Published in final edited form as:

J Endod. 2013 September ; 39(9): 1161–1166. doi:10.1016/j.joen.2013.04.005.

***In vitro* osteogenic/dentinogenic potential of an experimental calcium aluminosilicate cement**

Ashraf A. Eid, BDS, MSc¹, Li-na Niu, DDS, MS², Carolyn M. Primus, PhD³, Lynne A. Opperman, PhD⁴, Ikuya Watanabe, DDS, PhD¹, David H. Pashley, DMD, PhD⁵, and Franklin R. Tay, BDS (Hons), PhD^{5,6}

¹Department of Dental and Biomedical Material Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

²Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, China

³Primus Consulting, 7046 Owl's Nest Terrace, Bradenton, FL

⁴Center for Craniofacial Research and Diagnosis, and Department of Biomedical Sciences, Texas A&M University Baylor College of Dentistry, Dallas, TX

⁵Department of Oral Biology, Georgia Health Sciences University, Augusta, GA

⁶Department of Endodontics, Georgia Health Sciences University, Augusta, GA

Abstract

Introduction—Calcium aluminosilicate cements are fast-setting, acid-resistant, bioactive cements that may be used as root-repair materials. This study examined the osteogenic/dentinogenic potential of an experimental calcium aluminosilicate cement (Quick-Set) using a murine odontoblast-like cell model.

Methods—Quick-Set and white ProRoot MTA (WMTA) were mixed with the proprietary gel or deionized water, allowed to set completely in 100% relative humidity and aged in complete growth medium for 2 weeks until rendered non-cytotoxic. Similarly-aged Teflon discs were used as negative control. The MDPC-23 cell-line was used for evaluating changes in mRNA expressions of genes associated with osteogenic/dentinogenic differentiation and mineralization (qRT-PCR) alkaline phosphatase enzyme production and extracellular matrix mineralization (Alizarin red-S staining).

Results—After MDPC-23 cells were incubated with the materials in osteogenic differentiation medium for 1 week, both cements showed upregulation in *ALP* and *DSPP* expression. Fold increases in these two genes were not significantly different between Quick-Set and WMTA. Both cements showed no statistically significant upregulation/downregulation in *RUNX2*, *OCN*, *BSP* and *DMP1* gene expression compared with Teflon. Alkaline phosphatase activity of cells cultured on Quick-Set and WMTA were not significantly different at 1 week or 2 weeks, but were

© 2013 American Association of Endodontics. Published by Elsevier Inc. All rights reserved.

Corresponding author: Dr. Franklin Tay, Department of Endodontics, College of Dental Medicine, Georgia Health Sciences University, Augusta, Georgia, 30912-1129, USA. TEL: (706) 7212152; FAX: (706) 7218184; ftay@georgiahealth.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest statement:

Dr. Primus is the inventor of Quick-Set.

significantly higher ($p<0.05$) than Teflon in both weeks. Both cements showed significantly higher calcium deposition compared with Teflon after 3 weeks of incubation in mineralizing medium ($p<0.001$). Differences between Quick-Set and WMTA were not statistically significant.

Conclusions—The experimental calcium aluminosilicate cement exhibits similar osteogenic/dentinogenic properties to WMTA and may be a potential substitute for commercially-available tricalcium silicate cements.

Keywords

alizarin red-S staining; alkaline phosphatase; calcium aluminosilicate cement; quantitative reverse transcription polymerase chain reaction; MDPC-23 cells

INTRODUCTION

Since its introduction in 1993 in the form of mineral trioxide aggregate (MTA), tricalcium silicate hydraulic cements have gained worldwide acceptance as the material of choice for various endodontic applications [1]. The biocompatibility, osteogenicity and hydrophilicity of mineral trioxide aggregate made it ideal for use as a root-end filling material following surgical endodontics, perforation repair, vital pulp therapy including direct pulp capping and pulpotomy of immature teeth (apexogenesis), as an apical barrier in immature teeth with necrotic pulps (apexification), and as a coronal barrier in regenerative endodontic procedures in immature teeth with apical periodontitis [2]. Despite the favorable clinical outcome of tricalcium silicate cements, some tricalcium silicate products possess major clinical deficiencies such as suboptimal handling characteristics, poor washout resistance, slow setting reaction [3], uncontrolled expansion [4], incompatibility with current adhesive restorative materials [5] and initial cytotoxicity [6]. Although some of those drawbacks were addressed in more recent formulations of tricalcium silicate cements [7], the development of alternative biocompatible endodontic cements with overall improved properties is highly desirable.

