Crystalline phases involved in the hydration of calcium silicate-based cements: Semi-quantitative Rietveld X-ray diffraction analysis

Renata Grazziotin-Soares, MSc, PhD[1]; Mohammad H. Nekoofar, MSc, DoIBoE[2,3*]; Thomas Davies, PhD[4]; Roberto Hubler, €PhD[5]; Naghmeh Meraji, DDS, MSc, DoIBoE[2,*]; and Paul M.H. Dummer, MScD, PhD, DDSc, FDSRCS (Ed), FHEA[3]

1 College of Dentistry, University of Saskatchewan, Saskatoon, SK, Canada
2 Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
3 School of Dentistry, College of Biomedical & Life Sciences, Cardiff University, UK
4 School of Chemistry, Cardiff University, Cardiff, UK
5 Materials and Nanosciences Laboratory, School of Physics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Correspondence

Dr Naghmeh Meraji, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, North Karegar St, 14399-55991 Tehran, Iran.

Emails: meraji_n@yahoo.com & n-meraji@sina.tums.ac.ir and

Dr Mohammad H. Nekoofar, Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran & School of Dentistry, College of Biomedical & Life Sciences, Cardiff University, UK.

Emails: nekoofar@tums.ac.ir & nekoofarmh@cardiff.ac.uk

Abstract

Chemical comparisons of powder and hydrated forms of calcium silicate cements (CSCs) and calculation of alterations in tricalcium silicate (Ca_3SiO_5) calcium hydroxide ($Ca(OH)_2$) are essential for understanding their hydration processes. This study aimed to evaluate and compare these changes in ProRoot MTA, Biodentine and CEM cement. Powder and hydrated forms of tooth coloured ProRoot MTA, Biodentine and CEM cement were subjected to X-ray diffraction (XRD) analysis with Rietveld refinement to semi-quantitatively identify and quantify the main phases involved in their hydration process. Data were reported descriptively. Reduction in Ca_3SiO_5 and formation of $Ca(OH)_2$ were seen after the hydration of ProRoot MTA and Biodentine; how-ever, in the case of CEM cement, no reduction of Ca_3SiO_5 and no formation of $Ca(OH)_2$ were detected. The highest percentages of amorphous phases were seen in Biodentine samples. Ettringite was detected in the hydrated forms of ProRoot MTA and CEM cement but not in Biodentine.

Introduction

Mineral trioxide aggregate (MTA) is a calcium silicate– based cement (CSCs) used in dentistry with various clini-cal applications, such as vital pulp treatment modalities, endodontic surgery, one step orthograde root end clo-sure, sealing of perforations, orifice plugs and regenera-tive endodontic (1). Many disadvantages have been reported for this CSC such as difficult handling properties (1,2) and tooth discoloration (3,4). Several other CSCs have been developed with the aim of overcoming these disadvantages.

Biodentine (Septodont, Saint Maur des Fosses, France) is a fast setting CSC with high compressive strength (5)

that is marketed as a dentin replacement material (6). The powder is composed of tricalcium silicate, zirconium oxide and calcium carbonate, whereas the liquid supplied for mixing includes calcium chloride as an accelerator and a hydrosoluble polymer as a water-reducing agent (7).

Calcium-enriched mixture cement (CEM cement) (Bionique, Tehran, Iran) is a white CSC, with no discol-oration potential (3). It is composed of calcium oxide (CaO), sulphur trioxide (SO_3), phosphorus pentoxide (P_2O_5) and silicon dioxide (SiO_2).

X-ray diffraction (XRD) can be used to analyse and characterise the crystal phases in both powder and hydrated forms of CSCs (8,9). This technique recognises

specific diffraction peaks that are particular to the various crystalline phases present in each material (10) such as calcium hydroxide crystalline structures (8,9). Rietveld refinement, in turn, is used for the quantitative analysis of these detected crystalline phases (11).

The assessment, quantification and comparison of the crystalline phases in CSCs before and after hydration is important for improving our understanding of cement composition and changes occurring during the setting process (9). Therefore, the aim of this study was to quan-titatively evaluate the crystalline phases present in the powder and hydrated forms of tooth coloured ProRoot MTA, Biodentine and CEM cement using Rietveld-XRD analysis.

Materials and methods

This study has been approved by the Endodontology Research Group of Cardiff University, UK.

