Persistent Luminscence Material for Lamp-Free Photodynamic Therapy to control Lung Infection Bacteria

Light4Lungs

Partners involved

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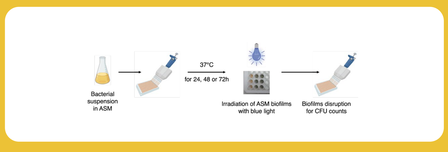
Objectives

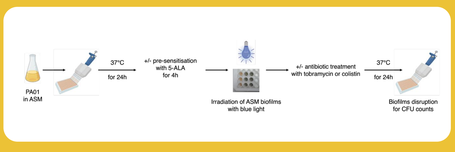
In patients with cystic fibrosis (CF), long term and persistent bacterial colonisation and infection of the lungs are a major problem due to the difficulty to clear the thick and viscous sputum that are produced in their airways. The accumulation of sputum promotes the formation of bacterial biofilms which are often resistant to antibiotic treatment. Antimicrobial photodynamic therapy is a promising approach to eradicate biofilms via the bactericidal activity of reactive oxygen species (ROS) produced in bacteria by the combination of visible light, oxygen and a photosensitiser. In this study we aimed to investigate the photokilling activity of blue light in *Pseudomonas aeruginosa* and Methicillin-Resistant *Staphylococcus* aureus (MRSA) biofilms. We also assessed the susceptibility of biofilms to antibiotics post light irradiation and whether pre-sensitisation of biofilms with a photosensitiser could enhance blue light and antibiotics efficacy.

Methodology

1- *P. aeruginosa* and MRSA biofilms were produced in vitro in the artificial sputum medium (ASM) that mimics the sputum found in the CF lung environment. At 24, 48 or 72h post inoculation, biofilms were irradiated with a blue light emitted by a LED at 415 nm. Biofilms were then disrupted to quantify the bacterial loads.



2- *P. aeruginosa* strain PAO1 biofilms produced in vitro in ASM for 24h were pre-sensitised with 5-aminolevulinic acid hydrochloride (5-ALA) for 4h. After incubation, biofilms were irradiated with blue light and then treated with tobramycin or colistin for 24h. Then biofilms were disrupted to quantify the bacterial loads.



Results

Figure 1: Blue light had an antibacterial photokilling effect on both *P. aeruginosa* and MRSA biofilms by inducing significant reduction of bacterial loads (fig. a and b) and altering the growth of remaining viable cells (fig. e) that leaded to a cell growth delay (fig. c and d).

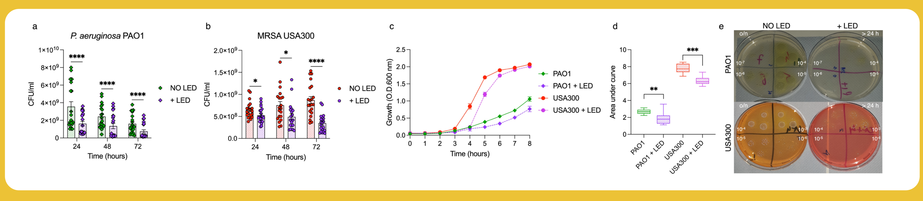
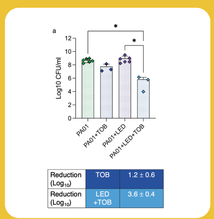
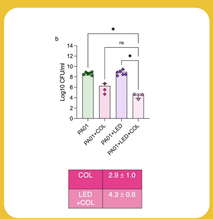


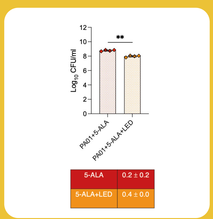
Figure 2: PAO1 biofilms appeared to have recovered from any inhibitory effect of light at 24h post irradiation as viable CFU counts were comparable to the untreated biofilms. Following treatment with tobramycin (TOB) or colistin (COL), PAO1 biofilms showed a slight reduction in viable cells; this effect was significantly enhanced when antibiotic treatment was combined with light irradiation (fig. a and b).
Pre-sensitisation with 5-ALA combined with blue light irradiation induced a significant reduction of PAO1 biofilms compared to pre-sensitised and no irradiated biofilms (fig. c).
Following 5-ALA addition and light irradiation, PAO1 biofilms treated with tobramycin displayed a greater reduction of CFU counts compared to biofilms treated with light and tobramycin only (fig. d).



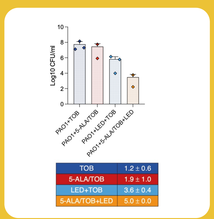
Reduction (Log_{10})	TOB	1.2 ± 0.6
Reduction (Log_{10})	LED +TOB	3.8 ± 0.4



COL	2.9 ± 1.0
LED +COL	4.3 ± 0.6



5-ALA	0.2 ± 0.2
5-ALA+LED	0.4 ± 0.0



TOB	1.2 ± 0.6
5-ALA/TOB	1.9 ± 1.0
LED+TOB	3.8 ± 0.4
5-ALA/TOB+LED	5.0 ± 0.0

Conclusions

Our results show that blue light has significant bactericidal activity on CF-like biofilms by altering bacterial growth and improving the bactericidal activity of antibiotics. Moreover, following blue light irradiation, biofilms were more susceptible to antibiotic treatment when they were pre-sensitised with the photosensitiser. Our next steps will be to i) assess the efficacy of antibiotics on MRSA biofilms following blue light irradiation in combination with the photosensitiser; ii) repeat our protocol to reach a total eradication of biofilms.

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