White Mineral Trioxide Aggregate Induces Migration and Proliferation of Stem Cells from the Apical Papilla

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Abstract

Introduction: Regenerative endodontic protocols recommend white mineral trioxide aggregate (WMTA) as a capping material because of its osteoinductive properties. Stem cells from the apical papilla (SCAP) are presumed to be involved in this regenerative process, but the effects of WMTA on SCAP are largely unknown. Our hypothesis was that WMTA induces proliferation and migration of SCAP. **Methods:** Here we used an unsorted population of SCAP (passages 3–5) characterized by high CD24, CD146, and Stro-1 expression. The effect of WMTA on SCAP migration was assessed by using transwells, and its effect on proliferation was determined by the WST-1 assay. Fetal bovine serum (FBS) and calcium chloride–enriched medium were used as positive controls. **Results:** The SCAP analyzed here showed a low percentage of STRO-1+ and CD24+ cells. Both set and unset WMTA significantly increased the short-term migration of SCAP after 6 hours ($P < .05$), whereas calcium chloride–enriched medium did after 24 hours of exposure. Set WMTA significantly increased proliferation on days 1–5, whereas calcium-enriched medium showed a significant increase on day 7, with a significant reduction on proliferation afterwards. SCAP migration and proliferation were significantly and steadily induced by the presence of 2% and 10% FBS. **Conclusions:** Collectively, these data demonstrate that WMTA induced an early short-term migration and proliferation of a mixed population of stem cells from apical papilla as compared with a later and longer-term induction by calcium chloride or FBS. *(J Endod 2014;40:931–936)*

Key Words

Calcium, chemotaxis, dental, MTA, SCAP, stem cells

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Interest in regenerative endodontics has increased substantially in recent years, encouraged by successful clinical case reports of treated immature necrotic teeth (1–3). A number of these cases have shown that after disinfection with triple antibiotic paste or calcium hydroxide, the use of mineral trioxide aggregate (MTA) as capping material over an induced blood clot will continue root development (2–4). Lovelace et al (1) have shown that the induction of bleeding at the apex results in a higher concentration of mesenchymal stem cells (MSCs) than is found in the systemic circulation. Although the exact source of these stem cells is as yet unknown, the apical papilla is the likely origin (1). The understanding of the effects of MTA on the stem cells from the apical papilla is critically important to optimizing clinical regenerative endodontics protocols.

Stem cells from the apical papilla (SCAP) have been isolated from immature permanent human teeth during root formation (5, 6). The apical papilla appears during the early stages of tooth development and plays an important role, moving apically to allow continued formation of the radicular dentin and dental pulp (7). SCAP are an excellent source of cells for regeneration of the pulp-dentin complex (8). Studies by Sonoyama et al (5) have shown that SCAP proliferate 2–3 times faster than other pulp cells, and they are as potent as MSCs for both osteogenic and odontogenic differentiation (5, 6). Recent studies have shown that MSCs and human dental pulp cells react favorably to the presence of some dental materials such as MTA (9–13).

The main components of unset white MTA (WMTA) are tricalcium silicate, dicalcium silicate, tricalcium aluminate, bismuth oxide, and calcium sulfate dehydrate. The composition of gray MTA is similar, except that it contains tetracalcium aluminoferrite (14, 15). The set composition of MTA is mostly calcium silicate hydrate and calcium hydroxide ($Ca(OH)_2$). A substantial release of calcium ions results from the dissociation of $Ca(OH)_2$ and the decomposition of the calcium silicate hydrate (14, 16, 17). The start of $Ca(OH)_2$ formation is greatest at 4–24 hours, with the highest total concentration occurring after 7 days. The formation of $Ca(OH)_2$ begins to decline at day 30 but continues during a period of 5 weeks (14, 17, 18). MTA has excellent biocompatibility and has been used in root end fillings, perforation repair, pulp capping, and apexification procedures (9, 14, 19). Calcium ions have been shown to be potent signaling molecules and to play a role in the regulation of most cellular activities (20–26). The migration of MSCs, bone marrow–derived progenitor cells, and tumor cells is affected by calcium ion concentrations (21–23). Human dental pulp cells increase proliferation when exposed to medium containing MTA and medium containing exogenous calcium ions (20). The mineralization potential of human periodontal ligament cells and adipose-derived stem cells is promoted with increased calcium ion concentrations (24, 25). The differentiation of pulpal cells into odontoblasts may also be influenced by the levels of calcium ions present in the surrounding environment (26). The ability of calcium ions to influence cell migration and proliferation makes it logical to explore its role element in regenerative procedures.

