

Dhvar5-Chitosan Nanogels Generated by Microfluidic Fighting Orthopedic Device-Related Infections

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Orthopedic Device-Related Infections (ODRIs) are a major medical challenge, due to the involvement of biofilm-encased and multidrug-resistant bacteria, and due to the inefficiency of current therapies based on antibiotic administration [1-2]. Therefore, there is the need for antibiotic-free alternatives [3]. Antimicrobial peptides (AMPs) are a promising solution due to their broad-spectrum of activity, high efficacy at very low concentrations, and low propensity to induce resistance [4]. We aim to develop a new AMP-based chitosan nanogel coating to prevent ODRIs. Chitosan was functionalized with norbornenes (NorChit) through the reaction with carbic anhydride [5] and then, a cysteine-modified AMP Dhvar5 was covalently conjugated to NorChit (NorChit-Dhvar5), through a thiol-norbornene photoclick chemistry, under UV-photoactivation [5]. Characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance spectroscopy (NMR) analyses, and a successful functionalization of chitosan with norbornenes and posterior Dhvar5 immobilization was proved. For NorChit-Dhvar5 nanogels production, the NorChit-Dhvar5 solution (0.15% w/v) and Milli-Q water were injected separately into a microfluidic system. The nanogels were characterized regarding size, concentration, shape, and charge, using Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and Dynamic light scattering (DLS). The nanogels antibacterial properties were assessed in Phosphate Buffer (PBS) for 6 h, against four relevant microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *S. aureus* methicillin-resistant (MRSA)), and in Muller-Hinton Broth (MHB), 50% (v/v) in PBS, supplemented with human plasma (1% (v/v)), for 6 and 24 h against MRSA. The obtained NorChit-Dhvar5 nanogels, presented a round-shaped, ~100 nm and positive charge. NorChit-Dhvar5 nanogels in a concentration of 10¹⁰ nanogels/mL in PBS were capable of reducing the initial inoculum of *E. coli* by 90%, *P. aeruginosa* by 99%, *S. aureus* by 99%, and *S. aureus* MRSA by 90%. These results were corroborated by a 99% *S. aureus* MRSA reduction, after 24 h in medium. Furthermore, NorChit-Dhvar5 nanogels do not demonstrate signs of cytotoxicity against osteoblastic MC3T3-E1 cells (a pre-osteoblast cell line) after 24 h, having high potential to prevent antibiotic-resistant infection in the context of ODRIs.

References

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