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

Marcela Borsatto Queiroz

Physicochemical and biological properties of tricalcium silicate-based reparative materials with alternative radiopacifiers and Biosilicate

Características físico-químicas e biológicas de materiais reparadores à base de silicato tricálcico associados à radiopacificadores alternativos e Biosilicato

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Orientador: Prof. Dr. Mário Tanomaru Filho

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ABSTRACT

Tricalcium silicate cements associated with radiopacifiers are used as repair materials. **Publication 1:** Evaluation of tricalcium silicate-based cements (TCS) associated with zirconium oxide (ZrO_2), calcium tungstate ($CaWO_4$) or niobium oxide (Nb_2O_5) radiopacifiers compared to MTA Repair HP (MTA HP). **Publication 2:** Evaluation of tricalcium silicate-based cements (TCS) associated with zirconium oxide (ZrO_2) radiopacifier with 10% or 20% of Biosilicate (TCS ZrO_2 + 10% Biosilicate and TCS ZrO_2 + 20% Biosilicate) compared to Biodentine. Setting Time (ST) and radiopacity were evaluated based on ISO 6876/2002 standard. Solubility was evaluated according to the method proposed by Carvalho-Júnior *et al.* (2007) modified. pH was measured at 3, 12 and 24 hours and 7, 14 and 21 days after immersion in distilled water. Cellular cytotoxicity and bioactivity were evaluated by methyltetrazolium (MTT), neutral red (NR), alkaline phosphatase (ALP), alizarin red (ARS) and real time PCR (qPCR) (Publication 1) assays in different periods of contact with eluates of the materials in Saos-2 cells. Antibacterial activity was evaluated by direct contact on *Enterococcus faecalis* in the planktonic form. For the physico-chemical and ARS tests, the data were submitted to ANOVA and Tukey tests; for MTT, NR and ALP tests the data were analyzed by the Two-Way ANOVA and Bonferroni tests; the antibacterial activity, were submitted to Kruskall-Wallis and Dunn tests ($\alpha = 0.05$). **Publication 1:** TCS + $CaWO_4$ presented the highest setting time and MTA HP the lowest ($p<0.05$). Except for TCS, all materials presented radiopacity above 3 mm Al. The materials presented solubility in accordance with ISO 6876/2002. The cements evaluated presented alkaline pH values in all periods. The materials were cytocompatible at the dilution of 1:8. The highest ALP activity occurred in 14 days for all the cements, especially TCS, TCS + ZrO_2 e TCS + $CaWO_4$ when compared with the negative control (NC). TCS + Nb_2O_5 presented higher formation of mineralization nodules in comparison with the NC ($p<0.05$). After 7 days, there was no statistically significant difference ($p>0.05$) in mRNA expression for ALP, when compared to NC. However, after 14 days there was na overexpressed ALP mRNA, especially TCS + Nb_2O_5 ($p<0.05$), in relation to the CN. All the materials TCS presented antimicrobial action against *E. faecalis*. **Publication 2**: TCS presented the highest setting time ($p<0.05$) and the other materials presented no statistical difference ($p<0.05$). TCS ZrO_2 + 10% Biosilicate and TCS ZrO_2 + 20% Biosilicate showed radiopacity and solubility in accordance with ISO 6876/2002. All materials presented alkaline pH in the different periods. In the MTT and NR assays, the materials presented no cytotoxic effects, except for Biodentine that presented lower cell viability compared with the NC at the lower dilutions (1:1 and 1:2). The highest ALP activity was observed in the period of 14 days, with emphasis on TCS cements and Biodentine. All the materials TCS and Biosilicate presented antimicrobial action against *E. faecalis*. It could be concluded that tricalcium silicate-based cement associated with different radiopacifiers presented proper physicochemical properties, bioactive potential and was non-cytotoxic in Saos-2, suggesting a potential use as a repair material (Publication 1). Biosilicate in two proportions, associated with TCS and ZrO_2, presented proper physicochemical properties and no cytotoxic effect. Therefore, this material showed perspectives for clinical application (Publication 2).

Key-words: Calcium silicate. Physical properties. Chemical properties. Cell culture techniques.