Sample preparation

Both powder and hydrated forms of three CSCs were investigated: tooth coloured ProRoot MTA (Dentsply Maillefer, Ballaigues, Switzerland); Biodentine (Septo-dont, Saint-Maur-des-Fosses, France); and CEM cement (BioniqueDent, Tehran, Iran).

To investigate the hydrated form of cements, mixing was performed as follows: i) for tooth coloured ProRoot MTA and CEM cement, the mixing of the material was standardised by placing 1 g of the powder in a sterilised amalgam capsule (2). Then, 0.33 g of their respective liq-uids, distilled water for MTA and phosphate-buffered solution (PBS) for CEM cement were added to the cap-sules. ii) For Biodentine, five drops of the liquid provided were added to the powder, which is packaged in a plastic capsule.

The encapsulated materials were then mixed mechani-cally for 30 s at 4500 rpm (2) using an amalgamator (Dentsply Caulk, York, PA, USA). Subsequently, mixed slurries were placed in three prefabricated cylindrical sili-cone moulds (Elite Double 22; Zhermack SpA, Rome, Italy) with dimensions of 6 0.1 mm length and 4 0.1 mm internal diameter and subjected to a con-stant vertical compaction force of 3.22 MPa applied for 1 min (12). The moulds were then incubated at 37°C in fully saturated conditions for 7 days.

Rietveld-XRD analysis

Both the dry powders and the hydrated forms of the mate-rials (2 g each group) were subjected to Rietveld-XRD analysis for evaluation of their crystalline structures.

The hydrated materials were crushed to fine powder before analysis. Phase compositions of specimens from each group were determined using an X-ray diffractome-ter (PANalytical X'Pert PRO, Almelo, Netherlands) and CuKa radiation (40 Kv and 40 mA). Scans were under-taken from 10° to 80° in the 2h range with 0.02° step. In order to identify crystalline compounds, all patterns were matched using the database of the International Centre for Diffraction Data (ICDD, Pennsylvania, PA, USA). The Rietveld refinement tool was used for the quantitative analysis of phases resulted in six diffractograms: three for the original powder and three for the hydrated form.

Data analysis

To semi-quantitatively analyse the main phases related to the CSC hydration process, Rietveld refinement with the PANalytical X'Pert PRO software was used to adjust XRD spectra (diffractogram) in each sample. For this numeri-cal simulation, the standard crystalline phases reported in the literature were used, according to the Inorganic Crys-tal Structure Database (ICSD), as follows: tricalcium sili-cate (Ca_3SiO_5) [64759], dicalcium silicate (Ca_2SiO_4) [166637], calcium hydroxide ($CaOH_2$) [91882], trical-cium aluminate ($Ca_3Al_2O_6$) [163579], the radiopacifiers bismuth oxide (Bi_2O_3) [15072], zirconium oxide (ZrO_2) [82543], and barium sulphate ($BaSO_4$) [167519]. Addi-tional phases detected in the samples were added to the spectra during the adjustments and are reported descrip-tively (%) with their respective ICSD codes.

Results

XRD patterns in the 10°–80° 2h range and Rietveld analy-sis results are shown in Figures 1−3. All samples consisted of tricalcium silicate and dicalcium silicate.

Figure 1 reveals the following:

(a) a diffractogram for tooth coloured ProRoot MTA, powder form (G1 powder): showing mainly tricalcium silicate (Ca_3SiO_5) and bismuth oxide (Bi_2O_3) peaks, as well as dicalcium silicate (Ca_2SiO_4). Rietveld analysis identified and quantified the following phases: 14.4% Bi_2O_3 [15072], 79.6% Ca_3SiO_5 [64759], 0.3% Ca_2SiO_4 [166637], 3.9% $CaAl_2O_6$ [163579] and 1.8% $CaSO_4$ [15876]; and

