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Physicochemical and biological properties of Biodentine associated with radiopacifiers

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Physicochemical and biological properties of Biodentine associated with radiopacifiers

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Physicochemical and biological properties of Biodentine associated with radiopacifiers

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“Experience life in all possible ways; goodbad, bitter-sweet, dark-light, summer-winter. Experience all the dualities. Do not be afraid of experience, because the more experience you have, the more mature you become”

Rajneesh

Ochoa-Rodríguez VM. Propriedades físico-químicas e biológicas do Biodentine associado a radiopacificadores [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

RESUMO

Biodentine^{TM} (BD) apresenta bioatividade, biocompatibilidade e propriedades físico-químicas adequadas; no entanto, não possui radiopacidade adequada. Os objetivos foram avaliar (1) a radiopacidade de BD e BD associado com 15% de tungstato de cálcio ($BDCaWO_4$) ou óxido de zircônio ($BDZrO_2$), empregando sistemas de radiografia convencional e digital; e (2) as propriedades físico-químicas de tempo de presa, pH e solubilidade, e as propriedades biológicas de citocompatibilidade e potencial para induzir mineralização desses cimentos. Para a avaliação da radiopacidade, cada corpo de prova foi radiografado ao lado de uma escada de alumínio usando filme oclusal, placa de fósforo ou sensores digitais. As radiografias convencionais foram digitalizadas por câmera fotográfica ou scanner. Os valores médios de cinza dos materiais foram expressos em milímetros de alumínio (mm Al). A solubilidade foi avaliada após 7 dias de imersão dos espécimes em água destilada e expressa em percentagem de perda de massa. O tempo de presa foi avaliado empregando a agulha de Gillmore (105 ± 0,5 g) e o pH foi mensurado com um medidor de pH. A citocompatibilidade e a bioatividade celular foram avaliadas em células de linhagem osteoblástica (Saos-2) utilizando os ensaios de metiltetrazólio (MTT), vermelho neutro (NR), atividade de fosfatase alcalina (ALP) e coloração de vermelho de alizarina. Os dados foram avaliados utilizando ANOVA de um fator e pós-teste Tukey ou ANOVA de dois fatores e pós-teste de Bonferroni ($\alpha=0,05$). A radiopacidade do BD foi inferior a 3 mm Al e do $BDZrO_2$ e $BDCaWO_4$ foi acima de 3 mm Al em todos os sistemas de radiografia utilizados. A solubilidade foi de 2,28% para BD, 2,27% para $BDZrO_2$ ($p>0,05$) e 3,63% para $BDCaWO_4$ ($p<0,05$). O tempo de presa foi de 27,5 min para BD, 33,5 minutos para BD ZrO_2 e 30 minutos para $BDCaWO_4$. Os ensaios MTT e NR revelaram que os extratos de cimentos, nas diluições 1: 2, 1: 4, 1: 8 e 1:12, apresentaram citocompatibilidade maior ($p<0,05$) ou similar ($p>0,05$) ao grupo controle (meio de cultura). A atividade de ALP nos grupos dos cimentos foi semelhante ($p>0,05$) ou maior ($p<0,05$) que o grupo controle aos 1, 3 e 7 dias. Aos 7 dias, a maior atividade de ALP foi detectada para o grupo BD seguido de $BDZrO_2$ ($p<0,05$) e do $BDCaWO_4$ ($p<0,05$). Não houve diferença significativa entre $BDCaWO_4$ e grupo controle ($p>0,05$). Todos os materiais induziram maior produção de nódulos mineralizados que grupo controle ($p<0,05$) sem diferença significativa entre eles. Em conclusão, a radiopacidade de BD foi inferior a 3 mm de Al em todos os sistemas radiográficos, e a adição de 15% de ZrO_2 ou $CaWO_4$ foi suficiente para aumentar a radiopacidade de BD para valores maiores que o mínimo recomendado pelo ISO 6876 (>3mm Al). BD associado a radiopacificadores mostrou propriedades adequadas do tempo de presa, pH e solubilidade, exceto $BDCaWO_4$, que apresentou maior solubilidade que BD e $BDZrO_2$. Todos os cimentos apresentaram citocompatibilidade e potencial de induzir mineralização em células Saos-2. Os resultados sugerem que a adição de 15% de ZrO_2 pode ser uma boa opção para aumentar a radiopacidade do BD sem alterar suas propriedades físico-químicas e biológicas.

