The Relationship of Surface Characteristics and Antimicrobial Performance of Pulp Capping Materials

Cher Farrugia, BChD, MSc,[*†] *Christie Y.K. Lung, BSc, MSc, PhD,*[‡] *Pierre Schembri Wismayer, MD, PhD,*[§] *Maria Teresa Arias-Moliz, BDS, PhD,*[‖] *and Josette Camilleri, BChD, MPhil, PhD, FADM, FIMMM, FHEA*[*¶]

Abstract

Introduction: Pulp capping materials need to be able to protect the pulp but also bond to the overlying restorative materials. Light-curable pulp capping materials bond better to restorative materials and are easier to place than most water-based cements. The aim of this study was to characterize new light-curable tricalcium silicate–based pulp capping materials and compare their surface and antimicrobial properties with clinically available Theracal (Bisco, Schaumburg, IL) and Biodentine (Septodont, Saint-Maur-des-Fossés, France). **Methods:** The surface characteristics of 3 light-curable pulp capping materials based on a resin and filled with tricalcium silicate and tantalum oxide radiopacifier and Theracal and Biodentine were assessed by scanning electron microscopy, X-ray diffraction, and contact angle measurement. The radiopacity was measured following ISO 6876 standards. The antimicrobial activity was determined by the direct contact test and the antibiofilm activity by the adenosine triphosphate assay and the confocal laser scanning Live/Dead assay (Invitrogen, Eugene, OR) using a polymicrobial culture. **Results:** The surface characteristics of the materials varied with the unfilled resin and Biodentine exhibiting a hydrophobic surface. Biodentine showed significantly higher antimicrobial properties in the direct contact test, but this property was absent in the antibiofilm activity tests. The resins filled with tricalcium silicate and Theracal showed higher antimicrobial activity than Biodentine in the adenosine triphosphate and live/dead assays. **Conclusions:** The surface characteristics of a material affect its antimicrobial properties. The experimental resin-modified materials exhibited comparable antimicrobial properties with other light-curable pulp capping agents. Further long-term studies on the materials' antimicrobial activity are required to assess whether they can result in better clinical outcomes. *(J Endod 2018;■:1–6)*

Key Words

Antimicrobial activity, light-curable pulp capping materials, surface micromorphology

Significance

Materials that possess antimicrobial properties are ideal for pulp capping. The surface characteristics may affect the bacterial attachment.

Pulp capping materials are used to seal pulp wounds; facilitate dentin bridge formation; and, as a result, preserve the dental pulp and prevent root canal therapy (1). Ideal pulp capping materials should induce dentin bridge formation and possess antibacterial properties (2), but many of the commercially available materials do not satisfy clinical requirements. With the current pulp capping systems, microgaps may be present at the pulp capping material–dentin interface as well as between the pulp capping material and the composite restorative material (3), potentially resulting in failure of the interface.

Mineral trioxide aggregate (MTA) is a tricalcium silicate–based material that has shown greater dentin bridge formation than the traditionally used calcium hydroxide. However, MTA-based materials are generally difficult to handle and have an extended setting time (4), making them clinically less attractive than materials that have controlled setting properties. Furthermore, bonding of a composite restoration to MTA may be problematic (5, 6).

Theracal (Bisco, Schaumburg, IL) (7, 8) and Biodentine (Septodont, Saint-Maur-des-Fossés, France) (9) have been extensively studied in the literature as possible alternatives for pulp capping. Theracal provided a more clinically efficient solution because of its light-curing properties but has shown less calcium ion release than Biodentine (10, 11). Biodentine exhibits good results both in *in vitro* testing and clinically because of its easier handling properties and biocompatibility but still demonstrates problems bonding to composite (12, 13), and etching of Biodentine results in deterioration of material properties and microleakage. A delay in the placement of the final restoration when using Biodentine is advised (7, 14). The aim of this study was to characterize new light-curable resin-based pulp capping materials. The study

From the *Department of Restorative Dentistry, Faculty of Dental Surgery and [§]Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta; [†]School of Clinical Dentistry, University of Sheffield, Sheffield, United Kingdom; [‡]Dental Materials Science, Faculty of Dentistry, The University of Hong Kong, Pokfulam, Hong Kong; [‖]Faculty of Dentistry, University of Granada, Granada, Spain; and [¶]School of Dentistry, College of Medical and Dental Sciences, Institute of Clinical Sciences, University of Birmingham, Birmingham, United Kingdom.

Address requests for reprints to Dr Josette Camilleri, School of Dentistry, College of Medical and Dental Sciences, Institute of Clinical Sciences, University of Birmingham, 5 Mill Pool Way Edgbaston, Birmingham B5 7EG, UK. E-mail address: J.Camilleri@bham.ac.uk

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compares the antimicrobial properties of the novel materials with those of 2 commercially available products using a polymicrobial biofilm.

Methods

The materials investigated in this study included the following:

1. Experimental light-curing resin (LC)
2. Experimental light-curing resin with tricalcium silicate filler in 60:40 proportion by volume (LC + TCS)
3. Experimental light-curing resin with tricalcium silicate filler in 60:40 proportion by volume radiopacified with tantalum oxide replacing the filler by 20% by volume (LC + TCS + TO)
4. Theracal
5. Biodentine

The experimental resins have been described in a previous publication (15). Dosage by volume was used for the experimental materials to determine the percentage replacement of the filler and radiopacifier because these had different densities; thus, dosage by weight would have led to inaccuracies. Experimental samples were light cured for 20 seconds using a light-emitting diode curing light (Bluephase 20i; Ivoclar Vivadent, Mississauga, Canada) with an approximate wavelength of 470 nm. The materials were cured in 1-mm increments to allow the curing light to penetrate the deeper layers. Theracal and Biodentine were prepared following the manufacturers' instructions.