(b) a diffractogram for tooth coloured ProRoot MTA, hydrated form (G1 hydrated): showing the same phases of the powder sample, that is, tricalcium sili-cate (Ca_3SiO_5), dicalcium silicate (Ca_2SiO_4), and bis-muth oxide (Bi_2O_3), plus calcium hydroxide (Ca $[OH]_2$) peaks and a small peak for ettringite (E). Riet-veld analysis found 15.8% Bi_2O_3 [15072], 44.9% Ca_3SiO_5 [64759], 6.6% Ca_2SiO_4 [166637], 17.9% Ca
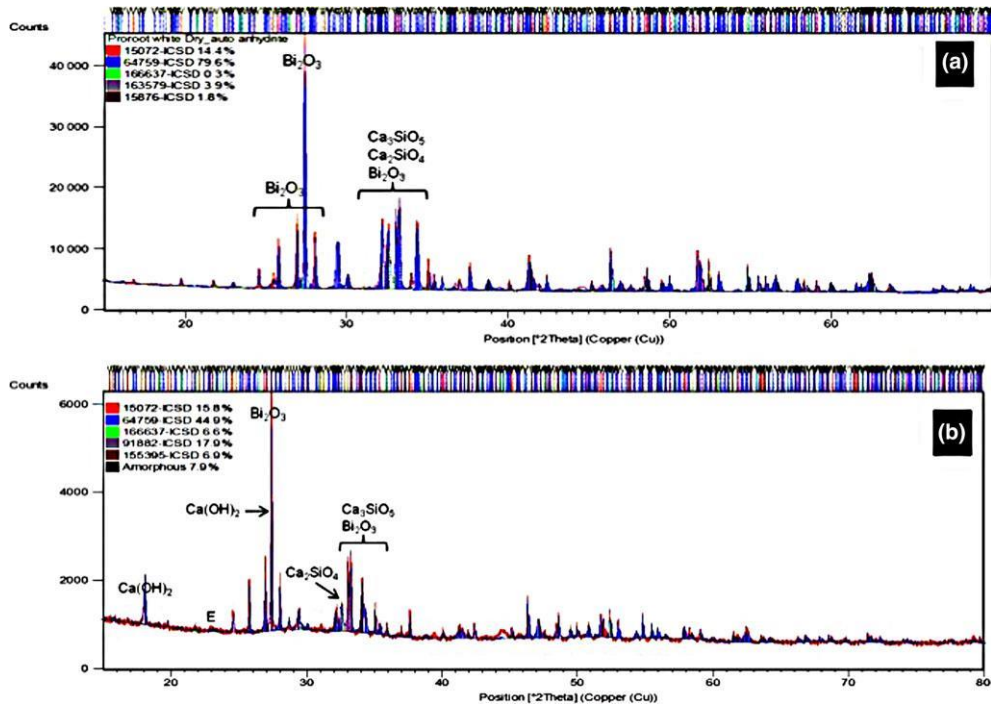
Figure 1 Diffractogram and Rietveld analysis for tooth coloured ProRoot MTA, (a) powder and (b) hydrated form.
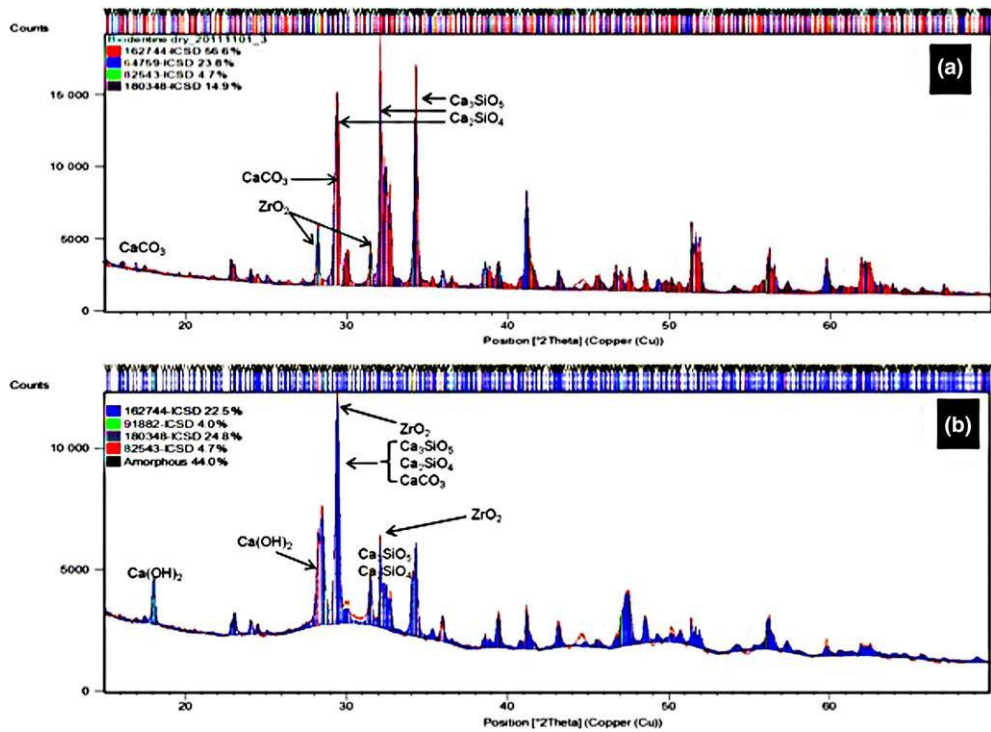

