Australian Endodontic Journal
The Journal of the Australian Society of Endodontology Inc., the Australian and New Zealand Academy of Endodontists and the Asian Pacific Endodontic Confederation

Aust Endod J 2017

ORIGINAL RESEARCH

Effect of blood contamination on the compressive strength of three calcium silicate-based cements

Mohammad S. Sheykhrezae,DDS, MSc, DoIBoE[1]; Naghmeh Meraji,DDS, MSc, DoIBoE[1]; Fatemeh Ghanbari,DDS[2]; Mohammad H. Nekoofar,DDS, MSc, DoIBoE, PhD[1,3,*]; Behnam Bolhari,DDS, MSc, DoIBoE[1]; and Paul M.H. Dummer,BDS, MScD, PhD, DDSc, FDSRCS(Ed), FHEA[3]

1 Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
2 General Dentist, Private practice, Tehran, Iran
3 School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, UK

Keywords
biodentine, blood contamination, CEM Cement, compressive strength, ProRoot MTA.

Correspondence
Dr Mohammad H. Nekoofar, School of Dentistry, North Kargar Avenue, Tehran, Iran.
Emails: nekoofar@tums.ac.ir and nekoofarmh@cardiff.ac.uk

doi: 10.1111/aej.12227

(Accepted for publication 27 June 2017.)

Abstract

The aim of this study was to investigate the effect of human blood exposure on the compressive strength of various calcium silicate-based cements. Two hundred and eighty-eight customised cylindrical moulds were randomly divided into three groups according to material used: ProRoot MTA, Biodentine or CEM cement (n = 96). Each group was divided into two subgroups according to exposure conditions: PBS or blood. Then, the compressive strength of the specimens was measured after 6 h, 24 h, 72 h and 7 days. The compressive strength of CEM cement could not be measured after 6 and 24 h regardless of the exposure conditions nor could the compressive strength of 6 h blood-exposed ProRoot MTA. The compressive strength of blood-exposed ProRoot MTA was only significantly lower after 6 h, but no difference was seen at other time intervals. Blood exposed did adversely affected the compressive strength of Biodentine. The compressive strength of all groups significantly increased over time ($P < 0.005$).

Introduction

Hydraulic calcium silicate cements (HCS cements) are often referred to as mineral trioxide aggregate (MTA) and MTA-like biomaterials (1). These materials have various and increasing clinical applications in endodontics, such as in vital pulp treatments, root-end fillings, revascularisation and repair of perforations (1).

Biodentine™ (Septodont, Saint Maur des Fosses, France) powder is composed of tricalcium silicate, calcium carbonate and zirconium oxide, and the material is, therefore, classified as a HCS cement. Its liquid consists of water and calcium chloride, as a setting accelerator (1,2). Biocompatibility (3), short setting time (1), high compressive strength (4) and ease of handling are reported as being the favourable properties of this biomaterial.

CEM Cement (BioniqueDent, Tehran, Iran), another HCS cement, consists mainly of calcium oxide, sulphur trioxide, phosphorus pentoxide and silicon dioxide (5). It has been claimed to have properties such as low cytotoxicity (6), bioactivity (7), calcium and phosphate ions release (8) and antibacterial activity (9).

Compressive strength is defined as the highest vertical compressive force a material tolerates before fracture (10). This physical property is correlated to the stage of hydration of cements (11); thus, it is considered to be one of the most important physical properties of hydraulic calcium silicate cements (12).

Contamination with physiological liquids such as blood and pus occurs in most clinical applications when HCS cements are used. It is, therefore, important that the properties of these cements should not be affected by such contamination (13,14). However, several studies have shown that contamination with blood (15) and physiological solutions with acidic pH (16) have an adverse effect on the physical properties of HCS cements.

The aim of this study was to compare the effect of blood contamination on the compressive strength of Biodentine (Septodont), tooth coloured ProRoot MTA (Dentsply Tulsa Dental, Johnson City, TN, USA) and CEM Cement (BioniqueDent). ProRoot MTA was

included as it remains the gold standard of HCS cements (1).

