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In Vitro Biocompatibility of Nanostructured Endodontic Materials Using *SCAP* Cells

SUMMARY

Background/Aim: *Lately, fully innovative sol-gel method with high-temperature self-propagating reaction was used for the synthesis of new nanostructured endodontic materials, in combination with different radiopacifiers: bismuth (ALBO-MPCA$_1$) and barium (ALBO-MPCA$_2$). The aim of this study was to investigate the biocompatibility of nanostructured endodontic materials based on highly active calcium silicates and mixed with different radiopacifiers in comparison to MTA$^+$ using human stem cells from the apical papilla- SCAP cells.* ***Material and Methods:*** *Morphology of the samples was studied by SEM. The tested materials were mixed with distilled water in a ratio 2:1 (m/m). Fifteen minutes fter the preparation, samples were used in the experiment. The biocompatibility of fresh materials, after 3h and 7 days, was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide- MTT test.* ***Results:*** *Samples mostly consisted of spherical and rode-like. The relative viability of cells increased following the exposure time.* ***Conclusion:*** *The biocompatibility of synthesized materials is comparable to the control material MTA$^+$, and therefore these materials can be recommended for for further clinical studies.*

Key words: Biocompatibility, Calcium Silicates, MTT, MTA, Biomaterials

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Introduction

The Food and Drug Administration (FDA) has identified a large number of substances used in the promotion of health, among which are drugs and medical devices. Most dental materials are classified into the group of medical devices, including filling materials, as well. Drugs differ from medical devices because they achieve their primary effects through a chemical reaction. Therefore, drugs require both safety checks and precise definition of their intended chemical effect. Medical devices, on the other hand, must provide only security checks[1]. Most dental products belong to Class I or Class II medical devices (dental cements)[1].

MTA is a calcium silicates based material composed of 75% refined PC, 20% bismuth oxide and 5% calcium sulfate dihydrate with traces of SiO_2, CaO, MgO, K_2SO4 and Na_2SO_4[2,3,4]. The whole family of calcium silicates based materials is called hydraulic silicate cements (HSC), since the binding reaction of these materials is based on hydration processes, meaning that these materials are bonded and stable in the presence of water[5]. This classification also provide difference from other silicate elements which setting is based on an acid-base reaction.

Since 2002, only one form of MTA material consisting of gray powder (GMTA) was available, and then the white MTA (WMTA) appeared on the market, in order to fulfill aesthetic demands[3,6]. Analyzes have confirmed that there is a big difference in the composition of GMTA and WMTA, primarily in concentrations of Al_2O_3, MgO and Fe_2O_3[2,7]. It was proven that WMTA contains 54.9% less Al_2O_3, 56.5% less MgO and 90.8% less Fe_2O_3, which led to the conclusion that the decrease in Fe_2O_3 is most likely the cause for the change in powder color[8]. WMTA also has a smaller particle size than GMTA, which defines the degree of hydration[9].

Similar to MTA, new nanostructured endodontic materials, based on highly active calcium silicates were synthesized using nowel sol-gel method with high-temperature self-propagating reaction[10]. This method

increases the hydration rate, reduces the setting time and enhances the biological properties of the materials. The investigated materials, ALBO-$MPCA_1$ and ALBO-$MPCA_2$, were prepared by mixing the active calcium silicate phases with Bi_2O_3 and $BaSO_4$ (radiocontrast agents), respectively[10].

The aim of this study was to investigate the biocompatibility of nanostructured endodontic materials based on highly active calcium silicates and mixed with different radiopacifiers in comparison to MTA^+ using *SCAP* cells.

Material and Methods

Scanning electron microscopy

Before the analysis, investigated samples were dried at 110°C and then vaporized with a thin layer of gold. At the voltage of 30 kV, morphology of the previously hydrated samples was scanned using scanning electron microscopy (SEM, JEOL, JSM-5300, Tokyo, Japan).

Cell Culture

Stem cells were isolated from the apical papilla of the teeth with incomplete root develompent (*SCAP cells*). Using the flow cytometry (CD 34, CD 73, CD 90- positive and CD 45, CD 105- negative markers), the detection and characterization of stem cells was confirmed. The cells were cultured in a medium (DMEM/F12) enriched with 10% fetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL). Incubation of cell cultures was carried out at 37^0C, in an atmosphere with 5% CO_2 to the formation of the confluent monolayered culture, confirmed by a light microscope (Boeko, Germany). The total number of cells cultued on the micro-plates was 2×10^4 (Bright-Line Hemacytometer, Hausser Scientific, Horsham, PA, USA).