Calcium aluminate hydraulic cements for biomedical uses are modifications of high-alumina cements used for refractory linings in furnaces and some civil engineering purposes. They were developed in the early 20th century as more chemically durable cements than Portland cement [8]. The hydraulic calcium phases typically contain 30 to 50% alumina, whereas Portland cements contain less than 5%. Commercial, non-biomedical, calcium aluminate cements are used as sewer pipe lining due to their inherent acid resistance to biogenic corrosion. This acid-resistant feature may be useful in infection-laden sites of the human body where the pH value drops significantly [9]. In the biomedical field, these inert, biocompatible, and sufficiently strong cements have been successfully used as bone cements and in orthopedic implants [10]. In dentistry, calcium aluminate powder has been incorporated into direct restorative materials (DoxaDent; Doxa AB, Uppsala, Sweden) [11] and in glass-ionomer based luting cement (Ceramir C&B, Doxa AB) [12]. Calcium aluminate with the addition of radiopacifier powders were mixed with water or salt solution to form an endodontic cement of similar indications as commercially-available tricalcium silicate cements (EndoBinder; Binderware, São Carlos, SP, Brazil) [13].

Capasio (Primus Consulting, Bradenton, FL) is an experimental calcium aluminosilicate cement designed for endodontic use. It is composed primarily of calcium aluminosilicate powder, dental glass and bismuth oxide as a radiopacifier, and is supplied with a water-based gel. This hydraulic calcium aluminosilicate cement was found to be slightly less basic (pH 10.9) than white MTA (pH 11.6) upon final set, had a setting time of 9 min, penetrated dentinal tubules, and demonstrated improved acid resistance and washout resistance when

compared with white MTA [3, 14]. Its *in vitro* biocompatibility and bone nodule formation had also been tested in rat osteoblasts [15]. Recently, Capasio powder has been refined and renamed as Quick-Set (Primus Consulting) and the cationic surfactant was removed from the liquid gel component, which was thought to interfere with its biocompatibility [15]. A recent biocompatibility study using a murine dental papilla-derived odontoblast-like cell line (MDPC-23) showed that Quick-Set exhibited similar *in vitro* cytotoxicity profiles as WMTA (performed with MTT assay, flow cytometry and vital cell staining) [6]. However, it is not known whether this experimental aluminosilicate endodontic cement possesses osteogenic/dentinogenic properties that may promote periapical bone healing and pulpal calcific barrier formation; these properties being prominent features in commercially-available tricalcium silicate cements [2]. Thus, the objective of the present study was to investigate the effect of Quick-Set on the osteogenic/dentinogenic differentiation potential of murine odontoblast-like cells. The null hypothesis tested was that no differences exist between the experimental calcium aluminosilicate cement and a commercially-available tricalcium silicate cement on the osteogenic/dentinogenic differentiation potential of murine odontoblast-like cells.

MATERIALS AND METHODS

Quick-Set and white ProRoot MTA (WMTA; Dentsply Tulsa Dental Specialties, Tulsa, OK) were mixed with the proprietary gel or deionized water, respectively, in a powder/water ratio of 3:1. The mixed materials were placed in pre-sterilized Teflon molds (5-mm diameter and 3-mm thick), covered with pre-sterilized Mylar sheets, and allowed to set completely in a 100% humidity chamber for 24 hours. Teflon disks of the same dimensions were used as the control. All materials were sterilized with ultraviolet light for 4 hours before testing. Prior to evaluating the osteogenic/odontogenic potential of test cements, the cement and control discs were rendered non-cytotoxic by immersing them in complete growth medium (Dulbecco's Modified Eagle's Medium (DMEM, Lonza, Wakersville, MD) for 2 weeks, as recommended by a previous study [6].

