Fatal attraction: chitosan microspheres decorated with MSI-78A kill *Helicobacter pylori*

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Helicobacter pylori chronic infection accounts for 80% of all diagnosed gastric cancers. This gastric pathogen is one of the sixteen bacteria that ranks higher in what concerns antibiotic resistance and as a threat to human health [1]. Antimicrobial peptides (AMPs) are an interesting alternative to antibiotics due to their broad-spectrum of activity and low propensity to induce bacterial resistance. MSI-78A, an analogue of Pexiganan, is one of the few reported bactericidal AMPs against *H. pylori* [2]. However, *in vivo*, AMPs can be less effective due to proteolytic degradation and aggregation with proteins. AMPs immobilization onto a biomaterial surface is an advocated strategy to overcome these drawbacks [3].
This work intends the development of biocompatible chitosan microspheres decorated with MSI-78A (AMP-ChMic) that, after oral administration, will cross the gastric mucosa and kill *H. pylori in situ*, i.e., at the surface of epithelial gastric cells where the majority of the bacteria are found.
Chitosan (Ch) was chosen as biomaterial since it is a natural, non-toxic cationic polysaccharide, with mucoadhesive properties and has been explored in approaches against *H. pylori* [4,5]. Ch (acetylation degree of 6%) was purified and crosslinked with genipin (2.5 mM) to prevent its dissolution in gastric acidic conditions. Ch microspheres (ChMic) were produced by spray drying technique (mean diameter around 3 µm). Afterwards, a heterobifunctional spacer (NHS-PEG-MAL) was added to the ChMic that allowed immobilizing the MSI-78A modified with a terminal cysteine on the C-terminal (MSI-78A-SH) in a controlled orientation.
AMP-ChMic were successfully engineered, corroborated by Fourier Transform Infrared technique and the immobilization reaction yield was around 77%. The microspheres were able to retain their integrity in both acidic (1.2, 2.6, 4.0 & 6.0) and neutral (7.0 & 7.4) pH, proving their pH-resistance, and validating this approach for gastric settings. AMP-ChMic efficacy against *H. pylori* J99 strain, (human highly pathogenic strain) was demonstrated in a concentration dependent manner. Effect was seen as soon as 2 h and it was maintained up to 6 h, with a decrease of 3logs (from 10^7 to 10^3 CFU/mL). This result indicates that MSI-78A can retain its activity when immobilized onto ChMic.
Overall, AMP immobilization onto ChMic was successfully achieved. The selected immobilization strategy promotes AMP exposure and interaction with *H. pylori*, boosting its bactericidal performance. AMP-ChMic demonstrated high potential for *H. pylori* infection management and should be further explored within the scope of non-antibiotic therapeutic strategies.

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