas indicações existem também duas propriedades que são altamente desejáveis: citocompatibilidade e a sua bioatividade. De forma que, o material é capaz de gerar um bom selo da interface entre o biomaterial e o tecido pulpar, a fim de manter a saúde da polpa, impedir a infiltração de bactérias e promover a formação de ponte de dentina (Hilton[38], 2009). No que diz respeito à sua bioatividade, em estudos de biomineralização, este refere-se à promoção da formação de tecido duro, que é induzido pelo material (Rathinam et al.[60], 2015) e, geralmente, os cimentos de silicato de cálcio ganharam uma atenção considerável devido a suas melhores propriedades bioativas do que os materiais dentários convencionais (Rathinam et al.[60], 2015). No entanto, apesar de muita investigação, não há compreensão completa de como os eventos acontecem que levam à formação de tecido duro após a aplicação destes cimentos (Rathinam et al.[60], 2015). A bioatividade, entendido como a capacidade de um material para permitir que o depósito de apatita na sua superfície, quando exposta a soluções fisiológicas, tem sido investigada nestes materiais (Camilleri et al.[15], 2013; Camilleri et al.[16], 2014; Kim et al.[44], 2014). Embora tenha sido observado que Biodentine apresenta melhores propriedades bioativas do que TheraCal, existem dúvidas sobre a estabilidade dos depósitos formados, só fosfatos de cálcio amorfo é observado e não apatita cristalina (Han et al.[35], 2013; Camilleri[13], 2014; Kim et al.[44], 2014).

Neste estudo foi proposto o uso de nanopartículas de vidro bioativo para melhorar a bioatividade de um cimento de silicato de cálcio, Biodentine. Foi demonstrado a viabilidade da preparação de nanocompósitos de cimento bioativo, com base na incorporação de vidro bioativo nano-dimensionado na matriz de Biodentine (nBG/BD). A avaliação da bioatividade do nanocompósito nBG/BD revelou uma formação acelerada da fase de apatita, quando comparada com a Biodentine não modificada. A análise DRX mostrou cristalização de apatita após de 3 dias em nBG/BD, com a aparência de uma das reflexões da estrutura de cristal de apatita (ICDD® PDF No: 9-432) mais características (a 25,90°, correspondente ao plano 002). O ATR-FTIR, de forma similar, confirmou a presença precoce de depósitos de apatita nos nanocompositos pela aparência das vibrações características de PO_4 da estrutura de apatite cristalina (Lin et al.[49], 2009). Consistentemente, as imagens em SEM apresentaram precipitados de superfície com morfologia globular nos compósitos nBD/BG, que apresentaram a aparência de depósitos de apatita (Luz et al.[50], 2011). Além disso, o valor Ca/P dos depósitos de nBG/BDs foi mais próximo do valor estequiométrico da estrutura de hidroxiapatita (1,67) do que os depósitos de Biodentine (Wang et al.[77], 2003). Em conjunto, as análises estruturais, químicas, morfológicas e elementares combinadas mostraram que os nanocompósitos possuem uma bioatividade melhorada, expressa por uma formação acelerada da fase apatita cristalina em sua superfície quando imersa em SBF, em comparação com Biodentine não modificada.

A bioatividade melhorada exibida pelos nanocompósitos foi atribuída diretamente à incorporação de nBGs na matriz de Biodentine. Vidro bioativo é um material bem conhecido por sua capacidade de formar uma camada de apatita na sua superfície através da dissolução mediada pela dissolução da estrutura de vidro (Hench[37], 2006; Jones[41], 2013). Portanto, os resultados neste estudo sugerem que quando nBG foi incorporado em Biodentine e imerso em solução, a capacidade bioativas das nanopartículas foi expressa. A dissolução da rede de silicatos de vidro bioativo em componentes iônicos forma uma solução supersaturada, resultando na nucleação de uma camada amorfa de fosfato de cálcio, que se transforma em apatite (Kokubo et al.[46], 2004). Este fenômeno foi observado nos nanocompósitos após períodos mais curtos de imersão em comparação com Biodentine, o que é devido à rápida dissolução dos nanopartículas de vidro bioativo que podem formar