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RESUMO

Cimentos de silicato tricálcico com radiopacificadores são utilizados como materiais reparadores. **Publicação 1:** Avaliação de cimento à base de silicato tricálcico (STC) associado aos radiopacificadores óxido de zircônio (ZrO_2), tungstato de cálcio ($CaWO_4$) ou óxido de nióbio (Nb_2O_5) em comparação ao MTA Repair HP (MTA HP). **Publicação 2:** Avaliação de material à base de silicato tricálcico (STC) e radiopacificador óxido de zircônio (ZrO_2) e 10% ou 20% de Biosilicato (STC ZrO_2 + 10% de Biosilicato e STC ZrO_2 + 20% de Biosilicato) em comparação ao Biodentine. Tempo de presa e a radiopacidade foram avaliados seguindo ISO 6876/2002. A solubilidade foi avaliada de acordo com o método proposto por Carvalho-Júnior *et al.* (2007) modificado. pH foi avaliado 3, 12 e 24 horas, 7, 14 e 21 dias após imersão em água destilada. A citotoxidade e bioatividade celular foram avaliadas pelos testes metiltetrazólio (MTT), vermelho neutro (VN), atividade de fosfatase alcalina (ALP), ensaio de vermelho de alizarina (ARS) e PCR em tempo real (qPCR) (Publicação1), em diferentes períodos de contato com eluídos dos materiais em células Saos-2. Atividade antimicrobiana dos materiais foi avaliada por meio do teste de contato direto com *Enterococcus faecalis* na forma planctônica. Para os testes físico-químicos e ARS, os dados foram submetidos aos testes ANOVA e Tukey; para os ensaios do MTT, VN e ALP e qPCR os dados foram analisados aos testes Two Way ANOVA e Bonferroni; os dados da atividade antimicrobiana foram submetidos aos testes Kruskall-Wallis e Dunn. (α=0.05). **Publicação 1:** STC + $CaWO_4$ apresentou o maior tempo de presa e MTA HP o menor ($p<0.05$). Com exceção do STC, todos os materiais apresentaram radiopacidade acima de 3 mm Al. Os materiais apresentaram solubilidade de acordo com a ISO 6876/2002. Os cimentos avaliados apresentaram valores de pH alcalino em todos os períodos. Os materiais foram citocompatíveis na diluição 1:8. A maior atividade de ALP ocorreu em 14 dias para todos os cimentos, com destaque para STC, STC + ZrO_2 e STC + $CaWO_4$ quando comparados ao controle negativo (CN) ($p<0.05$). Após 7 dias, não houve diferença estatística ($p>0.05$) na expressão gênica (mRNA) para ALP, quando comparados ao CN. Entretanto, após 14 dias houve um aumento do transcrito ALP, especialmente STC + Nb_2O_5 ($p<0.05$), em relação ao CN. Todos os materiais de STC apresentaram ação antimicrobiana contra *E faecalis***.** **Publicação 2**: STC apresentou o maior valor de tempo de presa ($p<0.05$) e os demais materiais não apresentaram diferença estatística ($p>0.05$). STC ZrO_2 + 10% de Biosilicato e STC ZrO_2 + 20% de Biosilicato apresentaram valores de radiopacidade e solubilidade de acordo com a norma ISO 6876/2002. Todos os materiais, nos diferentes períodos apresentaram valores de pH alcalino. Nos ensaios MTT e VN os materiais não apresentaram efeitos citotóxicos, com exceção do Biodentine que apresentou menor viabilidade celular que o CN nas menores diluições (1:1 e 1:2). A maior atividade de ALP foi observada no período de 14 dias, com destaque para os cimentos STC e Biodentine. Todos os materiais de STC e Biosilicato apresentaram ação antimicrobiana contra *E faecalis***.** Conclui-se que cimento de silicato tricálcico associado aos diferentes radiopacificadores apresentam propriedades físico-químicas adequadas, potencial bioativo e citocompatibilidade que sugere potencial para uso como material reparador (Publicação 1). O Biosilicato nas duas proporções, associado ao STC e ZrO_2, apresentou adequadas propriedades físico-químicas e não demonstrou efeitos citotóxicos. Portanto, este material mostrou perspectivas para aplicação clínica (Publicação 2).

Palavras-chave: Calcarea silicata. Propriedades físicas. Propriedades químicas. Técnicas de cultura de célula.

