Summary

The aim of the study was to check the potential of using silver nanoparticles and bacteriophages to combat bacteria isolated from patients with chronic rhinosinusitis (CRS) and the biofilm formed by them. In total, 873 bacterial isolates were cultured and identified from all samples. From swabs and mucosa samples of CRS patients, 767 bacterial isolates were cultured and identified. The ability of selected clinical isolates of bacteria to form a biofilm was assessed using the crystal violet (CV) method. 80.3% of isolates grown from CRS patients formed a strong or moderate biofilm. In addition, the results of the experiments showed that the isolates can differ significantly in terms of biofilm growth dynamics. The biocidal properties of 15 types of silver nanoparticles (AgNPs), prepared with the use of biologically active compounds, were tested on the reference bacterial strains *S. aureus* ATCC29213 and *E. coli* ATCC25922. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined. The lowest MIC and MBC values against reference species were observed for nanoparticles stabilized with tannic acid (TA-AgNP) and lysine. TA-AgNPs were selected for further research due to the documented antioxidant and bactericidal properties of tannic acid. MIC and MBC for clinical isolates were determined. MIC were recorded in the concentration range of TA-AgNP from 5 mg/L to 40 mg/L and MBC from 15 mg/L to 95 mg/L depending on the isolate. Bacteriophage typing was carried out with phages from the BIOPHAGE S.A. collection and MSA-6 phage. *S. aureus* isolates were selected for biofilm experiments because the biofilm formed by them seems to be of the greatest importance and adversely affecting the course of CRS. The cytotoxicity of TA-AgNP on the human nasal epithelial cell line HNEpC (PromoCell) was assessed in cooperation with the Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University. It was shown that high concentrations of nanoparticles and extended incubation time were required for cytotoxic effects. After 24 h of incubation, cell viability remained above 80%, up to a concentration of 75 mg/L TA-AgNP, and no morphological changes associated with cell death were observed. A methodology for examining the effects of the influence of the tested factors on the model biofilm during its maturation (after 3 h and 24 h) was developed. A CV test was performed to measure biofilm mass and an experiment was performed on the relative metabolic activity of bacteria in the biofilm, determined by the reduction of formazan crystals. The obtained results were analyzed in terms of the effectiveness of TA-AgNP, bacteriophages and both factors. Significant differences in the mass of biofilm created by individual isolates after treatment with bacteriophage for both 3h and 24h in relation to the

biofilm created by isolates without the presence of bacteriophage were observed. Taking into account the results obtained from the measurement of the biofilm mass and the metabolic activity of bacteria in the biofilm, it can be concluded that the bacteriophage inactivated the bacteria by the lytic route in almost 100%, but it did not cause complete degradation of the biofilm matrix. There was a statistically significant reduction in biofilm weight treated with TA-AgNP compared to the control biofilm (0mg/L) for each of the concentrations tested (25mg/L, 50mg/L, 100mg/L and 150mg/L). At a concentration of 50 mg/L, the effect of eradication of 50% of the biofilm mass was obtained after 3 hours for MSSA and a decrease in metabolic activity to 60%. For biofilm control, a concentration of 50 mg/L was used in combination with bacteriophage. Complete eradication of biofilm from CV was not achieved (absorbance measurements at 570 nm were at a median level of 0.4 - 0.5, with measurements for a blank control sample with a sterile TSB at 0.05), but the metabolic activity of bacterial cells in the biofilm after 24 h of action, it decreased to about 10% when acting on isolates sensitive to phage and TA-AgNP.