DENTAL MATERIALS XXX (2018) XXX–XXX

Available online at www.sciencedirect.com

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Freshly-mixed and setting calcium-silicate cements stimulate human dental pulp cells

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ARTICLE INFO

Article history:
Received 26 October 2017
Received in revised form
12 February 2018
Accepted 12 February 2018
Available online xxx

Keywords:
Portland cement
Tricalcium silicate
Pulpotomy
Cytotoxicity
Biocompatibility
Bioactive materials
Phosphopullulan

ABSTRACT

Objectives. To evaluate the effect of the eluates from 3 freshly-mixed and setting hydraulic calcium-silicate cements (hCSCs) on human dental pulp cells (HDPCs) and to examine the effect of a newly developed hCSC containing phosphopullulan (PPL) on HDPCs.

Methods. Human dental pulp cells, previously characterized as mesenchymal stem cells, were used. To collect the eluates, disks occupying the whole surface of a 12-well plate were prepared using an experimental hCSC containing phosphopullulan (GC), Nex-Cem MTA (GC), Biodentine (Septodont) or a zinc-oxide (ZnO) eugenol cement (material-related negative control). Immediately after preparing the disks (non-set), 3 ml of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) were added. The medium was left in contact with the disks for 24 h before being collected. Four different dilutions were prepared (100%, 50%, 25% and 10%) and cell-cytotoxicity, cell-proliferation, cell-migration and odontogenic differentiation were tested. The cell-cytotoxicity and cell-proliferation assays were performed by XTT-colorimetric assay at different time points. The cell-migration ability was tested with the wound-healing assay and the odontogenic differentiation capacity of hCSCs on HDPCs was tested with RT-PCR.

Results. Considering all experimental data together, the eluates from 3 freshly-mixed and setting hCSCs appeared not cytotoxic toward HDPCs. Moreover, all three cements stimulated proliferation, migration and odontogenic differentiation of HDPCs.

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https://doi.org/10.1016/j.dental.2018.02.005

Significance. The use of freshly-mixed and setting hCSCs is an appropriate approach to test the effect of the materials on human dental pulp cells. The experimental material containing PPL is non-cytotoxic and positively stimulates HDPCs.

1. Introduction

Direct pulp capping aims to seal the vital pulp tissue exposed by trauma or deep caries excavation, in an attempt to enable the pulp to heal and so to avoid a more invasive and often technically difficult root-canal treatment. Pulp exposure mostly involves odontoblast destruction and fibroblast injury [1,2]. A successful pulp-capping procedure requires a mild inflammatory reaction along with a supply of mainly calcium ions to stimulate recruitment and proliferation of dental pulp stem cells from their vascular niche [3,4]. These cells need to migrate and differentiate into mineralized tissue to form a hard-tissue barrier that will (re-)isolate the exposed pulp from the oral environment [5].

Thanks to the excellent biocompatibility and overall performance for diverse endodontic applications, hydraulic calcium-silicate cements (hCSCs) are becoming the material of choice for direct pulp capping [6,7]. Nevertheless, hCSC's properties are still far from the ideal requirements a pulp-capping agent should meet. While setting within a moist oral environment, disadvantages as difficult handling, long setting time and the risk on discoloration have been reported [8]. To overcome these drawbacks, new materials with improved characteristics have been developed, such as the resin-free hCSC Biodentine (Septodont, Saint Maurdes-Fosses, France) and resin-based hCSC Theracal (Bisco, Schaumburg, IL, USA) [3,9]. However, specific properties such as the low adhesion to dentin in case of the resin-free hCSC Biodentine and the lower bioactivity of the resin-based hCSC Theracal LC remain to be improved [10,11].

Based on new bioadhesive technology and knowledge gained from research in the field of dental adhesive technology, an experimental hCSC formulation (GC, Tokyo, Japan) has been developed by adding phosphorylated pullulan (PPL) [12]. Phosphorylation of the biodegradable polysaccharide pullulan enabled to combine adhesion potential to dentin with gradual dissolution of internalized bioactive components. The experimental hCSC formulation showed improved osseointegration and adhesion to dental tissues, thereby even having potential to serve as 'bioadhesive' for bone regeneration [13,14]. Today, little is known regarding this material in light of its application for pulp capping [15].

Although much research on pulp-capping potential of hCSCs has been conducted, the way most researchers apply hCSCs in laboratory experiments differs from how clinicians employ them. Most protocols leave the cement to set for some hours, ranging from 1 up to 72 h [16,17] prior to exposing them to HDPCs. HDPCs are commonly not exposed to 'freshly-mixed and setting' hCSCs, as is done clinically. Consequently, these data cannot directly be extrapolated to common clinical practice.

Therefore, the objectives of this research were (1) to evaluate the effect of the eluates from 'freshly-mixed and setting' hCSCs on cytotoxicity, migration, proliferation and odontogenic differentiation of human dental pulp cells (HDPCs), and (2) to compare these qualities for the experimental PPL-containing hCSC (Exp.-PPL) with those of the two commercial (reference) hCSCs Biodentine (Septodont) and Nex-Cem MTA (GC, Tokyo, Japan). Two null hypotheses were tested: (1) freshly-mixed and setting hCSCs are not suitable for *in vitro* testing their pulp-capping potential on HDPCs; (2) the extracts of Biodentine and Nex MTA are less cytotoxic and better promote cell proliferation, migration and odontogenic differentiation, when exposed to HDPCs, than the experimental Exp.-PPL hCSC.

2. Materials and methods

2.1. Materials

For the preparation of the culture media, all materials were purchased from Gibco (Carlsbad, CA, USA). Chemicals were obtained from Sigma–Aldrich Chemicals (St. Louis, MO, USA) unless otherwise stated.

2.2. Isolation of human dental pulp cells (HDPCs)

The isolation of HDPCs was performed according to the previously reported outgrowth method [18–20]. Briefly, healthy human third molars (from patients ranging 15–25 years old), extracted because of orthodontic reasons, were gathered as approved by the Commission for Medical Ethics of KU Leuven under the file number S54254. The teeth were rinsed in phosphate buffered saline (PBS) supplemented with 200 U/ml penicillin and 200 μg/ml streptomycin; the periodontal ligament was removed with a new #15 sterile blade (Swann Morton, Sheffield, UK), upon which the teeth were mechanically split to collect the pulp tissue. From each tooth, the pulp tissue was gently harvested using a sterile tweezers; the isolated pulp tissue was next cut with a new sterile blade into approximately 1-mm^3 fragments. These pulp-tissue fragments were seeded on the surface of 25-cm^2 cell-culture flasks (Costar, Cambridge, MA, USA) filled with 1 ml culture medium consisting of Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco). When the HDPCs reached 70–80% confluence, the cells were harvested using 0.25% Trypsin/EDTA (Sigma–Aldrich) and observed as passage 0. The HDPCs were cultured in 175-cm^2 cell-culture flasks (Costar) at 37 °C, 5% CO_2 and 95% humidity. Cells at passage 3–6 were used in this study. Twelve teeth from six different donors (two from each patient) were used for this study.

Please cite this article in press as: Pedano MS, et al. Freshly-mixed and setting calcium-silicate cements stimulate human dental pulp cells. Dent Mater (2018), https://doi.org/10.1016/j.dental.2018.02.005