Antifouling Coatings to Prevent Biofilm Formation

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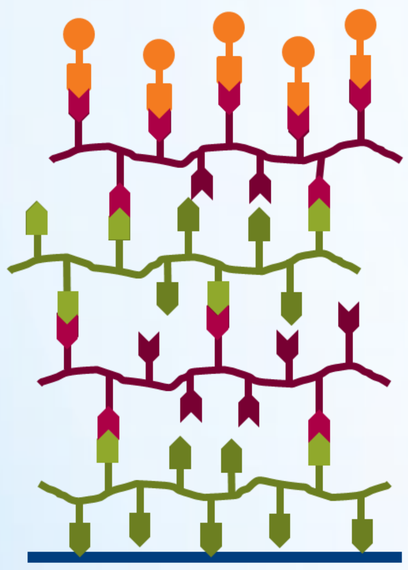
Abstract

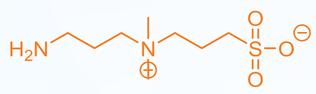
The prevention of biofilm formation remains an important challenge in the field of antimicrobial resistance (AMR). This project is aimed at using multi-layers of reactive polymers to coat and post-functionalise surfaces in order to prevent the formation of a biofilm. Reactive polymer coatings of controllable thickness were composed of multiple bilayers of poly(4,4-dimethyl-2-vinylazlactone) (pVDMA) and poly(ethyleneimine) (pEI). Reactive surfaces were functionalised using a range of different length oligo/poly(ethylene glycol) amines, a zwitterionic sulfobetaine amine, and perfluorodecylamine. Ellipsometry, contact angle, XPS, SIMS-TOF, and FT-IR were used to monitor the successful attachment of functional groups. To quantify the anti-fouling ability of these surfaces biofilms of fluorescent (YFP expressing) *Escherichia coli* were grown on coated glass surfaces. The degree of biofilm formation was determined through crystal violet staining (staining the entire film), measuring metabolically active bacteria using resazurin and through the amount of fluorescence emitted by surface-bound *E.coli*.

Methods and Results

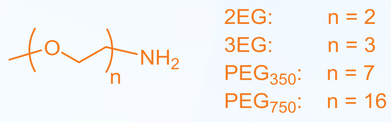
A) Synthesis and post-functionalisation of the reactive polymers

Cleaned glass slides were sequentially dipped into solutions of the polymers and amines



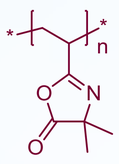


zwitterionic sulfobetaine amine

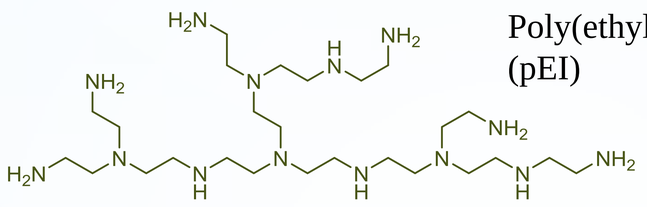


oligo/poly(ethylene glycol) amines

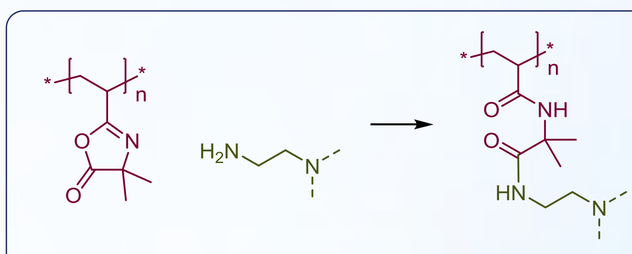
perfluorodecylamine



Poly(vinyl azlactone) (pVDMA)



Poly(ethylene imine) (pEI)