Preparation of fresh materials' samples

The tested materials were mixed with distilled water in a ratio 2:1 (m/m). Freshly prepared materials were placed in the plastic modules, 6 mm in diameter and 2 mm in depth. Fifteen minutes after the preparation, samples were plased in the culture medium for 3h and 7-days.

MTT test

After the culture medium was removed and 100 µl of fresh materials' solutions was added. The samples were incubated for 24h and after that 10 µl of *3-(4,5-dimethylthiazol-2-*yl)*-2,5-diphenyltetrazolium bromide* (5 mg/ml MTT solution, Sigma, Munich, Germany) was added per pool. After additional incubation, 100 µl of 10% sodium dodecyl sulfate (SDS) in 0.01 M HCl (Serva, Heidelberg, Germany) was added. An optical density was quantitated spectrophotometrically on ELISA plate reader (DV990/BV6, Roma, Italy) at a wavelength of 570 nm. The relative viability of cells (%M) was calculated based on the formula:

$$\%M = \frac{OD\ tested\ material}{OD\ negative\ control} \times 100$$

Statistical analysis

The data were evaluated by one-way ANOVA test using the statistical software IBM SPSS (IBM SPSS 20, IBM Corporation, New Orchard Road Armonk, New York, SAD). A level of significance of 5% was chosen to denote the difference between means.

Results

The particles of investigated materials ALBO-$MPCA_1$, ALBO-$MPCA_2$ and MTA^+ formed the agglomerates, spherical and rod-like (Figures 1, 2 & 3). Nano-particles were polygonal, elongated in one direction, with predominant calcium silicate phases.

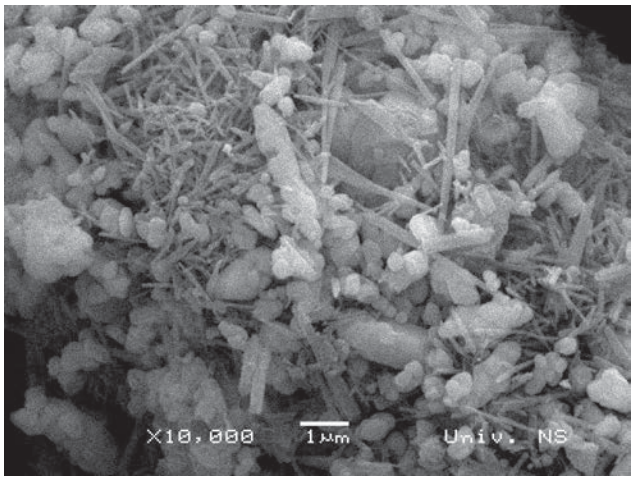

Figure 1. SEM micrograph of hydrated sample of ALBO-$MPCA_1$, maginication 10000×