SUMMARY

1 INTRODUCTION

Mineral Trioxide Aggregate (MTA) is a hydraulic calcium silicate-based material[1] developed for sealing communication between the pulp cavity and external tooth surface[2]. MTA is biocompatible and has sealing ability and bioactivity[3]. MTA is composed of 53.1% tricalcium silicate, 22.5% dicalcium silicate, 21.6% bismuth oxide (Bi_2O_3) and traces of calcium sulphate[4-6].

Tricalcium silicate-based cements have been proposed as alternative materials to MTA[7,8], due the tricalcium silicate phase that is responsible for the bioactive potential of this material. Tricalcium silicate hydration after reaction with water forms hydrated hydraulic calcium silicate and calcium hydroxide[9].

The presence of bismuth oxide Bi_2O_3 as radiopacifier in MTA reduces the release of calcium hydroxide, increases the solubility and harms the dimensional stability of the material[10]. Moreover, this radiopacifier increases the porosity of Portland Cement (PC)[11], and diminishes its compressive strength[11,5]. The presence of bismuth oxide[12] affects the color of MTA Angelus when it comes into contact with the dental structures. Therefore, the use of alternative radiopacifiers such as zirconium oxide (ZrO_2), niobium oxide (Nb_2O_5) or calcium tungstate ($CaWO_4$) have been indicated as potential substitutes for Bi_2O_3.

ZrO_2 has been shown to be inert[10], without changing the properties of MTA[13,14]. The association of tricalcium silicate with ZrO_2 leads to calcium ion release[15], promotes alkalinization and bioactive potential[16]. Furthermore, this radiopacifier does not undergo leaching when immersed in water or HBSS[15]. When hydraulic calcium silicate is associated with the radiopacifier ZrO_2, it has a setting time similar to that of MTA Angelus; low solubility; radiopacity higher than 3 mm Al, and alkaline pH[17]. Hydraulic calcium silicate cement with ZrO_2 and MTA present better bacterial sealing capacity when compared with zinc oxide and eugenol[18].

Biodentine (Septodont, Saint Maur des Fossés, France) is a hydraulic calcium silicate-based biomaterial with indications similar to those of MTA[19-23], and has a better consistency[24]. Biodentine consists of a powder that contains tricalcium and dicalcium silicate ($3CaO\ SiO_2$ and $2CaO\ SiO_2$), calcium carbonate ($CaCO_3$) and ZrO_2 as radiopacifier. The liquid is made up of calcium chloride ($CaCl_2\ 2H_2O$) and a water reducing agent in an aqueous solution with a mixture of polycarboxylate (a super plasticizing agent)[20,22]. MTA hydration is slower than that of Biodentine and tricalcium silicate associated with 20% ZrO_2, due to the smaller quantity of tricalcium silicate present in the material[7]. Biodentine has a shorter initial setting time than that of MTA Angelus, and radiopacity below the value of the ISO Standard 6876/2002[25]. MTA and Biodentine are biocompatible and show bioactivity in Saos-2 cell cultures transfected with the gene that codifies bone morphogenetic protein 2 (BMP-2)[26].

Nb_2O_5 has been used in bone implants due to its biocompatibility, resistance to corrosion and disintegration[27]. Nb_2O_5 used as micro and nano particulate radiopacifier in hydraulic calcium silicate cement in the proportion of 30% promoted radiopacity in accordance with the ANSI/ADA specifications[28,29]. The addition of Nb_2O_5 to hydraulic calcium silicate-based cement promoted a biocompatible material with adequate radiopacity and setting time[30].

Nb_2O_5 provides radiopacity and improves the biological properties of materials, well as their biocompatibility and bioactivity[31]. Nb_2O_5 and ZrO_2 associated with hydraulic calcium silicate cement in endodontic cement formulations promoted the deposition of hydroxyapatite crystals[32]. Nb_2O_5 associated with hydraulic calcium silicate cement shows cytocompatibility in Saos-2 cells, stimulates alkaline phosphatase activity[33], in addition to showing cellularbioactivity[34].

When $CaWO_4$ is associated with Portland cement (PC), it promotes higher pH and calcium ion release values similar to those of MTA[35], increases the compressive strength and diminishes the solubility of PC[35,36]. Moreover, it does not change its mechanical properties and final setting time[36]. When $CaWO_4$ is associated with PC it presents antimicrobial action[37] and is not cytotoxic[38]. When associated with PC in the proportion of 20%, it presents radiopacity equivalent to 3.11 mm Al[39]. $CaWO_4$ associated with hydraulic calcium silicate cement shows bioactive potential similar to that of MTA Angelus[40]; low solubility, and alkaline pH[17].

