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Dentin matrix components extracted with phosphoric acid enhance cell proliferation and mineralization

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ABSTRACT

Objective. Acids, such as those used in adhesive dentistry, have been shown to solubilize bioactive molecules from dentin. These dentin matrix components (DMC) may promote cell proliferation and differentiation, and ultimately contribute to dentin regeneration.

The objective of this study was to evaluate the potential for varying concentrations of DMC extracted from human dentin by phosphoric acid of a range of pHs to stimulate proliferation and mineralization of two different cultured pulp cell populations.

Methods. DMC were solubilized from powdered human dentin (7 days – 4 °C) by phosphoric acid of pH 1, 3, and 5 and also, EDTA. Extracts were dialyzed for 7 days against distilled water and lyophilized. Undifferentiated mouse dental pulp cells (OD-21) and cells of the odontoblast-like cell line (MDPC-23) were seeded in six-well plates (1×10^5) and cultured for 24 h in DMEM (Dulbecco's modified Eagle's medium) containing 10% (v/v) FBS (fetal bovine serum). The cells were washed with serum-free medium and then treated with different concentrations of DMC (0.01, 0.1, 1.0 and 10.0 μg/ml) daily in serum free medium for 7 days. After 3, 5 (MDPC-23 only), and 7 days of treatment, cell proliferation was measured using 10 vol% Alamar blue solution, which was added to each well for 1 h. Cell numbers were first measured by cell counting (Trypan blue; $n = 5$) and Alamar blue fluorescence to validate the assay, which was then used for the subsequent assessments of proliferation. Mineralization was assessed by Alizarin Red S assay after 12 days exposure to DMC ($n = 5$). Controls were media-only (DMEM) and dexamethasone (DEX; positive control). Results were analysed by ANOVA/Tukey's ($p \leq 0.05$).

Results. There was a linear correlation between cell counts and Alamar blue fluorescence ($R^2 > 0.96$ for both cell types) , verifying the validity of the Alamar blue assay for these cell types. In general, there was a dose-dependent trend for enhanced cell proliferation with higher concentration of DMC for both cell lines, especially at 10.0 μg/ml. DEX exposure resulted in significantly higher mineralization, but did not affect cell proliferation. DMC exposure demonstrated significantly greater mineralization than media-only control for 10 μg/ml for all extracts, and at lower concentrations for EDTA and pH 5 extracts.

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Significance. Human dentin matrix components solubilized by acids at pH levels found in commercial dentin adhesives enhanced cell proliferation and mineralization of mouse and rat undifferentiated dental pulp cells when presented in adequate concentration.

1. Introduction

The dental pulp contains progenitor/stem cells that can proliferate and differentiate into odontoblast-like cells that can replace damaged odontoblasts following dental caries and operative procedures. It has been suggested that morphogens released from the dentin matrix may play a role in the proliferation and differentiation of stem cells into odontoblasts-like cells [1–3]. Recent reports confirm that treating human dentin with dental materials, such as acids used in dental bonding agents, and basic compounds like calcium hydroxide and mineral trioxide aggregate, solubilize dentin and release a rich cocktail of potentially bioactive molecules formerly sequestered within the dentin [4,5].

The extracellular matrix (ECM) of dentin consists of hydroxyapatite, collagen, and non-collagenous matrix proteins. Dentin ECM contains a 3D scaffold that is formed of type I, III and V collagen fibrils [6,7]. Thin collagen fibrils form the predentin, which is comparable to the osteoid matrix of bone. Several ECM components (proteins and lipids) are involved in the control of the mineralization process. Non-collagenous proteins (NCPs) probably play a role in transforming predentin to dentin [8,9] and it has been proposed that some NCPs promote the growth of hydroxyapatite (HAP) crystals and their effects depend upon their concentration and conformational form [9,10].

Dentin contains a unique family of phosphoproteins, which includes dentin sialophosphoprotein (DSPP), dentin matrix protein 1 (DMP1), bone sialoprotein (BSP), matrix extracellular phosphorylated glycoprotein (MEPE), and osteopontin (OPN) [7,9,11]. It is reported that dentin mineralization was disrupted in DSPP null mutant mice and therefore, it is proposed that the DSPP gene products play a specific and crucial role in the formation of mineralized dentin [3]. Other mutation studies have also shown that NCPs are essential in the mineralization process of dentin and or bone [7,11,12].

Embery et al. have reported that glycosaminoglycans (GAG), part of the proteoglycans (PG), are important for collagen fibril maturation and proper mineralization [13]. Dentin also contains Fetuin-A, a serum protein produced in the liver and in mineralized tissues like dentin, that inhibits undesirable ectopic calcification without affecting the mineralization process in dentin or bone [14]. Dentin, as a mineralized tissue, contains growth factors, which have the ability to bind to hydroxyapatite [5]. These growth factors also can stimulate matrix production or regulate the production of specific matrix proteins or molecules that are important for the remineralization process [5,14]. The release of these molecules from dentin is therefore proposed to provide a source of bioactive molecules with significant potential for application in the repair of damaged or restored tooth structure [5,15].

Acids, such as those used in adhesive dentistry, have been shown to solubilize bioactive molecules from dentin [15]. DMC were solubilized from powdered human dentin by phosphoric acid of pH 1, 3, and 5, and EDTA [15]. There was a trend for greater DMC solubilization to occur with higher hydrogen ion concentrations, although the quantities of DMCs solubilized at different pH levels were not statistically significant. Assays confirmed the release of TGF-β_1, non-collagenous proteins (NCPs) and glycosaminoglycans (GAGs) at a range of pHs. It has been suggested that these DMC may promote cell proliferation, differentiation, and ultimately remineralization in the dentin–pulp complex.

The objective of this study was to evaluate the effects of varying concentrations of DMC extracted from human dentin by phosphoric acid of varying pH on two different cultured pulp cell lines. Our hypothesis was that DMC would stimulate cell proliferation and/or differentiation to produce mineral, and that the effect would be dose dependent.

2. Materials and methods

2.1. Dentin matrix component preparation

As previously described, DMC were solubilized from powdered human dentin (7 days – 4 °C) by phosphoric acid of pH 1, 3, and 5, and EDTA. Extracts were dialyzed for 7 days against distilled water, lyophilized, and stored at −20 °C [15].

2.2. Cell culture

Undifferentiated mouse dental pulp cells (OD-21) and cells of the rat odontoblast-like cell line (MDPC-23) were maintained in Dulbecco's modified Eagle's medium (DMEM), containing 4.5 g/L glucose, 10% (v/v) fetal bovine serum (FBS), and supplemented with an antibiotic solution (100 U/ml penicillin-G and 100 μg/ml streptomycin) and 0.594 g/l L-glutamine at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.3. Alamar blue assay

The Alamar blue assay was used to evaluate viability and proliferation of OD-21 and MDPC-23 cells treated with DMC. Cells were seeded in six-well plates at a density of 1×10^5 per well and cultured for 24 h in 1 ml DMEM containing 10% (v/v) FBS. The cells were washed with serum-free medium (DMEM without serum) and then treated daily for 7 days with different concentrations of DMC (0.01, 0.1, 1.0 and 10.0 μg/ml) in serum free medium. The concentrations were selected based on previous work that has demonstrated positive effects on cell proliferation and differentiation for DMC extracted by EDTA and calcium hydroxide [16–18]. After the third, fifth (MDPC-23