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Enhanced Antimicrobial Strategy against *Staphylococcus aureus*: Synergistic Action of Plasma-Activated Water and Silver Nanoparticles

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Extended Abstract

The rising prevalence of multidrug-resistant infections, particularly in chronic wounds of diabetic patients, necessitates the development of innovative antimicrobial approaches [1]. *Staphylococcus aureus*, a leading opportunistic pathogen in such cases, poses a significant challenge due to its antibiotic resistance and biofilm-forming capability [2].

This study investigates the combined antimicrobial efficacy of plasma-activated water (PAW), produced via a gliding arc plasma system, and silver nanoparticles (AgNPs), synthesized through a bottom-up chemical reduction method [3]. The primary objective was to determine whether the synergistic interaction between PAW-derived reactive species and AgNPs' broad-spectrum antimicrobial properties could enhance bactericidal activity against *S. aureus*.

Reactive species in PAW, including hydrogen peroxide (H_2O_2) and nitrite (NO_2^-), were quantified using spectrophotometric colorimetric assays. Absorbance measurements, obtained with a BioTek Synergy HT microplate reader at 410 nm (H_2O_2) and 543 nm (NO_2^-), demonstrated high linearity in standard calibration curves ($R^2 > 0.99$). Results revealed time-dependent variations in reactive species: H_2O_2 peaked at 15 minutes' post-activation, while nitrite concentrations reached maximum levels at 2 hours before gradually declining. These dynamics underscore the importance of precise timing for PAW's clinical application.

Antimicrobial testing employed standardized *S. aureus* inoculum, evaluating individual and combined treatments of PAW and AgNPs over 15- and 60-minute incubation periods. Bare AgNPs achieved complete bacterial inhibition using $2{,}6 \times 10^{11}$ particles/mL, while lower concentrations showed a number of particles-dependent reduction in viability. PAW alone exhibited time-dependent antimicrobial effects, with longer exposure (60 minutes) resulting in significantly greater bacterial inactivation compared to the 15-minute treatment. However, when PAW was combined with AgNPs, no significant difference was observed between the 15- and 60-minute treatment durations. Both conditions achieved similar inhibition of bacterial growth using low concentrations of AgNPs. These findings indicate that the synergistic antimicrobial effect of the PAW–AgNPs combination may compensate for shorter exposure times, enhancing efficacy even under reduced treatment durations. This synergistic effect is likely due to the combined mechanisms of oxidative stress induced by PAW's reactive species and bactericidal action by AgNPs.

In conclusion, the integration of PAW and AgNPs reduced *S. aureus* viability through complementary antimicrobial mechanisms. These results suggest a potential approach to support existing treatments for resistant infections, including those associated with chronic wounds. The combination of cold plasma and nanomaterials could pave the way for innovative antimicrobial solutions, offering enhanced support to conventional therapies in complex infection scenarios.

References

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