INTRODUCTION

Calcium silicate-based cements (CSCs), such as mineral trioxide aggregate (MTA), are widely used for pulp capping, perforation repair, apexification procedures, and root-end filling. CSCs are active biomaterials with an ability to produce calcium phosphate and apatite-like precipitates at the cement-dentine interface and within the dentinal tubules, triggering the formation of an interfacial hybrid layer with tag-like structures at the interface; this hybrid layer could be responsible for chemical and mechanical bonds. Calcium ions (Ca\(^{2+}\)) released from these cements react with phosphate ions (P\(^{−}\)) available in the tissue fluid forming calcium phosphate and apatite-like crystals on the cement surface. The apatite forming ability of CSCs is proportional to their ability to release Ca\(^{2+}\) and the presence of P\(^{−}\) in the tissue fluids. CSCs must interact with P\(^{−}\) to produce their remineralization ability, so it may be possible to improve the retention of MTA apical plugs to dentine by using a phosphate containing solution as an intra-canal dressing or as a final rinse in prepared root canals, as suggested by Martin et al. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a biocompatible milk-derived protein that has been used in dentistry as a source of phosphate ions (Ca\(^{2+}\) and P\(^{−}\) for caries control. It has been reported that the addition of CPP-ACP to CSCs improved Ca\(^{2+}\) and P\(^{−}\) release. The addition of CPP-ACP to CSCs may induce the precipitation of calcium phosphate salts and apatite-like precipitates at the cement-dentine interface and thus improve the dentinal bond strength. The aims of the present study were to determine whether the addition of CPP-ACP to CSCs would improve the dentinal push-out bond strength of the modified cement and to compare the push-out bond strength of a new trial MTA with commercially available CSCs.

MATERIALS AND METHODS

Commercially available Biodentine™ (BD, Septodont, Saint Maur des Fosses, France), Angelus® MTA (AMTA, Angelus Indústria de Produtos Odontológicos, Londrina, PR, Brazil) and a GC trial MTA (GCMTA, GC, Tokyo, Japan) were tested and their composition and lot numbers are listed in Table 1. Various concentrations of CPP-ACP were added to the powder of each test cement. For each cement, five groups with different CPP-ACP concentrations were tested: Group 1 (control); 0% w/w CPP-ACP, Group 2; 0.5% w/w CPP-ACP, Group 3; 1.0% w/w CPP-ACP, Group 4; 2.0% w/w CPP-ACP, Group 5; 3.0% w/w CPP-ACP. An analytical balance, accurate to 0.1 mg (Precisa Gravimetrics AG, Dietikon, Switzerland), was used to weigh the powder of the cements and CPP-ACP.

Sixty extracted single-rooted human teeth were collected under a protocol approved by the institutional ethics committee of Melbourne Dental School at the University of Melbourne, Melbourne, Australia (Ethics application number: 1339580). The teeth were disinfected in 1% chloramine-T (Sigma-Aldrich, St Louis, MO, USA) for up to 1 month at 4°C and then stored in deionized distilled water (DDW) at 4°C. The crowns of all the teeth were removed, the roots were mounted in epoxy resin (EpoFix, Struers, Ballerup, Denmark) blocks, and the middle thirds of the roots were sectioned horizontally using a 0.3 mm thick diamond cut-off wheel and slow speed rotary machine with water cooling system (Struers) to obtain three hundred 1.0±0.1 mm thick root sections. Each section was polished with 320-grit sand-paper (Norton Tuftak, Saint-Gobain Abrasives, Auckland, NZ). The thickness of each section was measured with a digital calliper (Mitutoyo, Kawasaki, Japan) to an accuracy of 0.01 mm. In each section the canal space was enlarged with #2 to #5 Gates-Glidden...
burs (Dentsply, Maillefer, Ballaigues, Switzerland) to obtain standardized cavities with 1.3 mm diameter according to the methodology of Vanderweele et al.\textsuperscript{11}. The slices were immersed in 1% sodium hypochlorite for 3 min to remove cutting debris and any remaining pulp tissue. The combination of sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA) was avoided because EDTA was reported to interfere with the hydration of MTA and decrease the mineral content of dentine\textsuperscript{12,13}. The samples were then immediately washed in DDW and dried with an air jet from a triplex syringe. For each test cement, 100 sections were divided randomly into 5 groups (n=20), and the cavities were filled with control and test cements. The cement for each group was mixed according to manufacturers' instructions. The cements were incrementally compacted with an endodontic plugger into the canal space of each root slice and the excess material was trimmed from the surface of each slice with a sharp scalpel. The root slices were wrapped in wet gauze and the cement was allowed to set for 24 h in an incubator at 37\degree C and relative humidity of at least 90%. The specimens of each group were stored in sterile plastic vials containing 15 mL of phosphate buffer solution (PBS) for 2 months (according to previous studies\textsuperscript{2,3}) at 37\degree C and the PBS was replaced every week.