Palavras-chave: Cimentos dentários. Endodontia. Teste de materiais.

Ochoa-Rodríguez VM. Physicochemical and biological properties of Biodentine associated with radiopacifiers [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

ABSTRACT

Biodentine^{TM} (BD) presents bioactivity, biocompatibility and suitable physicochemical properties; however, it does not have adequate radiopacity. The objectives were to evaluate (1) the radiopacity of BD and BD associated with 15% calcium tungstate (BDCaWO$_4$) or zirconium oxide (BDZrO$_2$), employing conventional and digital radiography systems; and (2) the physicochemical properties of setting time, pH and solubility, and biological properties of cytocompatibility and potential to induce mineralization of these cements. For radiopacity evaluation, each cement specimen was radiographed alongside an aluminum step-wedge using occlusal film, photostimulable phosphor plates or digital sensors. The conventional radiographies were digitized by digital photographic camera or scanner. Mean grey values of materials were expressed in millimeters of aluminum (mm Al). Solubility was evaluated after 7 days of specimens' immersion in distilled water and expressed as percentage of mass loss. Setting time was evaluated employing a Gillmore needle (105 ± 0.5 g) and pH was evaluated with pH meter. The cytocompatibility and cell bioactivity were evaluated in osteoblasts-like cells (Saos-2) using methyl-thiazol-tetrazolium (MTT), neutral red (NR), alkaline phosphatase (ALP) activity and alizarin red staining assays. The data were evaluated using one-way ANOVA and Tukey post-test or two-way ANOVA and Bonferroni post-test (α=0.05). BD radiopacity was below 3 mm Al and BDZrO$_2$ and BDCaWO$_4$ was above 3 mm Al in all radiography systems used. Solubility was 2.28% for BD, 2.27% for BDZrO2 ($p>0.05$) and 3.63% for BDCaWO$_4$ ($p<0.05$). All cements showed alkaline pH with no statistical difference between them ($p>0.05$). The setting time was 27.5 min. for BD, 33.5 min. for BDZrO$_2$ and 30 min. for BDCaWO$_4$. MTT and NR assays revealed that cements extract at dilutions of 1:2, 1:4, 1:8 and 1:12 had greater ($p<0.05$) or similar ($p>0.05$) cytocompatibility in comparison to control group (culture medium). The ALP activity of cements groups at 1, 3 and 7 days was similar ($p>0.05$) or greater ($p<0.05$) than the control group. At 7 days, the highest ALP activity was detected for BD group followed by BDZrO$_2$ ($p<0.05$) and BDCaWO$_4$ group ($p<0.05$). There was no significant difference between BDCaWO$_4$ and control group ($p>0.05$). All materials induced greater production of mineralized nodules than control group ($p<0.05$) without significant difference among them. In conclusion, BD radiopacity was below 3 mm Al in all radiography systems, and addition of 15% ZrO$_2$ or CaWO$_4$ was sufficient to increase the radiopacity of BD to values greater than the minimum recommended by ISO 6876 (> 3mm Al). BD associated with radiopacifiers showed suitable properties of setting time, pH and solubility, except BDCaWO$_4$, which exhibit a higher solubility than BD and BDZrO$_2$. All cements had cytocompatibility and potential to induce mineralization in Saos-2 cells. The results suggest that the addition of 15% ZrO$_2$ may be a good option to increase the radiopacity of BD without altering its physicochemical and biological properties.

