ABSTRACT

Chronic wounds are a serious problem worldwide directly correlated with the growth of obesity and diabetes cases, and the increase of life expectancy. The major part of these wounds is infected by pathogenic bacteria. These infections associated with the perpetual inflammatory environment on the wound might become life-threatening since currently-used treatments with conventional antibiotics are failing. The usual reasons for treatment failure are related to the evolution of mechanisms of antibiotic resistance in bacteria and the formation of biofilm structures on the biotic surface, augmenting their tolerance against the antibiotic activity. Hence, drug-free antimicrobial wound products are urgently required.

For the first section of the experimental work, antimicrobial and immunomodulation properties of clinically-applied biomaterials were explored. This preparatory work allowed to understand the insufficiently explored macrophage-biomaterial interactions. Additionally, it permitted a finer selection of the bacterial strains and eukaryotic cell lines related to chronic wounds for the subsequent part of the laboratory experiments. In this second section, an extensive investigation of new antibiotic-free antibacterial polymers was performed based on polyurethane antimicrobial-peptide biomimetics.

In the wound care market, silver-containing dressings are the most commonly developed antibiotic-free antibacterial materials. Hence, for the first section of the experimental work, in chapter 2, antimicrobial, cytocompatibility and macrophage immunomodulation studies were performed with different silver-based products: Atrauman® Ag, Biatain® Alginate Ag and PolyMem WIC Silver® Non-adhesive. Biatain® Alginate Ag and PolyMem WIC Silver® Non-adhesive induced an excellent antibacterial effect in broth dilution assays. All the dressings stimulated a macrophage response in 24 hours. M0 macrophages' common response to all silver-impregnated dressings was to increase the production of the anti-inflammatory cytokine TGF-β which indicates a polarization towards tissue-healing M2-like macrophages, as it is desired in a chronic wound.

For the second section of the laboratory results, in the investigation of the antibacterial susceptibility and biological safety of antimicrobial peptide-biomimetic polyurethanes, the most promising candidate for overcoming Gram-positive bacteria known to infect wounds was selected (chapter 3) and its mechanism of action was examined (chapter 4). The chosen antimicrobial polyurethane, named NHP407-g-Poly(ILM-Br), showed rapid bactericidal capacity and prevention of biofilm formation against sensitive and drug-resistant Gram-positive *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* strains.

Moreover, the determined selectivity index proved the polymer cytocompatibility on fibroblasts, keratinocytes and monocyte-derived macrophages. Through morphological characterization by scanning electron microscopy and a combination of 2D gel-based and liquid chromatography–mass spectrometry–based approaches it was possible to determine that NHP407-g-Poly(ILM-Br) is a bacterial membrane-active agent, like most of antimicrobial peptides. The newly developed antimicrobial peptide-biomimetic polyurethane NHP407-g-Poly(ILM-Br) is a very promising candidate to treat sensitive and drug-resistant Gram-positive bacteria infected wounds by mimicking the antimicrobial peptides' most common mode of action.