Similar to the previously reported biocompatibility study on Quick-Set [6], the osteogenic/dentinogenic potential of both cements was evaluated using the previously reported MDPC-23 cell line [16]. The MDPC-23 cells were plated at 1×10^4 cells/cm² and cultured in the aforementioned complete growth medium, supplemented with 10% fetal bovine serum (Gibco, Invitrogen Corp., Carlsbad, CA), 2 mmol/L L-glutamine and 100 U/mL penicillin-streptomycin until they exhibited ~70–80% confluence. The growth medium was then replaced by osteogenic differentiation medium, consisting of the original growth medium, supplemented with 50 µg/mL ascorbic acid, 10 mM β-glycerophosphate and 100 nM dexamethasone (Sigma-Aldrich, St. Louis, MO). The aged materials were placed in respective Transwell® inserts (Corning Inc. Life Sciences, Tewksbury, MA) and inserted into the culture wells with osteogenic differentiation medium. The latter was changed every 3 days and the effect of the test cements on the osteogenic/dentinogenic differentiation potential of the MDPC-23 cells was evaluated from three aspects.

Messenger RNA (mRNA) Expression of Genes Associated with Osteogenic/Dentinogenic Differentiation and Mineralization

Expression of osteogenic/dentinogenic differentiation markers was examined using quantitative reverse transcription polymerase chain reaction (qRT-PCR). This procedure was conducted after the MPDC-23 cells were incubated with aged cement discs in osteogenic differentiation medium for 1 week. Similarly-aged Teflon discs were used as the control.

The markers used for examination of differentiation of hard tissue forming cells and their mineralization were: alkaline phosphatase (*ALP*), runt-related transcription factor 2 (*RUNX2*), osteocalcin (*OCN*), bone sialoprotein (*BSP*), dentin sialophosphoprotein (*DSPP*)

and dentin matrix protein-1 (*DMP1*) [17, 18]. The qRT-PCR procedures were performed by isolating total RNA from the MDPC-23 cells. The total RNA was used as templates for synthesis of single-stranded complementary DNA (cDNA), using QIAshredder and RNeasy kit (Qiagen, Valencia, CA). The manufacturer's recommended protocol was followed for RNA purification from animal cells. The purity and quantity of the resultant RNA was then assessed from 2-μL samples using a NanoDrop1000 spectrophotometer (ThermoScientific, Wilmington, DE). Equal amounts of total RNA (0.1 mg RNA/mL) were then reverse-transcribed into single-stranded cDNA, using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA), in a thermal cycler using the recommended settings. The resultant cDNA was then amplified in real-time using TaqMan® Universal master mix and Ready-to-go format TaqMan® assays (Life Technologies, Grand Island, NY) for the designated target genes in a 7300 Real Time PCR system (Applied Biosystems). Each sample was run in triplicate. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the housekeeping gene. Relative quantification of gene expression was performed using the comparative threshold cycle method ($\Delta\Delta C_T$), where the level of each target gene expression was normalized to the *GAPDH* endogenous control used as an active reference. The data output was expressed as fold-changes of expression levels, given by $2^{-\Delta\Delta C_T}$ [19]. Expression profiles of the target genes were compared among test groups. As the data for each gene were normally distributed (Shapiro-Wilk test) and exhibited equal variance (modified Levene test), they were statistically analyzed using one-factor ANOVA, with statistical significance preset at $\alpha = 0.05$. The Tukey method of multiple comparisons was used to perform pair-wise comparisons of the three groups at each gene level. A Bonferroni-adjusted significance level of $0.05/3 = 0.017$ was used for each pair-wise comparison so that the family-wise error rate for each gene expression level could be controlled at the 0.05 level.

Alkaline Phosphatase Enzymatic Assay

Cells were cultured in osteogenic differentiation medium as described previously and incubated with the 3 materials (Quick-Set, WMTA, Teflon control) for 7 and 14 days (N=6 wells; different MPDC-23 cells examined at the two time-points). Alkaline phosphatase activity was determined using a QuantiChrom ALP assay kit (BioAssay Systems, Hayward, CA). Colorimetric determination was based on hydrolysis of *p*-nitrophenyl phosphate by ALP into inorganic phosphate and *p*-nitrophenol, a yellow-colored product [20]. After incubation, the cells were lysed with 0.2% Triton X-100 for 20 minutes, and the cell lysate was incubated with *p*-nitrophenyl phosphate at 37 °C for 1 hour. Absorbance of *p*-nitrophenol at 405 nm was recorded every minute for a maximum of 16 minutes using a microplate reader. The level of alkaline phosphatase activity of the cell lysate in IU/L was calculated from the equation:

$$\frac{(OD_{Sample\ t} - OD_{Sample\ 0}) \cdot Reaction\ Vol \times 35.3}{(OD_{Calibrator} - OD_{H_2O}) \cdot Sample\ Vol \cdot t}$$