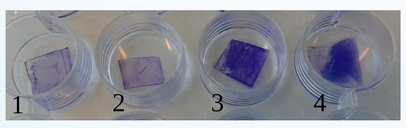


Addition reaction between pVDMA and amino groups producing a covalent linkage

B) Development and detection of a biofilm to evaluate coating

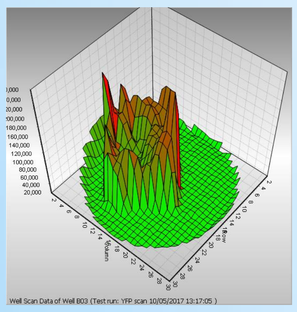
Pieces of functionalised polymer coated glass slides were incubated in a bacterial culture for 24 h at 25 °C. The planktonic (non attached) bacteria were removed and the biofilm/living bacteria were quantified:

Crystal violet staining
- Stains all components of a biofilm on surface
- 1- PEG350, 2- PEG750, 3-2EG, 4- uncoated control
- PEG shows less purple staining than the control

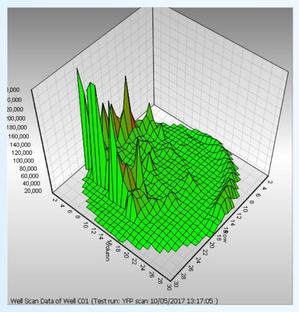


Resazurin staining
- Measures metabolically active bacteria
- Pink: bacteria, blue: no bacteria

Fluorescence scanning in a Clariostar plate reader
- Detects fluorescing (YFP expressing) bacteria

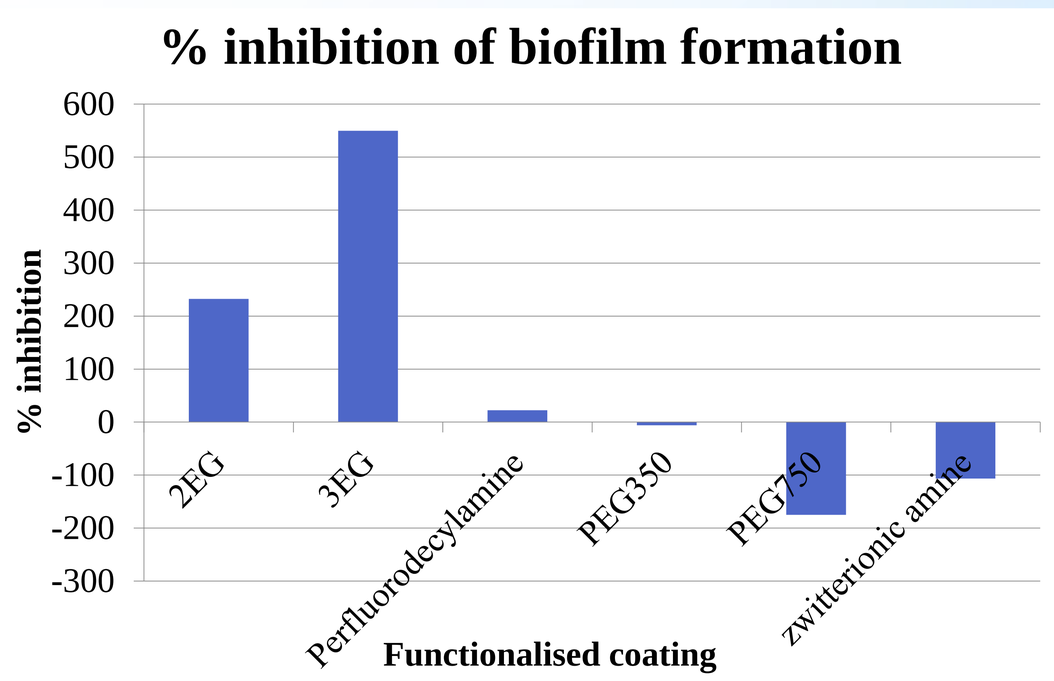


Uncoated glass



PEG 750 coated glass

The PEG750 coated glass shows less fluorescence signal than the uncoated glass due to the presence of less bacteria.



Using the optical density of the crystal violet stained biofilm on the surface the degree of biofilm inhibition due to the functionalised polymer was calculated. Data shows inhibition of biofilm development as a result of coating with PEG750 coating and zwitterionic amine.

Conclusion

Glass slides have been coated with multiple bilayers of reactive polymers and functionalised with different amines. Poly(ethylene glycol)750 and zwitterionic amine inhibited the development of the biofilm.