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Experimental tricalcium silicate cement induces reparative dentinogenesis

Xin Li[a,b,1], Mariano Simón Pedano[a,1], Bernardo Camargo[a,c], Esther Hauben[d], Stéphanie De Vleeschauwer[e], Zhi Chen[b], Jan De Munck[a], Katleen Vandamme[a], Kirsten Van Landuyt[a], Bart Van Meerbeek[a,*]

[a] KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT & UZ Leuven (University Hospitals Leuven), Dentistry, Leuven, Belgium
[b] Wuhan University, School and Hospital of Stomatology, Ministry of Education, The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine, Wuhan, PR China
[c] Federal University of Rio de Janeiro, Nuclear Engineering Program, Rio de Janeiro, Brazil
[d] Laboratory for Pathology, UZ Leuven & Department of Imaging and Pathology, Translational Cell and Tissue Research, KU Leuven, Leuven, Belgium
[e] Laboratory Animal Center, KU Leuven, Leuven, Belgium

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ABSTRACT

Objectives. To overcome shortcomings of hydraulic calcium-silicate cements (hCSCs), an experimental tricalcium silicate (TCS) cement, named 'TCS 50', was developed. *In vitro* research showed that TCS 50 played no negative effect on the viability and proliferation of human dental pulp cells, and it induced cell odontogenic differentiation. The objective was to evaluate the pulpal repair potential of TCS 50 applied onto exposed minipig pulps.

Methods. Twenty permanent teeth from three minipigs were mechanically exposed and capped using TCS 50; half of the teeth were scheduled for 7-day and the other half for 70-day examination (n = 10). Commercial hCSCs ProRoot MTA and TheraCal LC were tested as references (n = 8). Tooth discoloration was examined visually. After animal sacrifice, the teeth were scanned using micro-computed tomography; inflammatory response at day 7 and day 70, mineralized tissue formation at day 70 were assessed histologically.

Results. Up to 70 days, TCS 50 induced no discoloration, ProRoot MTA generated gray/black discoloration in all teeth. For TCS 50, 40.0% pulps exhibited a mild/moderate inflammation at day 7. No inflammation was detected and complete reparative dentin with tubular structures was formed in all pulps after 70 days. ProRoot MTA induced a similar response, TheraCal LC generated a less favorable response in terms of initial inflammation and reparative dentin formation; however, these differences were not significant (Chi-square test of independence: $p > 0.05$).

Significance. TCS 50 induced reparative dentinogenesis in minipig pulps. It can be considered as a promising pulp-capping agent, also for aesthetic areas.

* *Corresponding author at*: KU Leuven (University of Leuven), Department of Oral Health Sciences, BIOMAT, Kapucijnenvoer 7, blok a — box 7001, BE-3000 Leuven, Belgium.
E-mail address: bart.vanmeerbeek@kuleuven.be (B. Van Meerbeek).
[1] Equal first-author contribution.
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1. Introduction

Calcium hydroxide has long been considered as the gold standard for pulp capping [1–3]. Lately, the most well-known and most intensively investigated hydraulic calcium silicate cements (hCSCs), often referred to as MTA (mineral trioxide aggregate), have demonstrated a superior performance than calcium hydroxide with regard to inducing less pulpal inflammation and generating more predictably a hard-tissue barrier or so-called dentin bridge [4–6]. Nevertheless, some major drawbacks of hCSCs remain a concern, such as their relatively difficult handling [7,8], long setting time [9], release of heavy metal elements [10], and potential tooth discoloration [11].

In order to overcome these shortcomings, an experimental tricalcium silicate (TCS) based cement, being referred as TCS 50, has been developed. The powder of TCS 50 consists of 50 wt% TCS and 50 wt% zirconium oxide (ZrO_2); 1 M calcium chloride ($CaCl_2$) was employed as liquid to accelerate setting. A previous study revealed that TCS 50 possessed a comparable mini-fracture toughness to that of the commercial hCSC cement Biodentine (Septodont, Saint Maur des Fosses, France), which contains 80% TCS and dicalcium silicate as the main active ingredients, 5 wt% $Zr0_2$ as the radiopacifier. As compared to Biodentine (Septodont), the Ca release from TCS 50 was lower initially but reached a prolonged release thereafter [12]. Moreover, TCS 50 appeared more biocompatible to human dental pulp fibroblasts than Biodentine (Septodont) [12]. In addition, TCS 50 played no negative effect on the viability and proliferation of human dental pulp cells (HDPCs), and it induced odontogenic differentiation of HDPCs [13], which is crucial for pulpal repair [14,15].