Experimental cements with ZrO_2 and $CaWO_4$ as radiopacifiers have adequate hydration properties, high pH and calcium ion release values[41]. MTA HP is a cement similar to MTA developed by Angelus (Brazil), which has $CaWO_4$ as radiopacifier agent, in addition to a plasticizing agent in the liquid. Evaluation of the cytotoxicity, biocompatibility and biomineralization capacity has demonstrated that MTA HP has similar biocompatibility and biomineralization to that of White MTA (Angelus)[42].

Different materials, including metals, polymers, ceramics and composites have been used to repair or reconstruct parts of the injured musculo-skeletal system. According to Hench and Wilson[43], biomaterials are classified according to their type of interaction with live tissues. The concept of bioactive material was introduced by Hench: ‘‘A bioactive material generates a specific biological response at the interface, resulting in the formation of a bond between the tissue and material"[44]. More specifically, bioactive materials are materials which, when in contact with live tissue, produce a hydroxycarbonate apatite (HCA) in the superficial layer, thus promoting an extremely strong chemical bond between the tissue and implant[43,44].

Some glass-ceramic biomaterials with high bioactive potential have been developed. Thus, a completely crystallized bioactive glass-ceramic denominated Biosilicate (Federal University of São Carlos, São Carlos) has been developed by controlled heat treatment, for use in the medical and dental areas[45]. Biosilicate (Biosilicate, Vitrovita, São Carlos, SP, Brazil) has demonstrated bioactivity[46,47]. Biosilicate has positive characteristics for bone tissue regeneration: it is highly bioactive, osteoconductive, osteoinductive, non-cyctotoxic, non-genotoxic, and has antibacterial properties[48]. Martins *et al.*[49] observed that Biosilicate is a broad-spectrum antimicrobial agent, including anaerobic bacteria. The antimicrobial effect of Biosilicate may be explained by its highly crystalline bioactive phase, elevated superficial area and ultrafine particles (between 0.1 and 20 μm). Biosilicate is capable of increasing the pH of aqueous solutions[50,51]. The basic pH of the solution makes the bioglass more soluble due to the presence of OH^- that has the capacity to cleave the chains of silica[46,47,52].

Biosilicate has a stimulatory effect on bone cell metabolism[46]. Complete crystallization of bioactive glasses in Biosilicate can promote an increase in the formation of tissue similar to that of bone tissue, in vitro, in an osteogenic cell culture system[46]. Biosilicate promotes the bone regenerative process of surgically created defects in rat tibias[53]. Bone repair adjacent to conventional titanium implants placed in dental alveoli of dogs has demonstrated that filling the alveolus with Biosilicate particles preserved the alveolar bone height and allowed osseointegration[47]. Biosilicate shows osteogenic activity, accelerating bone repair, shown by the activating of immunomarkers, such as COX-2, BMP-9 and RANKL, related to tissue repair[54]. Biosilicate does not induce damage to DNA in fibroblast and osteoblast cell lineages[55]. Therefore, Biosilicate is used as a synthetic bone graft for replacing bone in various types of surgery[56]. Biosilicate has shown osteogenic activity and the capacity to accelerate bone matrix deposition in experimental models of bone defects[57]. However, the properties of manipulation and low dissolution of the material represent a disadvantage to the biological properties of Biosilicate[53].

The aim of the present study was to develop and evaluate tricalcium silicate cement associated with ZrO_2, Nb_2O_5 and $CaWO_4$ radiopacifiers, and with Biosilicate.

6 CONCLUSION

Based on the results obtained,

- The tricalcium silicate cements associated with the radiopacifiers ZrO_2, $CaWO_4$, Nb_2O_5 and with Biosilicate (10% or 20%), as well as MTA HP presented proper setting time, radiopacity, antimicrobial action and solubility.
- Biodentine was not in accordance to ISO 6876/2002 standards regarding solubility and radiopacity.
- All the materials evaluated presented alkaline pH.
- The materials TCS + ZrO_2; TCS + Nb_2O_5; TCS + $CaWO_4$; MTA HP, and Biodentine presented no cytotoxic effects and increased the biomineralization potential in Saos-2 cells.
- The association of Biosilicate (10% or 20%) with TCS + ZrO_2 demonstrated adequate physico-chemical properties and capacity to reduce setting time and solubility of TCS, in addition to presenting no cytotoxic effects.