Where *OD* is the optical density at 405 nm and *t* is the time in minutes. *OD_{Sample 0}* is the optical density at 0 minutes. The resultant data was plotted on a graph and the peak value for each material was selected and compared to the other materials. As the raw data obtained for the 3 materials and 2 time-points were not normally-distributed, they were logarithmically transformed and analyzed using two-factor ANOVA to examine the effect of materials and time-points, and the interaction of these two factors on ALP activity. The Tukey test was used for comparison among groups. For all tests, statistical significance was preset at $\alpha = 0.05$.

Extracellular Matrix Mineralization and Calcium Deposition (Alizarin Red-S Staining)

Cells were cultured in osteogenic differentiation medium as previously described, and exposed to the 3 materials (Quick-Set, WMTA, Teflon control) for 21 days (N = 18). Extracellular mineralization was qualitatively and quantitatively determined using the alizarin red-S assay [21]. Alizarin red-S will stain calcium-rich deposits (nodules) produced by cells in culture. The dye can be extracted from the stained nodules and assayed by spectrophotometry. The tissue culture dishes were washed with phosphate-buffered saline, the cells and nodules fixed in 10 % formaldehyde, and stained with alizarin red-S (40 mM solution, pH 4.2). Images of the specimens were taken for qualitative assessment of the stained nodules. The stained nodules were then incubated in 10% acetic acid for 30 minutes and neutralized with 10% ammonium hydroxide. Absorbance of the supernatants was determined at OD = 405 nm. The level of alizarin red-S staining in the samples ($\mu\text{g/L}$) was determined according to a linear regression equation derived from the pre-equilibration standard curve. As the data were not normally distributed, they were analyzed using Kruskal-Wallis ANOVA and Dunn's multiple comparison test at $\alpha = 0.05$.

RESULTS

mRNA Expressions

Fold changes of osteogenic differentiation markers in MDPC-23 cells after 1 week of incubation with Quick-Set, WMTA, and Teflon are collectively represented in Figure 1. There was little or no change of *RUNX2*, *OCN* and *DMP1* gene markers expression in both experimental groups; however, the results were not statistically significant from the Teflon control ($P = 0.583$ for *RUNX2*; $P = 0.263$ for *OCN*; $P = 0.138$ for *DMP1*). Expressions of *ALP* among the three groups were significantly different ($P = 0.003$); however there was no difference in the fold upregulation between Quick-Set and WMTA ($p = 0.662$). Expression of *DSPP* among the three groups were also significantly different ($P = 0.003$); however, there was no significant difference in the fold upregulation between Quick-Set and WMTA ($P = 0.072$). No significant difference in the fold changes between Quick-Set and Teflon could be detected after Bonferroni adjustment ($P = 0.044$). Expression of *BSP* appeared to have been downregulated in both experimental groups, but the fold changes were not significantly different from Teflon control ($P = 0.385$).

Alkaline Phosphatase Activity

A summary of the ALP activities of MDPC-23 cells, after 1 and 2 weeks of exposure to the test materials in osteogenic differentiation medium, is presented in Figure 2. Both materials ($P < 0.001$) and time-points ($P < 0.001$) significantly affected ALP activity. The interaction of these two factors was not statistically significant ($P = 0.177$). For each time-point, ALP activities exhibited by Quick-Set and WMTA were not significant different, but were significantly higher than that exhibited by the Teflon control ($P < 0.05$).