Figure 2 Diffractogram and Rietveld analysis for Biodentine, (a) powder and (b) hydrated form.
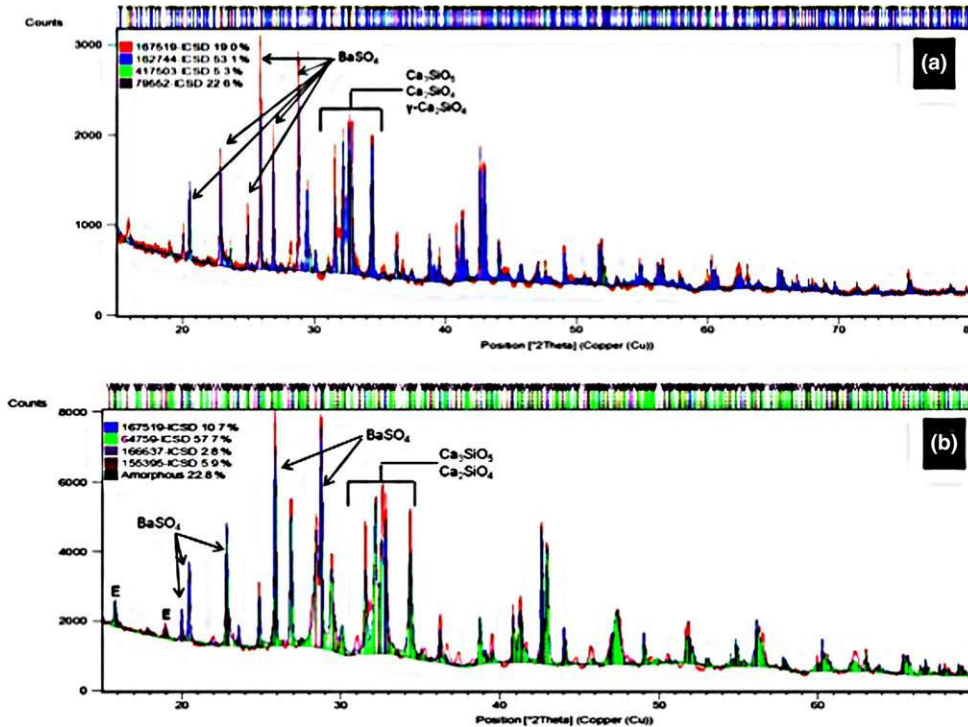
Figure 3 Diffractogram and Rietveld analysis for CEM cement, (a) powder and (b) hydrated form.

(OH)$_2$ [91882] and 6.9% Ca$_6$Al$_2$(SO$_4$)(OH)$_2$.$_{26}$H$_2$O [155395]. Material hydration revealed new crystalline phases, and the rearrangement caused some type of disorder, represented by 7.9% of an amorphous phase. Monoclinic and triclinic forms of tricalcium silicate were not distinguishable in MTA samples. Figure 2 reveals the following:

(a) a diffractogram for Biodentine, powder form (G2 powder): two different structures of tricalcium silicate (Ca$_3$SiO$_5$): triclinic [162744] and monoclinic [64759] were found. Rietveld analysis showed 56.6% Ca$_3$SiO$_5$ (triclinic structure), 23.8% Ca$_3$SiO$_5$ (monoclinic struc-ture), 4.7% ZrO$_2$ and 14.9% CaCO$_3$ [180348]; and

(b) a diffractogram for Biodentine hydrated form (G2 hydrated): the same phases of the powder sample, that is, tricalcium silicate (Ca$_3$SiO$_5$), dicalcium silicate (Ca$_2$SiO$_4$), calcium carbonate (CaCO$_3$), calcium hydroxide (Ca[OH]$_2$) and zirconium oxide (ZrO$_2$) peaks were found. Rietveld analysis showed 22.5% Ca$_3$SiO$_5$ [162744] (only the triclinic structure), 4.0% Ca(OH)$_2$ [91882], 24.8% CaCO$_3$ [180348], 4.7% ZrO$_2$ [82543] and 44.0% amorphous phase.

