Metal-enzyme nano-composites with bactericidal and antibiofilm efficacies

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Antimicrobial resistance (AMR) is causing 50 thousand deaths per year in Europe and North America, with a concomitant heavy economic burden. Bacteria have developed different specific mechanisms conferring antibiotic resistance altering or destroying the antibiotic molecules, decreasing the membrane permeability to the antibiotic or expulsing it through efflux pumps.[1] Besides these resistance mechanisms, the ability of bacteria to proliferate adhered to inert surfaces or living tissues forming biofilms aggravates the appearance of AMR. Bacteria encased in these structures avoid the immune system response and makes them highly tolerant to high concentrations of antimicrobials.[2] Furthermore, conventional antibiotics do not remove biofilms thus, despite a successful antibiotic treatment; remaining sessile cells may lead to an infection relapse.

Metallic nanoparticles (NPs) are increasingly used to target pathogenic agents as an alternative to antibiotics. Metallic NPs offer a platform against bacteria, viruses, fungus and protozoa, these nanoparticles present multiple biocidal mechanisms that occur at the same time[3] (oxidative stress induction, metal ion release, or non-oxidative mechanism) reducing and hindering the appearance of the microbial resistance. On the other hand, among anti-biofilm strategies, different enzymes can be applied to prevent the biofilm formation or its eradication once established.

In this study, we integrate metallic particles with antibiofilm enzymes in the same nano-entity to combine synergistically different antimicrobial agents. The enzymatic component disrupts the bacterial communication pathways, through the hydrolysis of small molecule signals that govern the biofilm formation.[4] On the other hand, the biocidal activity of the nano-composite is supplied by the metallic particles while functions as an active template for the grafting of the enzyme. This antimicrobial approach could be applied in the form of coatings on surfaces such as hospital textiles, water treatment membranes and implantable medical devices, ensuring a safer environment for both patients and healthy population.

Silver-chitosan NPs (AgCh NPs) were synthesised by silver ions reduction, using chitosan as a reducing and capping agent. After washing the particles, UV-vis spectrometry reveal the pick at 420 nm corresponding to the formation of Ag NPs. TEM images revealed spherical NPs of 25-30 nm of diameter. After that, the AgCh NPs were functionalized with the quorum quenching enzyme (QQE) acylase I. The TEM analysis showed that the AgCh NPs were embedded in a protein matrix-like structure forming a silver-chitosan-acylase I (AgChAcyl) composite. The particles containing acylase were able to hydrolyse N-acetyl-L-methionine during the ninhydrin assay, indicating that the enzyme maintained part of its activity after the cross-linking between the chitosan and the acylase I.

The antimicrobial efficacies of AgCh NPs and AgChAcyl NPs were tested towards *Psuedomonas aeruginosa*. Silver and chitosan are well-known antimicrobial agents.[5] However, the antimicrobial assay showed that the presence of acylase I enhanced the antimicrobial activity of AgCh NPs (Fig. 1A). In addition, the time-killing assay reveal that AgCh NPs and AgChAcyl NPs displayed a rapid antimicrobial effect. After 20 minutes incubation of the NPs with *P. aeruginosa*, the bacterial concentration was drastically reduced. Nonetheless, this reduction was faster when the nanoparticles contained the QQE enzymes acylase I (Fig. 1B). Acylase I by itself did not presented any antimicrobial effect, which indicated that there is a synergistic effect between the QQE and the antimicrobial agent.

P. aeruginosa was exposed to AgCh NPs and AgChAcyl NPs to test the capacity of composites to disrupt the biofilm formation. The biofilm was washed to remove the NPs and the non-attached bacteria. The establishment of the extracellular matrix was assessed by crystal violet assay and live-dead staining. Both assays confirmed that the presence of acylase I in the nanoparticles inhibits the formation of biofilm (Fig. 2). Acylase I is able to hydrolase N-butyryl-l-homoserine lactone, a quorum sensing molecule of *P. aeruginosa*, hindering the quorum sensing and affecting the processes that are related with this communication, such as the production of virulence factors and the biofilm formation.

Finally, the biocompatibility of the particles was assessed using different human skin cell lines. The toxicity towards these cell lines was reduced when the enzyme was present on the surface of the NPs reduced the toxicity of the composites towards mammal cells.