Material Characterization

After setting, the materials were stored in Hank's balanced salt solution (Sigma-Aldrich, St Louis, MO) for 24 hours; after which, they were retrieved, dried, and characterized. The surface micromorphology was assessed using scanning electron microscopy. Phase analysis was performed by glancing angle X-ray diffraction analysis at a fixed angle of incidence of 3° over a solid surface. The X-ray diffractometer (Rigaku, Tokyo, Japan) was operated in the grazing incidence asymmetric Bragg mode using CuKα radiation, an operating current of 40 mA, and a voltage of 45 kV for 15°–45° 2θ with a sampling width of 0.05° and a scan speed of 0.8°/min. Phase identification was accomplished using search-match software of the ICDD database (International Centre for Diffraction Data, Newtown Square, PA).

Contact Angle Measurements

Contact angle measurement was used to investigate the wettability of the material surfaces. Cylindrical specimens 10 mm in diameter and 2-mm high were prepared from each material type. A 20-μL drop of distilled water was placed on the surface of the samples, and the contact angle was measured using a contour measurement device comprising a leveled stage, a backlight source, and an optical imaging setup. Each experiment was repeated at least 3 times. The contact angle was measured using image processing and analysis in Java software (ImageJ; National Institutes of Health, Bethesda, MD).

Radiopacity Assessment

Three specimens 10 $\pm$ 1 mm in diameter and 1 $\pm$ 0.1 mm thick were prepared of each material type and radiographed on a photostimulable phosphor plate adjacent to a calibrated aluminum step wedge (Everything X-ray, High Wycombe, UK) with 3-mm increments using a standard X-ray machine with an exposure time of 0.50 seconds at 10 mA, tube voltage at 65 $\pm$ 5 kV, and a cathode-target film distance of 300 $\pm$ 10 mm. The gray pixel value of each step in the step wedge on the digital images was determined using an imaging program (Adobe Photoshop; Adobe Systems, San Jose, CA), and a graph of thickness of aluminum versus the gray pixel value on the radiograph was plotted with the best-fit logarithmic trend line. The equation of the trend line gave the gray pixel value of an object on the image as a function of the object's thickness in mm of aluminum. The gray pixel values of the cement specimens were then determined and the relevant thickness of aluminum calculated.

Antimicrobial Activity Analysis

Three antimicrobial methods were performed, the direct contact test (DCT), the adenosine triphosphate (ATP) assay, and the live/dead assay for antibiofilm activity, and observed using a confocal laser scanning microscope. The bacterial strains used were *Streptococcus mutans* ATCC 25175 (American Type Culture Collection, Manassas, VA), *Streptococcus gordonii* ATCC 33478 (American Type Culture Collection), and *Streptococcus sobrinus* ATCC 33399 (American Type Culture Collection). All bacterial suspensions were prepared in brain-heart infusion (BHI) broth (Scharlau Chemie SA, Barcelona, Spain) and adjusted using a turbidimeter (Densichek Plus; Biomeriuex, Boston, MA) to match an optical density of the 1.0 McFarland standard. This suspension was diluted 30-fold in broth to obtain a bacterial suspension of approximately 1×10^7 colony-forming units per milliliter. For multispecies bacterial suspensions, these single bacterial suspensions were mixed equally (1:1:1). In the antimicrobial activity tests, all samples were exposed to ultraviolet light for 1 hour for sterilization.

DCT

An area of established dimensions on 1 side of the wells of a 96-well microtiter plate (Nunclon Delta Surface; Nunc, Roskilde, Denmark) was delimited by measuring 2 points of the edge of the wells separated by 4 mm in order to ensure the same amount of each material on the vertical wall of the well. The area was coated with each material, with a thickness of 1 mm, using a sterile spatula and measured using a caliper (16). Once the material was set, samples were exposed to 10 μL of the mixed bacterial suspension for 1 hour at 37°C to ensure direct contact between the bacteria and the tested materials. Bacterial suspensions placed on the wall of the uncoated wells served as the positive control. After incubation, 220 μL sterile BHI was added to each well. The bacterial suspension was mixed for 1 minute, diluted serially, and plated for viable cell counting. Plates were incubated for 24 hours at 37°C under anaerobic conditions. Each group was tested twice, each time in triplicate (n = 6/group). Six wells of each material were inoculated using sterile BHI as a negative control to check the sterility of the samples. Results of the DCT were expressed as $\log_{10}$ (colony-forming units + 1/mL).

Antibiofilm Activity by ATP Assay

Samples with a 1-mm height and 6-mm diameter (n = 6/group) were exposed to 1.8 mL sterile BHI and 200 μL of the polymicrobial suspension in a 24-microtiter plate incubated on a rocking table (model Swing Sw 8 10000-00015; OVAN, Badalona, Spain) for 7 days at 37°C under anaerobic conditions. The BHI was refreshed every 2 days. After the incubation period, the disks were rinsed with 0.9% saline solution, and the biofilms formed on the materials' surface were recovered by placing the disks in Eppendorf tubes with 500 μL BHI and vortexing for 2 seconds followed by sonication for 10 minutes. The bacteria in the recovered suspension was evaluated using the ATP assay. Six specimens of each material exposed to sterile BHI were included as negative controls to check the sterility of the samples.

For the ATP assay, 100 μL of the bacterial suspension was added to 100 μL BacTiter Glow reagent (Promega, Madison, WI) according to the manufacturer's instructions followed by incubation at room temperature for 5 minutes. The luminescence produced was measured with a