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Photosensitization of *in vitro* biofilms by toluidine blue O combined with a light-emitting diode

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In natural ecosystems, micro-organisms grow preferentially attached to surfaces, forming matrix-enclosed biofilms. The aim of this study was to determine photodestruction levels in biofilms after subjecting them to photodynamic therapy. Biofilms of *Streptococcus mutans*, *S. sobrinus*, and *S. sanguinis* were grown on enamel slabs for 3, 5 or 7 d. Both the number of viable micro-organisms and the concentration of water-insoluble polysaccharides were analysed, and mineral loss (ΔZ) analyses were performed on the enamel slabs. The antimicrobial potential of toluidine blue O (0.1 mg ml^{-1}), associated with 85.7 J cm^{-2} of a light-emission diode, was evaluated on the viability of 5-d biofilms. Both the number of micro-organisms and the concentration of water-insoluble polysaccharide increased with the age of the biofilms. A significant reduction ($\approx$ 95%) in viability was observed for *S. mutans* and *S. sobrinus* biofilms following photosensitization, with a > 99.9% reduction in the viability of *S. sanguinis* biofilms. In conclusion, a biofilm model was shown to be suitable for studying changes in bacterial numbers and enamel mineralization and for demonstrating the potential value of photosensitization in the control of *in vitro* biofilms.

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