Antibacterial performance of bovine lactoferrin-fish gelatine electrospun nanocomposites

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The alarming increase of antibiotic resistant microorganisms urged the development and synthesis of novel antimicrobial biomaterials, to be employed in a broad range of applications, ranging from food casings to medical devices [1 – 3]. This work describes the processing and characterization of an innovative fully biobased eletrctrospun nanocomposite material displaying antibacterial properties. Its composition is exclusively comprised of proteins, with fish gelatine as the structural matrix and bovine lactoferrin as the antimicrobial agent.

Mainly obtained from the inedible components of the fishery processed catch, fish gelatine (FG) represents a viable alternative source for this highly demanded protein [4]. Electrospun fish gelatine possesses highly interesting properties, such as resilience, biocompatibility, and is stable in aqueous solutions after crosslinking through exposure to glutaraldehyde or genipin atmosphere [5, 6]. Bovine lactoferrin is a wide spectrum antimicrobial protein, exerting its action in numerous virus, bacteria and prokaryotic parasites. Moreover, lactoferrin bears immunoregulatory properties and anti-tumour activity. Specifically, the antibacterial activity of lactoferrin consists of several mechanisms, namely through deprivation of environmental iron, destabilization of Gram negative lipopolysaccharide outer membrane via calcium chelation, and surface charge disruption of Gram positive [7]. In order to confirm the bovine lactoferrin bactericidal efficiency, the minimal inhibitory concentration was determined using clinical isolates of *Escherichia coli* and *Staphylococcus aureus*, through microtitre broth dilution test.

Two distinctive methods were used to incorporate lactoferrin into the fish gelatine nanofibers: i. as a filler in the electrospinning formulation using concentrations of 2, 5 and 10 (%wt), and ii. through adsorption in a solution with 40 mg mL^{-1} of lactoferrin.

Fourier transform spectroscopy analysis revealed that the structure of both nanocomposite proteins remained intact through the electrospinning blending and crosslinking procedure. The increase in the concentration of lactoferrin as a filler diminished in approximately 50% the size of the fibres when compared to pristine gelatine.

The electrospun material with adsorbed LF displayed an antimicrobial activity similar to the fish gelatine fibres without LF, most likely due to the low uptake of LF. The nanocomposites bearing 5% of LF as a filler showed a bacterial reduction of approximately 90% when compared to the control (electrospun FG). In addition, films containing 10% of LF revealed a notable antibacterial performance, with 100% of contact killing capacity, representing above 6 log reduction in *E. coli* and *S. aureus* bacterial populations.

Keywords: electrospinning; fish gelatine; lactoferrin; bactericidal; nanocomposite

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