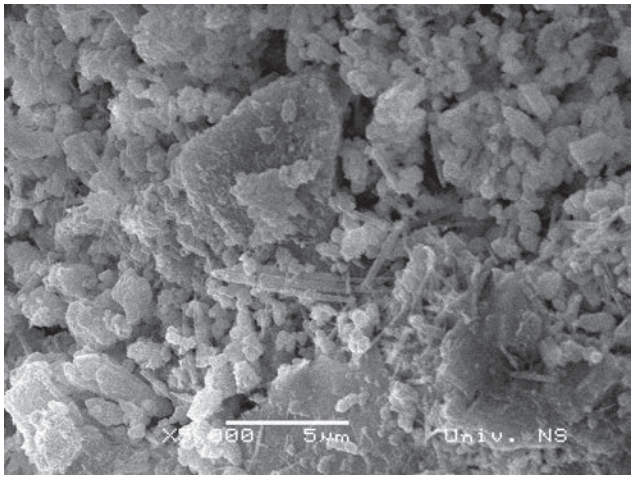

Figure 2. SEM micrograph of hydrated sample of ALBO-$MPCA_2$, maginication 5000×

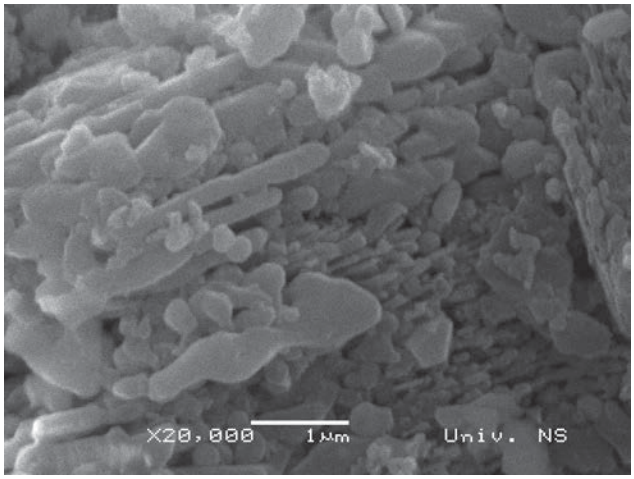

Figure 3. SEM micrograph of hydrated sample of MTA$^+$, maginication 20000×

The relative viablility of cells increased following the exposure time (Figure 4). Statistically significant differences between 3h and 7-day exposure time were observed respecting all investigated materials ($p<0.05$). Statistically significant differences between ALBO-$MPCA_2$ and MTA^+, and ALBO-$MPCA_2$ and ALBO-$MPCA_2$ after 3h were noted ($p<0.05$).

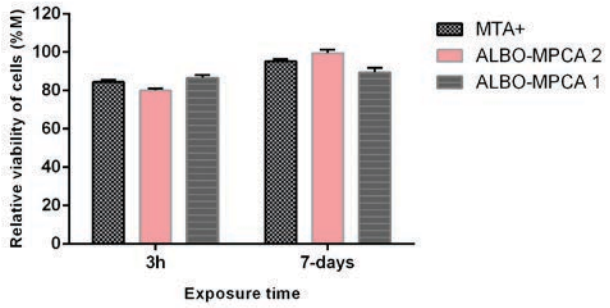

Figure 4. Relative viability of cells (%) following the exposure to fresh investigated materials

Discussion

Prior to the clinical use of new material, it is of utmost importance to conduct investigations on its *in vitro* and *in vivo* biocompatibility. It has been shown that nanostructured surface can affect the material's biocompatibility, as weel as its biological activity after the placement into the vital tissues[11]. Furthermore, material's biocompatibility may be influenced by its chemical nature and the dynamics of ion release[12].

The results of our study indicate that investigated materials ALBO-$MPCA_1$, ALBO-$MPCA_2$ and MTA^+ are consisted of polygonal nano-particles that form the agglomerates, spherical and rod-like with predominant calcium silicate phases. Such nanostructured surface is desirable because it mimics more closely the hierarchical organization of the bone tissue and may enhance cell differentiation and proliferation[11].

Results obtained by the MTT test showed that metabolic activity of cells increased with the exposure time for all investigated materials which can be considered as a good indicator of materials' biocompatibility. Since the MTT test speaks about the mitochondrial activity of cells, we can not conclude with completely certanty whether the cell proliferation in cell culture occured, but we can discuss about the relative viability of cells. None of the samples showed relative viability of cell below 70%, which is the limit defined by the ISO standard[13]. The lowest relative viability of cells observed in the case of ALBO-$MPCA_2$ after 3h can be related to initially the highest aluminium ion release and the highest pH values[14]. Although the mentioned study investigated the completely set materials, it is reasonable to expect that the concentration of released aluminium ion would be even higher.

The relative viability of cells increased with time, indicating that Bi_2O_3 used as radiocontrast agent in the case of ALBO-$MPCA_1$ does not signifficantly affect material's biocompatibility, as previously stated[15,16,17]. Although there are some attepts to use different radiopacifier, like ytterbium trifluoride[18] and zirconium oxide[19] that may improve some other properties, we decided to use Bi_2O_3 because they provide lower contrast. The same radiocontrast agent was used in the case of MTA^+. It can be also concluded that $BaSO_4$, used as radiocontrast agent in the case of ALBO-$MPCA_2$ does not influence its bicompatibility due to the highest observed relative viability of cells after 7-days.

According to our knowlegde there is no study that investigated the cytotoxic effects of freshly prepared materilas based on calcium-silicates. Due to the fact that the primary indication of calcium silicate based materials is the therapy of teeth with incomplete root development, the *SCAP* cell culture was chosen for the experimental model in this study.

Earlier studies showed that materials based on calcium silicates are biocompatible, using different experimental models, cell cultures and materials' elutes[20,21,22]. A certain percentage of cytotoxicity is expected due to the very high pH values of calcium silicate based materials, resulting from the release of calcium hydroxide that occurs during the hydration process of these materials[2]. However, such high pH values may be decreased in *in vivo* conditions due to buffer capacity of dentine that drops the pH level to physiological, and creates the optimum conditions for material's biological effect[23].

Conclusions

The synthesized materials are adequately designed, possessing the appropriate surface morphology, which is of importance for their bioactivity. The biocompatibility of investigated materials is comparable to the control material MTA$^+$, and therefore these materials can be recommended for for further clinical studies.

Note: The results of this paper were awarded for the oral presentation at the 22nd BaSS Congress.