Figure 3 reveals the following:

(a) a diffractogram for CEM Cement powder form (G3 powder): tricalcium silicate (Ca$_3$SiO$_5$), dicalcium sili-cate (Ca$_2$SiO$_4$), dicalcium silicate c–phase (c–Ca$_2$SiO$_4$) and barium sulphate (BaSO$_4$) peaks. Quantification with Rietveld analysis found 19.0% BaSO$_4$ [167519], 53.1% Ca$_3$SiO$_5$ [162744] (triclinic structure), 5.3% c–Ca$_2$SiO$_4$ [417503] and 22.6% Ca$_2$SiO$_4$ [79552]; and

(b) a diffractogram for CEM Cement hydrated form (G3 hydrated): tricalcium silicate (Ca$_3$SiO$_5$), dicalcium silicate (Ca$_2$SiO$_4$), barium sulphate (BaSO$_4$) and ettringite (E) peaks. Rietveld analysis showed 10.7% BaSO$_4$ [167519], 57.7% Ca$_3$SiO$_5$ [64759] (monoclinic structure), 2.8% Ca$_2$SiO$_4$ [166637], 5.9% Ca$_6$Al$_2$(-SO$_4$)$_3$OH$_{12}$.H$_2$O [155395] and 22.8% amorphous phase.

Discussion

Investigating the crystalline phases of the hydrated forms of CSCs and comparing it with their unhydrated powder and calculation of reduction in tricalcium sili-cate and formation of calcium hydroxide powder form was an attempt to better understand the hydration and setting of these cements (8,9,13). Differences in the for-mation of calcium hydroxide may result in various bio-logical properties (14,15). The wide range of applications of CSCs (16,17) and the current scarcity of evidence regarding the hydration process of Biodentine and CEM Cement highlight the clinical importance of crystalline phase analysis and comparison of these materials.

According to the results of this study, as expected Ca_3SiO_5 was the predominant phase detected in all evalu-ated non-hydrated CSCs. During the initial stages of cement hydration, this phase reacts with water producing calcium hydroxide (7,9). Triclinic and monoclinic poly-morphic phases of Ca_3SiO_5 were only distinguishable in Biodentine and CEM cement and not in ProRoot MTA. The triclinic form was predominant in Biodentine and this was consistent with the study by Grech and coworkers (18). The powder form of CEM cement only contained the triclinic form. After hydration, no triclinic form could be detected in CEM cement and only the monoclinic form was detected. After hydration of Biodentine, both forms of Ca_3SiO_5 reduced and no monoclinic form was found. The temperature used during the sintering process of cement production and presence of impurities determine the exist-ing polymorphic phases of Ca_3SiO_5 (19,20), therefore indi-cating differences in the manufacturing temperatures used in the cements evaluated (21).

In the hydrated form of all three CSCs, an increase in the amorphous phase was seen. Greater amounts of amorphous phase tend to increase the hardness of mate-rials (13). The highest percentage of amorphous phase was seen in Biodentine (44%), with the percentage of this phase being 22.8% and 7.9% for CEM cement and tooth coloured ProRoot MTA, respectively. This greater percentage in Biodentine may indicate an advanced hydration in relation to the other two materials. In this regard, it is important to point out that the liquid of Bio-dentine contains calcium chloride as a setting accelerator (18,22). Furthermore, this cement contains calcium car-bonate, which acts as a nucleation site for C-S-H, reduc-ing the duration of the induction period and consequently causing the setting reaction to start a few minutes after mixing (10).

In this study, calcium hydroxide crystalline structures were only detected in the hydrated forms of ProRoot MTA and Biodentine. These changes occur consequent to the hydration of calcium silicate, which begins soon after the powder and liquid are combined and produces cal-cium silicate hydrate gel (C-S-H) and calcium hydroxide. Interestingly, in the case of CEM cement, no crystalline calcium hydroxide was detected after the hydration of CEM cement.

