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In vitro photodynamic effect of methylene blue versus mesoporous silica nanoparticles with methylene blue on Staphylococcus aureus and Pseudomonas aeruginosa

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Background: *Staphylococcus aureus* and *Pseudomonas aeruginosa* are commonly involved jointly producing skin and soft tissue mixed infections. Due to the increased ability to resist the currently used antibiotics, these infections persist over time causing chronic ulcers. Antimicrobial photodynamic therapy (aPDT), based on the application of a photosensitizer (PS) activated by visible light to generate reactive oxygen species that are cytotoxic, could be an alternative treatment. Methylene Blue (MB) is a very active PS for aPDT and nanoparticles (NPs) can be a better way to reach a target. We have synthesized mesoporous silica nanoparticles with methylene blue (MSNP-MB).

The aim of this study is to evaluate the efficacy of MSNP-MB vs. MB in the photoinactivation of *S. aureus* and *P. aeruginosa.*

Material/methods: *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 suspensions containing >10^7cells/mL were prepared. Different concentrations two-fold serial dilution of MSNP-MB (from 0.08 µg/ml to 10 µg/mL) and MB (from 0.08 µg/mL to 80 µg/mL) were added. After several times of pre-incubation (0, 0.5, 2 and 24 hours), the samples were irradiated with a red light-emitting diode lamp (625 nm, 0.007 W/cm^2) at 18 and 36 J/cm^2. The resultant suspensions were sub-cultured onto blood agar to determine the viable bacteria by colony-forming units counting (CFU/mL). Dark controls of MSNP-MB and MB, in addition to irradiated controls were performed.

Results: A reduction of 6 $\log_{10}$ CFU/mL in *P. aeruginosa* was reached with 10-40 µg/mL of MB, with or without any time of pre-incubation, at a fluence of 18 J/cm^2, or 10-20 µg/mL of MB at 37 J/cm^2. NP with a maximum concentration of 10 µg/mL MB achieved a reduction of 4.5-6 $\log_{10}$ CFU/mL at a fluence of 18 or 37 J/cm^2.

In the case of *S. aureus*, 0.6-2.5 µg/mL of MB or MSNP-MB and a fluence of 18 or 36 J/cm^2 were needed with or without pre-incubation to decrease the concentration to 6 $\log_{10}$ CFU/mL.

In both bacteria, the use of the different times of pre-incubation or fluence of irradiation does not result in significant differences in the amount of PS needed to achieve the same $\log_{10}$ reduction. Regarding

to the effect of MB alone vs. MSNP-MB, no significant differences in terms of photoinactivation of both bacteria under the tested conditions were found.

Conclusions: aPDT based on MB has a bactericidal effect on *S. aureus* and *P. aeruginosa* and this study supports its clinical use for mixed infections. MSNP-MB shows the same efficacy as MB against both bacteria but the maximum concentration of MSNP-MB synthesized is insufficient for photoinactivate *P. aeruginosa* completely.

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