Key words: Dental cements. Endodontics. Materials testing.

SUMMARY

1 INTRODUCTION

MTA is mainly composed of Portland cement (PC) and contains 53.1% of tricalcium silicate, 22.5% of dicalcium silicate, 21.6% of bismuth oxide (Bi_2O_3) as radiopacifier and traces of calcium sulfate (Torabinejad, White[87], 1998; Camilleri et al. [16], 2005; Camilleri[20], 2007; Camilleri[19], 2008). It is considered the gold standard material for diverse treatments in endodontics, such as root perforation, root-end filling, among others (Hwang et al.[46], 2011; Torabinejad et al.[88], 2018), due to its sealing capability, biocompatibility and ability to induce mineralization (Parirokh, Torabinejad[66], 2010; Tanomaru-Filho et al.[83], 2017; Rodrigues et al.[73], 2017). However, MTA is difficult to manipulate and insert into cavities (Parirokh, Torabinejad[66], 2010), low compressive strength (Parirokh, Torabinejad[65], 2010), has a long setting time (Parirokh, Torabinejad[65], 2010; Tanomaru-Filho et al. [86], 2012) and causes tooth discoloration (Belobrov, Parashos[8], 2011; Akbari et al.[1], 2012; Felman, Parashos [32], 2013; Kang et al.[51], 2015) derived from the chemical reaction between the collagen in the dentin matrix and Bi_2O_3 (Marciano et al.[59], 2014). There is evidence that Bi_2O_3 causes structural damages capable of compromising the longevity of the material, increasing the porosity degree, and consequently reducing the compressive strength (Coomaraswamy et al.[27], 2007).

Tricalcium silicate, the principal active component in MTA (Camilleri[16], 2005), has been used with or without additives as bone cement (Huan, Chang[42], 2008; Zhao et al.[97], 2008), posterior restorative material (Laurent et al.[56], 2008) and reparative dental material (Wang et al.[93], 2008; Camilleri et al. [17], 2013). It has shown suitable physicochemical properties (Wang et al. [93], 2008; Huan, Chang[42], 2008), bioactivity and biocompatibility (Peng et al.[69], 2011; Camilleri et al.[17], 2013; Tanomaru-Filho et al.[83], 2017), besides promoting odontoblastic differentiation of human dental pulp cells (Peng et al.[69], 2011). The hydration of the tricalcium silicate after chemical reaction with tissue fluids forms hydrated calcium silicate gel and calcium hydroxide, thus, being the tricalcium silicate phase responsible for the bioactivity of this material (Camilleri[18], 2011; Khalil et al.[54], 2016). Dental materials based on tricalcium silicate have been developed. These materials are synthesized in the laboratory from high purity raw materials unlike the Portland cement in MTA. One such formulation is Biodentine^{TM} – BD (Septodont, Saint-Maurdes-Fossés, France) which was developed for use as a bioactive dentin substitute and has been indicated for coronal and radicular restorations, pulp capping, pulpotomy, root and furcation perforations, apexification, root resorption and as root-end filling (Rajasekharan et al.[70], 2014). BD is composed of a powder and liquid system. The powder contains 80% tricalcium silicate (main component), 15% calcium carbonate (filler material), 5% zirconium oxide (radiopacifier), dicalcium silicate (traces), calcium oxide (traces), iron oxide (traces). The mixing liquid is an aqueous solution of a hydrosoluble polymer (water reducing agent) with calcium chloride, which decreases the setting time of the cement (Septodont)[76]. Studies show that this cement has biocompatibility (Fonseca et al.[33], 2016), bioactivity (Grech et al.[39], 2013), with better handling conditions (Butt et al.[13], 2014) and lower setting time in relation to MTA (Kaup et al.[53], 2015).

