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Disinfection of Silicone Surfaces using Photo-Activated Disinfection

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INTRODUCTION:

As current antimicrobial agents are gradually being rendered ineffective by resistance developing in target organisms there is an urgent need for alternative antimicrobial approaches. Toluidine Blue O (TBO) is a light-activated antimicrobial agent which has been shown to be effective against a wide range of bacteria. The aim of this investigation was to determine the efficacy of TBO against biofilms grown on silicone surfaces when impregnated into the substrate and when applied externally.

METHODS:

Silicone discs were impregnated with 1mg/ml TBO by swelling the discs with chloroform for two hours and then applying the TBO solution (or water for controls) for 16 hours. The discs were then rinsed of excess TBO and dried. Biofilms of *Proteus mirabilis* and *Staphylococcus epidermidis* were grown by seeding the silicone discs for four hours with the appropriate culture in TSA at 37^{o}C ($1x10^5$ CFU/ml). The culture was then removed from each disc and replaced with fresh medium. The discs were then incubated for a further 44 hours.

PAD was initiated by activating the biofilms for 15 minutes with red light from a diode laser (wavelength: 633 ± 2nm) to apply a total energy does of 59 Joules. In an alternative treatment protocol discs were washed with a TBO solution (25µg/ml) followed by light activation as before.

Following treatment biofilm viability was assessed by removal of adherent bacteria and enumeration by viable counts.

RESULTS:

For *S. epidermidis* biofilms washing with TBO led to a 3.2 log reduction in cell numbers. Impregnated discs when not exposed to red light resulted in a 1.1 log reduction and when exposed to red light a 1.2 log reduction was observed. Washing of biofilms grown on impregnated discs gave a 1 log reduction in the non-light-activated control and a 2.4 log reduction when exposed to red light (wavelength: 633nm ± 2).

For *P.mirabilis* biofilms washing alone led to a 1.1 log reduction in cell numbers when compared to the controls. Impregnated discs when not exposed to red light gave a 0.4 log reduction and when exposed to red light no further reduction was observed. Washing *P.mirabilis* biofilms on impregnated discs gave no significant reduction in the control (0.1 log) and a 1 log reduction when exposed to red light.

DISCUSSION & CONCLUSIONS:

Successful disinfection according to the British Standard (BS EN 1276)[1] occurs when there is a five log reduction in cell number within 5 minutes. This did not occur with any of the treatment protocols described here.

When the two organisms are compared, it can be clearly demonstrated that *S. epidermidis* is more susceptible to disinfection using PAD than *P. mirabilis*. However, this was to be anticipated as most research suggests that Gram negative bacteria are more resistant to this mode of killing than Gram positive bacteria[2].

However, it was unexpected that the discs which had been impregnated with TBO showed a reduced kill level when compared to biofilms which had only been washed with TBO.

Two main conclusions can be drawn from this investigation:

- Gram positive organisms are more susceptible to killing using singlet oxygen than Gram negative organisms.
- Treating biofilms with an externally applied solution of TBO is a more successful method of applying PAD technology than release of impregnated TBO.

REFERENCES:

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