An universal one-pot microfluidic system to produce nanoparticles decorated with antimicrobial compounds

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Antibiotic resistance is a main public health challenge, being described by the World Health Organization as “one of the biggest threats to global health & development today”. The use of antimicrobial peptides (AMPs) is gaining relevance as an alternative to antibiotics. PexigananA (MSI-78A) is an analog of Pexiganan with reported bactericidal activity against *Helicobacter pylori* (Hp), a gastric bacterium that causes several gastric disorders and accounts for 90% of all diagnosed gastric cancers (5th most common & 4th deadliest worldwide)[1-3]. AMPs immobilization onto biomaterials overcomes the drawbacks usually associated with their performance *in vivo*, as it avoids their proteolytic degradation and aggregation with proteins, enhancing the bactericidal effect as they become effective at concentrations lower than in solution[4].

Here, a versatile, cost-effective, and environmentally friendly “one-pot” microfluidics system suitable for nanoparticles (NPs) production and bioconjugation of any ligand containing a thiol group (e.g., cysteine amino acid) is proposed. MSI-78A was directly grafted onto chitosan nanoparticles (AMP-NP, 113 ± 2 nm) surface using a microfluidic system that allows peptide bioconjugation *in situ* using the Thiol–Norbornene “Photoclick” Chemistry. The reaction yield was ~40% (analysis of amino acids - direct method), and grafting was confirmed by Fourier-transform infrared spectroscopy (FTIR), where the characteristic absorption bands of the AMP appeared at 1660 cm^{-1} (amide I) and 1530 cm^{-1} (amide II).

AMP-NP (10^{11} NP/mL) had a fast-bactericidal effect against Hp 26695 strain, reaching full eradication in 30min, while for the Hp J99 strain, the same bactericidal effect was achieved after 24h. These results demonstrated that MSI-78A maintained its activity after surface grafted onto NP, in which the amount of grafted peptide (96 µg/mL) was lower than the minimal inhibitory concentration (MIC) & minimal bactericidal concentration (MBC) of the free peptide (256 µg/mL). The high Hp–chitosan affinity could further improve the killing effect. After exposure to AMP (AMP-NP), Hp membrane presented irregularities, the formation of vesicles and changes/release in the cytoplasm.

Furthermore, AMP-NP at bactericidal concentration were cytocompatible against human gastric adenocarcinoma cell lines (AGS & MKN74. ATCC®), in accordance with ISO 10,993–5;12.

Overall, the designed AMP-NPs boosted the activity of MSI-78A and are promising for Hp eradication.

Also, a straightforward system to obtain AMP-conjugated chitosan nanoparticles was developed. Its main advantage is the possibility to simultaneously produce, crosslink and immobilize different thiolated-compounds (due to thiol-ene chemistry) in the same device. This system can be further explored with other biomaterials and for different applications.

References

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