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Effect of Saliva and Blood Contamination on the Bi-Axial Flexural Strength and Setting Time of Two Calcium-Silicate Based Cements: Portland Cement and Biodentine

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Abstract - *This study evaluated the effect of contamination with saliva and blood on the bi-axial flexural strength and setting time of pure gray Portland cement and Biodentine (Septodont, Allington, UK). A one-way ANOVA showed that contamination caused no significant difference between the cements in bi-axial flexural strength (P> 0.05). However, there was a significant difference in setting time (P<0.001), with Portland cement taking longer than Biodentine, regardless of the contaminant, and contamination with blood increased the setting time of both materials. Biodentine was similar in strength to Portland cement, but had a shorter setting time for both contaminated and non-contaminated samples.*

KEY WORDS: Biodentine, bi-axial flexural strength, MTA, surgical endodontics

INTRODUCTION

The introduction of mineral trioxide aggregate (MTA) in 1993 was a significant advance in endodontics[1]. This material has since been used for achieving an apical seal in peri-radicular surgery, repair of perforations, apexification and for pulp capping. Prior to the introduction of MTA, amalgam was used but this had the disadvantages of initial leakage, the need for mechanical retention, corrosion and the presence of mercury and tin. MTA is considered to be a bio-active material, forming apatite structures which encourage bone deposition at surgical sites[2]. The growth of cementum and periodontal ligament is also encouraged at surgical sites[3]. However, MTA has a long setting time (initial set in approximately 3 hours), which is a particular disadvantage in the repair of perforations, and has a degree of solubility in contact with body fluids[2].

A new material, Biodentine dentine substitute (Septodont Ltd, Allington, UK), was introduced in 2010 to attempt to overcome the disadvantages of MTA. Biodentine has an initial setting time of 12 minutes and is produced in a capsulated form. Both Biodentine and MTA are calcium silicate-based materials.

The effect of contamination of both of these materials with blood is clearly relevant as they are often used in surgical sites where complete haemostasis may not be possible. Contamination with saliva is also possible in surgical sites, although less likely than with blood. The effects of contamination on the properties of both white and gray MTA with blood has previously been investigated and found to be detrimental to their physical properties[4]. However, Biodentine has not been investigated in this regard.

The aim of this investigation was to test the effect of contamination with saliva and fresh blood on the setting time and bi-axial flexural strength of MTA and Biodentine. MTA is similar in composition to Portland cement, both being calcium silicate-based materials, although MTA has less gypsum and aluminium[5] and bismuth oxide is added to improve radio-opacity. It was therefore decided to use a fine pure form of gray Portland cement (Ferrocrete, Lafarge Cement UK, Birmingham, UK) in place of MTA in this investigation.

MATERIALS AND METHOD

A total of 30 disc-shaped specimens (2 mm thick and 12 mm diameter) were prepared from Biodentine and Portland cement using a silicone mould. All the materials were mixed according to the manufacturer's instructions and the mould was filled to slight excess before a glass plate was placed over the material before setting to generate a smooth surface. For each material, at the time of mixing, 10 specimens were not contaminated, 10 were contaminated with saliva and 10 were contaminated with fresh human blood. Saliva and blood were taken from the investigator preparing the samples on the same day that the samples were prepared.

In the case of Biodentine, this was mixed according to the manufacturer's instructions and one drop of the contaminants was added to the cement before gently mixing in with the material and placing in the mould. The single drop of contaminant was dispensed using a Biodentine dropper bottle in a consistent manner in all specimens in

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an attempt to minimise variation. For contaminated Portland cement specimens, 1.00 g of the powder was mixed with 0.40g of water. The amount of water was more than the amount recommended by the manufacturer in order to ease the handling and provide a smooth surface for testing. The contaminants were added in the same way as they were for Biodentine.

A Universal Testing Machine (Model 4469, Instron Ltd., High Wycombe, UK), at a crosshead speed of 1 mm min^{-1}, was used to determine the bi-axial flexural strength of the discs. This testing was carried out at least one week after preparation of the specimens. Each disc was placed on three circumferentially arranged fixed ball bearings, spaced every 120^0 around the perimeter of a circle of radius 4 mm on a specially constructed jig. Load, to fracture, was applied perpendicular to the specimen's surface, at its centre, through a stainless steel rod of radius 1 mm at the point of specimen contact. The load at fracture was recorded and for each specimen bi-axial flexural strength was calculated using the following formula[6]:

$$S = 0.2387\ F\ (X-Y)/d^2$$

where S is the maximum tensile stress (MPa) in the centre of the disc, F is the total load (N) resulting in fracture, d is the thickness of the specimen (mm) at the site of applied load and X and Y are products of the following formulae:

$$X = (1+v)1n(r_2/r_3)^2 + [(1-v)/2](r_2/r_3)^2$$

$$Y = (1+v)\ [\ln\ (r_1/r_3)^2] + (1-v)\ (r_2/r_3)^2$$

where r_1 is the radius of the supporting circle (4 mm), r_2 is the radius of the loaded area (1 mm) and r_3 is the radius of the specimen disc (6 mm). v is the Poisson's ratio for the material under test as derived from the Engineering Toolbox (www.engineering toolbox.com) with 0.20 being used for both materials as they are primarily based upon Portland cement.