REFERENCES*

1. Darvell BW, Wu RC. "MTA"- an hydraulic silicate cement: review update and setting reaction. Dent Mater. 2011; 27(5): 407-22.
2. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod. 1993; 19(11): 541-4.
3. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part I: chemical, physical, and antibacterial properties. J Endod. 2010; 36(1): 16-27
4. Camileri J. Hydration mechanisms of mineral trioxide aggregate. Int Endod J. 2007; 40(6): 462-70.
5. Camilleri J. Characterization of hydration products of mineral trioxide aggregate. Int Endod J. 2008; 41(5): 408–17.
6. Gandolfi MG, Taddei P, Tinti A, Prati C. Apatite- forming ability (bioactivity) of ProRoot MTA. Int Endod J. 2010b; 43(10): 917–29.
7. Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. Dent Mater. 2013; 29(5): 580-93.
8. Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. Dent Mater. 2013; 29(2): 20-8.
9. Camilleri J, Cutajar A, Mallia B. Hydration characteristics of zirconium oxide replaced Portland cement for use as a root-end filling material. Dent Mater. 2011; 27(8): 845–54.
10. Camilleri J, Mallia B. Evaluation of the dimensional changes of mineral trioxide aggregate sealer. Int Endod J. 2011; 44(5): 416-24.
11. Coomaraswamy K, Lumley PJ, Hofmann MP. Effect of bismuth oxide radioopacifier content on the material properties of an endodontic Portland cement–based (MTA-like) System. J Endod. 2007; 33(3): 295-8.

* De acordo com o Guia de Trabalhos Acadêmicos da FOAr, adaptado das Normas Vancouver. Disponível no site da Biblioteca: http://www.foar.unesp.br/Home/Biblioteca/guia-de-normalizacao-atualizado.pdf

12. Marciano MA, Costa RM, Camilleri J, Mondelli RF, Guimarães BM, Duarte MA. Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. J Endod. 2014; 40(8): 1235-40.
13. Cutajar A, Mallia B, Abela S, Camilleri J. Replacement of radiopacifier in mineral trioxide aggregate; characterization and determination of physical properties. Dent Mater. 2011; 27(9): 879–91.
14. Antonijevic D, Medigovic I, Zrilic M, Jokic B, Vukovic Z, Todorovic L. The influence of different radiopacifying agents on the radiopacity, compressive strength, setting time, and porosity of Portland cement. Clin Oral Investig. 2014;18 (6):1597-604.
15. Camilleri J, Grech L, Galea K, Keir D, Fenech M, Formosa L, *et al.* Porosity and root dentine to material interface assessment of calcium silicate-based root-end filling materials. Clin Oral Investig. 2014; 18(5): 1437-46.
16. Camilleri J. Evaluation of the effect of intrinsic material properties and ambient conditions on the dimensional stability of white mineral trioxide aggregate and Portland cement. J Endod. 2011; 37(2): 239-45
17. Bosso-Martelo R, Guerreiro-Tanomaru JM, Viapiana R, Berbert FL, Duarte MA, Tanomaru-Filho M. Physicochemical properties of calcium silicate cements associated with microparticulate and nanoparticulateradiopacifiers. Clin Oral Investig. 2016; 20(1): 83-90
18. Espir CG, Guerreiro-Tanomaru JM, Spin-Neto R, Chávez-Andrade GM, Berbert FL, Tanomaru-Filho M. Solubility and bacterial sealing ability of MTA and root-end filling materials. J Appl Oral Sci. 2016; 24(2): 121-5.
19. Laurent P, Camps J, De Meo M, Dejou J, About I. Induction of specific cell responses to a Ca(3) SiO(5)-based posterior restorative material. Dent Mater. 2008; 24(11): 1486–94.
20. Laurent P, Camps J, About I. Biodentine (TM) induces TGF-beta1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012; 45(5): 439-48.
21. Koubi S, Elmerini H, Koubi G, Tassery H, Camps J. Quantitative evaluation by glucose diffusion of microleakage in aged calcium silicate-based open-sandwich restorations. Int J Dent. 2012; 2012: 105863.

