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Cytotoxicity and osteogenic potential of silicate calcium cements as potential protective materials for pulpal revascularization

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ABSTRACT

Objectives. In pulpal revascularization, a protective material is placed coronal to the blood clot to prevent recontamination and to facilitate osteogenic differentiation of mesenchymal stem cells to produce new dental tissues. Although mineral trioxide aggregate (MTA) has been the material of choice for clot protection, it is easily displaced into the clot during condensation. The present study evaluated the effects of recently introduced calcium silicate cements (Biodentine and TheraCal LC) on the viability and osteogenic differentiation of human dental pulp stem cells (hDPSCs) by comparing with MTA Angelus.
Methods. Cell viability was assessed using XTT assay and flow cytometry. The osteogenic potential of hDPSCs exposed to calcium silicate cements was examined using qRT-PCR for osteogenic gene expressions, alkaline phosphatase enzyme activity, Alizarin red S staining and transmission electron microscopy of extracellular calcium deposits. Parametric statistical methods were employed for analyses of significant difference among groups, with $\alpha = 0.05$.
Results. The cytotoxic effects of Biodentine and TheraCal LC on hDPSCs were time- and concentration-dependent. Osteogenic differentiation of hDPSCs was enhanced after exposure to Biodentine that was depleted of its cytotoxic components. This effect was less readily observed in hDPSCs exposed to TheraCal LC, although both cements supported extracellular mineralization better than the positive control (zinc oxide–eugenol-based cement).

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Significance. A favorable tissue response is anticipated to occur with the use of Biodentine as a blood clot-protecting material for pulpal revascularization. Further investigations with the use of *in vivo* animal models are required to validate the potential adverse biological effects of TheraCal LC on hDPSCs.
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1. Introduction

Treatment of immature teeth with non-vital pulps and apical periodontitis is fraught with challenges [1]. Traditionally, these teeth are managed by apexification to induce a natural calcific apical barrier [2], or by creating an artificial apical plug to facilitate conventional root canal treatment [3]. However, these procedures do not promote root development and result in thin canal walls that are susceptible to root fracture [4,5]. Revascularization of immature necrotic dental pulps has been proposed as a biologically based alternative treatment strategy to conventional root canal treatment [6–13]. The revascularization process consists of meticulous asepsis of the root canal space *via* the use of antibiotics or disinfecting irrigants, induction of bleeding to create a blood clot for homing of undifferentiated mesenchymal stem cells, insertion of a collagenous matrix over the blood clot for controlled placement of a biocompatible material to an optimal level to protect the blood clot, and placement of a leak-free restoration to serve as a coronal seal to prevent leakage and resulting reinfection [9,14–16].

Because of it excellent sealing properties and biocompatibility, mineral trioxide aggregate (MTA) has been the material of choice for covering the blood clot [6,9,17,18]. However, placing MTA over a soft blood clot can be a formidable task; MTA may collapse into the newly formed clot during condensation [19]. This, in turn, may lead to islands of dystrophic calcification within the revascularized dental pulp [20]. Although placement of a collagen membrane between the clot and cement is a feasible alternative to reduce this displacement risk [9], it is legitimate to look for calcium silicate materials that are easier to handle than MTA for pulpal revascularization

New calcium silicate cements have been commercialized because of MTA's clinical success. Biodentine (Septodont, Saint Maurdes-Fosses, France), a tricalcium silicate (Ca_3SiO_5)-based cement, is marketed as a dentin substitute [21]. Cell cultures studies indicate that Biodentine has minimal cytotoxicity [22] and is biocompatible when placed in contact with immortalized murine pulp cells [23]. The material's bioactivity was demonstrated by its ability to stimulate release of growth factor and to induce dental pulp mineralization [24]. TheraCal LC (Bisco Inc., Schamburg, IL, USA) is a light-cured resin-modified calcium silicate material designed for direct and indirect pulp capping [25]. The material was reported to be less cytotoxic than other resin-based light-cured liners [26] and released more calcium ions than the other dental cements [27].

These new tricalcium silicate cements appear to possess superior handling characteristics, making them potential candidates for pulpal revascularization procedures to prevent displacement of the cement into the freshly formed blood clot. Calcium silicate cements should demonstrate excellent biocompatibility before they can be adopted as clot-protecting materials. Accordingly, the objective of the present study was to compare the *in vitro* biocompatibility and osteogenic potential of Biodentine and TheraCal, after exposure of these materials to human dental pulp stem cells (hDPSCs). Two null hypotheses were tested: (1) there is no difference in the survivability of undifferentiated hDPSCs after their exposure to Biodentine, TheraCal LC or a commercial MTA cement and (2) Biodentine, TheraCal and a commercial MTA cement are equally adept at augmenting the osteogenic potential of the hDPSCs after the cements are rendered non-cytotoxic *via* elution of their cytotoxic components.

2. Materials and methods

2.1. Materials

Biodentine, TheraCal LC and MTA Angelus (Angelus Dental Solutions, Londrina, PR, Brazil) were evaluated. The compositions of these calcium silicate-based materials are shown in Table 1. Biodentine and MTA Angelus were mixed with deionized water or the liquid supplied, using the liquid/powder ratio recommended by the respective manufacturer. These materials were placed in pre-sterilized Teflon molds (5-mm diameter and 3-mm thick), covered with pre-sterilized Mylar sheets, and allowed to set in a 100% humidity chamber for 24 h. TheraCal LC was supplied by the manufacturer in pre-mixed syringes and required no preparation before use. The resin-based calcium silicate cement was dispensed in increments into the Teflon mold and polymerized with a light-emitting diode-type light-curing unit for 20 s per increment. The last increment was covered with a pre-sterilized Mylar sheet prior to light-curing to prevent the formation of an oxygen inhibition layer. Untreated cells were used as the negative control. Disks of similar dimensions to the test cements were prepared with zinc oxide-eugenol cement (Intermediate Restorative Material [IRM], Dentsply Caulk, Milford, DE, USA; one drop of liquid to one level scoop of powder) and used as the positive control. The set materials were sterilized with ultraviolet light for 4 h prior to testing.

2.2. Cell culture

Previously characterized hDPSCs with $CD90^+/CD105^+/CD34^-/CD45^-$ immunophenotype [28] were employed in the present study. The cells were previously obtained from young healthy patients (18–25 years old; 10 teeth from 6 patients) according to a protocol approved by the Ethics Committee of the Fourth Military Medical University, Xi'an,

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