References

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Figures

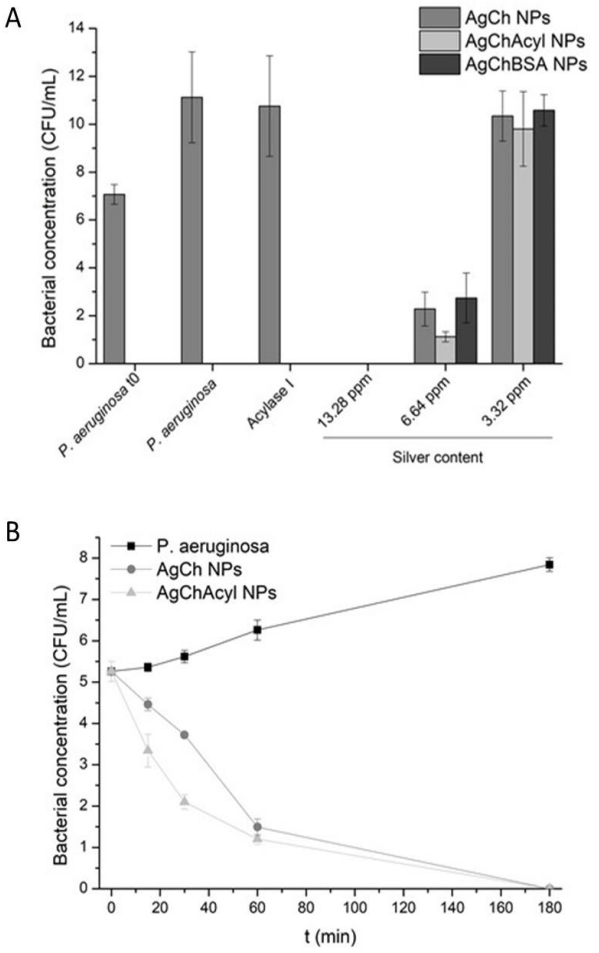
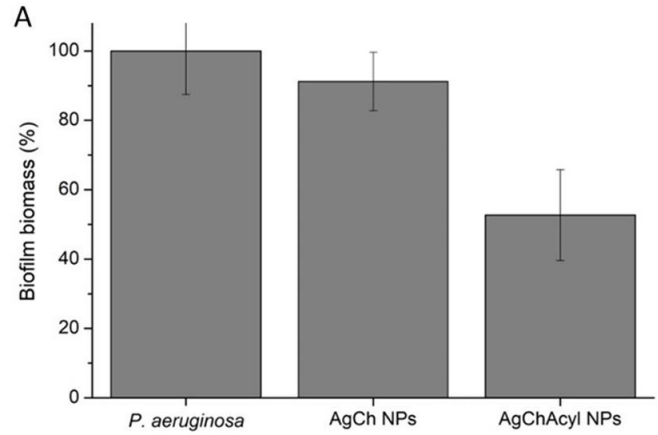


Figure 1. A) Evaluation of the antimicrobial activity of AgCh NPs, AgChAcyl NPs and AgChBSA NPs. The particles were tested using the same silver content. Acylase I was tested at the initial concentration of the synthesis of the particles. *P. aeruginosa* t_0 indicates the initial bacterial concentration of the test and *P. aeruginosa* value indicates the final concertation of bacterial without treatment B) Time-kill curves of AgCh NPs (dark grey) and AgChAcyl NPs (light grey) towards *Pseudomonas a*eruginosa. *P. aeruginosa* was incubated with 13. 28 ppm of silver and the bacteria surviving the treatment were estimated after 15, 30, 60 and 180 min.



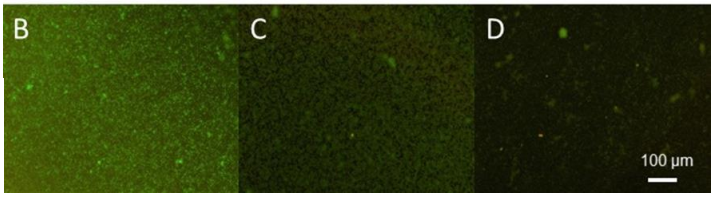


Figure 2. Evaluation of the biofilm inhibitory activity of AgCh NPs and AgChAcyl NPs. A) Assessment by crystal violet assay of *P.aeruginosa* biofilm inhibition, incubated for 24 h with AgCh NPs and AgChAcyl NPs. B) Fluorescence microscopy images of *P. aeruginosa* biodfilm after the treatment with C) AgCh NPs and D) AgChAcyl NPs.