The biological properties of BD have been studied, showing positives responses. BD presents cytocompatibility (Chang et al.[24], 2014; Daltoe et al.[30] 2016; Rodrigues et al.[74], 2017) and in vitro potential to induce mineralization (Gomes- Cornélio et al.[37], 2017) higher than MTA (Collado-González et al.[25], 2017; Rodrigues et al.[74], 2017). In vivo, BD promotes formation of collagenous capsules when implanted in the subcutaneous tissue of rats (Fonseca et al.[33], 2016) and induces the formation of mineralized tissue when used as pulp-capping material in human and dog teeth (Nowicka et al.[63], 2013; De Rossi et al.[31], 2014; Cuadros-Fernández et al.[28], 2016) or when used for the sealing of furcation perforations (Silva et al.[80], 2017).

The physicochemical properties of BD have benefit in relation to MTA. The initial setting time ranges from 9 minutes (Septodont)[76] to 16 minutes (Lucas et al.[58], 2017) and the final setting time from 35 minutes (Lucas et al.[58], 2017) to 85.6 minutes (Kaup et al.[53], 2015), which is lower than MTA (Parirokh et al.[67], 2018). The polycarboxylate-based hydrosoluble polymers in the liquid of BD acts as water reducing agent and allows low water/powder ratio. As a result, BD has lower porosity and, consequently, higher compressive strength than MTA (Camilleri et al.[17], 2013; Lucas et al.[58], 2017). BD presents alkaline pH similar to MTA (Lucas et al.[58], 2017). This pH is derived from the hydration reaction of tricalcium silicate which forms calcium hydroxide and calcium silicate hydrate gel (Camilleri et al.[17], 2013; Khalil et al.[54], 2016).

Despite of the good properties, some in vitro studies, using conventional film (Lucas et al.[58], 2017) or photostimulable phosphor plates (Tanalp et al.[82], 2013), have shown that BD presents lower radiopacity than that recommended by the International Standards Organization (ISO 6876)[48]. According to ISO standard, the endodontic sealers must have a radiopacity equivalent to not less than 3 mm Al (ISO 6876)[48]. Moreover, researchers, who have used BD as a retrograde obturation material in human teeth, have reported that low radiopacity is the primary clinical limitation of BD, which makes radiographic assessment of treatment and follow-up difficult (Bachoo et al.[5], 2013; Caron et al.[22], 2014). Considering the appropriate properties of tricalcium silicate-based cements associated with zirconium oxide

(ZrO_2) and calcium tungstate - $CaWO_4$ (Cutajar et al.[29], 2011; Gomes-Cornélio et al.[38], 2011; Húngaro Duarte et al.[44], 2012; Camilleri et al.[17], 2013; Bosso-Martelo et al.[11], 2015; Silva et al.[79], 2017), an option to improve BD's radiopacity is to associate it with these radiopacifiers.

$CaWO_4$ has being used as an alternative radiopacifier to Bi_2O_3 for calcium silicate-based cements (Marciano et al.[60], 2016). Studies have reported that $CaWO_4$ associated with Portland cement, promotes alkaline pH (Húngaro-Duarte et al.[44], (2012), decreases the solubility, increases the compressive strength, does not affect the final setting time (Tanomaru-Filho et al.[86], 2012) and is not cytotoxic for periodontal and osteoblast-like cells (Gomes-Cornélio et al.[38], 2011). $CaWO_4$, associated with calcium silicate-based cement, presents bioactivity (Bosso-Martelo et al.[11], 2015) and maintains physicochemical properties similar to MTA (Bosso-Martelo et al.[12], 2016).

