Hydration of Biodentine, Theracal LC, and a Prototype Tricalcium Silicate–based Dentin Replacement Material after Pulp Capping in Entire Tooth Cultures

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Abstract

Introduction: The calcium-releasing ability of pulp-capping materials induces pulp tissue regeneration. Tricalcium silicate–based materials produce calcium hydroxide as a by-product of hydration. Assessment of hydration and calcium ion leaching is usually performed on samples that have been aged in physiological solution for a predetermined period of time. The hydration and activity of the materials *in vivo* may not be similar to those displayed *in vitro* because of insufficient fluid available in contact with dentin. The aim of this research was the assessment of hydration of Biodentine, Theracal LC, and a prototype radiopacified tricalcium silicate–based material after pulp capping and to compare it with direct hydration in an aqueous solution. **Methods:** The extent of hydration of Biodentine, Theracal LC, and a prototype radiopacified tricalcium silicate–based material with a similar composition to Biodentine but not incorporating the additives was assessed by scanning electron microscopy and energy dispersive spectroscopy of polished specimens after being allowed to hydrate in Hank's balanced salt solution for 14 days. The extent of hydration was compared with material hydration when used as direct pulp capping materials by using a tooth culture model. Material activity was also assessed by x-ray diffraction analysis to investigate the deposition of calcium hydroxide by the materials, and calcium ion leaching in Hank's balanced salt solution was assessed by ion chromatography. **Results:** Biodentine and the prototype tricalcium silicate cement hydrated and reaction by-products were deposited in the cement matrix both after pulp capping and when incubated in an aqueous solution. Calcium hydroxide was formed, and calcium ions were leached in solution. Theracal LC hydration was incomplete because of the limited moisture diffusion within the material. Thus, no calcium hydroxide was produced, and a lower calcium ion leaching was recorded. **Conclusions:** Theracal LC had a heterogeneous structure with large unhydrated particles because not enough moisture was present to allow hydration to proceed. Biodentine composition was shown to be optimized, and the environmental conditions did not affect material microstructure. Biodentine exhibited formation of calcium hydroxide and calcium ion leaching, which are beneficial to the dental pulp. *(J Endod 2014;40:1846–1854)*

Key Words

Biodentine, hydration, Theracal LC, tooth culture pulp capping, tricalcium silicate

Pulp-capping materials are necessary to protect the pulp from thermal, chemical, and other noxious stimuli. Calcium hydroxide has been the material of choice for pulp capping for several years. More recently with the introduction of mineral trioxide aggregate (MTA) for clinical use, tricalcium silicate–based materials have become indicated for use as pulp-capping materials (1). MTA produces calcium hydroxide as a by-product of hydration (2). Both materials solubilize bioactive molecules such as transforming growth factor β1 from the dentin (3, 4). This factor is involved in pulp progenitor cell migration to its production site and in the subsequent odontoblastic differentiation and dentin bridge formation under tricalcium silicate–based materials (5). Thus, the mode of action of MTA can be assumed to be similar to that of calcium hydroxide. When used as a pulp-capping agent, MTA exhibited comparable (6, 7) or better (8, 9) clinical and radiographic success rates to calcium hydroxide. The main disadvantages of MTA are the long setting time and the material incompatibility with other dental materials when layered (10, 11).

Second-generation calcium silicate–based materials indicated for use as pulp-capping materials are modified and exhibit a reduced setting time, thus making them more suitable in clinical use. Biodentine is composed of tricalcium silicate cement, zirconium oxide, and calcium carbonate, which, when mixed with water, calcium chloride, and a water-soluble polymer, sets in 12 minutes (12) and forms calcium hydroxide as a by-product of hydration (13). Biodentine is bioactive because it increases murine pulp cell proliferation and biomineralization (14). Furthermore, it induces transforming growth factor β1 release from human pulp cells and early dental pulp mineralization (5). In contact with animal pulps, Biodentine induced cell proliferation and formation of mineralization foci, which were strongly positive for osteopontin. At longer time points, the formation of a homogeneous dentin bridge at the injury site, secreted by cells displaying an odontoblastic phenotype, was observed. These observations were similar to those for MTA, but calcium hydroxide showed porous organization, suggesting a reparative process different from those induced by calcium silicate cements (15, 16).

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In a recent clinical study, Biodentine exhibited complete dentinal bridge formation and an absence of inflammatory pulp response. Layers of well-arranged odontoblast and odontoblast-like cells were found to form tubular dentin under the osteodentin (17).

Another calcium silicate–based material indicated for use as a pulp-capping material is Theracal LC. It is light-curable and indicated for use as liner under composite restorations aiming to achieve a bond between the different layers of materials, thus reducing microleakage. Theracal LC is a resin-modified Portland cement–based material that has demonstrated release of more calcium than ProRoot MTA and Dycal and thus was able to alkalinize the surrounding fluid (18). This is in contrast to a recent study evaluating the hydration of Theracal LC compared with Biodentine where Theracal LC exhibited low calcium ion release and a slower reaction rate than Biodentine. In addition, no calcium hydroxide was formed as a by-product of hydration (19). Contact of Theracal LC with pulp cells resulted in a reduction in cell metabolism, a reduced protein expression, and cell toxicity (20).