uma camada de apatita em períodos de 24 horas (Covarrubias et al.[20], 2015). A bioactividade do vidro bioativo também foi confirmada em outros estudos in vivo e in vitro, demonstrando a formação de uma camada de apatita semelhante a osso na superfície e a ligação ao osso através desta camada (Hench[37], 2006; Jones[41], 2013). Outras tentativas de incorporar vidro bioativo em materiais dentários restauradores, tais como adesivo de resina e cimentos de ionômeros de vidro, foram relatados (Yli-Urpo et al.[82], 2004; Yli-Urpo et al.[81], 2005; Osorio et al.[55], 2012). No entanto, até à data, não há estudos publicados que avaliem os efeitos da sua incorporação em materiais à base de silicato de cálcio. Além disso, as partículas de incorporadas em adesivos de resina e cimentos de ionômero de vidro foram usando partículas tradicionais de vidro bioativo de tamanho micrométrico (Yli-Urpo et al.[82], 2004; Yli-Urpo et al.[81], 2005; Osorio et al.[55], 2012), em contraste com o vidro bioativo nanodimensionado usado neste trabalho.

Além disso, foi formada uma camada interfacial rica em minerais dentro da dentina em contato com Biodentine e os cimentos nanocompósitos, que era mais espessa e tinha maior absorção de Si na dentina tratada com cimento modificado com nanopartículas de vidro bioativo. Isto pode indicar que os nanocompósitos têm uma capacidade de mineralização mais proeminente do que a Biodentine não modificada. A capacidade de cimentos de silicato de cálcio para mineralização da dentina quando imerso em fluidos foi relatada anteriormente (Reyes-Carmona et al.[61], 2009; Han et al.[34], 2011). Acredita-se que a formação desta camada interfacial pode estar relacionada ao bom selo marginal de cimentos de silicato de cálcio (Han et al.[34], 2011), apoiado por relatos onde a imersão em fluidos diminuiu o vazamento marginal (marginal leakage) (Martin et al.[52], 2007) e o aumento das forças de puxão (push-out strength) (Reyes-Carmona et al.[62], 2010). Para Biodentine, esta camada interfacial foi denominada "zona de infiltração mineral", é foi atribuída aos efeitos duplos de um ataque alcalino seguido de troca mineral (mineral exchange) (Kim et al.[44], 2014). Isso leva à crença de que os depósitos minerais poderiam reduzir o vazamento preenchendo espaços ao longo da interface e através de interações com dentina, como a deposição de apatita intrafibrilares (Han et al.[35], 2013). O papel preciso da absorção de Si permanece obscuro, mas pensa-se que a absorção de sílica na dentina pode aumentar a resistência ácida e a força física (Han et al.[34], 2011).