ZrO_2 was initially introduced as a biomaterial for use in joint implants in orthopedic surgery. In restorative dentistry, ZrO_2 is used to replace the metal framework in crown and bridges and as radiopacifier in glass ionomer cements (McCabe et al.[61], 2003). It is commonly used in combination with tricalcium silicate cements for endodontic use (Viapiana et al.[91], 2014; Tanomaru et al.[85], 2017) including BD. ZrO_2 does not participates in the hydration process of Portland cement thus being inert when compared to Bi_2O_3 (Camilleri et al.[14], 2011; Camilleri et al.[17], 2013). The association of Portland cement with 30% ZrO_2 resulted in a material with physicochemical properties comparable to those of MTA (Cutajar et al.[29], 2011). ZrO_2 in association with white Portland cement induced lower inflammatory reaction than Bi_2O_3 (Silva et al.[78], 2014), fibroblast proliferation and accelerated the regression of the inflammatory reaction when compared to MTA (Silva et al.[79], 2017) in subcutaneous rat tissue.

Radiopacity of endodontic materials should be sufficient to allow distinction from dentin or cortical bone (American National Standard/American Dental Association - ANSI/ADA)[3]. For quantifying the radiopacity of endodontic materials, specimens should be prepared in standard discs and radiographed along with an aluminum (Al) step-wedge reference with at least 98% pure, using type D or E occlusal films (ISO 6876)[48]. Values in terms of Al equivalent thickness minimize the influence of exposure time and film development time (Rasimick et al[71]., 2007; Akcay et al.[2], 2012). ISO standard recommend that radiopacity must be evaluated in conventional radiographic films using an optical densitometer (ISO 6876)[48]. However, nowadays, the radiopacity of dental materials has been performed using digital images obtained by indirect (Akcay et al.[2], 2012; Siboni et al.[77], 2017) or direct technique (Baksi et al.[6], 2007; Akcay et al.[2], 2012; Khalil et al.[54], 2016, Versiani et al.[90], 2016). In the indirect technique, the conventional radiographic image is converted into digital sign using radiographic scanner (Tanomaru-Filho et al.[84], 2007; Akcay et al.[2], 2012; Siboni et al.[77], 2017) or digital photographic camera (Húngaro Duarte et al.[43], 2009; Candeiro et al.[21], 2012; Wang et al.[94], 2014). In the direct technique, digital radiography is obtained using digital sensors or photostimulable phosphor plates (Baksi et al.[7], 2008; Akcay et al.[2], 2012; Grech et al.[40], 2013; Khalil et al.[54], 2016, Versiani et al.[90], 2016).

Although several studies have assessed the radiopacity of endodontic materials by using digital systems (Rasimick et al.[71], 2007; Baksi et al.[7], 2008; Akcay et al.[2], 2012; Grech et al.[40], 2013, Camilleri et al.[17], 2013; Khalil et al.[54], 2016, Versiani et al.[90], 2016), there is no consensus on how digital radiography influences the radiopacity of materials. Rasimick et al.[71] (2007) reported that barium-containing materials tended to be 13% more radiopaque in radiographs obtained by digital sensor than on the conventional film type. On the other hand, other endodontic materials appeared less radiopaque on digital radiography, ranging from 7% to 20% difference between conventional and digital radiography obtained by photostimulable phosphor plates (Baksi et al.[7], 2008). Therefore, it is important to evaluate the radiopacity of BD and BD associated with radiopacifiers using conventional and digital radiography systems. In addition, it is important to evaluate the effect of the addition of the radiopacifiers on the physicochemical and biological properties of BD.

6 CONCLUSIONS

BD radiopacity was lower than 3 mm Al in the conventional and digital radiography systems, and addition of 15% ZrO_2 or $CaWO_4$ was sufficient to increase the radiopacity of BD to values greater than the minimum recommended by ISO 6876 (> 3 mm Al). BD associated with radiopacifiers showed suitable properties of setting time, pH and solubility, except BD $CaWO_4$, which exhibit higher solubility than BD and BDZrO_2. All cements evaluated had citocompatibility and potential to induce mineralization in Saos-2 cells. The results suggest that the addition of 15% ZrO_2 may be a good option to increase the radiopacity of BD, allowing its radiograph detection in clinical practice, without altering its physicochemical and biological properties.

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