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Poster 3

Antimicrobial photodynamic inactivation of *Pseudomonas aeruginosa* biofilms: a synergistic approach with berberine, gentamicin, and colistin

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Abstract

Background: Chronic wound infections caused by *Pseudomonas aeruginosa* biofilms are highly resistant to conventional treatments, necessitating innovative therapeutic strategies [1]. Antimicrobial photodynamic inactivation (aPDI) has proven to be a promising alternative [2]. However, its efficacy against Gram-negative bacteria remains suboptimal due to the restricted penetration of light and photosensitizers through the biofilm matrix, which hinders its full antimicrobial potential [3]. **Objective:** This study explores the potential of colistin (Col) at subinhibitory concentrations to enhance the photodynamic activity of a berberine-gentamicin (Ber-Gen) combination against *P. aeruginosa* biofilms. **Methods:** *P. aeruginosa* ATCC 10145 strain from the American Type Culture Collection was used. Col-induced membrane permeability changes were assessed by flow cytometry, and synergistic interactions within the Ber-Gen-Col combination were determined by checkerboard assay. Biofilms were exposed to blue light (420 nm, 30 mW/cm^2, 10 min) after one or three aPDI cycles (administered at 24 h intervals). Treatment efficacy was evaluated by quantifying biomass (crystal violet), metabolic activity (resazurin), and culturability (CFU/cm^2). The mechanism of action was examined by ROS production (fluorometry), membrane damage (microscopy), and biofilm structural changes (optical coherence tomography). **Results:** Colistin (4 µg/mL) increased membrane permeability by 30 %, significantly enhancing the photodynamic action of Ber-Gen-Col. The triple combination led to a nearly complete eradication of biofilm cells, achieving a 7-log CFU/cm^2 reduction and over 90 % decreases in biomass and metabolic activity. However, sustained suppression of biofilm regrowth was observed only after multiple irradiation cycles, while single-cycle treatments allowed biofilm recovery within 24 h. Regarding the mechanism of action of photoactivated Ber-Gen-Col, a significant disruption of the biofilm structure, increased reactive oxygen species (ROS) generation, and extensive membrane damage were observed. **Conclusion:** These findings demonstrate that integrating subinhibitory Col concentrations with repeated aPDI cycles represents a promising strategy for effectively eliminating *P. aeruginosa* biofilms in chronic wound infections.

Keywords: antimicrobial photodynamic inactivation; berberine-gentamycin-colistin combination; *Pseudomonas aeruginosa* biofilms

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