22. Tran XV, Gorin C, Willig C, Baroukh B, Pellat B, Decup F, et al. Effect of a calcium-silicate-based restorative cement on pulp repair. J Dent Res. 2012; 91(12): 1166-71.
23. Koubi G, Colon P, Franquin JC, Hartmann A, Richard G, Faure MO, *et al.* Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth - a prospective study. Clin Oral Investig. 2013; 17(1): 243-9.
24. Butt N, Talwar S, Chaudhry S, Nawal RR, Yadav S, Bali A. Comparison of physical and mechanical properties of mineral trioxide aggregate and Biodentine. Indian J Dent Res. 2014; 25(6): 692-7.
25. Lucas CP, Viapiana R, Bosso-Martelo R, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M. Physicochemical properties and dentin bond strength of a tricalcium silicate-based retrograde material. Braz Dent J. 2017; 28(1): 51-6.
26. Rodrigues EM, Gomes-Cornélio AL, Soares-Costa A, Salles LP, Velayutham M, Rossa-Junior C, et al. An assessment of the overexpression of BMP-2 in transfected human osteoblast cells stimulated by mineral trioxide aggregate and Biodentine. Int Endod J. 2017; 50(2): e9-e18.
27. McMahon RE, Ma J, Verkhoturov SV, Munoz-Pinto D, Karaman I, Rubitschek F, et al. A comparative study of the cytotoxicity and corrosion resistance of nickel-titanium and titanium-niobium shape memory alloys. Acta Biomater. 2012; 8(7): 2863-70.
28. Guerreiro Tanomaru JM, Storto I, Da Silva GF, Bosso R, Costa BC, Bernardi MI, et al. Radiopacity, pH and antimicrobial activity of Portland cement associated with micro- and nanoparticles of zirconium oxide and niobium oxide. Dent Mater J. 2014; 33(4): 466–70.
29. Guerreiro-Tanomaru JM, Vázquez-García FA, Bosso-Martelo R, Bernardi MI, Faria G, Tanomaru-Filho M. Effect of addition of nano-hydroxyapatite on physico-chemical and antibiofilm properties of calcium silicate cements. J Appl Oral Sci. 2016; 24(3): 204-10.
30. Silva GF, Tanomaru-Filho M, Bernardi MI, Guerreiro-Tanomaru JM, Cerri OS. Niobium pentoxide as radiopacifying agent of calcium silicate-based material: evaluation of physicochemical and biological properties. Clin Oral Investig. 2015; 19(8): 2015-25.

31. Wang Z, Lee Y H, Wu B, Horst A, Kang Y, Tang YJ, et al. Anti-microbial activities of aerosolized transition metal oxide nanoparticles. Chemosphere. 2010; 80(5): 525-9.
32. Viapiana R, Flumignan DL, Guerreiro-Tanomaru JM, Camilleri J, Tanomaru-Filho M. Physicochemical and mechanical properties of zirconium oxide and niobium oxide modified Portland cement-based experimental endodontic sealers. Int Endod J. 2014; 47(5): 437-48.
33. Mestieri LB, Tanomaru-Filho M, Gomes-Cornelio AL, Salles LP, Bernardi MI, Guerreiro-Tanomaru JM. Radiopacity and cytotoxicity of Portland cement associated with niobium oxide micro and nanoparticles. J Appl Oral Sci. 2014; 22(6): 554-9.
34. Mestieri LB, Gomes-Cornélio AL, Rodrigues EM, Faria G, Guerreiro-Tanomaru JM, Tanomaru-Filho M. Cytotoxicity and bioactivity of calcium silicate cements combined with Niobium Oxide in different cell lines. Braz Dent J. 2017; 28(1): 65-71.
35. Húngaro Duarte MA, Minotti PG, Rodrigues CT, Zapata RO, Bramante CM, Tanomaru Filho. Effect of diferente radiopacifying agentes on the physicochemical properties of white Portland cement and white mineral trioxide aggregate. J Endod. 2012; 38(3): 394-7.
36. Tanomaru-Filho M, Andrade AS, Rodrigues EM, Viola KS, Faria G, Camilleri J, et al. Biocompatibility and mineralized nodule formation of NeoMTA Plus and an experimental tricalcium silicate cement containing tantalum oxide. Int Endod J. 2017; 50(2): e31-e39.
37. Guerreiro-Tanomaru JM, Vázquez-García FA, Bosso-Martelo R, Bernardi MI, Faria G, Tanomaru M Filho. Effect of addition of nano-hydroxyapatite on physico-chemical and antibiofilm properties of calcium silicate cements. J Appl Oral Sci. 2016; 24(3): 204-10.
38. Gomes-Cornélio AL, Rodrigues EM, Salles LP, Mestieri LB, Faria G, Guerreiro-Tanomaru JM, et al. Bioactivity of MTA Plus, Biodentine and an experimental calcium silicate-based cement on human osteoblast-like cells. Int Endod J. 2017; 50(1): 39-47.
39. Húngaro Duarte MA, de Oliveira EI, Kadre GD, Vivan RR, Guerreiro Tanomaru JM, Tanomaru Filho M. Radiopacity of portland cement associated with different radiopacifying agents. J Endod. 2009; 35(5): 737-40.

