MICROSTRUCTURE OF CHITOSAN-BASED, ZnO-DOPED ANTIBACTERIAL COMPOSITE

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Introduction

Biomaterials for regenerative medicine, are subjected to continuous improvement, by i.e. amending their integration with body tissues or giving them bactericidal properties [1,2]. The porosity and sizes of pores enables appropriate migration of cells and ensures easy delivery of nutrients within the implant, appear to be of significant importance [3]. Literature reports suggest that natural and synthetic, biostable and biodegradable polymers as well as calcium phosphates (e.g. hydroxyapatite, TCP) or Hench's bioglass [4], which have no antibacterial properties, are currently used in tissue engineering. Chitosan is also widely used in medicine. Its main characteristics include biocompatibility, antimicrobial and hemostatic properties, and biodegradability [5]. Due to the osteoconductive properties it is suitable for hard tissue engineering [6]. Silver is most commonly used as an antibacterial agent, but other metal ions (i.e. Zn^{2+}) are also characterized by bactericidal properties [7].
The purpose of the study was to determine the effect of glass composition, amount of polymer used and preparation method on microstructure of chitosan-based antibacterial composites, suitable for use in regenerative medicine.

Materials and Methods

Porous biocomposites based on chitosan solution and 1wt% or 2wt% ZnO doped bioglass from CaO-SiO_2-P_2O_5 system were fabricated using lyophilization method. Composite microstructure was controlled by the appropriately selected amount of bioglass in relation to the polymer, its appropriate grain size and by the amount of the solvent present in lyophilizated dispersions.
The morphologies of the resulting composites were determined by scanning microscope observations. Their bioactivity was assessed by comparing SEM-EDS analysis of chemical compositions before and after contact with SBF solution. By measuring the specific surface area using BET method, the effect of the dispersion composition on development of the surface of composites was determined. The evaluation of cytotoxicity was performed according to PN-EN 10993-5 "Biological evaluation of medical devices - Part 5: Tests for cytotoxicity: *in vitro* methods" after contact with fibroblast like cells L929. Studies of antimicrobial activity were performed by a dilution method, using precultures of test bacterial strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Results and Discussion

All obtained composites showed random distribution of bioglass grains in polymer matrix with no visible agglomerates. The cross-section SEM image of the P5Zn1gCH_1:1 composite (FIG. 1) shows the structure of internally connected open pores of sizes in range of 40–300 μm. The increase of bioglass amount in relation to polymer results in a more distorted microstructure of the composite. Also, the presence of bioglass of larger grains disrupts composite microstructure and causes deformation of the pores. The change of solvent amount affects the stability of the dispersions in lyophilization, the shape and the size of composite pores, as well as its rigidity. Specific surface area that determines the kinetics of ion release, which indirectly affects bioactivity, for P5Zn1gCH_1:1 composite was 127.68 ±0.02 m^2/g. Changes in intensity of Si, Ca and P signals on EDS spectra indicate that apatite layer formation starts after composites incubation in SBF solution.

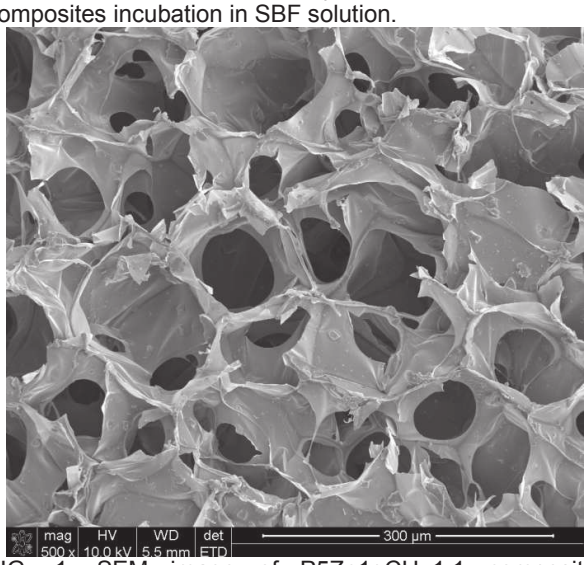

FIG. 1. SEM image of P5Zn1gCH_1:1 composite microstructure.

Morphological images of cell cultivations in the indirect method, after a contact of L929 with eluates of examined composites, were correct, and proliferation indicated after 48 h was tending to increase. Results of antimicrobial activity indicate that all of obtained materials caused the reduction of the number of bacteria. The best results were obtained for P5Zn2CH_1:1 composite doped with 2% of ZnO.

Conclusions

Chitosan-based, ZnO-doped antibacterial biocomposites have an optimal mean pore size for bone tissue ingrowth as well as exhibit antibacterial activity against the strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The composites microstructure could be controlled by changing of the preparation parameters.

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References

[1] L. Ciołek, J. Karaś *at al.*, Engineering of Biomaterials, vol. XI, nr 77-80, (2008) 25-27.
[2] H.Zhu, Ch Hu., *at al.*, Mater. Sci. Eng., C, 42 (2014) 22-30.
[3] BS Kim, IK Park *et al*., Prog. Polym. Sci. 36(2011) 238-268.
[4] AM El-Kady, EA Saad *et al*., Ceram. Int.,36(2010) 995-1009.
[5] A. Di Martino, M. Sittinger *et al*., Biomaterials, 26(2005) 5983-5990.
[6] PR. Sivashankari, M. Prabaharan *et al.,* Int. J. Biol. Macromol., B. 93(2016) 1382-1389.
[7] T.N. Kim, Q.L. Feng, *at al.*, J. Mat. Sci – Mater. Med., 9 (1998) 129-134.