No presente estudo, esta camada interfacial foi consideravelmente mais espessa quando o Biodentine foi carregado com nanopartículas de vidro bioativo, possivelmente devido ao efeito adicional das espécies iônicas bioativas produzidas pela dissolução das nanopartículas de vidro bioativo, que pode difundir ao longo dos túbulos da dentina e induzir a formação de depósitos de apatita. Esta nova camada de remineralização poderia ser formada pela penetração do biomaterial nos túbulos dentinários abertos e consequente transformação em uma fase de apatita por contato com o meio fisiológico. A sílica é uma componente chave das nanopartículas de vidro bioativo e, após a dissolução, poderia atuar como um local de nucleação para a precipitação de íons de cálcio e fosfato dissolvidos para formar hidroxiapatita (Curtis et al.[21], 2010). Isso se correlaciona bem com o mapa de EDX, onde um maior conteudo de Si é observado na fase mineral formada por nBG/BD. A presença desta camada morfologicamente diferente ocluindo túbulos previamente abertos sugeriu que o cimento com nanopartículas de vidro bioativo se tornasse alojado dentro dos túbulos, onde ocorreu a formação de apatita. Curtis et al. relataram efeito similar escovando micro e nano-vidro bioativo na dentina com túbulos expostos. Verificou-se que quando o nano-vidro bioativo é aplicado, uma estrutura de apatita semelhante a uma haste é formada dentro dos túbulos, enquanto que apenas uma camada superficial de apatita na abertura do túbulo foi detectada com micro-vidro bioativo (Curtis et al.[21], 2010). Portanto, o uso de vidro bioativo com dimensões nanométricas favorece fortemente a difusão de vidro bioativo nos túbulos e sua conseqüente transformação em fase de apatita. Além disso, a dentina desmineralizada pode ser mais rápida remineralizada por nanopartículas de vidro bioativo do que com vidro bioativo de tamanho micrométrico como conseqüência da taxa substancialmente maior de dissolução das nanopartículas de vidro bioativo (Vollenweider et al.[76], 2007; Covarrubias et al.[20], 2015).

Seria interessante estudar a estabilidade a longo prazo desta camada interfacial em condições que imitam a dinâmica de suas possíveis aplicações clínicas. Quando o vidro bioativo foi adicionado a outros veículos, como pastas dentífricas, demonstrou-se que a camada de oclusão do túbulo da dentina formada é resistente ao desafio ácido (Farooq et al.[25], 2015) e reduz significativamente a permeabilidade da dentina em ambiente oral simulado (Zhong et al.[84], 2015). Além

disso, sugeriu-se que as hastes de apatite formadas em túbulos de dentina após a escovação com suspensões de nanopartículas de vidro bioativo teriam excelente retenção (Curtis et al.[21], 2010). Isto com base na observação de que as hastes de apatita observadas estão fortemente ligadas aos túbulos da dentina, portanto, a retenção mecânica dessas hastes pode ser assegurada à medida que os contornos do túbulo dentinário evitarão a deslocamento desta interface (Curtis et al.[21], 2010). No entanto, seria de interesse o estudo da estabilidade da camada mineralizada observada quando nBG/BD foram aplicados na dentina.

A excelente capacidade do cimento BD modificado com nBGs para acelerar a formação de apatita cristalina dentro dos túbulos da dentina poderá ter consequências clínicas favoráveis. O cimento modificado com nBG poderia gerar um selo fortemente mineralizado quando o controle de umidade é difícil ou poderia remineralizar o tecido dentinário em terapias restauradoras.

5 CONCLUSÃO

• Os cimentos de silicato de cálcio são atualmente uma boa alternativa para tratamentos de capeamento pulpar direto e indireto. Novos cimentos de silicato de cálcio cimentos como Biodentine e TheraCal LC com propriedades melhoradas têm aparecido, no entanto, devido à sua aparição mais recente, não há muitos estudos clínicos, mas estudos in vitro mostram resultados promissores.

• Os cimentos de silicato de cálcio melhorados, Biodentine e TheraCal LC apresentam diferente composição química com distintos agentes radiopacos, este é reflexado em diferencias em suas radiopacidades. A radiopacidade de TheraCal LC é menor do que Biodentine.

• A incorporação de nanopartículas de vidro bioativo em Biodentine aumenta as propriedades bioativas in vitro do Biodentine, acelerando a formação de uma camada de apatita cristalina na sua superfície após um curto período de imersão em solução.

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ANEXO 1

Universidad de Chile

Camila María Corral Núñez <camila.corral@u.uchile.cl>

Permiso para utilizar artículo en tesis de doctorado

4 mensajes

Camila María Corral Núñez <camila.corral@u.uchile.cl> 18 de abril de 2017, 10:19
Para: Revista Facultad de Odontologia Universidad de Antioquia <revistaodontologia@udea.edu.co>

Estimado Dr. Julio Roberto Saldarriaga Molina
Editor de Revista Facultad de Odontologia Universidad de Antioquia,

Junto con saludarlo, me gustaría solicitar permiso para incluir el artículo "Revisión del estado actual de cementos de silicato de calcio en odontología restauradora" (publicado en vol. 27 n°2, primer semestre, 2016) en mi tesis de doctorado.

