Biomimetic Calcium-Silicate Cements Support Differentiation of Human Orofacial Mesenchymal Stem Cells

Maria Giovanna Gandolfi, DBiol, DSc, MSc, PhD, Sara N. Shah, BA,† Ruoxue Feng, BS,† Carlo Prati, MD, DDS, PhD,* and Sunday O. Akintoye, BDS, DDS, MS†*

Abstract

Introduction: Human orofacial bone mesenchymal stem cells (OFMSCs) from maxilla and mandible have robust osteogenic regenerative properties on the basis of our previous reports that demonstrate phenotypic and functional differences between jaw and axial bone mesenchymal stem cells in same individuals. Furthermore, a combination of OFMSCs with bioactive calcium-releasing cements can potentially improve OFMSC multilineage differentiation capacity, but biocompatibility of calcium-silicate cements with OFMSCs is still unclear. We tested the hypothesis that material extracts of calcium-releasing calcium-silicate cements support biomimetic microenvironment for survival and differentiation of human OFMSCs. **Methods:** Two experimental calcium-silicate cements, (1) calcium-silicate mineral powder (wTC) containing dicalcium and tricalcium-silicate, calcium sulfate, and calcium chloride and (2) wTC doped with alpha-tricalcium phosphate (wTC-αTCP), were designed and prepared. Cement setting times were assessed by Gilmore needles, ability to release calcium and hydroxyl ions was assessed by potentiometric methods, and OFMSC attachment to calcium-silicate discs was assessed. Calcium-silicate material extracts were tested for ability to support OFMSC survival and *in vitro/in vivo* differentiation. **Results:** Fewer OFMSCs attached to calcium-silicate discs relative to tissue culture plastic (P = .001). Extracts of calcium-silicate cements sustained OFMSC survival, maintained steady state levels of vascular cell adhesion molecule-1, alkaline phosphatase, and bone sialoprotein while up-regulating their respective gene transcripts. Adipogenic and *in vivo* bone regenerative capacities of OFMSCs were also unaffected by calcium-silicate extracts. **Conclusions:** Ion-releasing calcium-silicate cements support a biomimetic microenvironment conducive to survival and differentiation of OFMSCs. Combination of OFMSCs and calcium-silicate cement can potentially promote tissue regeneration in periapical bone defects. *(J Endod 2011;37:1102–1108)*

Key Words

Alpha-tricalcium phosphate, bone cements, bone regeneration, calcium-silicate, endodontic, orofacial stem cells

Orofacial bone mesenchymal stem cells (OFMSCs) from maxilla and mandible are postnatal mesenchymal stem cells with inherently higher survival and osteogenic properties than bone mesenchymal stem cells (BMSCs) from non-oral bones (1, 2). We have previously shown that in same individuals, BMSCs in the jaw (ie, OFMSCs) are skeletally site-specific on the basis of proliferation, survival, differentiation, and *in vivo* regenerative properties (1–3). A combination of the biology of the local microenvironment and circulating levels of soluble calcium and inorganic phosphates has been correlated with bone regeneration (4, 5); therefore, calcium-silicate cements, also known as mineral trioxide aggregate (MTA) cements, are used clinically in oral surgery and root canal therapy. Calcium-silicate cements have attractive chemical, physical, and biological properties that include ability to set in the presence of moisture, the release of calcium ions, and ability to form apatite in the presence of phosphate-containing simulated body fluids (6–11).

The bioactive properties of calcium-silicate cements combined with bio-interactivity (12) with local microenvironment might be conducive to cell survival and bone regeneration (13), but there is paucity of information on effects of calcium-silicate cements on jaw-specific mesenchymal stem cells (14). Alpha-tricalcium phosphate (αTCP) is a reactive compound that can serve as a source of phosphate (15) and calcium ions (16) for apatite formation (17) and tissue regeneration. Although calcium-silicate cements are more resorbable than hydroxyapatite-tricalcium phosphate commonly used to promote bone regeneration, they can potentially stimulate appropriate biomimetic microenvironments for OFMSC differentiation because they have been shown to promote attachment and proliferation of osteoblast-like cells and dental pulp stem cells (DPSCs) (5, 18). DPSCs share similar embryologic origin with OFMSCs (1, 19), but OFMSCs display higher osteogenic differentiation (2). A combination of superior osteogenesis of OFMSCs with biomimetic properties of calcium-silicate cements is attractive for bone regeneration, but biocompatibility of calcium-silicate material extracts with survival and regenerative properties of OFMSCs is still unclear.

