Advanced Antibacterial Wound Dressing Produced with Natural-origin Materials

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Introduction: The failure of a wound to heal within the expected time frame may result in a chronic wound.[1] Additionally, the presence or easy access of pathogenic bacteria can hinder the healing process. Chitosan (Ch) nanofiber mesh (NFM) is a material with natural characteristics favoring its use as human wound dressing.[2] Liposomes, a nanoparticle release system made by phospholipids, hold tremendous promise as release systems. The present work proposes the immobilization of Gentamicin (Gent)-loaded liposome at the surface of electrospun Ch NFMs to promote its antibacterial activity.

Methods: Ch NFMs were produced by electrospinning. Gent-loaded liposome (Table 1) were produced by ethanol injection method: formulation F1 was used to study the encapsulation efficiency (EE), and formulation F2 was used to perform the release studies of Gent from the liposomes immobilized at the surface of electrospun Ch NFMs and for the antimicrobial assays.

Table 1- Liposome formulations

	DPPC	Chol	DSPE-PEG-Mal	PE-Rho
F1	2	1	-	-
F2	2	1	0.1	0.002

Particle size distribution and ζ-potential were determined by Zetasizer instruments. An indirect spectrophotometric method was used to quantify the released Gent, using o-phtaldialdehyde as derivatizing agent (Abs at 332nm). Ch NFMs were functionalized with thiol groups, and Gent-loaded liposomes were covalently immobilized by the reaction of the SH groups with maleimide. The disk diffusion and broth dilution assays were used to test the susceptibility of *S. aureus* (ATCC 25913), *E. coli* (STCC 434), and *P. aeruginosa* (ATCC 27853) against Gent-loaded liposomes immobilized at the surface of electrospun Ch NFMs. All the assays were adapted from the European Committee for Antimicrobial Susceptibility Testing recommendations.

Results: Gent was successfully encapsulated into the liposomes with an efficiency of 17%. Gent-loaded liposomes were uniformly distributed at the surface of the Ch NFMs and the drug release kinetic showed a sustained release of Gent during 16 h, achieving a steady state at 24 h (Figure 1). Ch NFM and Gent-free liposomes immobilized at the surface of Ch NFM (NFM_F2) presented no antibacterial activity against all the bacterial strains (Figure 2). Inversely, all the bacterial strains were susceptible to Gent-loaded liposomes immobilized at the surface of Ch NFM (NFM_F2-G). Specifically, it was observed a log reduction of 3.87 ± 0.33 for *S. aureus*, 4.87 ± 0.21 for *E. coli*, and 4.20 ± 0.24 for *P. aeruginosa*, representing more than 99.9% kill rate success for all the strains.

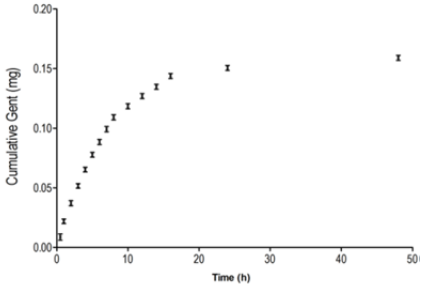

Figure 1 – *In vitro* cumulative Gent release (mean±SD) from liposomes immobilized at the surface of electrospun Ch NFM.

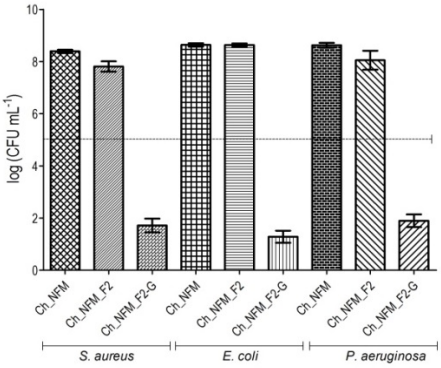

Figure 2 – *In vitro* antibacterial activity of electrospun Ch NFM, Gent free liposomes immobilized at the surface of electrospun Ch NFM (NFM_F2), Gent-loaded liposomes immobilized at the surface of electrospun Ch NFM (NFM_F2-G) against the bacterial strains *S. aureus*, *E. coli* and *P. aeruginosa*. The dot line represents the initial bacteria inoculum.

Conclusions: We conclude that Gent-loaded liposomes immobilized at the surface of Ch NFM inhibited the growth of common phatogenic bacteria strains. Therefore, these results show that the developed nanostructured delivery system could be successfully used in local applications in the eradication of these pathogens, which are a common cause of local and systemic infections.

References:

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