Extracellular Matrix Mineralization and Calcium Deposition

Figures 3A-3C are representative images of alizarin red-S stained calcium deposits produced by the MDPC-23 cells cultured in osteogenic medium, after exposure for 3 weeks to Quick-Set, WMTA and Teflon, respectively. Nodular calcific deposits of varying stain intensities could be observed macroscopically. Significant differences were observed in the amount of alizarin red-S staining among the three groups ($p < 0.001$). The amounts of dye expressed in Quick-Set and WMTA were not significantly different ($p > 0.05$), but were both significantly higher than the amount of dye expressed in Teflon ($p < 0.05$). Concentrations ($\mu\text{g/mL}$) of alizarin red-S extracted from the stained calcium deposits in the three groups are summarized in Figure 3D.

DISCUSSION

Osteogenicity and dentinogenicity are highly desirable properties for any endodontic cement. Osteogenicity of commercially-available endodontic cements has been demonstrated previously [2]. However, reliable dentinogenesis following vital pulp therapy remain an elusive goal [22]. In the present study, a cause/effect, time-dependent strategy was employed to study the osteogenicity/dentinogenicity of the test cements. According to this strategy, the mRNA expression profiles of target genes were evaluated during the first week. This was followed by assessment of the enzymatic activity of ALP in the 1st and 2nd week. Qualitative and quantitative expressions of calcific deposits and extracellular matrix mineralization were subsequently examined in the third week. Freshly mixed test cements were found in a previous study to affect mitochondrial activity and induce apoptosis and necrosis in MDPC-23 cells [6]. These cytotoxic effects were transient and decreased with elution of the cytotoxic components from the materials with time. Our time-dependent strategy took into consideration the cytotoxicity profile of test cements and its inhibitive effects on the mineralization potential of such cements [6]. Accordingly, all cement samples were rendered non-cytotoxic prior to testing their mineralization potential by immersing them in complete growth medium for 2 weeks. The MDPC-23 odontoblast-like cells were employed in the present study as their partially-differentiated status enabled them to differentiate into either osteogenic or dentinogenic lineage [18].

On the gene expression level, several gene markers associated with osteogenic/dentinogenic differentiation were examined; among which is *ALP*, which has been shown to be expressed in the earlier stages of bone formation [23]. Likewise, *RUNX2*, is upregulated in immature osteoblasts, but downregulated in mature osteoblasts [24]. *RUNX2* inhibits osteoblast maturation and mature bone formation, and was also found to inhibit terminal differentiation of odontoblasts, and induces transdifferentiation of odontoblasts into osteoblasts [18]. *OCN* is involved in bone remodeling by exerting a role in the resorption process and adhesion of osteoclasts [25]. The function of *BSP* in mineralized tissues is unknown, although it may act as a nucleus for the formation of the first apatite crystallites [26, 27]. Both Quick-Set and WMTA did not alter the gene expressions of *RUNX2*, *OCN* or *BSP* to statistically significant levels when compared with the Teflon control. However, both cements significantly upregulated the expression of *ALP* gene when compared with the Teflon control.

Both *DSPP* and *DMP1* are gene markers that are most closely associated with dentinogenesis [28]. Cleaving of *DSPP* into dentin phosphoprotein (*DPP*) and dentin sialoprotein (*DSP*) occurs during dentin mineralization. While *DSP* may be involved in the initiation of dentin mineralization but not in the maturation of this tissue [28], the functions of *DSP* are presently undefined [29]. *Dentin phosphoprotein (DPP)*, the most abundant extracellular matrix protein component in dentin, has been found to play a role in nucleation and growth of hydroxyapatite crystallites during dentin mineralization; it has a high affinity for type I collagen, and has been shown to activate the SMAD pathway [30]. *Dentin matrix protein 1* belongs to the SIBLING (Small Integrin Binding N-linked Glycoproteins) family, which are associated with mineralized tissues [31]. After post-translational proteolytic cleavage, *DMP1* exists within the extracellular matrix of bone and dentin as a NH₂-terminal fragment, a COOH-terminal fragment, and the proteoglycan form of the NH₂-terminal fragment (DMP1-PG) [32]. Biochemical analysis of bovine teeth showed that predentin is rich in DMP1-PG, whereas mineralized dentin primarily contains the COOH-terminal fragment [33], and so, it is suggested that the C-terminal 57 kDa fragment (COOH terminal) of *DMP1* is primarily responsible for the function of this protein in dentin biomineralization [33].

