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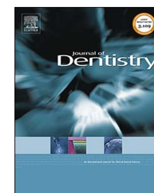

Antimicrobial and biological activity of leachate from light curable pulp capping materials

Maria Teresa Arias-Moliz[a], Cher Farrugia[b], Christie Y.K. Lung[c], Pierre Schembri Wismayer[d], Josette Camilleri[b,*]

[a] Department of Microbiology, Faculty of Dentistry, University of Granada, Granada, Spain
[b] Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Malta
[c] Dental Materials Science, Faculty of Dentistry, The University of Hong Kong, Hong Kong
[d] Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Malta

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ABSTRACT

Objectives: Characterization of a number of pulp capping materials and assessment of the leachate for elemental composition, antimicrobial activity and cell proliferation and expression.

Methodology: Three experimental light curable pulp-capping materials, Theracal and Biodentine were characterized by scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction. The elemental composition of the leachate formed after 24 h was assessed by inductively coupled plasma (ICP). The antimicrobial activity of the leachate was determined by the minimum inhibitory concentration (MIC) against multispecies suspensions of *Streptococcus mutans* ATCC 25175, *Streptococcus gordonii* ATCC 33478 and *Streptococcus sobrinus* ATCC 33399. Cell proliferation and cell metabolic function over the material leachate was assessed by an indirect contact test using 3-(4,5 dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The hydration behavior of the test materials varied with Biodentine being the most reactive and releasing the highest amount of calcium ions in solution. All materials tested except the unfilled resin exhibited depletion of phosphate ions from the solution indicating interaction of the materials with the media. Regardless the different material characteristics, there was a similar antimicrobial activity and cellular activity. All the materials exhibited no antimicrobial activity and were initially cytotoxic with cell metabolic function improving after 3 days.

Conclusions: The development of light curable tricalcium silicate-based pulp capping materials is important to improve the bonding to the final resin restoration. Testing of both antimicrobial activity and biological behavior is critical for material development. The experimental light curable materials exhibited promising biological properties but require further development to enhance the antimicrobial characteristics.

1. Introduction

The aim of vital pulp therapy is maintenance of pulp vitality and preservation of its functional and biological activities. The long term success of this therapy will depend mainly in the formation of an optimal hard tissue bridge which will provide a natural protection against the infiltration of bacteria and chemical products [1,2].

The repair process of the dentin-pulp complex after oral pulp exposure depends on the pulp status and the application of an effective pulp capping material. This material should be able to induce tissue healing and reparative dentinogenesis, exert antimicrobial activity, biocompatibility and sealing capabilities [3]. Calcium hydroxide has been the material of choice for pulp capping. However its solubility plus the multiple tunnel defects and cell inclusions observed in dentine bridges [4], have led to the use of alternative materials.

Calcium silicate-based cements are bioactive materials that have the ability to release hydroxyl and calcium ions thus creating an adequate environment to promote healing and repair enabling odontoblastic cell differentiation responsible for the production of the reparative dentine bridge. Mineral trioxide aggregate (MTA) is a tricalcium silicate based material that has shown greater dentine bridge formation [5] and similar clinical outcomes to calcium hydroxide after capping pulp exposures [6,7]. The major disadvantages of using MTA as a pulp capping material are the difficulties in handling and its extended setting time.

* Corresponding author at: Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Medical School, Mater Dei Hospital, Msida, MDS 2090, Malta.
E-mail address: josette.camilleri@um.edu.mt (J. Camilleri).

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Biodentine (Septodont, Saint-Maur-des-Fosses, France) is an interesting alternative to MTA. Its main advantages over MTA include easy handling, shorter setting time and improved physicochemical properties [8]. Besides it has been shown to have similar biological effects to MTA in *in vitro* experiments [9–11] and clinical trials [12]. The main drawback with using MTA and Biodentine is their water-based chemistry and thus poor bonding as the bond is mainly micromechanical to the overlying resin restoration [13]. It has been suggested to delay the tooth restoration in order not to affect their setting [14]. The physicochemical properties alter when acid etched favoring microleakage [15,16]. To overcome this limitation, new light-curable resin modified tricalcium silicate materials are being formulated for pulp capping procedures. TheraCal (Bisco, Schaumburg, IL, USA) contains polymerizable methacrylate monomers that are responsible for the adequate shear bond strength to the resin composite with either the self-adhesive or the etch and rinse system once it is light cured [17]. It presents a low solubility and short setting time. Theracal also induces odontoblastic differentiation and mineralization effect [18] although it releases less calcium ions than Biodentine [19]. Its biocompatibility has been questioned as it decreases significantly the cell activity after 7 days [20].

