Biocompatibility of Accelerated Mineral Trioxide Aggregate on Stem Cells Derived from Human Dental Pulp

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Abstract

The aim of this study was to evaluate the effects of several additives on the setting time and cytotoxicity of accelerated-set mineral trioxide aggregate (MTA) on stem cells of human dental pulp. ProRoot white MTA (WMTA) (Dentsply Tulsa Dental, Johnson City, TN) was mixed with various additives including distilled water, 2.5% disodium hydrogen phosphate (Na_2HPO_4) (Merck, Darmstadt, Germany), K-Y Jelly (Johnson & Johnson, Markham, ON, Canada), and 5% and 10% calcium chloride ($CaCl_2$) (Merck). The setting times were evaluated using a Vicat apparatus (Alsa Lab, Istanbul, Turkey). Human dental pulp stem cells were isolated and seeded into 48-well plates at 2×10^3 cells per well and incubated with MTA samples for 24 hours, 3 days, and 7 days. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. MTA mixed with 10% $CaCl_2$ showed the lowest setting time ($P < .05$). According to the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium results on the 1st, 3rd, and 7th days, a statistically significant difference was found ($P < .05$) between MTA groups and the control group. MTA mixed with K-Y Jelly in all groups showed the lowest cell viability at all time points ($P < .05$). The cell viability of MTA mixed with distilled water, 5% $CaCl_2$, 10% $CaCl_2$, and Na_2HPO_4 increased significantly through time ($P < .05$). This *in vitro* study found MTA mixed with 5% and 10% $CaCl_2$ and Na_2HPO_4 is biocompatible with dental pulp stem cells in terms of cell viability. Further *in vitro* and *in vivo* investigations are required to prove the clinical applications of MTA mixed with various additives. (*J Endod 2016;42:276–279*)

Key Words

Accelerators, biocompatibility, dental pulp stem cell, mineral trioxide aggregate, setting time

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Mineral trioxide aggregate (MTA) is a hydrophilic cement that consists of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, and other mineral oxides (1). MTA has been used primarily as a root-end filling material, but MTA is now most widely used in endodontics for pulp capping, pulpotomy, repair of root perforations, root canal filling, and apical barrier formation in teeth with necrotic pulps and open apices (2, 3). MTA has been extensively used because of its excellent biocompatibility and sealing ability (4). MTA has shown significantly greater frequency of dentin bridge formation, thicker and less porous dentin, and less pulp inflammation compared with calcium hydroxide and other hydraulic calcium silicate cements such as Biodentine (Septodont, Saint-Maur-des-Fossés, France) for endodontics applications (2, 5).

The dental pulp contains several niches of potential progenitor/stem cells, which can proliferate and differentiate into hard tissue–forming odontoblastlike cells. The research has shown that whenever MTA was placed in direct contact with human dental pulp stem cells (DPSCs), it promoted proliferation and differentiation of the DPSCs into the cells similar to odontoblasts (6, 7).

Although *in vitro* biocompatibility studies have been conducted and have shown favorable biological properties of MTA, it has some disadvantages, such as long setting time, poor handling properties, and high material cost (8, 9). One of the major drawbacks of the material is its slow initial setting time, which, in turn, necessitates multiple visits by patients (8–10). Therefore, in recent studies, various accelerants such as calcium chloride ($CaCl_2$), calcium formate, citric aside, sodium hypochlorite gel, disodium hydrogen orthophosphate (Na_2HPO_4), calcium lactate gluconate, and K-Y Jelly (Johnson & Johnson, Markham, ON, Canada) have been added into MTA to improve its physicochemical properties (8, 10–14). However, some researchers have reported the cytotoxicity effects of MTA on the various cell lines, including osteosarcoma, mouse lymphoma, and human endothelial cells (15–17). Nonetheless, the effects of accelerated MTA on DPSCs have never been studied thoroughly to investigate the biocompatibility of different accelerants added into MTA.

In vitro cytotoxicity assays are simple, reproducible, cost-effective, relevant, and suitable for the evaluation of basic biological aspects relating to biocompatibility. In this kind of analyses, the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay; Promega, Madison, WI) has shown some significant advantages such as simplicity, rapidity, and precision (18). The aim of this study was to evaluate the effects of several additives on setting time and the cytotoxicity of white MTA (ProRoot MTA; Dentsply Tulsa Dental, Johnson City, TN) mixed with selected accelerants on stem cells isolated from human dental pulp.

Materials and Methods

Preparation of the Materials

The materials were mixed using a spatula and glass plate. The powder/liquid ratio used for all tested materials was 3:1. White ProRoot MTA powder was mixed with various additives. $CaCl_2$ and Na_2HPO_4 were dissolved in the distilled water before mixing with the MTA powder.

Materials were divided into the following groups: in group 1 (control), MTA was mixed with sterile distilled water, and in groups 2–5, MTA was mixed with 10% $CaCl_2$

(Merck, Darmstadt, Germany), 5% $CaCl_2$ (Merck), 2.5% Na_2HPO_4 (Merck), and K-Y Jelly (0.25 mL), respectively, in the same weight ratio.

Setting Time

Setting time was determined with a Vicat apparatus (Alsa Lab). The Vicat apparatus was assembled with an indenter needle that has a mass of 100 ± 0.5 g and a flat-end diameter of 2 mm ± 0.1 mm (19). This experiment has been based on American National Standards Institute/American Dental Association specification #57 (20).

The materials were mixed and inserted in metallic ring molds (10-mm diameter and 2-mm thick). A 1.0-mm-diameter flat-end indenter was used with a 300-g load and carefully lowered vertically to the surface of the tested material. This procedure was repeated every 5 minutes. The needle was cleaned between each test. The MTA setting times were measured 2 times for each group.

