COMPOSITES BASED ON MAGNESIUM-SUBSTITUTED HYDROXYAPATITE AND POLYSACCHARIDES

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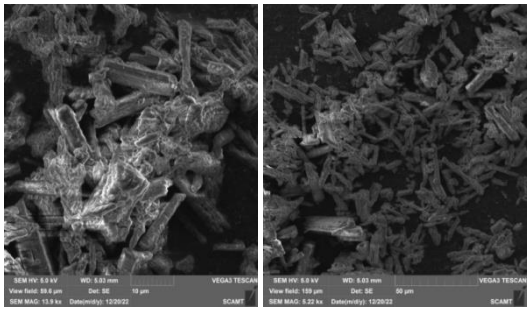
Abstract

Magnesium-substituted hydroxyapatites (Mg-HA) were synthesized in the presence of a chitosan or chitin polymer matrix by the aqueous solution method. The compounds obtained were studied for phase, spectroscopic and thermal properties. It was found that all the samples have identical morphologies. The results of cytotoxicity tests using the MMT assay revealed low effects of the samples on the cultured cells. The cell viability improved with a longer period of incubation. The composites that dissolve in physiological solutions with a medium rate have a stable positive effect on cells. The composites obtained are promising as non-toxic materials for the acceleration of implant bioresorption, which takes place with the participation of osteoclasts *in vivo*.



Key words: magnesium-substituted hydroxyapatite, chitosan, chitin, composites, bone tissue, cytotoxicity, MMT test.

Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a versatile hard biomaterial with a stoichiometric composition close to natural bone. Natural apatite has better physical and biological properties than synthetic hydroxyapatite due to a significant content of ion-substituted impurities [1]. The application of a polymer matrix makes it possible to change the characteristics of an inorganic filler and thereby create the compositions with specified osteoinductive, osteoconductive and resorption properties [2]. Both anion and cation of HA can be replaced by ions of other metals. Metal substitution alters the physical (*i.e.*, crystallinity, lattice parameters, and stability) as well as biological properties of HA [3]. Owing to the progress in tissue and cell engineering, the production of biopolymers suitable for creating a matrix on which cells can form tissue structures for subsequent implantation in places of damaged organs is rapidly developing. Matrices are used for effective treatment of cells as a substrate on which they can be fixed [4, 5]. Chitosan, collagen, and chitin, which contain biologically active calcium compounds are used as porous composite matrices.

The goal of this work was to synthesize HA composites and determine the effect of organic biopolymers on the composition and morphology of doped hydroxyapatite.

Results and discussion

Hydroxyapatite composites Mg-HA-chitosan and Mg-HA-chitin were obtained by the precipitation from an aqueous solution at room temperature in the presence of chitosan or chitin [5].

Magnesium-containing powders were obtained using the HA–polymer composite method. It was previously found that the process of hydroxyapatite crystallization in chitosan matrices slows down at a molar ratio of calcium to magnesium ions in the solution of 1:9 [5].

The study of the microstructure and surface features of the resulting composites was carried out using a JSM-6390/6390LV scanning electron microscope (JEOL, Japan). The cytotoxic activity was evaluated by the conventional MMT assay. The powder composites weighing 1 g were preliminarily sterilized with ozone for 90 min. After sterilization, HA powder was filled with 5 mL of the complete nutrient medium (DMEM/F12 (Gibco) containing 10% (v/v) of heat-inactivated fetal bovine serum (FBS) (HyClone, USA), 1% L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin). Mg-HA-chitosan/Mg-HA-chitin powders with the complete nutrient medium were stored in an incubator at 37 °C in CO_2 atmosphere for 4 to 6 days. The morphological examination of cell cultures was performed using an inverted microscope (Nicon, Germany).

The MTT test is based on the reduction of MTT-reactive cellular enzymes—oxyreductases. As a result of regeneration, water-insoluble formazan is formed, the amount of which correlates with the number of viable metabolically active cells [6–8]. The incubation period for the contact of the nutrient medium of cells with powders was 4 to 6 days. The culture status was examined after 1 and 3 days of culturing fetal mesenchymal stromal cells (FetMSCs) in the nutrient medium after 4 days of incubation with the composites (Figs. 1, 2). The micrograph of the control sample after 1 day of cultivation shows that the culture is in good condition, the cells are distributed practically on the entire surface, the shape of the

cells is mainly spindle-shaped, with distinct contours and pronounced processes. The micrograph taken in 3 days of cultivation is similar but the number of cells on the surface of the cup is approximately 2 times higher. For the experimental wells containing the medium, cells, and composites, the micrographs are identical to the control samples: the cells are in the medium, there is no zone of alteration, the growth is generally uniform. The largest number of cells was noted on the micrographs of composite **1**. Small particles of HA were noted in the nutrient medium in all samples.

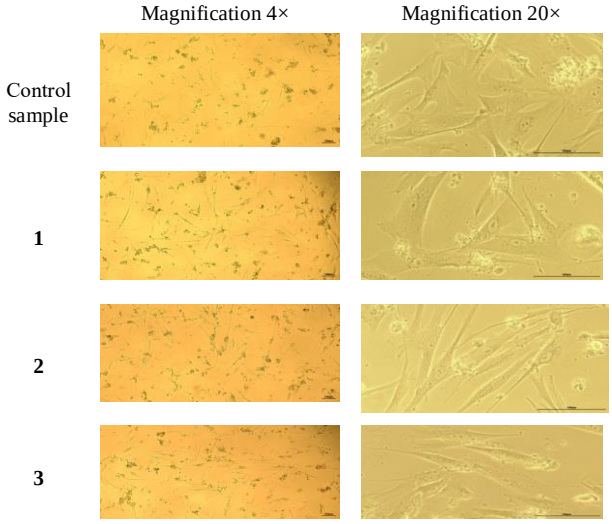


Figure 1. Micrographs of FetMSCs after 1 day of cultivation in the nutrient medium followed by the incubation with HA particles for 4 days.