To measure the setting time, 15 samples of Biodentine and 15 of Portland cement were prepared. The 15 samples in each group were divided into 3 sub-groups of 5. The first sub-group were the non-contaminated samples, the second were contaminated with saliva while the third were contaminated with blood. The mixing of the materials was carried out in the same way as for bi-axial flexural strength testing and the same moulds were used to form the specimens. For Biodentine, the moulds were 12 mm in diameter and 2 mm deep. One gram of Portland cement was mixed with 0.35 gram of water. For the contaminated groups, one drop of each contaminant was added to each gram of powder. The contaminants were added after the material was mixed, as previously described. The samples were prepared and left to set at room temperature, as the temperature in a site isolated by a rubber dam and requiring perforation repair, or indeed a surgically-exposed root apex, is likely to be variable and unlikely to be as high as the body core temperature of 37^0C.

Only the initial setting time of the cements was tested. Biodentine specimens were tested every 5 minutes, while Portland cement samples were tested every 10 minutes. The testing was performed using a Gilmore needle, which had a 2mm flat end and a 100 gram mass. The needle was placed onto the surface of the specimen for approximately 30 seconds and the material was considered to have reached initial set when the needle was supported and failed to sink or leave a mark on the surface of the sample. The Gilmore needle has been shown to adequately determine clinical handling times[7] and also care was taken to ensure consistency in the manner in which this test was applied.

RESULTS

The biaxial flexural strength of the materials conformed to a normal statistical distribution (Table 2) and so a one-way analysis of variance (ANOVA) was undertaken. This showed that there were no statistically significant differences ($P = 0.72$) in bi-axial flexural strengths between Biodentine and Portland cements in all states of preparation.

The mean setting times also conformed to a normal distribution and may be seen in Table 3. When tested by a one-way ANOVA, significant differences were found ($P < 0.0001$) and therefore post hoc testing was carried out using Tukey's Multiple Comparison Test (Table 4). The lowest mean setting time among the non-contaminated Biodentine groups was 30 minutes. However, the difference between the non-contaminated Biodentine group and the saliva-contaminated group was not significant ($P > 0.05$). The setting time for the Biodentine group contaminated with saliva was 31 minutes. The longest setting time within the Biodentine groups was for the blood-contaminated samples, where the mean was 16 minutes greater than for the non-contaminated samples.

The blood- and saliva-contaminated specimens of Portland cement had significantly longer setting times compared to the non-contaminated groups ($P < 0.001$). The mean setting time of the non-contaminated Portland cements was 74 minutes. The longest setting time was recorded for blood-contaminated Portland cement, which was 114 minutes. However, the setting time of saliva-contaminated Portland cement was 108 minutes, which was not significantly different from the blood-contaminated samples.

DISCUSSION

In endodontic surgery, the setting time and the effect of contaminants are important factors to consider when selecting a material to achieve an apical seal. It is difficult to avoid contamination in this field, particularly when the setting time is prolonged. However, there have been very few studies of the effect of contamination on the strength and setting time of Biodentine, in particular.

One of the difficulties in this study was the question of exactly how to contaminate the cement. It was decided that the best way to replicate the clinical situation was to mix the cement as instructed by the manufacturer and then add a single drop of contaminant and blend with the mixture. However, there are alternative ways that this could have been done. For instance, after mixing the cements according to the manufacturer's instruction and placing the cement in the mould, a drop of contaminant could have been added to cover the surface. One also can consider replacing some of the mixing fluid with drops of contaminants. The latter means that the contaminant is a part of the mixing fluid and does not act as extra moisture as occurs in the first two techniques. However, the third method may be difficult in determining the precise measurements and arguably our chosen method best replicates the clinical situation.