In the present study, both cements stimulated the upregulation of *DSPP*. Although there was a tendency of *DMP1* by both cements compared with Teflon, the level of upregulation was not statistically significant. The lack of statistically significant upregulation of *DMP1* may be caused by small sample size that resulted in large standard deviations observed for the experimental groups. It has been suggested that *DSPP* may be upregulated by *DMP1* during dentinogenesis [34]. This speculation is further supported by another study [35] showing that the COOH-terminal end of *DMP1*, which is localized in the nucleus during early differentiation of the odontoblasts, is able to bind specifically with the rat *DSPP* promoter in the region between nt -450 and +80 and to activate the transcription of *DSPP*. The upregulated expressions of *DSPP* in Quick-Set and WMTA suggest that both cements may favor dentinogenic differentiation of odontoblast-like cells. This is of clinical interest in cases such as direct pulp capping and vital pulpotomy, where deposition of osteodentin or bone-like tissues is the inevitable outcomes [22]. This conclusion is also supported by the decreased expression of the more osteoblast-specific differentiation markers. We speculate that inclusion of an epithelial component in the cell culture model may elicit a more profound dentinogenic response, since the resultant epithelial-mesenchymal interactions might induce a more dentinogenic-favorable crosstalk between both cellular components. Research in this direction is underway.

Compared with the qRT-PCR data, the ALP enzymatic activities yielded more definitive results that are indicative of increased matrix mineralization as a result of exposure of MDPC-23 cells to aged Quick-Set and WMTA cements. Alkaline phosphatase activity for both cements was significantly higher than the control, indicating increased osteoblastic/odontoblastic activity and increased mineralization turnover. Alizarin red-S assay results also suggest increased extracellular calcium deposition by MDPC-23 cells after exposure to Quick-Set and WMTA.

Within the limits of the present study, it may be concluded that the *in vitro* osteogenic/dentinogenic differentiation potential of Quick-Set is not different from that of MTA after elution of their cytotoxic components. Thus, the null hypothesis that no differences exist between the experimental calcium aluminosilicate cement and a commercially-available tricalcium silicate cement on the osteogenic/dentinogenic differentiation potential of murine odontoblast-like cells can not be rejected. It will be of interest to see if the favorable *in vitro* response exhibited by Quick-Set can be replicated in a large animal study, to test the effect of Quick-Set aluminosilicate cement on healing of periapical lesions, as well as its effect on the quality and quantity of calcific barrier formation in vital pulp therapy.

Acknowledgments

This study was supported by grant R44 DE20204-02 from the National Institute of Dental & Craniofacial Research. The authors report that a financial affiliation exists for this paper: Dr. Primus is the inventor of Quick-Set. The MDPC-23 cell line utilized in the present study was a generous gift from Professor Jacques Nör, University of Michigan Dental School, Ann Arbor, MI. The authors thank Mrs. Petra Lockwood for her assistance in cell culture.

REFERENCES

1. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endod.* 1993; 19:591–595. [PubMed: 8151252]
2. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--part III: clinical applications, drawbacks, and mechanism of action. *J Endod.* 2010; 36:400–413. [PubMed: 20171353]
3. Porter ML, Berto A, Primus CM, Watanabe I. Physical and chemical properties of new-generation endodontic materials. *J Endod.* 2010; 36:524–528. [PubMed: 20171376]