40. Bosso-Martelo R, Guerreiro-Tanomaru JM, Viapiana R, Berbert FL, Tanomaru-Filho M. Calcium silicate-based cements associated with micro- and nanoparticle radiopacifiers: physicochemical properties and bioactivity. Int Sch Res Notices. 2015; 23: 874283.
41. Marciano MA, Duarte MA, Camilleri J. Calcium silicate-based sealers: assessment of physicochemical properties, porosity and hydration. Dent Mater. 2016; 32(2): 30-40.
42. Cintra LTA, Benetti F, de Azevedo Queiroz ÍO, de Araújo Lopes JM, Penha de Oliveira SH, et al. Cytotoxicity, biocompatibility, and biomineralization of the new high-plasticity MTA material. J Endod. 2017; 43(5): 774-8.
43. Hench LL, Wilson J. Surface-active biomaterials. Science. 1984; 226(4675): 630-6.
44. Hench LL. The story of Bioglass. J Mater Sci Mater Med. 2006; 17(11): 967-78.
45. Zanotto ED, Ravagnani C, Peith Filho O, Panzeri H, Guimarães LEH; Universidade Federal de São Carlos, Universidade de São Paulo. Process and compositions for preparing particulate, bioactive or resorbable biosilicates for use in the treatment of oral ailments. BR WO2004/074199 A1. 2004 Feb 20.
46. Moura J, Teixeira LN, Ravagnani C, Peitl O, Zanotto ED, Beloti MM, et al. In vitro osteogenesis on a highly bioactive glass-ceramic (Biosilicate). J Biomed Mater Res A. 2007; 82(3): 545-57.
47. Roriz VM, Rosa AL, Peitl O, Zanotto ED, Panzeri H, de Oliveira PT. Efficacy of a bioactive glass-ceramic (Biosilicate) in the maintenance of alveolar ridges and in osseointegration of titanium implants. Clin Oral Implants Res. 2010; 21(2): 148-55.
48. Kido HW, Tim CR, Bossini PS, Parizotto NA, de Castro CA, Crovace MC et al. Porous bioactive scaffolds: characterization and biological performance in a model of tibial bone defect in rats. J Mater Sci Mater Med. 2015; 26(2): 74.
49. Martins CH, Carvalho TC, Souza MG, Ravagnani C, Peitl O, Zanotto ED, et al. Assessment of antimicrobial effect of Biosilicate® against anaerobic, microaerophilic and facultative anaerobic microorganisms. J Mater Sci Mater Med. 2011; 22(6): 1439-46.
50. Stoor P, Söderling E, Salonen JI. Antibacterial effects of a bioactive

glasspaste on oral microorganisms. Acta Odontol Scand. 1998; 56(3): 161-5.