References

1. Wataha JC. Predicting clinical biological responses to dental materials. Dent Materi, 2012;28:23-40.
2. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Pitt Ford TR. The constitution of mineral trioxide aggregate. Dent Mater, 2005;21:297-303.
3. Dammaschke T, Gerth HUV, Zuchner H, Schafer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater, 2005;21:731-738.
4. Sarkar NK, Caidedo R, Tirwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod, 2005;31:97-100.
5. Fridland M, Rosado R. MTA solubility: a long term study. J Endod, 2005;31:376-379.
6. De Vasconcelos BC, Bernardes RA, Luna Cruz SM, Duarte MAH, De Magalhaes Padilha P, Bernardineli N, et al. Evaluation of pH and calcium ion release of new root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2009;108:135-139.
7. Belio-Reyes IA, Bucio L, Cruz-Chavez E. Phase composition of ProRoot mineral trioxide aggregate by X-ray powder diffraction. J Endod, 2009;35:875-878.
8. Asgary S, Parirokh M, Egbbal MJ, Brink F. Chemical differences between white and gray mineral trioxide aggregate. J Endod, 2005;31:101-103.
9. Camilleri, J. Hydration mechanisms of mineral trioxide aggregate. Int Endod J, 2007;40:462-470.
10. Jokanovic V, Colovic B, Mitric M, Markovic D, Cetenovic B. Synthesis and properties of a new endodontic material based on nanostructured highly active calcium-silicates and calcium carbonates. Int J Appl Ceram Techol, 2014;11:57-64.
11. Xu JL, Khor KA, Sui JJ, Zhang JH, Chen WN. Protein expression profiles in osteoblasts in response to differentially shaped hydroxyapatite nanoparticles. Biomater, 2009;30:5385-5391.
12. Maeno S, Niki Y, Matsumoto H, Morioka H, Yatabe T, Funayama A et al. The effect of calcium ion concentration on osteoblast viability, proliferation and differentiation in monolayer and 3D culture. Biomater, 2005;26:4847-4855.
13. International Standards Organization. Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. ISO 10993-5, 3st ed. 2009-06-01.
14. Cetenovic B, Prokic B, Vasilijic S, Dojcinovic B, Magic M, Jokanovic V et al. Biocompatibility investigation of new endodontic materials based on nanosynthesized calcium silicates combined with different radiopacifiers. J Endod, 2017;43:425-432.
15. Chen C, Hsieh SC, Teng NC, Kao CK, Lee SY, Lin CK et al. Radiopacity and cytotoxicity of Portland cement containing zirconia doped bismuth oxide radiopacifiers. J Endod, 2014;40:251-254.
16. Kim EC, Lee BC, Chang HS, Lee W, Hong CU, Min KS. Evaluation of the radiopacity and cytotoxicity of Portland cements containing bismuth oxide. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2008;105:e54-e57.
17. Gomes Cornélio AL, Salles LP, da Paz MC, Cirelli JA, Guerreiro-Tanomaru JM, Filho MT. Cytotoxicity of Portland cement with different radiopacifying agents: A cell death study. J Endod, 2011;37:203-210.
18. Antonijevic D, Jeschke A, Colovic B, Milovanovic P, Jevremovic D, Kisic D, et al. Addition of a Fluoride-containing Radiopacifier Improves Micromechanical and Biological Characteristics of Modified Calcium Silicate Cements. J Endod, 2015;41:2050-2057.
19. Bosso-Martelo R, Guerreiro-Tanomaru JM, Viapiana R, Berbert FL, Duarte MA, Tanomaru-Filho M. Physicochemical properties of calcium silicate cements associated with microparticulate and nanoparticulate radiopacifiers. Clin Oral Investig, 2016;20:83-90.
20. Margunato S, Taşli PN, Aydin S, Karapınar Kazandağ M, Şahin F. In Vitro Evaluation of ProRoot MTA, Biodentine, and MM-MTA on Human Alveolar Bone Marrow Stem Cells in Terms of Biocompatibility and Mineralization. J Endod, 2015;41:1646-1652.
21. Petrović V, Opačić-Galić V, Živković S, Nikolić B, Danilović V, Miletić V et al. Biocompatibility of new nanostructural materials based on active silicate systems and hydroxyapatite: in vitro and in vivo study. Int Endod J, 2015;48:966-975.
22. Modareszadeh MR, Chogle SA, Mickel AK, Jin G, Kowsar H, Salamat N, Shaikh S, Qutbudin S. Cytotoxicity of set polymer nanocomposite resin root-end filling materials. Int Endod J, 2011;44:154-161.
23. Wang JD, Hume WR. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. Int Endod J, 1988;21:17-26.

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