Study of the biological activity of lanthanide and transition metal ions as well as nanosized hydroxyapatite co-doped with those ions against *Pseudomonas aeruginosa*

Summary

Pseudomonas aeruginosa is a human opportunistic pathogen characterized by the presence of many virulence factors, high antibiotic resistance and the ability to cause infections at implant sites. For this reason, there is a need to search for implantation materials with an additional antibacterial effect. A good candidate is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), a non-toxic, highly biocompatible material with osteoregenerative potential, which naturally occurs in the human body as a component of bones and teeth. It is possible to modify it by partial substitution of calcium ions (Ca^{2+}), which allows obtaining compounds with specific physicochemical and biological properties. Calcium substitution with transition metal ions (silver Ag^{+}, zinc Zn^{2+} or copper Cu^{2+}) allows to obtain materials with antimicrobial activity. They can also be modified by the addition of lanthanide ions (Ln^{3+}) due to their luminescence ability which can be used in bioimaging. While the effect of transition metal ions on bacterial cells is well described in the literature, little is known about the effect of lanthanide ions.

One of the main objectives of the study was to assess whether subinhibitory concentrations of lanthanide ions (Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}) affect the virulence of *P. aeruginosa* (biofilm, production of pyocyanin, pyoverdine, elastase, swimming, swarming and twitching motility). The influence of selected ions on the surface properties of the cell (surface charge, hydrophobicity, membrane permeability) was also examined and their cytotoxicity was determined on the model of erythrocytes. Additionally, the influence of the presence of these ions on the antibacterial effect of ciprofloxacin and gentamicin as well as copper ions (Cu^{2+}) was evaluated. The studies showed that the presence of lanthanide ions in subinhibitory concentrations significantly increased the production of bacterial biofilm, slightly changed the surface properties of cells and caused a change in the permeability of cell membranes. In addition, it was shown that the concentrations used (0.5MIC) were cytotoxic and caused visible changes in the morphology of erythrocytes and their agglomeration, without leakage of hemoglobin.

In the second part of the work, the antibacterial activity of hydroxyapatites doped and co-doped with transition metal ions and Eu^{3+} ions was examined. The influence of the presence of

different forms (powdered material, colloid, pressed material (tablets), materials introduced into the hydrogel matrix and their eluates) on the survival, growth and formation of biofilms by *P. aeruginosa* was checked. It was also determined how the initial release of materials into the medium affects their antibacterial activity and, for selected compounds, the cytotoxicity was determined on the model of erythrocytes and a mouse osteoblast cell line. Studies have shown high antibacterial activity of nanomaterials doped with Ag^{+} ions, especially in the form of tablets. In the case of Zn^{2+} ions as dopants, a decrease in the number of bacteria after incubation in the presence of colloids made of materials modified by substitution of phosphate groups with silicate groups was demonstrated. Antibacterial activity was also demonstrated for materials doped and co-doped with Cu^{2+} ions. In the case of nanomaterials doped and co-doped with Cu^{2+} and Eu^{3+} ions, significant differences were found in the actual amount of incorporated ions, compared to the assumed amount, and differences in the release of ions to the substrate, which probably results from their altered antibacterial activity and cytotoxicity. Pre-release of ions into the culture medium has been shown to improve their activity, which may explain the better performance of eluates compared to powdered materials. The tested nanomaterials, especially those doped with Cu^{2+} ions, showed toxicity towards erythrocytes and osteoblasts. However, there was no significant effect of Eu^{3+} ions on the antibacterial activity of Cu^{2+} ions.

The obtained results allow to conclude that although the presence of lanthanide ions significantly affects the virulence and surface properties of *P. aeruginosa* cells, they can be used as dopants in nanomaterials with potential application in implantology. This is especially supported by the fact that their concentrations in hydroxyapatites are much lower than the concentrations of 0.5MIC, for which the effects presented in this thesis were observed.