Biocompatibility and Osteogenic/Calcification Potential of Casein Phosphopeptide-amorphous Calcium Phosphate Fluoride

Alaa E. Dawood, PhD, David J. Manton, PhD, Peter Parashos, PhD, Rebecca H. Wong, PhD, William Singleton, MSc, James A. Holden, PhD, Neil M. O'Brien-Simpson, PhD, and Eric C. Reynolds, PhD

Abstract

Introduction: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and CPP-ACP with fluoride (CPP-ACFP) have been shown to provide bioavailable ions to promote mineralization. Hence, the aim of this study was to evaluate the materials' biocompatibility and osteogenic/calcification potential for endodontic applications. **Methods:** Human and mouse osteoblast-like and fibroblast-like cell lines were incubated with 0.05%–3.0% w/v CPP-ACP and CPP-ACFP, and toxicity, proliferation, alkaline phosphatase, interleukin (IL)-1α, and IL-6 production, collagen type I, osteocalcin, and osteopontin production, and mineralization/calcification were determined. **Results:** CPP-ACP and CPP-ACFP were non-toxic and had no significant effect on proliferation or production of the inflammatory cytokine IL-1α. Alkaline phosphatase activity of the osteoblast-like cells was significantly increased ($P < .05$) by CPP-ACP and CPP-ACFP, as was the production of the osteotropic cytokine IL-6, the formation of calcium mineral deposits, and the secretion of mineralization-related proteins (collagen type I and osteocalcin). **Conclusions:** CPP-ACP and CPP-ACFP are biocompatible and have the potential to induce osteoblastic differentiation and mineralization. Potential applications include apexification, perforation repair, vital pulp therapy, and regenerative endodontic procedures. *(J Endod 2017;■:1–6)*

Key Words

Biocompatibility, CPP-ACFP, CPP-ACP, differentiation, endodontics, proliferation

Significance

The results of this study suggest that both cell-mediated biomineralization (osteogenesis) and spontaneous calcium precipitation (calcification) were promoted by CPP-ACP/CPP-ACFP. Hence, CPP-ACP and CPP-ACFP may have potential application in contemporary endodontics, including apexification, perforation repair, vital pulp therapy, and regenerative endodontic procedures.

The mechanism of action of endodontic repair cements that are based on calcium hydroxide and calcium silicate, such as mineral trioxide aggregate, depends on their bioactivity represented by their ability to release ions such as calcium (Ca^{2+}) and hydroxide (OH^-). Calcium silicate–based cements that release Ca^{2+} enhance hard tissue–forming cell viability, proliferation, and differentiation, and the OH^- release increases the alkalinity of the environment that supports hard tissue repair and active calcification (1, 2). Released Ca^{2+} from endodontic repair cements stimulates the expression of mineralization-associated genes such as bone morphogenetic protein-2, collagen 1, and osteocalcin (3–5). The release of high concentrations of Ca^{2+} from the repair cement at the pulpal site activates the migration of pre-odontoblasts located in the central pulp (6, 7). Ca^{2+} released from repair cements may serve as a bioactive signal that promotes the process of cell-based tissue repair. However, the high pH of calcium hydroxide may not be optimal for cell-based repair mechanisms, and modification of cements to improve their physical and biological properties shows promising results (2).

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is a phosphopeptide stabilized Ca^{2+} and phosphate ion (P_i) complex that provides bioavailable Ca^{2+} at neutral pH and promotes remineralization of enamel and dentin (8). A fluoride-containing version (casein phosphopeptide-amorphous calcium fluoride phosphate [CPP-ACFP]) has superior remineralizing efficacy compared with CPP-ACP (9). *In vitro*, calcium-enriched CPP induced Ca^{2+} uptake by osteoblast-like cells, promoted osteoblastic differentiation, increased the level and activity of alkaline phosphatase (ALP), and enhanced the formation of calcified nodules (10, 11). Therefore, CPP-ACP may play a role in promoting osteogenesis and calcification. Furthermore, the addition of CPP-ACP to calcium silicate–based cements has been shown to improve ionic release (1); therefore, CPP-ACP added to these materials may help promote dental hard tissue repair.

From the Melbourne Dental School, Oral Health CRC, Bio21 Institute, The University of Melbourne, Melbourne, Australia.

Address requests for reprints to Prof Eric. C. Reynolds, Melbourne Dental School, Oral Health CRC, Bio21 Institute, The University of Melbourne, Melbourne, Victoria 3010, Australia. E-mail address: e.reynolds@unimelb.edu.au