Me encuentro terminando mi programa de doctorado el cual puede concluir con una tesis incluyendo tres artículos, por ello me interesa contar con la autorización de la revista para incluirlo.

De antemano muchas gracias

Camila Corral Núnez
Docente Dpto Odontología Restauradora
Facultad de Odontología
Universidad de Chile

Revista Facultad de Odontologia Universidad de Antioquia <revistaodontologia@udea.edu.co> 25 de abril de 2017, 10:01
Para: Camila María Corral Núñez <camila.corral@u.uchile.cl>

Buenos días estimada autora.

Teniendo en cuenta que el artículo ya ha sido publicado en nuestra Revista, su contenido solo puede ser incluido en otro trabajo académico a modo de citación, atendiendo las normas de citación requeridas para la entrega de su tesis doctoral (se sugiere citar tal y como se indica en la primera página del artículo, justo después de las palabras clave). El uso de los textos del artículo en otro trabajo académico sin la correpondiente citación a la publicación original, aun siendo de los mismos autores, incurre en la figura denominada autoplagio, y el correspondiente conflicto con los derechos patrimoniales de la Revista y la Universidad de Antioquia sobre el artículo en cuestión.

Por lo anterior, puede usar el artículo en su tesis doctoral siempre que se cite el número en que fue publicado.

Muchas gracias por su atención; quedamos atentos ante cualquier inquietud adicional.

Cordialmente,

Juan Camilo Villegas Echavarría
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Camila María Corral Núñez <camila.corral@u.uchile.cl> 25 de abril de 2017, 10:36
Para: Revista Facultad de Odontologia Universidad de Antioquia <revistaodontologia@udea.edu.co>

Buenos días,

muchas gracias por la respuesta.

Para confirmar, el artículo sería inserto en la tesis con la citación completa de la revista, año, volumen, página, citada en cada una de las páginas. Esto sería aceptado?

Saludos

Camila Corral Núnez
Docente Dpto Odontología Restauradora
Facultad de Odontología
Universidad de Chile

[El texto citado está oculto]

Revista Facultad de Odontologia Universidad de Antioquia 25 de abril de 2017, 12:04
<revistaodontologia@udea.edu.co>
Para: Camila María Corral Núñez <camila.corral@u.uchile.cl>

Desde luego. Muchas gracias!

Cordialmente,

Juan Camilo Villegas Echavarría
Bibliotecólogo - Editor Ejecutivo
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Medellin, Colombia
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ANEXO 2

Dear Camila,

You have the permission.
Best regards,
Karin Neppelenbroek

----- Mensagem encaminhada -----
De: Camila María Corral Núñez <camila.corral@u.uchile.cl>
Para: karinep@usp.br
Enviadas: Terça-feira, 18 de Abril de 2017 10:14
Assunto: Permission to include article in thesis

Dear Prof. Dr. Karin Hermana Neppelenbroek
Editor-in-Chief Journal of Applied Oral Science,

I would like to ask for permission to include the article "Enhanced bioactive properties of Biodentine modified with bioactive glass nanoparticles", which was recently published in Journal of Applied Oral Sciences (2017;25(2):177-85), in my doctoral thesis.

I am finally writing my thesis, which is in the format of three articles, therefore I would appreciate the permission to include it.

Best regards,

Camila Corral Núnez
Assistant Professor
Restorative Dentistry Department
Faculty of Dentistry
Universidad de Chile

Não autorizo a publicação deste trabalho pelo prazo de 2 anos após a data de defesa

Araraquara, 5 de Dezembro de 2017.

Camila María Corral Núñez