Abstract

The aim of the doctoral dissertation was to investigate the potential use of enzymes immobilized on bacterial cellulose (BC) membranes as agents for degrading polysaccharide components of *Pseudomonas aeruginosa* biofilm matrix and supporting the process of antibiotic therapy.

In recent years, there has been significant progress in the utilization of both natural and synthetic polymers as biomedical materials. Among natural polymers, BC has gained particular interest due to its efficient synthesis by various strains of *Komagataeibacter* bacteria. This biopolymer exhibits unique material properties, such as high crystallinity and mechanical strength, which make BC highly suitable for biomedical applications as an excellent wound dressing material or as a carrier for various active substances. However, despite these advantages, the utilization of BC is still limited by its costly production process and the lack of bactericidal and anti-biofilm properties.

During the research conducted in this doctoral thesis, the potential of silicone polyether as an additive to the Hestrin-Schramm (HS) growth medium was examined to enhance the efficiency of BC production by the *Komagataeibacter xylinus* strain. It was shown that the modification of the physicochemical properties of the cultivation medium, achieved by significantly reducing its surface tension, had a beneficial impact on the yield of BC synthesis process. Furthermore, it was demonstrated that the biopolymer obtained using the modified growth medium exhibited improved water parameters and demonstrated non-cytotoxicity towards eukaryotic cells, confirming its suitability for biomedical applications.

This study also demonstrated that the immobilization of glycoside hydrolase $PelA_h$ or alginate lyase, enzymes capable of degrading key polysaccharide components of the biofilm matrix, on the surface of BC membranes can effectively destabilize the process of biofilm formation by *P. aeruginosa*. Furthermore, it was shown that immobilizing alginate lyase on BC significantly increases the sensitivity of *P. aeruginosa* cells to gentamicin, allowing for a reduction in the therapeutic dose and enhancing the safety of the applied antibiotic therapy.

Modifications of the BC structure, altering its physicochemical properties, can expand its application as a dry carrier for delivering active substances. Therefore, in current research the surface modification of BC using low-pressure argon plasma with argon as the working gas (LPArP) was conducted. It was observed that through surface modification with LPArP and understanding the structural properties of enzymes, the release process from the carrier surface can be controlled. Furthermore, it was also demonstrated that the anti-biofilm activity of $PelA_h$ and $PslG_h$ enzymes was not significantly affected by their immobilization on the LPArP-modified BC.

In conclusion, the conducted research has demonstrated that the unique structure of bacterial cellulose enables its use as a carrier for immobilizing specific enzymes, such as $PelA_h$, $PslG_h$, and alginate lyase, conferring it with anti-biofilm properties. This innovative approach can potentially aid in combating wound infections caused by *P. aeruginosa* by inhibiting the biofilm formation process and enhancing the susceptibility of these pathogenic microorganisms to antibiotics.

31.07.2023

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