Materials and method

A total of 288 customised open-ended polymethylmethacrylate cylindrical moulds having an internal diameter of 4 mm and height of 6 mm (according to ISO 9917-1; 2003) were used (17). The moulds were randomly divided into three major experimental groups of 96 on the basis of material type: (i) tooth coloured ProRoot MTA; (ii) Biodentine and (iii) CEM cement.

Each group was divided into two subgroups of 48 according to the exposure of surfaces of specimens to phosphate buffered saline (PBS) and/or human blood. The compressive strength of the specimens was evaluated after incubation for 6 h, 24 h, 72 h and 1 week (n = 12).

Preparation of samples

One gram of tooth coloured ProRoot MTA powder or CEM cement was placed in an empty, clean plastic capsule, and 0.33 ml of PBS was added as described by Nekoofar *et al.* (13). It should be noted that several studies have reported that PBS improves the properties of MTA (16,18) and have suggested mixing this cement with PBS instead of distilled water (18); therefore, in this study, the mixture of tooth coloured ProRoot MTA with PBS was considered as the control group. Biodentine powder is delivered in a capsule and its liquid is supplied in a single-dose container. Each capsule was mixed with five drops of the liquid container. The encapsulated materials were then mechanically mixed for 30 s at 4500 rpm (15) using an amalgamator (Silamat; Ivoclar Vivadent AG, Liechtenstein).

In order to obtain defibrinated human (DH) blood, fresh whole blood was collected from a healthy consented volunteer by a trained medical nurse. The procedure was approved by the Ethics Committee in the School of Dentistry, Tehran University of Medical Sciences, Iran. The blood was then transferred to a glass container containing a clamp and sealed. The container was placed on a laboratory rotator for 6 min. The plastic clamp was surrounded by blood clot which was then removed with forceps. The resultant liquid was DH blood.

The cylindrical moulds were placed on glass slabs. Those used for the blood exposed specimens were filled with (DH) blood and those used for PBS exposed specimens were filled with PBS with the excessive being aspirated after 20 s from all.

Afterwards, the mixed materials were packed into the moulds with minimum pressure. The materials were then subjected to ultrasonic energy for 30 s at a power scale of 5 using a BUC-1 Spartan tip (Obtura Spartan, Fenton, MO, USA) attached to a Suprasson_P5 Booster (Satelec, Merignac, France) (13,15).

Specimens were then placed in Eppendorf tubes containing PBS or DH blood to expose the lower surface of specimens to PBS or DH blood, respectively. A moist cotton pellet was then placed above the specimens without contacting them.

After incubation at 37°C and in a fully saturated humidity, the specimens were removed from the moulds. The samples were placed vertically on the steel plate of a universal testing machine (Lloyd LR MK1 machine; Lloyd Instruments, Fareham, UK). The upper plate moved at a speed of 1 mm in^{-1}. Samples were subjected to compression loads until fracture. The fracture load was recorded and compressive strength was calculated in terms of megapascals (MPa) using the following formula:

$$\mathrm{CS} = \frac{4P}{\pi \mathrm{d}^2}$$

CS = compressive strength, P (N) = fracture load, d (mm) = diameter.

Statistical analysis was undertaken using SPSS 11.5. Since the effect of material type and time was nonparametrical, analysis was performed by the Kruskal–Wallis test and the effect of media was analysed by the Mann–Whitney test. The level of significance was considered as 5%.