A universal testing machine (Model 5544, Instron\textsuperscript{8}, MA, USA) was used to measure the push-out bond strength values. Each section was carefully positioned, with the aid of a (×10) microscopic magnification (Leica DML, Leica Microsystem Welzlar, Wetzlar, Germany), on a metal slab with a 1.5 mm central hole to allow free motion of the Instron’s plunger. A load was applied by exerting a downward pressure on the surface of the cement by using a 1.1 mm diameter cylindrical stainless steel plunger at a crosshead speed of 0.5 mm/min. The plunger diameter was smaller than the canal diameter to ensure contact with the cement only. The maximum load applied for dislodging the cement was recorded in Newtons. To express the bond strength in megapascals (MPa), the recorded value was divided by the adhesion surface area of root canal filling calculated by the following formula: \(2\pi rh\), where \(r\) is the root canal radius and \(h\) is the thickness of the root dentine slice in millimeters. The sections were then examined under the microscope at ×40 magnification to determine the nature of the bond failure. Each section was allocated into one of three failure modes: adhesive failure at the cement-dentine interface, cohesive failure within the cement, or mixed failure (Figs. 1a–c).

### Table 1 Composition of the cements used

| Cement       | Composition according to manufacturer                                      | Lot No. |
|--------------|-----------------------------------------------------------------------------|---------|
| Biodentine\textsuperscript{"} | Powder: tricalcium silicate, dicalcium silicate, calcium carbonate, calcium oxide, zirconium oxide. Liquid: water, calcium chloride, modified polycarboxylate (Plasticising agent). | B05594  |
| Angelus\textsuperscript{®} MTA | Powder: 80% Portland cement with no calcium sulphate (gypsum), 20% bismuth oxide. Liquid: distilled water | 26333   |
| GC trial MTA | Powder: Portland cement, bismuth oxide. Liquid: distilled water            | 1303291 |

Fig. 1 Inspection of the samples under a stereomicroscope at ×40 magnification and various failure modes of the samples.

(a) Adhesive failure; note the clean canal wall. (b) Cohesive failure; within cement.
(c) Mixed failure; there are remnants of cement inside the canal.
Table 2  Means and standard deviations of push-out bond strength values of the various groups

| CPP-ACP (%) | N   | BD       | AMTA     | GCMTA    |
|-------------|-----|----------|----------|----------|
| Gp1 (0%)    | 20  | 11 (1.5)A,a | 7 (1.7)B,a | 8.1 (1.5)B,a |
| Gp2 (0.5%)  | 20  | 13.9 (1.8)b  | 11.5 (2.2)b  | 11.3 (2.1)b  |
| Gp3 (1.0%)  | 20  | 16 (1.7)c  | 11.6 (2.5)b  | 13.3 (1.4)c  |
| Gp4 (2.0%)  | 20  | 15.2 (1.6)b,c | 11.2 (1.5)b  | 10.6 (2.6)b  |
| Gp5 (3.0%)  | 20  | 13.9 (2.3)b  | 10.3 (1.6)b  | 9.9 (2.2)b   |

N=Number of specimens, SD=Standard deviation. Values marked with different capital letters (A–C) indicate a significant difference between the different cements with the same CPP-ACP concentration. Values marked with different small letters (a–e) indicate a significant difference between the groups of the same cement (p<0.05).

Statistical analysis
The data were subjected to statistical analysis using SPSS ver. 11.5.0 (SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov (K-S) test was used to test normality and data were found to be normally distributed and therefore, parametric statistical tests were performed (one way analysis of variance followed by Tukey’s test for multiple comparisons). The level of statistical significance was set at p<0.05.

RESULTS
Mean values and standard deviations of the push-out bond strength of the control and test groups are shown in Table 2. There were statistically significant differences between the various cements and the various subgroups of each cement (p<0.001). The addition of CPP-ACP to BD, AMTA and GCMTA significantly increased the mean values of the push-out bond strength in comparison with the control group (p<0.05). However, this increase was not consistent with the concentration of the added CPP-ACP and there were also variations in the pattern of increased values from one cement to the others. The addition of 1.0% and 2.0% CPP-ACP to BD showed the highest push-out bond strength mean values, in comparison with the other groups of BD, with no statistically significant difference between these two concentrations. There was no statistically significant difference between 0.5% and 3.0% CPP-ACP containing subgroups of BD nor between the CPP-ACP containing subgroups of AMTA. The addition of 1.0% CPP-ACP to GCMTA showed the highest push-out bond strength mean values, in comparison with the other groups of GCMTA (p<0.05). There were no statistically significant differences between 0.5%, 2.0% and 3.0% CPP-ACP containing subgroups of GCMTA. The push-out bond strength of BD was higher than that of AMTA and GCMTA (p<0.001). There was no statistically significant difference between the push-out bond strength mean values of AMTA and GCMTA except at 1.0% CPP-ACP-containing groups, where the push-out bond strength of GCMTA was significantly higher than that of AMTA (p<0.05). The distribution of the failure modes for each test cement is shown in Table 3. The addition of CPP-ACP to the test cement did not create a noticeable effect on the failure modes of the subgroups of each cement.

Table 3  Failure modes of each test cement

| Test cements | N   | Failure modes, % (A/C/M) |
|--------------|-----|-------------------------|
| BD           | 100 | 2/69/29                 |
| AMTA         | 100 | 40/32/28                |
| GCMTA        | 100 | 37/29/34                |

N=Number of specimens, A=adhesive failure, C=cohesive failure, M=mixed failure.