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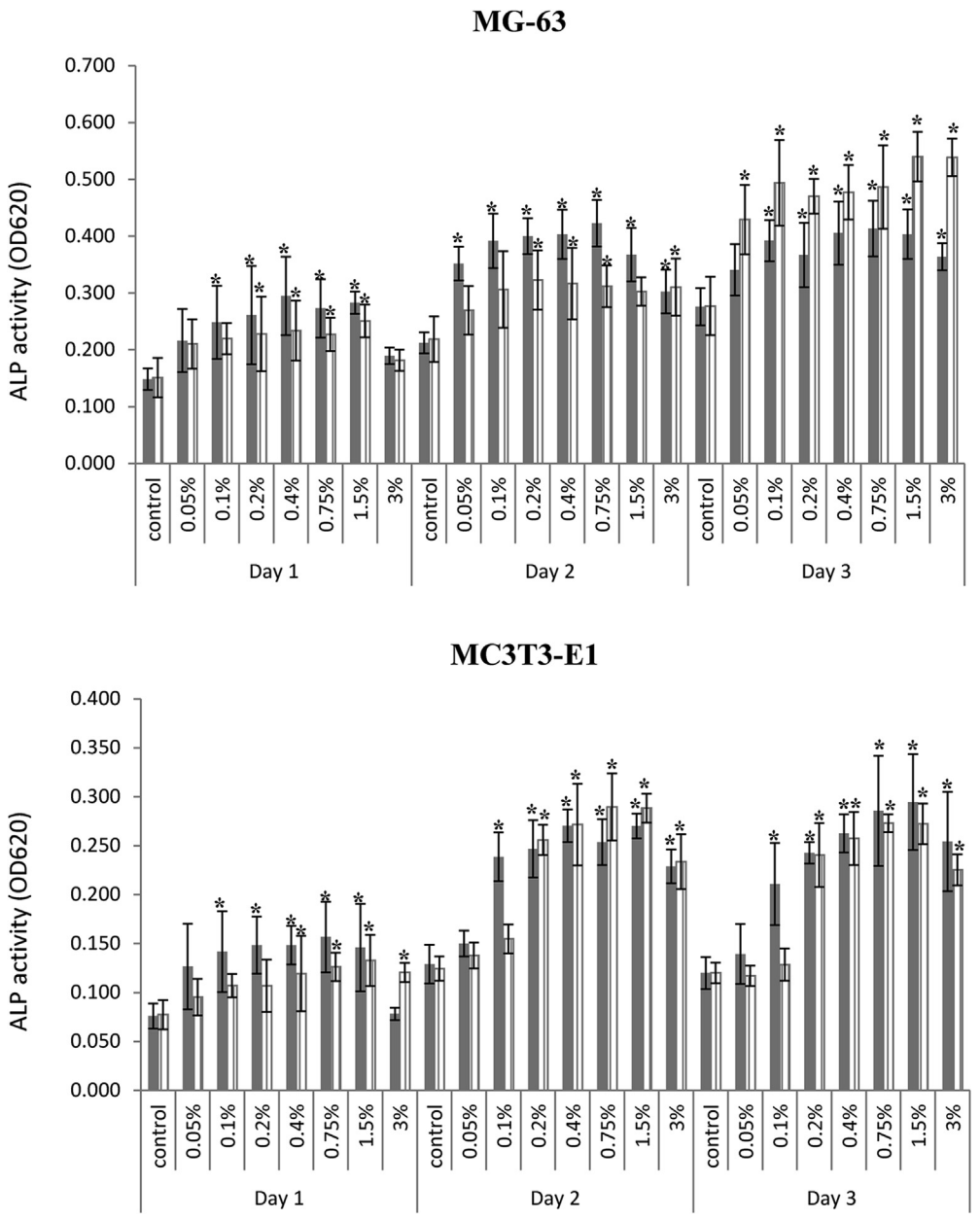

Figure 1. Effect of CPP-ACPF on production of ALP activity by the osteoblast-like cell lines MG-63 and MC3T3-E1. CPP-ACP, CPP-ACFP. *Denotes significant differences ($P < .05$) compared with controls (cells in medium alone). OD, optical density.

Therefore, the aims of this study were to examine the effects of CPP-ACP and CPP-ACFP on osteoblast-like and fibroblast-like cell lines to evaluate biocompatibility and osteogenic/calcification potential.

Materials and Methods

Cell Culture

Human (MG-63) and mouse (MC3T3-E1) osteoblast-like cell lines (Sigma-Aldrich, Castle Hill, NSW, Australia) and human (HGF-1) and mouse (NIH3T3) fibroblast-like cell lines (ATCC, Manassas, VA) were cultured in complete alpha minimum essential medium (complete α-MEM), composed of α-MEM supplemented with 10% v/v fetal calf serum, 1 U/mL penicillin, 0.1 mg/mL streptomycin, 1 mmol/L sodium pyruvate, and 2 mmol/L L-glutamine (Sigma-Aldrich). For the mineralization assay, the complete α-MEM was supplemented with 10 mmol/L glycerophosphate and 50 μg/mL ascorbic acid (Sigma-Aldrich) as an osteogenic medium. Cells were maintained in a humidified Heracell 150 incubator (Thermo Fisher Scientific, Lombard, IL) at 37°C and 5% v/v CO_2.

Preparation of CPP-ACP and CPP-ACFP Solution

The soluble CPP-ACP and CPP-ACFP powders (9) were mixed with complete α-MEM by magnetic stirring for 24 hours and sterilized through a 0.22-μm filter (Corning Incorporated Life Sciences, Bedford, MA). Stock solutions of 3.0% w/v CPP-ACP and 3.0% w/v CPP-ACFP were prepared and serially diluted to 7 concentrations (0.05%, 0.1%, 0.2%, 0.3%, 0.7%, 1.5%, and 3.0% w/v).