The aim of this study was to determine the biocompatibility of OFMSCs with biomimetic properties of 2 calcium-silicate preparations: (1) calcium-silicate mineral powder (wTC) containing dicalcium and tricalcium-silicate, calcium sulfate, and calcium chloride and (2) wTC doped with αTCP (wTC-αTCP). We hypothesized that

From the *Laboratory of Biomaterials and Oral Pathology, Department of Odontostomatological Sciences, University of Bologna, Bologna, Italy; and †Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

This project was supported in part with funds from United States Public Health Service/National Institutes of Health/National Cancer Institute (USPHS/NIH/NCI) research grant 5K08CA120875-05.

Address requests for reprints to Dr Sunday O. Akintoye, Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania, The Robert Schattner Center Room 211, 240 South 40th Street, Philadelphia PA 19104. E-mail address: akintoye@dental.upenn.edu

0099-2399/$ - see front matter

Copyright © 2011 American Association of Endodontists.

doi:10.1016/j.joen.2011.05.009

calcium ions and other minerals (silicon and phosphorous) in material extracts released by calcium-silicate cements will provide supportive biomimetic microenvironment for survival and differentiation of human OFMSCs.

Materials and Methods

Preparation of Calcium-Silicate Cements

Experimental wTC containing dicalcium and tricalcium-silicate, calcium sulfate, calcium chloride; and wTC-αTCP were designed and produced. The ground mineral powders were mixed by using 1 g of cement per 0.300 mL of calcium and magnesium-free Dulbecco phosphate-buffered saline, pH 7.4 (Lonza Walkersville Inc, Walkersville, MD; Cat #17-512) on a glass plate with stainless steel spatula. A homogeneous paste was achieved within 30 seconds and packed into a circular polyvinyl chloride mold (8 mm internal diameter and 1.6 mm thickness) to obtain a smooth surface.

Setting Times of Cements

Calcium-silicate cement (wTC and wTC-αTCP) discs covered on upper and lower surfaces with wet gauze to prevent dehydration were placed in a hermetically sealed curing chamber (37°C and 98% relative humidity) before evaluating setting times. Gilmore needles were used to assess setting times according to American Society for Testing and Materials International C266-03 and International Organization for Standardization 9917-1 (20, 21). Briefly, Gilmore initial setting time was the elapsed time (in minutes) between the mixing of the cement with liquid and the first penetration measurement that does not mark the specimen surface with a complete circular impression. As either the initial or final setting times approached (ie, no indentation), the specimens were tested every minute to determine the exact Gilmore setting time. After initial setting time, the specimens were assessed every 5 minutes until final setting time was determined. Weight/diameter of Gilmore needle used were 113.4 g/2.12 mm and 453.6 g/1.06 mm to test initial and final setting times, respectively (20). Setting time testing was performed on 3 replicates for each material, and each sample was used for only 1 indentation test.

Calcium Release and Alkalinizing Activity of Cements

Calcium-silicate cement (wTC and wTC-αTCP) discs (n = 10 for each material) were immediately immersed in 10 mL of deionized water (pH, 6.8) in polypropylene sealed containers at 37°C. Soaking water was collected at 3 and 24 hours and after 7, 14, and 28 days to assess pH and calcium content. Calcium released in soaking water was measured by using a calcium probe (calcium ion electrode; Eutech Instruments Pte Ldt, Singapore) after addition of 0.200 mL (2%) of ionic strength adjuster (4 mol/L KCl; WTW, Weilheim, Germany). The pH of soaking water was measured by using a selective temperature compensated electrode (Sen Tix Sur; WTW) and a previously calibrated multi-parameter laboratory meter (inoLab 750; WTW). The probes were inserted into the soaking medium at room temperature (24°C) with continuous stirring. Each measurement was repeated 3 times.