In a mixed population of pulp cells, MTA stimulates hard tissue formation and induces migration, proliferation, and differentiation into odontoblast-like cells (9, 10, 12). WMTA increases gene expression in human pulp cells exposed for 72 hours, with the up-regulation of 109 genes and down-regulation of 69 genes (11). Seo et al (27) have recently shown up-regulation of genes related to migration in dental pulp stem cells from permanent teeth. Although how MTA activates pulp cells remains unclear, the high calcium ion release from MTA suggests that calcium initiates the biological response (20). The purpose of this study was to determine whether WMTA plays a role in the migration and proliferation

of a mixed population of SCAP and to determine whether calcium chloride or fetal bovine serum (FBS) has the same effect.

Materials and Methods

Cell Culture

The SCAP used for all experiments were provided by Dr Songtao Shi (University of Southern California, Los Angeles, CA). These human cells were obtained and experiments carried out with the approval of the University of Southern California Institutional Review Board. Cells were cultured in minimal essential medium Eagle alpha (α-MEM) (Invitrogen, Carlsbad, CA). This medium was used for all the experimental conditions in its plain form or supplemented with 2% or 10% FBS, 250 g/ml L-glutamine, and 1% penicillin/streptomycin (Gibco, Grand Island, NY), and cells were incubated at 37°C in 5% CO_2 and 100% humidity. Cells used were between passages 3 and 5.

Flow Cytometric Analysis

SCAP from passages 2, 4, and 10 were evaluated by flow cytometry to characterize the cell population at different passages. Five $\times$ 10^5 cells were collected, and antibody staining was performed in the dark with human CD146-APC antibodies (Milenyi Biotec, Auburn, CA), PE mouse anti-human CD24 antibodies (BD Pharmingen, San Jose, CA), and mouse anti-STRO-1 primary antibodies (Invitrogen Corp) with fluorescein isothiocyanate rabbit anti-mouse immunoglobulin G conjugate antibodies (Invitrogen). Controls of untreated cells and cells stained with either the primary STRO-1 or conjugate fluorescein isothiocyanate antibody were used. Cells were incubated in triplicates in the dark on ice for 30 minutes. Four independent experiments were run. The samples were read on a BD FACSAria3 flow cytometry machine by using the BD FACSDiva software (BD Falcon, San Jose, CA) at 10,000 events.

MTA Preparation

WMTA (ProRoot; Dentsply Endodontics, Tulsa, OK) was mixed following the manufacturer's instructions. WMTA pellets of 10 mg were formed by using a ratio of 100 mg WMTA powder and 35 μL sterile water under a laminar flow hood in a sterile dappen dish (Keystone Industries, Cherry Hill, NJ) for approximately 1 minute. Pellets were formed by using a Lee MTA pellet-forming block (G. Hartzell & Son, Concord, CA). The pellets were allowed to set for 1 hour or 24 hours at 37°C in 5% CO_2 and 100% humidity under sterile conditions.

Calcium-enriched Medium

The calcium-enriched medium was prepared with anhydrous $CaCl_2$ (1 mol/L) dissolved in α-MEM (Invitrogen). Serial dilutions of 3.0, 0.3, and 0.03 mmol $CaCl_2$ were made.