Results

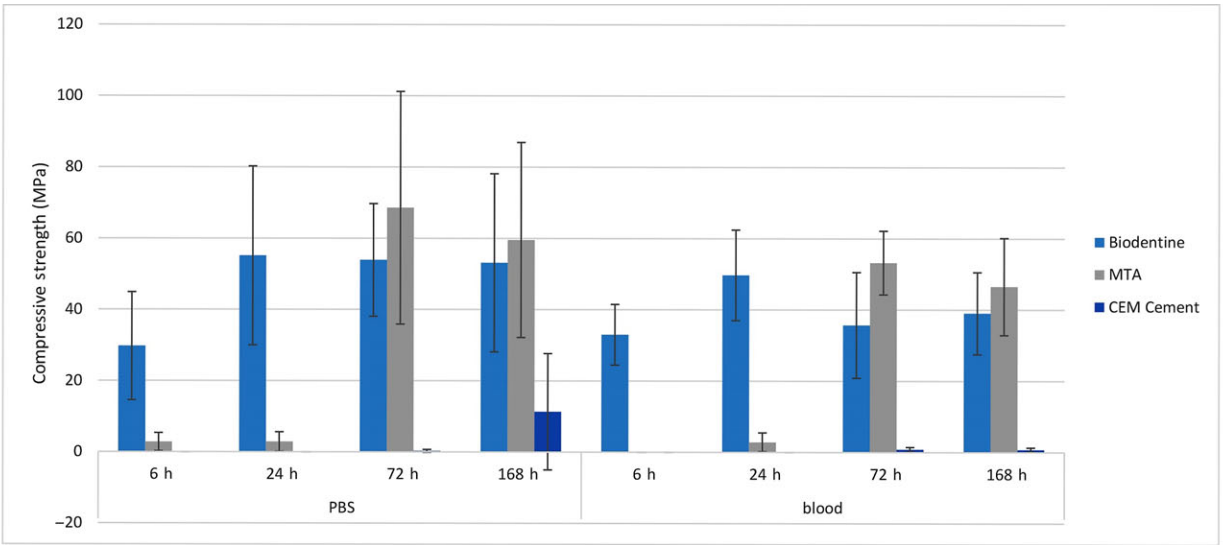
The mean compressive strength values for the experimental groups are shown in Figure 1. The 6 and 24 h specimens of CEM cement and blood-contaminated 6 h specimens of ProRoot MTA were not completely set; therefore, their compressive strength was not measurable at these time intervals.

Effect of material

Biodentine had significantly higher and CEM cement significantly lower compressive strength values at all time intervals and under all exposure conditions ($P < 0.005$).

When comparing ProRoot MTA with CEM cement at different incubation time intervals and exposure environments, compressive strength values of ProRoot MTA were higher than CEM cement; however, significant difference were only seen between the two materials at 72 h and 1 week regardless of the exposure environment ($P < 0.005$).

When comparing Biodentine and ProRoot MTA, compressive strength values of ProRoot MTA were less than



	PBS contaminated				Blood contaminated			
	6 h	24 h	72 h	168 h	6 h	24 h	72 h	168 h
Biodentine	29.73 ± 15.17	55.11 ± 25.12	53.88 ± 15.82	53.12 ± 25.01	32.97 ± 8.52	49.71 ± 12.71	35.69 ± 14.86	39.02 ± 11.52
ProRoot MTA	2.80 ± 2.55	2.87 ± 2.74	68.54 ± 32.66	59.54 ± 27.38	0.00 ± 0.00	2.83 ± 2.66	53.21 ± 8.93	46.52 ± 13.63
CEM Cement	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.41	11.33 ± 16.32	0.00 ± 0.00	0.00 ± 0.00	0.83 ± 0.61	0.77 ± 0.55

Figure 1 Mean compressive strength and standard deviation of different experimental groups (MPa).

Biodentine, but statistically significant difference was only seen between the two cements at 6 h and 24 h regardless of the exposure environment ($P < 0.05$).

When comparing Biodentine and CEM cement, the compressive strength of CEM cement was significantly lower than Biodentine at all time intervals regardless of the exposure environment ($P < 0.05$).

Effect of exposure environment

In the case of Biodentine, no significant difference was seen between the compressive strength of PBS and blood-contaminated groups at each time interval except after 72 h that had significantly higher compressive strength values in PBS ($P < 0.05$).

Regarding tooth coloured ProRoot MTA, a significant difference was only seen between the compressive strength values of the PBS and blood-contaminated groups in samples after 6 h ($P < 0.05$). No significant difference was seen between the compressive strength of PBS and blood-contaminated specimens at other time intervals.