4. Storm B, Eichmiller FC, Tordik PA, Goodell GG. Setting expansion of gray and white mineral trioxide aggregate and Portland cement. *J Endod.* 2008; 34:80–82. [PubMed: 18155499]
5. Eid AA, Komabayashi T, Watanabe E, Shiraishi T, Watanabe I. Characterization of the mineral trioxide aggregate-resin modified glass ionomer cement interface in different setting conditions. *J Endod.* 2012; 38:1126–1129. [PubMed: 22794220]
6. Wei W, Qi YP, Nikonov SY, Niu LN, Messer RL, Mao J, et al. Effects of an experimental calcium aluminosilicate cement on the viability of murine odontoblast-like cells. *J Endod.* 2012; 38:936–942. [PubMed: 22703657]
7. Modareszadeh MR, Di Fiore PM, Tipton DA, Salamat N. Cytotoxicity and alkaline phosphatase activity evaluation of endosequence root repair material. *J Endod.* 2012; 38:1101–1105. [PubMed: 22794214]
8. Neville, AM.; Wainwright, PJ. High alumina cement concrete. Lancaster: Construction Press Ltd; 1975.
9. Ye RC. The relationship of pH of the granulation tissue and the take of the skin graft. *Plast Reconstr Surg.* 1957; 19:213–217.
10. Oh SH, Choi SY, Lee YK, Kim KN. Preparation of calcium aluminate cement for hard tissue repair: effects of lithium fluoride and maleic acid on setting behavior, compressive strength, and biocompatibility. *J Biomed Mater Res.* 2002; 62:593–599. [PubMed: 12221708]
11. van Dijken JW, Sunnegardh-Gronberg K. A calcium aluminate cement as restorative material in Class V cavities. *Swed Dent J.* 2004; 28:111–118. [PubMed: 15506688]
12. Jefferies SR, Pameijer CH, Appleby DC, Boston D, Galbraith C, Loof J, et al. Prospective observation of a new bioactive luting cement: 2-year follow-up. *J Prosthodont.* 2012; 21:33–41. [PubMed: 22040467]
13. Jacobovitz M, Vianna ME, Pandolfelli VC, Oliveira IR, Rossetto HL, Gomes BP. Root canal filling with cements based on mineral aggregates: an in vitro analysis of bacterial microleakage. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009; 108:140–144. [PubMed: 19540451]
14. Bird DC, Komabayashi T, Guo L, Opperman LA, Spears R. In vitro evaluation of dentinal tubule penetration and biomineralization ability of a new root-end filling material. *J Endod.* 2012; 38:1093–1096. [PubMed: 22794212]
15. Washington JT, Schneiderman E, Spears R, Fernandez CR, He J, Opperman LA. Biocompatibility and osteogenic potential of new generation endodontic materials established by using primary osteoblasts. *J Endod.* 2011; 37:1166–1170. [PubMed: 21763915]
16. Moghaddame-Jafari S, Mantellini MG, Botero TM, McDonald NJ, Nör JE. Effect of ProRoot MTA on pulp cell apoptosis and proliferation in vitro. *J Endod.* 2005; 31:387–391. [PubMed: 15851935]
17. Hao J, Zou B, Narayanan K, George A. Differential expression patterns of the dentin matrix proteins during mineralized tissue formation. *Bone.* 2004; 34:921–932. [PubMed: 15193538]
18. Miyazaki T, Kanatani N, Rokutanda S, Yoshida C, Toyosawa S, Nakamura R, et al. Inhibition of the terminal differentiation of odontoblasts and their transdifferentiation into osteoblasts in Runx2 transgenic mice. *Arch Histol Cytol.* 2008; 71:131–146. [PubMed: 18974605]
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods.* 2001; 25:402–408. [PubMed: 11846609]
20. Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum. *J Biol Chem.* 1946; 164:321–329. [PubMed: 20989492]
21. Gregory CA, Gunn WG, Peister A, Prockop DJ. An Alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Anal Biochem.* 2004; 329:77–84. [PubMed: 15136169]
22. Okiji T, Yoshida K. Reparative dentinogenesis induced by mineral trioxide aggregate: a review from the biological and physicochemical points of view. *Int J Dent.* 2009; 2009:464280. [PubMed: 20339574]
23. Golub EE, Boesze-Battaglia K. The role of alkaline phosphatase in mineralization. *Curr Opin Orthop.* 2007; 18:444–448.
24. Komori T. Regulation of osteoblast differentiation by Runx2. *Adv Exp Med Biol.* 2010; 658:43–49. [PubMed: 19950014]