Cell Culture and Biocompatibility Test

The human permanent teeth used in this study were extracted from healthy patients (12–18 years of age) as part of a prophylactic treatment for orthodontic reasons. Written informed consent was obtained from the patients and their parents after receiving approval by the Ethics Committee of the Medical Faculty of Marmara University, Istanbul, Turkey.

The pulps were minced into small pieces (approximately 0.1 mm in diameter) with a sterile scalpel in growth medium containing Dulbecco modified essential medium (Gibco Invitrogen, Grand Island, NY) supplemented with 10% fetal bovine serum (Sigma-Aldrich, St Louis, MO), 100 U/mL penicillin, and 100 mg/mL streptomycin. Upon mincing, the small tissue pieces were then transferred into 6-well plates containing growth medium. After 10 to 15 days of incubation at 37°C in a humidified atmosphere of 5% CO_2 in the incubator, the cells were transferred to a new culture flask when they reached confluency. For cell characterizations, the cells were incubated with fluorescein-labeled antibodies (FITC) antihuman CD 24 (BD Biosciences, San Jose, CA), phycoerythrin (PE) antihuman CD45, FITC antihuman CD44, PE antihuman CD90, and PE antihuman CD117 antibodies for 45 minutes at room temperature. After washing to remove the excess antibodies, the cells were analyzed by a FACS Calibur Flow Cytometer (BD Biosciences).

ProRoot MTA was mixed according to the manufacturer's instructions. The mixed materials were inserted into 48-well plates. To prevent bacterial contamination, specimens were exposed to ultraviolet light for 30 minutes. The cytotoxicity of MTA samples was measured using the MTS assay. The cells were seeded at a concentration of 2000 cells/well in 48-well plates in growth medium. After 1, 3, and 7 days of incubation, MTS assay solution was added into each well according to the manufacturer's instructions. After 2 hours in culture, the cell viability has been detected quantitatively based on the optical density at 490 nm using an enzyme-linked immunosorbent assay plate reader (Elx800; BioTek, Winooski, VT).

TABLE 1. Setting Times for Mineral Trioxide Aggregate Mixed with the Various Additives

Additives	Setting time (mean ± SD)
10% $CaCl_2$	27 ± 2.83 min
K-Y Jelly	30 ± 1.41 min
Na_2HPO_4	32 ± 2.83 min
Distilled water	140 ± 2.83 min
5% $CaCl_2$	35 ± 2.83 min

SD, standard deviation.

Scanning Electron Microscopic Examination

Cell-seeded MTA samples were washed with cacodylate buffer (0.1 mol/L, pH = 7.4) and fixed with glutaraldehyde (2.5%) for 1 hour at room temperature. Fixed samples were then washed with cacodylate buffer and air dried completely. Samples were coated (SCD 005; Bal-Tec, Wetzlar, Germany) with gold (10 nm) and observed with a scanning electron microscope (EVO Scanning Electron Microscope; Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

Cell experiments were performed in triplicate. The data collected were analyzed using Statistical Package for the Social Sciences 16.0 (SPSS Inc, Chicago, IL) using the Mann-Whitney *U* and Kruskal-Wallis tests with the level of significance set at $P < .05$. The mean and standard error of the mean were reported.

Results

Setting Time

The working properties of MTA mixed with K-Y Jelly were not easy to manipulate in comparison with distilled water. The results of the setting time for MTA groups are reported in Table 1. MTA mixed with 10% $CaCl_2$ showed the lowest setting time ($P < .05$).

Characterization of Human DPSCs

DPSCs were characterized for mesenchymal stem cell surface markers CD24, CD45, CD44, CD90, and CD117 using flow cytometry. According to the data obtained from the FACS Calibur Flow Cytometer, DPSCs were shown to be positive for CD44 and CD90 and negative for CD24, CD45, and CD117 antibodies.

Cell Viability by MTS Assay

The relative cell viability of dental pulp stem cells after 1, 3, and 7 days of incubation is presented in Figure 1. A statistically significant difference was found between MTA groups and the control group in the 1st, 3rd, and 7th day values of the MTS assay ($P < .05$). MTA mixed with distilled water showed significantly higher cell viability than the other MTA groups on days 1 and 3 ($P < .05$). The cell viability of MTA mixed with 5% $CaCl_2$ and Na_2HPO_4 was similar to that of pure

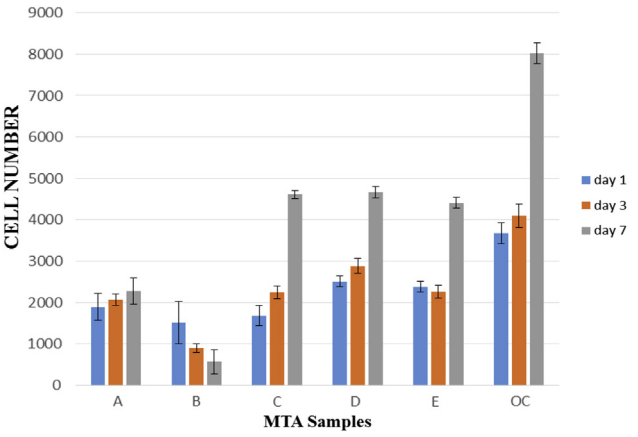

Figure 1. The cell viability of DPSCs after 1, 3, and 7 days of incubation using the MTS assay. Experimental materials and control groups represent statistically significant differences between the time points ($P < .05$). Initial cell seeding was 2000 cells/well. A, 10% $CaCl_2$; B, K-Y Jelly; C, Na_2HPO_4; D, distilled water; E, 5% $CaCl_2$; OC, only stem cell (control).