The effectiveness of a pulp-capping agent should not be predicted solely based on cellular responses researched *in vitro*. *In vivo* laboratory animal models have been widely used to evaluate pulpal repair potential [16–26]. Non-rodent mammals, such as monkeys, dogs, ferrets or minipigs are suitable animal models recommended by the ISO 7045-2008 standard [27]. However, the biological reactions of pulp tissues in dogs appeared not very alike as those found clinically in humans [28]. The success rate of pulp capping with calcium hydroxide appeared lower in dog than human pulps [28]. The dentition of monkeys and minipigs more closely resembles that of humans in terms of anatomical and physiological characteristics [16,22–26,29,30]. As minipigs are easier to handle and cheaper to maintain, they were selected as laboratory animal model in the current study.

The objective of this study was therefore to evaluate the pulpal repair potential of TCS 50 applied onto exposed minipig pulps. The pulpal response was evaluated 7 and 70 days after pulp capping. The market-representative resin-free hCSC ProRoot MTA (Dentsply Sirona, Konstanz, Germany) and the resin-based hCSC TheraCal LC (Bisco, Schaumburg, IL, USA) were selected as references. The hypotheses tested were (1) that exposed pulps capped with TCS 50 reveal no tooth discoloration after 7 or 70 days, (2) that exposed pulps capped with TCS 50 reveal no inflammatory response after 7 or 70 days, (3) that the exposed pulps capped with TCS 50 show mineralized tissue formation after 70 days, and (4) that there is no difference in inflammatory response or mineralized tissue formation of exposed pulps capped with the experimental hCSC TCS 50 and the reference cements ProRoot MTA (Dentsply Sirona) and TheraCal LC (Bisco).

2. Materials & methods

2.1. Minipigs

All experimental protocols complied with the ARRIVE guidelines, and were approved by the Ethical Committee for Animal Experimentation of KU Leuven under the file number P016/2015. The procedures were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. Three female Göttingen minipigs (Ellegaard, Dalmose, Denmark) at the age of 33–35 months and with a weight of 44–60 kg and intact permanent dentitions were group-housed on wood shavings with ad libitum access to water; they were fed twice a day with a minipig special diet (Welzijnskorrel minivarkens, AVEVE, Wilsele, Belgium).

2.2. Dental procedures

To ensure that all teeth were in a healthy state and the roots were fully developed, both the mandible and maxilla of each minipig were pre-operatively scanned using computed tomography (CT; SOMATOM Force, Siemens Healthcare, Erlangen, Germany).

Pulp capping was carried out at two times, in line with the two scheduled examination moments, namely 7 and 70 days after pulp capping, as recommended by the ISO 7045-2008 standard. Twenty-six permanent teeth (11 incisors, 3 canines, 6 premolars and 6 molars) on the left side of the 3 minipigs were treated in the first phase; sixty-three days later, 26 permanent teeth (11 incisors, 3 canines, 6 premolars and 6 molars) on the right side received pulp-capping treatment.

Prior to pulp capping, the minipigs were anesthetized with a combination of xylazine (1 mg/kg; XYL-M 2%, VMD, Arendonk, Belgium) and zolazepam/tiletamine (2.5–3 mg/kg; Zoletil 100, Virbac, Fort Worth, TX, USA). After endotracheal intubation, general anesthesia was maintained with isoflurane (1–1.5%), while the animals were ventilated with a tidal volume of 8–10 ml/kg at a frequency of 10–12 times per min. All teeth on the left side were ultrasonically cleaned (MiniMaster Ultrasonic Scaler, Electro Medical Systems, Chemin de la Vuarpilliere, Switzerland), rotary polished using Zircate Prophy Paste (Dentsply Sirona) and a rubber cup, and disinfected with 10% povidone iodine (iso-Betadine Dermicum, Meda Pharma, Brussels, Belgium). Under local anesthesia using 2% lidocaine, butt-joint class-V cavities were prepared on the buccal surface of incisors, canines and premolars; class-I cavities were made on the occlusal surface of molars. The cavities were prepared using a sterile round diamond bur (1.1 mm in diameter; Endo Access Bur Size 1, A 0164 300 001 00, Dentsply Sirona) at an ultra-high speed with copious water spray. Subsequently, the pulp tissue was mechanically exposed using a sterile round carbide bur (1.0 mm in diameter; H1SE.205.010, Komet, Lemgo, Germany) at a high speed with sterile saline (Fresenius Kabi, Bad Homburg, Germany) employed as coolant.

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