Soluble $Ca(OH)_2$ is released from the cement surface of CSCs during hydration (23), improving the alkalinity of the surrounding environment (24) and resulting in antibacterial properties (25). The reaction of soluble Ca $(OH)_2$ with phosphate present within body fluids and/or the phosphate present within the cement's liquid can result in the formation of hydroxyapatite (26,27). There-fore, the bioactivity and biocompatibility of CSCs are enhanced by releasing $Ca(OH)_2$ (27). Furthermore, when

soluble $Ca(OH)_2$ reaches saturated concentrations, crys-talline $Ca(OH)_2$ will be formed (14,28). Therefore, in the case of CEM cement, lack of crystalline $Ca(OH)_2$ may contribute to either early hydroxyapatite formation or sub-saturated concentrations of soluble $Ca(OH)_2$. Consid-ering that no reduction was seen in the peaks of trical-cium silicate in CEM cement, the latter is more likely to be the cause but more research is required regarding this matter.

Ettringite was detected in the hydrated forms of Pro-Root MTA and CEM cement as aluminate is present in their composition. This crystalline complex contains cal-cium, aluminium, silicon and sulphur and is responsible for resistance and hardening in the early hydration stage (9). The absence of ettringite peaks in the hydrated form of Biodentine may be a consequence of the reduction (or absence) of the aluminate phase in its powder form (10). The reduction/absence of the aluminate phase is an added benefit with respect to the workability of the fresh cement paste (10). It is associated with biological improvements, for example, avoiding the undesirable effects of aluminium (e.g. risks of Parkinson's and Alzhei-mer's disease) (29) and avoiding the leaching of trace ele-ments into surrounding tissues (30).

The alternative radiopacifiers detected in Biodentine (ZrO_2) and in CEM cement ($BaSO_4$) are an attempt to improve their chemical and mechanical properties. Bi_2O_3 has the potential to increase the setting time, decrease mechanical strength and increase the porosity of CSCs (13,22). It has been suggested that Bi_2O_3 present in Pro-Root MTA, unlike other radiopacifiers, is not inert during the cement hydration process (9,21). Rather, it has been reported to be present in the hydrated phase, forming a structure comprised of calcium silicate bismuth hydrate (C-S-H-Bi), which would affect $Ca(OH)_2$ precipitation and, consequently, the bioactivity of the hydrated mate-rial (9,21). In addition, a reduced amount of C-S-H will produce a poorer and slower hydration reaction, result-ing in a longer setting time and deterioration of mechani-cal properties (13), factors that would impact negatively on the clinical use of ProRoot MTA. On the contrary, the findings of this study revealed no reduction in the amount of Bi_2O_3 detected. This inconsistency may be due to different methodologies. For instance, in the study by Camilleri *et al.* (9,21), analysis was conducted 1 month after cement preparation. Further research is suggested to evaluate the amount of Bi_2O_3 at different time intervals after cement preparation.

Conclusion

The hydrated form of CEM cement had a different beha-viour in relation to tricalcium silicate and $Ca(OH)_2$ peaks

when compared with the hydrated forms of Biodentine and ProRoot MTA. In CEM cement, no reduction in tri-calcium silicate occurred and formation of calcium hydroxide was not detected. Biodentine did not have ettringite in its hydrated form, in contrast to the other cements. The highest percentage of amorphous phase was seen in Biodentine.

Author Contribution

All authors have contributed significantly and are in agreement with the manuscript.

Disclosure of interests

Authors deny any conflict of interest.

References

1. Kaup M, Schafer E, Dammaschke T. An in vitro study of different material properties of Biodentine compared to ProRoot MTA. Head Face Med. 2015; 11: 16.

2. Basturk FB, Nekoofar MH, Gunday M, Dummer PM. The effect of various mixing and placement techniques on the compressive strength of mineral trioxide aggregate. J Endod. 2013; 39: 111–4.

3. Esmaeili B, Alaghehmand H, Kordafshari T et al. Coronal discoloration induced by calcium-enriched mixture, min-eral trioxide aggregate and calcium hydroxide: a spec-trophotometric analysis. Iran Endod J. 2016; 11: 23–8.

4. Kohli MR, Yamaguchi M, Setzer FC, Karabucak B. Spec-trophotometric analysis of coronal tooth discoloration induced by various bioceramic cements and other endodontic materials. J Endod. 2015; 41: 1862–6.

5. Butt N, Talwar S, Chaudhry S et al. Comparison of physi-cal and mechanical properties of mineral trioxide aggre-gate and biodentine. Indian J Dent Res. 2014; 25: 692–7.

