Nano-Encapsulated colistin sulphate to fight *Pseudomonas aeruginosa* infections

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Pseudomonas aeruginosa frequently infects the respiratory tract of cystic fibrosis (CF) patients. Multidrug resistant phenotypes and a high capacity to form stable biofilms are common[1]. Recent studies have described the emergence of colistin –resistant isolates in CF patients treated with long-term inhaled colistin[2]. Currently, new delivery strategies such as the use of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) containing antimicrobials could overcome pre-existing drug resistance mechanisms, including decreased uptake and increased efflux of drug as well as biofilm formation[3, 4]. Lipid nanoparticles loaded with aminoglycosides are significantly more effective than free formulations against *P.aeruginosa* clinical isolates[5]. The aim of this study was to explore the antimicrobial activity of nanoencapsulated colistin (in both SLN and NLC) versus free drug against *P.aeruginosa* clinical isolates from CF patients and to investigate the efficacy of these novel formulations in the eradication of biofilms. The effect of the storage temperature of nanoparticles over time and susceptibility of planktonic bacteria to antimicrobials were examined by using the broth microdilution method and time-kill kinetic curves. Minimal Biofilm Eradication Concentration (MBEC) and Biofilm Prevention Concentration (BPC) were determined to assess antimicrobial susceptibility of sessile bacteria. We used atomic force microscopy (AFM) to visualize treated and untreated biofilms and to determine surface roughness and other relevant parameters. Our results showed that NLC were more stable than SLN and that they can be employed in a wider range of storage temperatures without relevant modifications on their antimicrobial activity. Colistin nanoparticles presented the same *in vitro* antimicrobial activity as free drug against planktonic bacteria. Nevertheless, nanoencapsulated colistin was much more efficient in the eradication of biofilms than free colistin. Thus, these formulations have to be considered as an improved therapeutic option to treat *P. aeruginosa* infections.

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