The response of Pseudomonas Aeruginosa biofilm to a novel glass polyalkenoate cement.

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Introduction: Proliferation of *Pseudomonas Aeruginosa* can be inhibited by zinc (Zn^{2+}) and silver (Ag^{+}) ion release from glass polyalkenoate cements (GPCs) [1]. Zn^{2+} inhibits glycolysis, transmembrane protein translocation and acid tolerance [2]. Ag^{+} binds to negatively charged components in proteins and nucleic acids, causing structural changes in bacterial cell walls, membranes and nucleic acids [3]. This study evaluates whether such ion release can also inhibit growth of a biofilm related to *Ps. Aeruginosa*.

Materials & Methods: A GPC was prepared by mixing 0.5 g glass ($56.04SiO_2$, $32.76ZnO$, $0.33Ag_2O$, Na_2O10.87 mol%, <25µm particles) with 0.2 g PAA (Mw, 210,000) and 0.25 ml distilled water. Cement constructs (4 mmØ, 6 mm ht) were matured (37˚C, 24 h) and ion release was measured from these by atomic absorption spectrometry (AAS). A biofilm assay was developed using *Ps. Aeruginosa* in a 96-well Microplate (Costar, Corning, NY, USA). The blank was 200 µl of fresh lysogeny broth. Two controls were used; 200 µl of *Ps. aeruginosa* was pipetted in 16 wells each. These were checked after 12h and 24 h. 200 µl of the culture was incubated (in 16 wells) for 12 h to form biofilm, the GPC was then added aseptically and biofilm levels were checked after another 12 h (24hr in total). The microplate was incubated at 30°C (24 h), after which, contents were emptied and the wells rinsed out with distilled water. 250µl of 0.1% crystal violet stain (Sigma Aldrich, Dublin, Ireland) was pipetted into each of the wells (residence time, 5min). The contents were then rinsed out. 276 µl of a 20:80% acetone:ethanol mix was then pipetted into the wells to solubilize the attached biofilm. The absorbance of the wells was tested at 595nm to determine if there was a difference in the absorbance between the contents of the wells containing, and those free of, GPC at the time intervals.

Results & Discussion: The majority of Zn^{2+} (1.5 ppm) and Ag^{+} (0.2 ppm) release occurred in the first 24 hours post mixing of the cement.

The absorbance (figure 2) of the wells containing biofilm after 12hr was 0.571 (SD 0.206) and after 24 h, 0.924 (SD 0.219). The wells containing biofilm and GPC (added after 12hr growth) had an absorbance of 0.302 (SD 0.076). Thus, a significant statistical difference (T-test; $p<0.001$) was observed at 12 h between wells containing biofilm and GPC and those containing only biofilm. The experiment was repeated with the biofilm left to grow for 24 h before the addition of the GPC, which shows absorbance values of the wells containing the biofilm and GPC (added after 24 h growth) had an absorbance of 2.02 (SD 0.411)

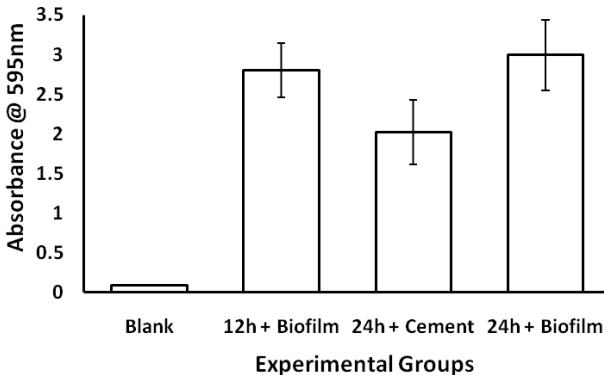

Figure 1: Absorbance levels of the growth of biofilm in the well plates containing biofilm alone and those containing biofilm and cement.

Conclusion

The reduction in absorbance in the wells that contained the GPC indicates that it partially eliminated biofilm presence and inhibited the formation of more. This study has shown that a novel Zn-Ag-GPC can inhibit biofilm formation and partially reduce the levels of biofilm *in vitro* indicating that it may be possible to produce biofilm inhibitory coatings or cements from this novel GPC.

References

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