The compressive strength of CEM cement was not measurable after 6 and 24 h due to lack of setting. Only the 1-week incubation time samples exposed to PBS had significantly higher compressive strength values than that of the blood-contaminated ones ($P < 0.001$). No significant difference was seen between the compressive strength of CEM cement exposed to different environments at other incubation time intervals.

Effect of time

The compressive strength of all groups significantly increased over time ($P < 0.005$).

Discussion

This study evaluated the effect of PBS and blood exposure on the compressive strength of three HCS cements.

The compressive strength of blood-contaminated ProRoot MTA was not measurable at 6 h. It has been shown that blood and haemoglobin increase porosity and air entrapment in Portland which inhibits the hydration

process (19). This may also occur in MTA due to its similarities with Portland cement in terms of its composition and hydration. Previous studies have reported interference with the formation of acicular crystalline structures in MTA, characteristic of the ettringite phase, due to blood contamination (20). Furthermore, the ettringite phase of MTA during hydration is also sensitive to blood contamination (15). These may be reasons why the compressive strength of this cement when contaminated with blood is reduced.

The compressive strength of ProRoot MTA at 72 h and 1 week was significantly higher than at 6 and 24 h regardless of the exposure environment. Increased compressive strength over time was also shown by Kayahan *et al.* (21). Therefore, postponing restorative procedures that involve compaction of materials subsequent to the use of this cement for at least 72 h is suggested.

The compressive strength of Biodentine was not influenced by blood contamination. This may be due to its short setting time due to the presence of calcium chloride, a component in the cement's liquid which is an accelerator (1). Furthermore, the calcium carbonate particles in this cement act as nucleation sites that allow the formation of reaction rims around them, thus enhancing the hydration of the cement (22). In addition, calcium sulphate, one of the components of MTA which is incorporated in its ettringite phase, is absent in Biodentine, so it may be concluded that the formation of ettringite phase is not crucial in crystallisation and maturation of Biodentine. Considering that this phase is sensitive to blood contamination (13,15,20), the material may be less susceptibility to blood contamination. Aggarwal *et al.* (23) evaluated the effect of blood contamination on the push-out bond strength of Biodentine and reported that this property of Biodentine was not affected by blood contamination.

The Biodentine specimens exposed to PBS had a significantly higher compressive strength values than the blood-contaminated specimens after 72 h incubation time. This may be due to a higher rate of hydroxylapatite crystalline production when it is in contact with PBS. Colon *et al.* (24) concluded that reaction between Biodentine and PBS led to hydroxylapatite formation.

The compressive strength of CEM cement specimens after 6 and 24 h was not measurable because they had not set. The lowest mean compressive strength values were recorded for CEM cement after 72 h and 1 week. Furthermore, calcium silicate–based materials react with water and produce calcium silicate hydrate gel (CSH gel) and calcium hydroxide (25). CSH gel is an adhesion factor in these cements (26) and sulphate can destroy it (27), which may reduce the strength of the material. As CEM cement contains more sulphur ions than MTA (7), it may be concluded that this cement consists of more ettringite phase. The compressive strength of the PBS-exposed group of CEM cement was significantly higher than the blood-contaminated group. This may be the result of greater hydroxylapatite crystalline production as a result of the contact with PBS and the adverse effect of blood on the hydration process and the ettringite phase of CEM cement. More research is required regarding this subject.

Conclusion

When blood becomes in contact with CEM cement, its compressive strength was reduced, but this adverse effect was not seen with Biodentine and ProRoot MTA. Thus, the compressive strength of Biodentine and ProRoot MTA are not affected by blood contamination. As a consequence, in clinical conditions where blood contamination is likely, ProRoot MTA and Biodentine may be more advisable.

Acknowledgements

The authors acknowledge Dr. Kharazi fard MJ, Mrs Mahjub M and Mr Mahmudi M for their valuable corporation in this project. This study was supported by a grant from Tehran University of Medical Sciences (grant no. 21593).

Authorship declaration

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

Disclosure statement

Authors deny any conflict of interest.

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