Table 1. *The constituents of the materials tested in this study.*

Biodentine	
Powder:	*Liquid:*
Tricalcium silicate	Water
Dicalcium silicate	Calcium chloride
Zirconium oxide	Polycarboxilate
Calcium carbonate	
Portland cement	
Tricalcium silicate	
Dicalcium silicate	
Tricalcium aluminate	

Table 2. *The mean and standard deviation of bi-axial flexural strength values (MPa)*

	Biodentine Non-contaminated	*Biodentine & Saliva*	*Biodentine & Blood*	*Portland Non contaminated*	*Portland & Saliva*	*Portland & Blood*
Mean	9.49	9.10	8.94	7.91	8.78	7.61
Standard Deviation	2.90	2.45	2.44	4.08	2.91	3.02

Table 3. *Mean and standard deviation of setting times (minutes)*

	Biodentine non-contaminated	*Biodentine & saliva*	*Biodentine & blood*	*Portland non-contaminated*	*Portland & saliva*	*Portland & blood*
Mean	30.00	31.00	46.00	74.00	108.0	114.0
Standard Deviation	0.0	6.51	8.21	5.47	4.47	5.47

Table 4. *The result of Tukey's Multiple Comparison Test of Means upon setting times (where N.S = Non significant, P>0.05; * = P <0.05; ** = P<0.01; *** = P<0.001)*

	Biodentine & Saliva	*Biodentine & Blood*	*Portland non-contaminated*	*Portland & Saliva*	*Portland & Blood*
Biodentine non-contaminated	N.S	**	***	***	***
Biodentine & Saliva		**	***	***	***
Biodentine & Blood			***	***	***
Portland				***	***
Portland & Saliva					N.S

There is also a difficulty in judging the quantity of contaminant to add to the cement. This may be another reason for having a non-significant difference in the strength. In this study the amount of the contaminants was the same for all the samples. However, in the clinical situation the amount of contaminant may vary considerably from one patient to another. It would be interesting in a further study to vary the quantity of contaminant and test the effect on setting time and strength.

These calcium-silicate based materials are brittle and by their nature contain flaws that precipitate failure under load. A uniaxial test is less searching of such defects and therefore the biaxial flexural strength is preferred to conventional flexural testing[8]. Although in the biaxial flexural strength test the load is applied to the surface, the resultant failure is a product of the internal flaws of the specimen and therefore is not a purely surface-driven phenomenon. Although the bi-axial flexural strength of all of the groups was similar, with no significant differences, the maximum strength was recorded for non-contaminated Biodentine. Surprisingly, the highest strength for Portland cement was for the group contaminated with saliva. The likely explanation for this result for Portland cement is that it has been found that any rise in the total amount of fluid added to the powder is in direct proportion to the flexural strength[9], as it has been found that the highest strength was when the specimens were exposed to moisture from both sides for no more than 24 hours. However, neither the mould nor the applied force was the same as in this present study and saline was used to moisten the samples rather than saliva.

It could be argued that biaxial flexural strength might be not critical for root-end filling materials because they are

not exposed to direct biting force. However, this parameter is important if the cement is used in other situations, such as repair of perforations or in pulp capping. In these cases, the final restoration might be placed directly on the cement. For this reason, it is important to know the flexural strength of both MTA and Biodentine. It has been said that the compressive condensation stress of amalgam is between 6 and 9 MPa[9], which is similar to the mean bi-axial flexural strength found in the present investigation for both cements, regardless of the contaminants. It would therefore be prudent to ensure sufficient bulk of material is present to prevent inducing crack propagation in either MTA or Biodentine.

The results showed that the setting time was significantly different and prolonged for both Portland cement and Biodentine when contaminants were added. The non-contaminated groups for both materials had the shortest setting time, however the contamination of Biodentine with saliva made no significant difference. There were no significant differences between saliva- and blood-contaminated specimens of Portland cement.

The setting time of non-contaminated Biodentine in this study was 30 minutes, which is longer than the 12 minutes stated by manufacturer[10]. The reason might be the over manipulation of the cement during placement in the mould, as this might break the crystal structure of the hydration process and prolong the setting time. It is for this reason that the manufacturer recommends waiting for 6 minutes after placement before shaping and carving

For the contaminated Biodentine samples, there are two possibilities for the prolonged setting time. First, the addition of contaminants after the normal mix by the amalgamator will almost certainly cause over manipulation. Second, increasing the amount of fluid mixed with the powder is expected to increase the setting time, according to the manufacturer.

There are several reasons for the long setting time of the Portland cement, including lack of additives, temperature of the water and insufficient mixing.

The setting time of the materials is very important. A short setting time is preferable because it reduces the possibility of washing out of the material from the cavity, especially when the cement is used as a retrograde root filling. Moreover, if the material is applied coronally, a prolonged setting time will prevent the insertion of the final restoration at the same visit.

From the outcomes of this study, and if further assessment of the strength and setting time is going to be carried out, it is advised that a drop of contaminant should be applied to the surface of the cement after it has been placed in the mould. This should avoid any effect on the setting reaction. Moreover, the use of different ratios of contaminants is recommended since it may better simulate the clinical conditions. Bearing in mind that this study did not perfectly replicate the clinical situation, the conclusions were:

There was no significant difference in bi-axial flexural strength between Portland cement and Biodentine.

Biodentine had a shorter setting time than Portland cement.

The setting time of Biodentine was less affected by the contaminants in comparison to Portland cement.

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