6. Camilleri J. Investigation of biodentine as dentine replace-ment material. J Dent 2013; 41: 600–10.

7. Camilleri J. Hydration characteristics of biodentine and theracal used as pulp capping materials. Dent Mater 2014; 30: 709–15.

8. Guven Y, Tuna EB, Dincol ME, Aktoren O. X-ray diffrac-tion analysis of MTA-Plus, MTA-Angelus and DiaRoot BioAggregate. Eur J Dent. 2014; 8: 211–5.

9. Camilleri J. Characterization of hydration products of mineral trioxide aggregate. Int Endod J 2008; 41: 408–17.

10. Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium sili-cate cement biodentine and MTA angelus. Dent Mater 2013; 29: 580–93.

11. Walenta G, Fullmann T. Advances in quantitative XRD analysis for clinker, cements, and cementitious addition. Powder Diffr. 2004;19:40–4.

12. Nekoofar MH, Adusei G, Sheykhrezae MS et al. The effect of condensation pressure on selected physical properties of mineral trioxide aggregate. Int Endod J 2007; 40: 453– 61.

13. Grazziotin-Soares R, Nekoofar MH, Davies TE et al. Effect of bismuth oxide on white mineral trioxide aggregate: chemical characterization and physical properties. Int Endod J 2014; 47: 520–33.

14. Nekoofar MH, Davies TE, Stone D et al. Microstructure and chemical analysis of blood-contaminated mineral tri-oxide aggregate. Int Endod J 2011; 44: 1011–8.

15. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental trauma-tology. Int Endod J 2011; 44: 697–730.

16. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end fill-ing material. J Endod. 1993; 19: 591–5.

17. Haghgoo R, Asgary S, Mashhadi Abbas F, Montazeri Hede-shi R. Nano-hydroxyapatite and calcium-enriched mix-ture for pulp capping of sound primary teeth: a randomized clinical trial. Iran Endod J. 2015; 10: 107–11.

18. Grech L, Mallia B, Camilleri J. Characterization of set intermediate restorative material, biodentine, bioaggre-gate and a prototype calcium silicate cement for use as root-end filling materials. Int Endod J 2013; 46: 632– 41.

19. De Noirfontaine M-N, Courtial M, Dunstetter F et al. Tri-calcium silicate Ca3SiO5 superstructure analysis: a route towards the structure of the M1 polymorph. Zeitschrift fur Kristallographie. 2012; 227: 102–12.

20. Dunstetter F, De NM-N, Courtial M. Polymorphism of tri-calcium silicate, the major compound of Portland cement clinker: 1. Structural data: review and unified analysis. Cem Concr Res 2006; 36: 39–53.

21. Camilleri J. Hydration characteristics of calcium silicate cements with alternative radiopacifiers used as root-end filling materials. J Endod. 2010; 36: 502–8.

22. Formosa LM, Mallia B, Camilleri J. The effect of curing conditions on the physical properties of tricalcium silicate cement for use as a dental biomaterial. Int Endod J 2012; 45: 326–36.

23. Cavenago BC, Pereira TC, Duarte MA et al. Influence of powder-to-water ratio on radiopacity, setting time, pH, calcium ion release and a micro-CT volumetric solubility of white mineral trioxide aggregate. Int Endod J 2014; 47: 120–6.

24. Amini Ghazvini S, Abdo Tabrizi M, Kobarfard F et al. Ion release and pH of a new endodontic cement, MTA and Portland cement. Iran Endod J. 2009; 4: 74–8.

25. Estrela C, Sydney GB, Bammann LL, Felippe JO. Mecha-nism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J. 1995; 6: 85–90.

26. Lazic S. Microcrystalline hydroxyapatite formation from alkaline solutions. J Cryst Growth 1995; 147: 147–54.

27. Sarkar NK, Caicedo R, Ritwik P et al. Physicochemical basis of the biologic properties of mineral trioxide aggre-gate. J Endod. 2005; 31: 97–100.

28. Niu LN, Jiao K, Wang TD et al. A review of the bioactivity of hydraulic calcium silicate cements. J Dent 2014; 42: 517–33.

29. Forbes WF, Gentleman JF. Risk factors, causality, and pol-icy initiatives: the case of aluminum and mental impair-ment. Exp Gerontol 1998; 33: 141–54.

30. Camilleri J, Kralj P, Veber M, Sinagra E. Characterization and analyses of acid-extractable and leached trace ele-ments in dental cements. Int Endod J 2012; 45: 737–43.