Transwell Migration Assay

SCAP migration was assessed by using a transwell migration assay after the cells were pre-stained with Cell Tracker Green (Invitrogen). One $\times$ 10^5 cells in 250 μL plain medium were seeded into the upper chambers of transwell 8-μm pore FluoroBlok of 24 multi-well system membrane filters (BD Falcon) and allowed to attach for 4 hours before exposure to test conditions. The lower chambers of the 24 multi-well system contained the following test conditions: plain α-MEM, plain α-MEM with 1-hour set WMTA or 24-hour set WMTA, plain α-MEM with 3.0 mmol $CaCl_2$, 0.3 mmol $CaCl_2$, or 0.03 mmol $CaCl_2$, 2% FBS α-MEM with 1-hour set WMTA or 24-hour set WMTA, 2% FBS α-MEM, or 10% FBS α-MEM. One pellet of WMTA was placed into each well of the test groups. Cells were cultured at 37°C in 5% CO_2 and 100% humidity. Cells that migrated to the bottom of the FluoroBlok inserts were read by fluorescence at 485/585 nm by using a microplate reader (Genius; Tecan, Grödig, Austria) at 0.5, 1, 3, 6, 12, 24, 48, and 72 hours.

WST-1 Proliferation Assay

Proliferation was evaluated by WST-1 proliferation assay. Four $\times$ 10^3 SCAP were seeded into 12-well companion plates (BD Falcon) with 1.5 mL α-MEM in the same test conditions as used for the migration assay. A 3-μm-pore size transwell cell culture insert (BD Falcon) with 2 pellets of either 1-hour or 24-hour set WMTA was used. The plates were incubated at 37°C in 5% CO_2 and 100% humidity, with the medium changed every 2–3 days. The plates were evaluated at 1, 3, 5, 7, 9, 11, and 14 days with WST-1 reagent (Roche, Mannheim, Germany). WST-1 reagent and medium in 1:10 ratio were added to each well in culture plate and incubated at 37°C in 5% CO_2 and 100% humidity for 1 hour. The supernatants were then transferred to a 96-well plate (BD Falcon), and the absorbance (450–685 mm) was determined in a microplate reader (Genius; Tecan).

Statistical Analysis

Four independent experiments were run in triplicates for each condition. The statistical analysis was completed with the support of the University of Michigan's Center for Statistical Consultation and Research. All data were analyzed by using 1-way analysis of variance with a Bonferroni comparison and a P value $\leq$.05 as statistically significant. The analysis was carried out by using SigmaStat 2.0 Software (Systat Software, San Jose, CA).

Results

SCAP Morphology Was Not Affected by the Presence of WMTA

To select the conditions used for each test, the SCAP were cultured in plain α-MEM, 2% or 10% FBS α-MEM, or 2% FBS α-MEM with WMTA. After 7 days, SCAP grown in plain α-MEM survived but did not show the confluency seen in SCAP grown in α-MEM with 2% FBS with or without WMTA or 10% FBS (Fig. 1*A*). SCAP grown with WMTA in α-MEM with 2% FBS showed similar morphologic features as those cells grown in α-MEM with 2% or 10% FBS (Fig. 1*A*). A significant effect on SCAP proliferation was found by WST-1 assay after 1 week in culture with α-MEM and FBS at 2% or 10% as compared with plain α-MEM (Fig. 1*B*).

SCAP Lose Stemness by Passage 10

To characterize the SCAP used in the experiments, the expression of putative stem cell markers was evaluated by flow cytometry. SCAP were cultured in 10% FBS α-MEM up to 10 passages, and evaluations of CD24, STRO-1, and CD146 positive cells were done at passages 2, 4, and 10. The percentage of CD24+ cells at passage 2 was 3.9%, at passage 4 was 5.9%, and decreased to 0% at passage 10. Only 5.1% of the cell population was STRO-1+ at passage 2, 6% at passage 4, and decreased to 0.8% at passage 10. At passage 2, 73.2% of the cells were CD146+, 75.6% at passage 4, and decreased to 60% at passage 10 (Fig. 1*C*).

WMTA Increases Early and Short-term SCAP Migration

The migration of SCAP started after 1 hour and continued up to 6 hours after exposure to 1-hour or 24-hour set WMTA in plain α-MEM. There was a significant difference at 6 hours for both groups as compared with the plain α-MEM group (P < .038). In these groups the proliferation was reduced gradually after 12, 24, and 48 hours of