Biocompatibility Investigation of New Endodontic Materials Based on Nanosynthesized Calcium Silicates Combined with Different Radiopacifiers

Bojana Cetenovic, DDS, PhD, Bogomir Prokic, DVM,† Sasa Vasilijic, PhD,‡ Biljana Dojcinovic, PhD,§ Marko Magic, DDS,‖ Vukoman Jokanovic, PhD,¶ and Dejan Markovic, DDS, PhD**

Abstract

Introduction: The aim of this article was to analyze biocompatibility and bioactivity of new endodontic materials on the basis of nanosynthesized calcium silicates (ALBO-MPCA$_1$ and ALBO-MPCA$_2$) combined with different radiopacifiers in comparison with MTA$^+$. **Methods:** Morphology of the samples was studied by scanning electron microscopy, and the pH and ion release analysis were also assessed. Biocompatibility of materials' eluates (24-hour, 7-day, and 21-day) was conducted by using MTT test. Twelve New Zealand white rabbits were used for intraosseous implantation. Four calvarial defects per animal were created and filled with freshly prepared investigated materials. **Results:** Samples mostly consisted of agglomerates built up from nanoparticles, preferably spherical and rod-like. There was no significant difference among pH values of materials' eluates after 24 hours ($P > .05$). The amount of calcium and aluminum ion release decreased, whereas the amount of magnesium and bismuth (ALBO-MPCA$_1$, MTA$^+$) and barium (ALBO-MPCA$_2$) increased during 21-day period. The metabolic activity of cells increased after the extraction time, except in case of undiluted elutes of ALBO-MPCA$_2$ and ALBO-MPCA$_1$ (21-day). Histologic analysis of the samples revealed newly formed bone tissue with moderate inflammation for all investigated materials, which subsided during 90-day period to mild. Both MTA$^+$ and ALBO-MPCA$_1$ were in direct contact with the newly formed bone tissue. After 90 days, statistically significant difference in hard tissue formation was observed in comparison of MTA$^+$ and ALBO-MPCA$_1$ with control group ($P < .05$). **Conclusions:** Experimental materials ALBO-MPCA$_1$ and ALBO-MPCA$_2$ possess both biocompatibility and bioactivity. Because ALBO-MPCA$_1$ provokes favorable biological response, it is especially good candidate for further clinical investigations. *(J Endod 2017;43:425–432)*

Key Words

Biocompatibility, biomaterials, calcium silicates, intraosseous implantation, MTA, MTT

Significance

Distinct bioactivity of investigated materials ALBO-MPCA1 and ALBO-MPCA2 is provided by their three-hierarchical organization and physiological limited alkalinity. Because ALBO-MPCA1 provokes favorable biological response comparable with the control material MTA+, it is especially good candidate for further clinical investigations.

Biocompatibility is interpreted as the material's ability to act within adequate host response in a specific situation (1). Immediately after material's implantation into living tissue, blood and intercellular fluid proteins adhere to its surface, thus enabling the body to recognize the material as a foreign body (2).

The host's response to foreign body depends on its chemical reactivity and surface structure. Furthermore, material's bioactivity may be affected by its topography. It has been shown that nanostructured surface mimics the hierarchical organization of the bone tissue more closely in comparison with microstructured by increasing the concentration of active biomolecules that may affect cell migration, adhesion, differentiation, and proliferation (3, 4).

During the 1990s, mineral trioxide aggregate (MTA) was presented as a material of choice for retrograde fillings because of its lower apical microleakage, setting in the presence of moisture and high pH (5). Despite favorable biological properties, MTA exhibits dry consistency, low flowability, and long setting time, which limit its clinical use (6). Thus, many investigations have been focused on finding new MTA formulations to improve the above-mentioned physicochemical disadvantages without affecting the biological properties (7, 8).

Similar to MTA, new endodontic material, mineral polyoxide carbonate aggregate ALBO-MPCA, which is based on calcium silicates, has been synthesized by using the

From the *Clinic for Pediatric and Preventive Dentistry, School of Dentistry, University of Belgrade, Belgrade, Serbia; †Department of Surgery, Orthopedy and Ophtalmology, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; ‡Institute for Medical Research, Military Medical Academy, Faculty of Medicine, University of Defense, Belgrade, Serbia; §Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia; ‖Department of Oral Surgery, School of Dentistry, University of Belgrade, Belgrade, Serbia; and ¶Department of Atomic Physics, Vinca Institute of Nuclear Sciences, Belgrade, Serbia.

Address requests for reprints to Dr Bojana Cetenovic, Clinic for Pediatric and Preventive Dentistry, School of Dentistry, University of Belgrade, Dr. Subotica 11, 11000 Belgrade, Serbia. E-mail address: bojanacetenovic@hotmail.com

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combination of fully innovative sol-gel method with high-temperature self-propagating reaction (9). This method of material synthesis enables the increase in hydration rate and consequently the reduction of setting time, as well as the enhancement of material's biological properties should occur.

The aim of this study was to estimate the biocompatibility and bioactivity of new nanostructured materials that are based on calcium silicates combined with different radiopacifiers in comparison with MTA^+ by using *in vitro* and *in vivo* model.

Materials and Methods

Chemical Characterization of Investigated Materials

Briefly, stoichiometric quantities of $CaCl_2 \times 5\ H_2O$ and silica sol were used for the synthesis of calcium silicate phases, 2β-$CaSiO_4$ (β-C_2S) and Ca_3SiO_5 (C_3S), whereas calcium chloride tetrahydrate was used as the precursor for production of $CaCO_3$ (9). The investigated materials, ALBO-$MPCA_1$ and ALBO-$MPCA_2$, were prepared by mixing calcium silicate phases and calcium carbonate with Bi_2O_3 and $BaSO_4$ (radiocontrast agents), respectively, in the ratio 1:2:2. MTA^+ (Cercamed, Stalowa Wola, Poland) consisting of calcium hydroxide with oxides of silicon, iron, aluminum, sodium, potassium, bismuth, magnesium, and calcium phosphate was used as a control material.