The release of calcium hydroxide is very important not only because it plays a role in cell differentiation and dentine bridge formation but also because it is responsible for the antimicrobial activity of these tricalcium silicate-based pulp capping materials mainly due to the strong alkaline environment created. Microorganisms are known to interfere with the pulpal reaction to capping materials by stimulating the pulpal inflammatory response and reducing the area of dentin bridge formation [21,22]. To date, there is no clear information in the literature about the amount of calcium that pulp capping materials need to release in order to enable an adequate antimicrobial activity and enhance biocompatibility. Toxic effects to oral microbes may disrupt cell function. The aim of this study was to formulate an experimental light curable pulp capping material. This experimental material together with two other prototypes which contain variants to the experimental material and Biodentine and Theracal were characterized and the leachate was tested for elemental composition, antimicrobial activity and cell proliferation and expression.

2. Methodology

The materials investigated in this study included the following

- Experimental light curing resin (LC)
- Experimental light curing resin with tricalcium silicate filler in 60:40 proportion by volume (LC-TCS)
- 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)
- Theracal
- Biodentine

The dosage by volume was used since the filler and radiopacifier had different densities thus dosage by weight would have led to inaccuracies. Once prepared after completion of setting for the Biodentine the materials were stored in Hank's balanced salt solution (HBSS; Sigma Aldrich, Gillingham UK) for 24 h after which they were retrieved, dried and characterized.

2.1. Material characterization

Characterization was performed by scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and X-ray diffraction analyses. For SEM analyses the materials were dried and impregnated in resin (Epoxyfix, Struers GmbH, Ballerup, Denmark) and the resin blocks were then ground and polished using an automatic polisher (Tegramin 20, Struers GmbH, Ballerup, Denmark). The specimens were mounted on aluminium stubs, carbon coated and viewed under the SEM in back-scatter mode at 2 K and 5 K magnifications. EDS analysis was also performed. For X-ray diffraction analysis, specimens were dried and crushed into a very fine powder using an agate mortar and pestle. The diffractometer (Bruker D8 Advance, Bruker Corp., Billerica, MA, USA) was operated in Bragg–Brentano theta-2 theta configuration using Cu Kα radiation at 40 mA and 45 kV and the detector was rotated between 15–45° with a step of 0.02°2θ and a step time of 0.8 s. Phase identification was accomplished using search-match software utilizing ICDD database.

2.2. Assessment of material leachate

Discs measuring 4 mm in diameter and 2 mm high were prepared and immersed in HBSS for 24 h. Materials were prepared in triplicate. After the 24-h soaking the discs were retrieved and discarded and the leachate was tested for pH and elemental composition.

2.2.1. pH measurement

The pH meter (Hanna HI 3221, Hanna Instruments, Woonsocket, RI, USA) and single-junction (Ag/AgCl) ceramic pH electrode (HI 1131, Hanna Instruments) using a glass probe (HI 1230 Hanna Instruments) was calibrated at three points (pH 4.00, 7.00 and 10.00; using standard calibrating solutions (Scharlau, Scharlab, Sentimenat, Spain) with temperature compensation (HI 7662, Hanna Instruments). Calibration was performed prior to measurement of the pHs of the soaking solutions. The results in triplicate were recorded and the mean and standard deviation of each was calculated.

2.2.2. Chemical analyses of leachate

Prior to immersion in HBSS the specimens were weighed to an accuracy of 0.0001 g. After the 24-h contact the leachate was assessed for the presence of calcium, silicon, aluminium, phosphorus, barium, strontium, zirconium and tantalum by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8300, Perkin Elmer). Blank solution of HBSS was also analyzed and the elements present in HBSS were deducted from the test leachates. The elemental concentration was calculated in mg/L taking into consideration the sample weight and the volume of solution used.

2.2.3. Antimicrobial activity analysis of leachate

Discs measuring 6 mm in diameter and 1 mm high were prepared, sterilised and immersed in sterile HBSS for 24 h. After the 24-h soaking, the discs were discarded and the antimicrobial activity of the leachate was tested by means of the minimal inhibitory concentration (MIC).

The bacterial strains used were *Streptococcus mutans* ATCC 25175, *Streptococcus gordonii* ATCC 33478 and *Streptococcus sobrinus* ATCC 33399. They were routinely cultured anaerobically in brain-heart infusion (BHI) (Scharlau Chemie S.A., Barcelona, Spain) at 37 °C. Two different multispecies bacterial suspensions of 1×10^6 and 1×10^4 colony forming units per milliliter (CFU/mL) were prepared in BHI broth and adjusted using a turbidimeter (Densichek Plus, Biomeriuex, Boston, USA). For this purpose the single suspensions were mixed equally (1:1:1). Two tests were performed, each one with a different bacterial suspension. Three replicates were done for each materiaĺs leachate.

Six serial two-fold dilutions of the eluent were prepared in BHI broth and 1 mL of each one was exposed to 1 mL of each bacterial suspension. After 24 h of incubation at 37 °C, the presence of turbidity was recorded. The MIC is equivalent to the lowest concentration of the eluent where no growth was seen.

2.2.4. Assessment of cell growth and proliferation

Discs measuring 6 mm in diameter and 1 mm high were prepared and sterilised. They were then immersed in sterile Dulbecco's modified Eagle medium (DMEM) for 24 h. After the 24-h soaking, the discs were

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