25. Chenu C, Colucci S, Grano M, Zigrino P, Barattolo R, Zambonin G, et al. Osteocalcin induces chemotaxis, secretion of matrix proteins, and calcium-mediated intracellular signaling in human osteoclast-like cells. *J Cell Biol.* 1994; 127:1149–1158. [PubMed: 7962073]
26. Qin C, Brunn JC, Jones J, George A, Ramachandran A, Gorski JP, et al. A comparative study of sialic acid-rich proteins in rat bone and dentin. *Eur J Oral Sci.* 2001; 109:133–141. [PubMed: 11347657]
27. Hunter GK, Goldberg HA. Modulation of crystal formation by bone phosphoproteins: role of glutamic acid-rich sequences in the nucleation of hydroxyapatite by bone sialoprotein. *Biochem J.* 1994; 302(Pt 1):175–179. [PubMed: 7915111]
28. Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, et al. Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. *Matrix Biol.* 2009; 28:221–229. [PubMed: 19348940]
29. Boskey A, Spevak L, Tan M, Doty SB, Butler WT. Dentin sialoprotein (DSP) has limited effects on in vitro apatite formation and growth. *Calcif Tissue Int.* 2000; 67:472–478. [PubMed: 11289697]
30. Prasad M, Butler WT, Qin C. Dentin sialophosphoprotein in biomineralization. *Connect Tissue Res.* 2010; 51:404–417. [PubMed: 20367116]
31. Fisher LW, Fedarko NS. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res.* 2003; 44(Suppl 1):33–40. [PubMed: 12952171]
32. Beniash E, Deshpande AS, Fang PA, Lieb NS, Zhang X, Sfeir CS. Possible role of DMP1 in dentin mineralization. *J Struct Biol.* 2011; 174:100–106. [PubMed: 21081166]
33. Maciejewska I, Cowan C, Svoboda K, Butler WT, D'Souza R, Qin C. The NH₂-terminal and COOH-terminal fragments of dentin matrix protein 1 (DMP1) localize differently in the compartments of dentin and growth plate of bone. *J Histochem Cytochem.* 2009; 57:155–166. [PubMed: 18854597]
34. Ye L, MacDougall M, Zhang S, Xie Y, Zhang J, Li Z, et al. Deletion of dentin matrix protein-1 leads to a partial failure of maturation of predentin into dentin, hypomineralization, and expanded cavities of pulp and root canal during postnatal tooth development. *J Biol Chem.* 2004; 279:19141–19148. [PubMed: 14966118]
35. Narayanan K, Gajjaraman S, Ramachandran A, Hao J, George A. Dentin matrix protein 1 regulates dentin sialophosphoprotein gene transcription during early odontoblast differentiation. *J Biol Chem.* 2006; 281:19064–19071. [PubMed: 16679514]

Figure 1.

Quantitative real-time polymerase chain reaction (qRT-PCR) results showing fold changes in target genes expressed by MDPC-23 cells, after they were exposed to the test materials for 1 week in osteogenic differentiation medium. Gene markers examined were: alkaline phosphatase (*ALP*), runt-related transcription factor 2 (*RUNX2*), osteocalcin (*OCN*), bone sialoprotein (*BSP*), dentin sialophosphoprotein (*DSPP*) and dentin matrix protein 1 (*DMP1*). Fold changes of the target genes in the two experimental group and the Teflon negative control were performed using the $\Delta\Delta C_T$ method. Values >1 indicate upregulation while values <-1 indicate downregulation. Any value between 1 (dotted line) and -1 (not shown) indicates no fold change. Only genes with significant differences among the 3 groups are labeled. For each of those genes, columns labeled with the same descriptor are not significantly different ($p > 0.05$).

Figure 2.

Alkaline phosphatase (ALP) enzymatic activities of MDPC-23 cells, after 1 and 2 weeks of exposure to the test materials in osteogenic differentiation medium. Data are expressed in international units per liter (IU/L). Each data column represents mean and one standard deviation. For each time-point (Week 1 or Week 2), material columns connected with a horizontal bar are not significantly different ($P < 0.05$). For each material, columns for the two time-points that are labeled with different designators (Quick-Set – upper case letters; WMTA – lower case letters; Teflon control – numeric) are significantly different ($P < 0.05$).

Figure 3.

A-C. Representative images of alizarin red S-stained calcium deposits produced by the MDPC-23 cells cultured in osteogenic medium, after exposure for 3 weeks to Quick-Set (**A**), WMTA (**B**) and Teflon (**C**). **D.** Concentrations (μg/mL) of Alizarin red-S extracted from the stained calcium deposits in the three groups. Each data column represents mean and one standard deviation. For each material, columns labeled with different upper case letters are significantly different ($p < 0.05$).