Assessment of hydration and material activity of calcium silicate–based cements is usually performed *in vitro* in standardized conditions, thus enabling researchers to compare the data obtained. It is difficult to relate *in vitro* conditions to *in vivo* situations because the replication of environmental conditions is not possible. Biodentine has demonstrated bioactivity, enhanced material hydration, and calcium ion release *in vitro* (13, 19, 21). The *in vitro* properties reflect well with the findings of material activity at the cellular (5, 14), histologic (15, 16), and clinical (17) levels. Although Theracal LC is certified as suitable for clinical use as a pulp-capping material, as yet the interaction of the material with the pulp is not so well-reported. Although Theracal LC releases calcium ions in solution (18), the pulpal response was not promising (20). Theracal LC is resin-modified, and the resin monomers may cause the adverse pulp reactions. Furthermore, Theracal LC does not include water for material hydration. It depends on the water taken up from the environment and its diffusion within the material.

The aim of this research was to assess the hydration of Biodentine and Theracal LC and a prototype radiopacified tricalcium silicate–based material after their application as pulp-capping materials for 14 days and to compare it with direct hydration for 14 days in an aqueous solution.

Materials and Methods

The materials used in this study included a prototype material composed of tricalcium silicate cement (Mineral Research Processing, Meyzieu, France) with 20% zirconium oxide (ZrO_2; Sigma-Aldrich, Buchs, Germany) that was mixed with water at a water-to-powder ratio of 0.35 (TCS-Zr-20) developed at the University of Malta, Biodentine (Lot no. B05128; Septodont, Saint Maur-des-Fosses, France) that was mixed according to manufacturer's instructions namely by adding the liquid provided in the vial to the powder in the capsule and triturating in an amalgam mixer for 30 seconds, and TheraCal LC (Lot no. 1200006458; Bisco Inc, Schaumburg, IL), which was dispensed from a syringe and light-cured with an LED light-curing unit (Woodpecker Zhengzhou Smile Dental Equipment Co, Ltd, Zhengzhou, Henan, China) for 20 seconds per increment.

Characterization of Set Materials

Assessment of Hydration of Materials Stored in Aqueous Solution. Six cylindrical specimens measuring 10 mm in diameter and 2 mm high were prepared from each material type. The materials were allowed to harden: 1 hour for the prototype material, 12 minutes for Biodentine, and immediately for Theracal because it hardened on application of light from light-curing device. Once set, the materials were immersed in 5 mL Hank's balanced salt solution (HBSS) (H6648; Sigma-Aldrich, St Louis, MO) for 14 days at 37°C. The composition of the HBSS was (g/L) 0.4 KCl, 0.06 KH_2PO_4 anhydrous, 0.35 $NaHCO_3$, 8.0 NaCl, 0.05 Na_2HPO_4 anhydrous, and 1.0 d-glucose. The specimens were then retrieved from the soaking solution, vacuum desiccated, and embedded in resin (Epoxyfix; Struers GmbH, Ballerup, Denmark), and polished specimens were attached to aluminum stubs, carbon coated, and viewed under the scanning electron microscope (Zeiss MERLIN Field Emission SEM; Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs of the different material microstructural components at different magnifications in backscatter electron mode were captured, and energy dispersive spectroscopy was carried out. The degree of hydration was assessed by observation of deposition of reaction by-products around the unhydrated cement particles and in the cement matrix.

Direct Pulp Capping by Using an Entire Culture Tooth Model and Assessment of Hydration after Direct Pulp Capping

Human third molars extracted for orthodontic reason and collected in agreement with French legislation (informed patients' and parents' consent and Institutional Review Board approval of the protocol used) were used. Immediately after extraction, each tooth was stored at 4°C in minimum essential medium supplemented with 300 IU/mL penicillin, 300 μg/mL streptomycin, and 0.75 μg amphotericin B (Lonza, Vervier, Belgium). Eighteen teeth with immature roots and wide open apices were selected, cleaned, dipped for 10 seconds in a 0.2% aqueous chlorhexidine solution, and rinsed for 30 seconds in phosphate-buffered saline.

A large and deep occlusal cavity with pulp exposure was prepared in each tooth with a round diamond bur mounted on a high-speed handpiece under sterile water cooling. Pulp exposure was controlled, and the cavity was gently air-dried. Then 6 teeth were capped with Biodentine, 6 with TheraCal LC, and 6 with the experimental tricalcium silicate material. Biodentine and the experimental material were applied in bulk, and 2 mm was placed over the pulp. TheraCal LC was applied in 2 layers of 1 mm in depth, and each layer was light-cured for 20 seconds. During the setting time, the apical part of the teeth was dipped into sterile absorbent cotton soaked with the culture medium to avoid any desiccation during the preparation procedure. At the end of this period, the same diamond bur used for the cavity preparation was used to eliminate the residual cement on the enamel-dentin walls.

The teeth were restored by application of self-etching adhesive resin (Xeno III, Lot no. 1302001227; Dentsply DeTrey GmbH, Konstanz, Germany) and restoration with SDR (Dentsply DeTrey GmbH).

The roots were suspended into the minimum essential medium supplemented with 200 IU/mL penicillin, 200 μg/mL streptomycin, and 0.50 μg amphotericin B (Fig. 1). The cultured teeth were incubated for 14 days, and media were changed every other day. At the end of the 14-day contact of the materials with the pulp, the teeth were removed from medium, vacuum desiccated, embedded in resin, and sectioned longitudinally under copious water irrigation through the mesial to distal axis, followed by polishing. The material hydration was assessed under the scanning electron microscope in back-scatter mode. A schematic drawing of the areas assessed for hydration is shown in Figure 1*E*.

Assessment of Material Activity

X-ray Diffraction Analysis. Phase analysis was carried out by using x-ray diffraction. Materials were allowed to set in HBSS for 14