Innovative phosphate-based bioresorbable and antibacterial bioglasses

E. Restivo[1, 2], D. Pugliese[3], D. Gallichi-Nottiani[3], G. Bruni[4], L. Cucca[5], D. Janner[3], D. Milanese[6], **L. Visai**[1, 2]
[1] University of Pavia, Biochemistry Unit, Department of Molecular Medicine; Center for Health Technologies; UdR INSTM, Pavia, Italy
[2] ICS Maugeri, IRCCS, Clinical-Specialized Medicine, UOR5 Laboratory of Nanotechnology, Pavia, Italy
[3] Politecnico di Torino, Department of Applied Science and Technology; UdR INSTM, Torino, Italy
[4] University of Pavia, Department of Chemistry, Physical Chemistry Section; Center for Colloid and Surfaces Science, Pavia, Italy
[5] University of Pavia, Department of Chemistry, Pavia, Italy
[6] University of Parma, Department of Engineering and Architecture; UdR INSTM, Parma, Italy

Introduction

Infectious diseases are one of the most important causes of death in the World. The constant use of antibiotics has led to the development of new very resistant bacterial strains, which are a threat for public health. For this reason, noticeable efforts from different scientific fields have been devoted to achieve solutions that might contribute to attenuate this problem. In this context, research on new antibacterial materials has become a current and important goal in materials science. Several metals are well-known in literature for their antibacterial properties, both because of direct contact with the surface and the release of ions into the solution (indirect contact). Among these metals, the most extensively used in different applications are silver and copper. They show a broad spectrum of action against bacteria both upon exposure to surfaces and ions in solution. The advantage of copper resides in its lower toxicity to mammalian cells than silver. Indeed, Cu^{2+} ions in small quantity facilitate the activity of different enzymes and help cross-linking of collagen and elastin of bones, whereas in high concentration it can inhibit osteoblast proliferation. Therefore, it is crucial to control copper concentration for biomedical applications.

The aim of this work is to evaluate the antibacterial effects and the biocompatibility of un-doped (CPG) and Cu^{2+}-doped calcium-phosphate glasses (CPG_Cu) which have shown in previous studies interesting degradative, mechanical and optical properties to be used in biophotonic and medical applications.

Experimental Methods

CPG and CPG_Cu were analyzed with inductively coupled plasma – optical emission spectroscopy (ICP-OES) to evaluate their content of Ca, P, Na, Mg, B, Si and Cu. The antimicrobial effect of CPGs against four of the most common causing-infections bacteria, namely *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* PAO1 and *Klebsiella pneumoniae* were investigated; the biocompatibility was performed using NIH-3T3 cells. These viability assays in both bacteria (after 24h) and eukaryotic cells (after 24 and 48h) were evaluated through the quantitative 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test, which was performed in both direct and indirect contact and measures dehydrogenase activity as an indicator of the metabolic state. Moreover, we investigated the viability of adherent cells with MTT and confirmed it with scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

Results and Discussion

The obtained results demonstrated the promising antibacterial properties of Cu^{2+}-doped CPG with respect to un-doped CPG, in particular against Gram-negative bacteria. The particular efficacy against *E. coli*, *P. aeruginosa* and *K. pneumoniae* is due to the different composition of bacterial surface. The preliminary quantitative and qualitative (SEM and CLSM) studies performed using 3T3 cells showed that CPG and CPG_Cu were not toxic.

Conclusion

The results suggested that the tested bioresorbable bioglasses showed biocompatible and antibacterial properties useful for medical applications.

Acknowledgement

D.P. and D.J. acknowledge the Interdepartmental Center “PhotoNext” of Politecnico di Torino for the partial support of this research effort. The study was supported by a grant of the Italian Ministry of Education, University and Research (MIUR) to the Department of Molecular Medicine of the University of Pavia under the initiative “Dipartimenti di Eccellenza (2018–2022)”. L.V. and E.R. acknowledge the following members of University of Pavia: Prof. Dr. Roberta Migliavacca for providing bacterial strains; Dr. Patrizia Vaghi for CLSM analyses; Stefano Iervese and Dr. Federico Bertoglio for performing some experiments.

References

[1] Ceci-Ginistrelli, E, Pugliese, D, Boetti, NG, Novajra, G, Ambrosone, A, Lousteau, J, Vitale-Brovarone, C, Abrate, S, Milanese, D 2016, 'Novel biocompatible and resorbable UV-transparent phosphate glass based optical fiber', *Optical Materials Express, 6, 2040-2051*. Doi: 10.1364/OME.6.002040

[2] Ghosh, R, Swart, O, Westgate, S, Miller, BL, Yates, MZ 2019, 'Antibacterial Copper-Hydroxyapatite Composite Coatings via Electrochemical Synthesis', *Langmuir, 35, 5957-5966.* Doi: 10.1021/acs.langmuir.9b00919

[3] Luo, J, Hein, C, Ghanbaja, J, Pierson, JF, Mücklich, F 2019, 'Bacteria accumulate copper ions and inhibit oxide formation on copper surface during antibacterial efficiency test', *Micron, 127, 102759*. Doi: 10.1016/j.micron.2019.102759

[4] Pinto, RJB, Daina, S, Sadocco, P, Neto, CP, Trindade, T 2013, 'Antibacterial Activity of Nanocomposites of Copper and Cellulose', *BioMed Research International*, 2013*, 280512*. Doi: 10.1155/2013/280512

[5] Bari, A, Bloise, N, Fiorilli, S, Novajra, G, Vallet-Regí, M, Bruni, G, Torres-Pardo, A, González-Calbet, JM, Visai, L, Vitale-Brovarone, C 2017, 'Copper-containing mesoporous bioactive glass nanoparticles as multifunctional agent for bone regeneration', *Acta Biomaterialia, 55, 493-504*. Doi: 10.1016/j.actbio.2017.04.012

Keywords: phosphate-glass, copper, antibacterial