DISCUSSION
The push-out bond test is a reliable method for measuring the dentinal bond strength of endodontic cements and the test’s loading closely simulates clinical stresses. It also represents a true and pure shear bond strength in comparison with the conventional shear test because, with the push-out test, fracture only occurs parallel to the cement-dentine interface with no possible torque that may occur with the conventional shear test. Furthermore, fewer stresses are generated at the bonding interface during sample preparation for the push-out test in comparison with the conventional shear test. For the present study, the roots were sectioned then the canal spaces were prepared and filled for the push-out test. A major disadvantage of sectioning roots after performing conventional root fillings is that natural roots are not perfectly straight and this will result in sections with canal space that is not perfectly perpendicular to the applied load and this will generate...
frictional resistance during the push-out test\textsuperscript{18}. The canal diameter will also vary between coronal to apical sections unless the sectioning occurs first and the canal spaces are prepared with the same drills to obtain a standardized canal diameter.

Although an attempt was made to control all of the variables, a few limitations existed such as the variation in root size and shape as well as variation in the setting expansion of the tested cements. The setting expansion may contribute to residual compressive stress and subsequently increase the bond strength values\textsuperscript{19,20}. The present findings indicated that the push-out bond strength values of BD were higher than AMTA and GCMTA. This could be attributed to the higher biomineralization ability of BD which might have triggered the formation of tag-like structures at the cement-dentine interface and increased the dislodgement resistance of BD as compared with MTA\textsuperscript{21,22}. The higher content of calcium-releasing products in BD than in MTA may contribute to higher biomineralization and higher bond strength\textsuperscript{22-24}. The higher biomineralization ability of BD may also explain the failure modes in the present study. The failure modes of AMTA and GCMTA were predominately adhesive, but predominately cohesive for BD. These findings are in agreement with previous studies that reported adhesive bond failure with MTA and cohesive bond failure with BD\textsuperscript{24,25}. The differences in bond strengths and failure modes among the tested cements may also be explained by the differences in the particle sizes, which may have an effect on the penetration of cement into dentinal tubules\textsuperscript{25}. Compared to the naturally derived MTA, the standardized components of BD as a manufactured material along with smaller particle size may be conducive to the formation of tag-like structures and better micromechanical adhesion to dentine\textsuperscript{23}. The similarity in the bond strengths and failure modes between AMTA and GCMTA may be due to the similarity in their basic composition according to the manufacturers’ information.

Interestingly, the addition of CPP-ACP to glass-ionomer cement improved the dentinal micro-tensile bond strength in a similar manner to what has occurred in the current study with CSCs\textsuperscript{26}. Although it was reported that the presence of CPP-ACP in CSCs improves Ca\textsuperscript{2+} and P, release, it is not recommended to add more than 1% CPP-ACP into BD and more than 0.5% into MTA to avoid compromising the mechanical properties\textsuperscript{10}. According to the present results, it may be possible to give similar recommendations for BD and AMTA because the highest push-out bond strength was associated with 1% CPP-ACP-modified BD in comparison with the subgroups of the same material and there were no significant differences between the CPP-ACP containing subgroups of AMTA.

The addition of CPP-ACP to CSCs might have increased calcium phosphate and apatite-like forming capacity of the modified cements that might have contributed to better biomineralization ability\textsuperscript{27,28}. Tay and Pashley\textsuperscript{29} reported the biomineralization of acid demineralized dentine by CSCs with a P\textsubscript{i} source and that the deposition of amorphous calcium phosphate within the demineralized collagen matrix was guided by extracellular matrix protein analogues. The precipitation of amorphous calcium phosphate and apatite-like crystals at the cement-dentine interface fills the microscopic gaps between the cement and dentine and increases mechanical retention resulting in improved MTA-dentine bond strength and MTA sealing ability\textsuperscript{25,30}. It has been reported that the application of CPP-ACP paste (Tooth Mousse, GC, Tokyo, Japan) onto the surface of acid etched dentine sections can induce mineralization of dentine through the formation of apatite-like crystals along and between dentine collagen fibers\textsuperscript{31}. A significant decrease in the permeability of dentine adhesives has been reported after dentine pre-treatment with the CPP-ACP paste and this was attributed to the ability of CPP-ACP to act as a source of Ca\textsuperscript{2+} and P\textsubscript{i} for dentine remineralization and dentinal tubules occlusion\textsuperscript{22}.

CONCLUSION

Within the limitations of this in vitro study it can be concluded that CPP-ACP increases the displacement resistance of CSCs.

ACKNOWLEDGMENTS

This project was supported by a research grant from Melbourne Dental School of The University of Melbourne, Australia. Further financial support was also received from the higher committee for education development in Iraq (HCED Iraq; the sponsor of Dr Alaa E. Dawood who was undertaking a PhD at Melbourne Dental School). The authors would like to thank GC, Japan and Septodont, France for supplying the trial MTA and Biodentine\textsuperscript{TM} used in this study.

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