51. Allan I, Newman H, Wilson M. Antibacterial activity of particulate bioglass against supra- and subgingival bacteria. Biomaterials. 2001; 22(12): 1683-7.
52. Jabur RO, Barros VMR, Trivelatto AE, Peitl O. Osseointegration of Ti implants in buccal alveolar bone defect filled with bioactive glasses in dog mandible. Oral Surg. 2010; 2(4): 161-6.
53. Granito RN, Rennó AC, Ravagnani C, Bossini PS, Mochiuti D, Jorgetti V, et al. In vivo biological performance of a novel highly bioactive glass-ceramic (Biosilicate®): a biomechanical and histomorphometric study in rat tibial defects. J Biomed Mater Res B Appl Biomater. 2011; 97(1): 139-47.
54. Pinto KN, Tim CR, Crovace MC, Matsumoto MA, Parizotto NA, Zanotto ED, et al. Effects of biosilicate® scaffolds and low-level laser therapy on the process of bone healing. Photomed Laser Surg. 2013; 31(6):252-60.
55. Kido HW, Oliveira P, Parizotto NA, Crovace MC, Zanotto ED, et al. Histopathological, cytotoxicity and genotoxicity evaluation of Biosilicate® glass-ceramic scaffolds. J Biomed Mater Res A. 2013; 101(3): 667-73.
56. Hench LL, Jones JR. Bioactive glasses: frontiers and challenges. Front Bioeng Biotechnol. 2015; 3: 194.
57. Kido HW, Brassolatti P, Tim CR, Gabbai-Armelin PR, Magri AM, Fernandes KR *et al.* Porous poly (D, L-lactide-co-glycolide) acid/biosilicate(®) composite scaffolds for bone tissue engineering. J Biomed Mater Res B Appl Biomater. 2017; 105(1): 63-71.
58. Schembri M, Peplow G, Camilleri J. Analyses of heavy metals in mineral trioxide aggregate and Portland cement. J Endod. 2010; 36(7): 1210-5.
59. Tanomaru JM, Tanomaru-Filho M, Hotta J, Watanabe E, Ito IY. Antimicrobial activity of endodontic sealers based on calcium hydroxide and MTA. Acta Odontol Latinoam. 2008; 21(2): 147-51.
60. Tanomaru-Filho M, Luis MR, Leonardo MR, Tanomaru JM, Silva LA. Evaluation of periapical repair following retrograde filling with different root-end filling materials in dogteeth with periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 102(1): 127-32.
61. Saghiri AM, Orangi J, Asatourian, James LG, Garcia-Godoy F,Lofti M, et al. Calcium silicate-based cements and functional impacts of various constituents. Dent Mater J. 2017; 36(1): 8-18.

62. Camilleri J. Characterization and hydration kineties of tricalcium silicate cement for use a dental biomaterial. Dent Mater. 2011; 27(8): 836-44.
63. Camilleri J. Sealers and warm gutta-percha obturation techniques. J Endod. 2015; 41(1): 72-8.
64. Prati C, Gandolfi MG. Calcium silicate bioactive cements biological perspectives and clinical applications. Dent Mater. 2015; 31(4): 351-70.
65. Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview - part I: vital pulp therapy. Int Endod J. 2018; 51(2): 177-205.
66. Tanalp J, Karapınar-Kazandağ M, Dölekoğlu S, Kayahan MB. Comparison of the radiopacities of different root-end filling and repair materials. ScientificWorldJournal. 2013; 23: 594950.
67. Kaup M, Schäfer E, Dammaschke T. An in vitro study of different material properties of Biodentine compared to ProRoot MTA. Head Face Med. 2015; 11: 16.
68. Dawood AE, Manton DJ, Parashos P, Wong R, Palamara J, Stanton DP, et al. The physical properties and ion release of CPP-ACP-modified calcium silicate-based cements. Aust Dent J. 2015; 60(4): 434-44.
69. Singh S, Podar R, Dadu S, Kulkarni G, Purba R. Solubility of a new calcium silicate-based root-end filling material. J Conserv Dent. 2015; 18(2): 149-53.
70. Camilleri J. Evaluation of the physical properties of an endodontic Portland cement incorporating alternative radiopacifiers used as root-end filling material. Int Endod J. 2010; 43(3): 231–40.
71. Huffman BP, Mai S, Pinna L, Weller RN, Primus CM, Gutmann JL, et al. Dislocation resistance of ProRoot Endo Sealer, a calcium silicate-based root canals sealer, from radicular dentine. Int Endod J. 2009; 42(1): 34–46.
72. Chang SW, Lee SY, Ann HJ, Kum KY, Kim EC. Effects of calcium silicate endodontic cements on biocompatibility and mineralization-inducing potentials in human dental pulp cells. J Endod. 2014; 40(8): 1194-200.