Calcium-Silicate Disc and Extract Preparations for Cell Culture

Calcium-silicate cement (wTC and wTC-αTCP) discs for OFMSC culture were prepared as above by using a circular polypropylene mold (12 mm internal diameter and 1.6 mm thickness). To obtain partial setting, cements were kept for 1 hour at 37°C and 95% relative humidity to allow for hydration and setting reactions. Cement discs were de-molded and sterilized for 2 hours in antibiotic medium consisting of α-modified minimum essential medium (MEM), 100 U/mL penicillin, 100 mg/mL streptomycin sulfate, and 25 μg/mL amphotericin B. Discs were washed free of antibiotic medium with sterile phosphate-buffered saline before using for cell attachment assay. Cement discs prepared in the similar manner as above were allowed to set for 1 hour at room temperature instead of 37°C, transferred to a 24-well culture plate, and sterilized with antibiotic medium as above. Discs were incubated in 1.5 mL fresh α-MEM containing 100 U/mL penicillin, 100 mg/mL streptomycin sulfate, and 2 mmol/L glutamine at 37°C in humidified atmosphere for 24 hours to allow for release of calcium and silicate ions into the medium. The supernatant collected was referred to as material extract. Both wTC and wTC-αTCP material extracts were passed through separate 0.22-μm filters, pH recorded with colorimetric strips, and kept at 4°C until used for cell culture.

Culture of Orofacial BMSCs

Human OFMSCs were expanded from primary cultures of human maxilla and mandible primary BMSCs previously isolated under an existing protocol approved by the University of Pennsylvania Institutional Review Board. The cells were cultured in growth medium consisting of α-MEM supplemented with 10% fetal bovine serum (Equitech Bio Inc, Kerrville, TX), 100 U/mL penicillin, 100 mg/mL streptomycin sulfate, and 2 mmol/L glutamine (BioSource International, Camarillo, CA) and incubated at 37°C in a humidified atmosphere of 5% CO_2 and air as previously described (1). Passage 3 OFMSCs were used for all experiments.

Attachment of OFMSCs to Calcium-Silicate Discs

Sterile 12-mm diameter wTC and wTC-αTCP discs prepared as above were transferred into separate wells of a 24-well culture plate containing α-MEM growth medium. Then 2.0×10^4 OFMSCs were seeded on triplicate wTC and wTC-αTCP discs and in blank wells so that tissue culture plastic served as control. After 24 hours, the discs were transferred to another 24-well plate. Attached cells were released with trypsin/ethylenediaminetetraacetic acid (EDTA) and cell scraper before counting with a hemocytometer.

OFMSC Survival in Calcium-Silicate Extracts

OFMSCs were seeded in 96-well plate at 3.2×10^4 cells per well. After 24 hours, α-MEM growth medium was changed so that 3 sets of 11 wells received extract of wTC or wTC-αTCP or fresh α-MEM growth medium. After another 72 hours in culture, surviving cells were assessed by using colorimetric WST-1 cell proliferation assay (Roche Applied Science, Indianapolis, IN) according to manufacturer's instructions.

Lineage Differentiation of OFMSCs in Calcium-Silicate Extracts

Osteogenesis. OFMSCs seeded at 9.5×10^4 cells per well in duplicate wells of a 6-well plate were kept in culture for 24 hours before exposure to either wTC or wTC-αTCP material extract prepared as above. Control cells received fresh α-MEM growth medium. After 48 hours, the cells were replenished with osteogenic medium consisting of α-MEM, 10% fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin sulfate, 2 mmol/L glutamine, 10^{-8} mol/L dexamethasone, and 100 μmol/L L-ascorbic acid phosphate magnesium-hydrate. After another 48 hours, total protein was collected by using Mammalian Protein Extraction Reagent (Pierce Biotechnology, Rockford, IL). In

Download English Version:

https://daneshyari.com/en/article/3148978

Download Persian Version:

https://daneshyari.com/article/3148978

Daneshyari.com