Scanning electron microscopy (SEM) (JEOL, JSM-5300, Tokyo, Japan) was used to study the morphology and agglomerate size distribution of the previously hydrated samples. Before analysis, the samples were dried at 110°C and then vaporized with a thin layer of gold. Prepared samples were transferred into the chamber of the instrument and observed at a voltage of 30 kV.

Inductively Coupled Plasma-optical Emission Spectroscopy and pH Analysis

Investigated materials ALBO-$MPCA_1$ and ALBO-$MPCA_2$ were mixed with ultrapure water in the ratio 2:1 (m/m), and MTA^+ was prepared according to the manufacturer's instructions and left in plastic molds (5 mm in diameter and 5 mm high) to set. After complete setting, samples were placed into 20 mL ultrapure water (pH = 5.76 ± 0.51). Ultrapure water of conductivity of 0.055 μS/cm (Barnstead GenPure Pro; Thermo Scientific, Karlsruhe, Germany) was changed after 24 hours, 7 days, and 21 days. The concentrations of elements in solution samples were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (n = 3). ICP-OES measurement was performed by using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, United Kingdom).

The pH measurements of completely set materials (24 hours, 7 days, and 21 days) were assessed in triplicates by pH-meter (pH-vision Microcomputer 6071; JENCO Electronics Ltd, Linkou Shiang, Taiwan) combined with the HI-type electrode 1131 (Hanna Instruments WTW GmbH, Woonsocket, RI). Calibration of the pH-meter was performed with certified buffers (pH 4.01 and pH 7.00) (Carlo Erba, Milan, Italy).

Material Preparation for MTT Cytotoxicity Test

Freshly prepared investigated materials were placed into plastic molds 5 mm in diameter and 5 mm deep and incubated for 24 hours at 37°C in an atmosphere with humidity of 95%, allowing their complete setting. For each of the investigated materials, 4 samples were prepared. The samples were sterilized with UV light for 120 minutes on both sides and then placed into 24-well plates in Dulbecco modified Eagle medium (DMEM) (PAA Laboratories GmbH, Pasching, Austria). The total mass to volume ratio was 100 mg/mL, and surface to volume ratio was approximately 43.96 mm^2/mL. The samples were incubated in atmosphere with 5% CO_2 at 37°C for 21 days. Cytotoxicity of undiluted (100%) and diluted (50%) eluates of investigated materials after 24 hours, 7 days, and 21 days was tested. Eluates were centrifuged for 15 minutes at 3000 rpm before conducting the testing.

Cell Culture

Cytotoxicity tests were carried out by using mouse fibroblasts (L929) cultured in DMEM supplemented with 10% fetal bovine serum, 2 mL L-glutamine, and 100 IU/mL penicillin/streptomycin. Cell cultures were incubated at a temperature of 37°C in an atmosphere with 5% CO_2 (MRC Scientific Instruments, Holon, Israel) to the formation of monolayer cultures, as confirmed by light microscopy (Model Lambda LQT 2; ATTO Instruments Co, Hong Kong, China). The cells of fourth passage were washed with phosphate-buffered saline, separated by using 0.25% trypsin, centrifuged, and resuspended in culture medium. Cell viability was checked by using 0.4% trypan blue solution, and the number of cells was determined by the hemacytometer (Bright-Line Hemacytometer; Hausser Scientific, Horsham, PA). The number of cells seeded in 96-well plates was 1×10^4.

MTT Cytotoxicity Assay

After 24 hours of incubation, the culture media were removed by aspiration from microtiter plates, and 100 μL of undiluted and diluted elutes of set materials was added. Microtiter plates were then incubated at a temperature of 37°C in an atmosphere with 5% CO_2 for 24 hours. After incubation, 10 μL MTT solution in a concentration of 5 mg/mL in a phosphate buffer was added per pool. Microtiter plates were then incubated under the same conditions for 4 hours, after which 100 μL 10% sodium dodecylsulfate in 0.01 mol/L HCl was added. After 24 hours an optical density was quantified spectrophotometrically on enzyme-linked immunosorbent assay plate reader (Behring ELISA Processor II, Heidelberg, Germany) at a wavelength of 570 nm.

The MTT assay was conducted in triplicate. The percentage of the cell metabolic activity (% M) was calculated on the basis of the formula:

$$\%\mathbf{M} = \frac{\mathbf{OD\ cell\ culture\ with\ samples} - \mathbf{OD\ samples\ without\ cell\ culture}}{\mathbf{OD\ cell\ culture\ without\ samples} - \mathbf{OD\ control\ medium}} * \mathbf{100}$$

Surgical Procedure

All the animal experiments were conducted according to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications no. 8023, revised 1978) and with the approval of the local Ethics Committee (Protocol No. 36/7, February 20, 2013).

The experiment procedure was carried out on 12 male New Zealand white rabbits, 4 months old with an average weight of 2.5 kg, under general dissociative anesthesia consisting of ketamine (ketamine 500 mg/mL; Laboratorio Sanders SA, Santiago, Chile) at a dose of 40 mg/kg and acepromazine (acepromezine 50 mL; Boehringer Ingelheim Vetmedica, Inc, St Joseph, MO) at a dose of 0.75 mg/kg.