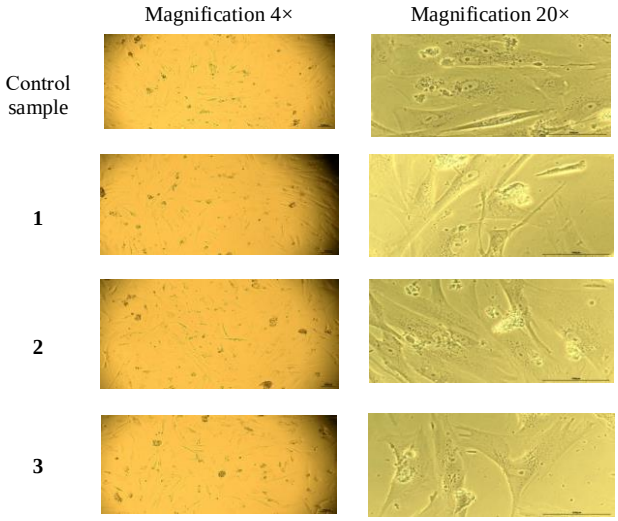
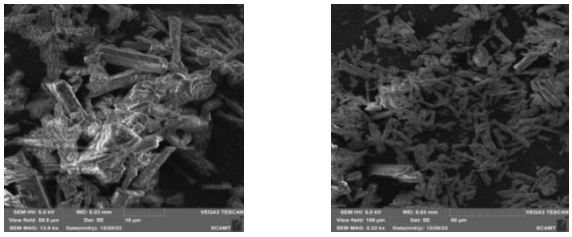


Figure 2. Micrographs of FetMSCs after 3 days of cultivation in the nutrient medium followed by the incubation with HA particles for 4 days.

It can be seen that, during the incubation of the particles of Mg-HA-chitosan/Mg-HA-chitin composites in the nutrient medium, they practically do not exert cytotoxic effects on the cultured cells. The metabolic activities of the cells in wells with the powders compared to that of the control sample were 73–85%. Sample **1** demonstrated the lowest toxic effect on cells when incubated for 4 days. Well-absorbed composite **1** showed a stable positive effect on the cells at different incubation times. Thus, the experiments on cell cultures revealed insignificant cytotoxicity and positive cell adhesion of the Mg-HA-chitin/chitosan composites.

Using optical microscopy, it was revealed that the Mg-HA-chitosan and Mg-HA-chitin aggregates have a lamellar elongated shape, characteristic of magnesium-containing hydroxyapatite crystals (Fig. 3).



a ***b***

Figure 3. Micrographs of the Mg-HA composites with chitosan (***a***) and chitin (***b***).

Conclusions

Hence, the magnesium-substituted hydroxyapatite composites in the polysaccharide matrices were synthesized. It was shown that the composites have a negligible cytotoxic effect on cells. The viability of cell cultures during incubation with the composites was 73–85%. The resulting composites can be used as materials which stimulate the regeneration of bone tissue in orthopedics and stomatology and as nanocarriers of drugs.

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References

1. N. Eliaz, N. Metoki, *Materials*, **2017**, *10*, 334. DOI: 10.3390/ma10040334
2. W. Yanhua, H. Hao, Y. Li, S. Zhang, *Colloids Surf., B*, **2016**, *140*, 297–306. DOI: 10.1016/j.colsurfb.2015.12.056
3. M. Hidouri, S. V. Dorozhkin, N. Albeladi, *J. Inorg. Organomet. Polym. Mater.*, **2019**, *29*, 87–100. DOI: 10.1007/s10904-018-0969-6
4. I. D. Reshetnikova, G. Sh. Isaeva, T. A. Savitskaya, L. T. Bayazitova, Yu. A. Tyurin, E. V. Khaldeeva, E. V. Agafonova, S. N. Kulikov, *Kazan Med. J.*, **2020**, *101*, 944–954. DOI: 10.17816/KMJ2020-944
5. A. I. Nikitina, O. A. Golovanova, *Russ. J. Inorg. Chem.*, **2022**, *67*, 131–138. DOI: 10.1134/S0036023622020115
6. D. G. Boeckel, R. S. A. Shinkai, M. L. Grossi, E. R. Teixeira, *Oral Surg., Oral Med., Oral Pathol. Oral Radiol.*, **2014**, *117*, e423–e428. DOI: 10.1016/j.oooo.2012.07.486
7. A. Yu. Prilepskiy, A. S. Drozdov, V. A. Bogatyrev, S. A. Staroverov, *ITMO Univ.*, **2019**.
8. S. P. Mohan, A. Palaniappan, M. K. K. Nawaz, R. Kripamol, R. Seenuvasan, P. R. A. Kumar, *J. Pharm. BioAllied Sci.*, **2023**, *15*, S677–S682. DOI: 10.4103/jpbs.jpbs_63_23