FLUID FLOW AND SUBINHIBITORY CONCENTRATIONS OF Ag RELEASED FROM Ag:CxHyOz COATINGS ENHANCE *icaA* GENE EXPRESSION IN *STAPHYLOCOCCUS EPIDERMIDIS*

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Introduction

Although infection remains one of the major impediments to the long-term use of many implanted and intravascular devices, and bacterial adhesion and biofilm formation seem to be the essential initial steps, the assessment of biomaterial susceptibility to infection relies mainly on the analysis of macroscopic bacterial responses to bacteria-material interactions, usually under static conditions. In this study we combined both conventional phenotypic analysis, using microscopies, and genotypic analysis, using the relative (r) reverse transcription Real-Time Polymerase Chain Reaction (RT-PCR), to examine the interaction of bacteria with antifouling and antimicrobial surfaces under dynamic flow conditions. In this direction Polyethylene oxide (PEO)–like coatings with and without nanoclusters of silver (Ag) were deposited by means of plasma assisted technologies on PET.

Materials and Methods

Substrates: the plasma-enhanced chemical vapor deposition (PE-CVD) was used to deposit the PEO-like and Ag/PEO-like coatings in RF (13.56 MHz) Glow Discharges fed by a mixture of 1% Diethyleneglycol di-methyl ether (DEGDME) and Ar. A simultaneous sputtering process from a silver-coated (2 mm thick lamina) cathode was used to obtain a dispersion of Ag clusters (25%) into the PEO-like coating for Ag/PEO-like coatings. RF power of 60 W and a pressure of 50 mTorr were used for both the deposition of PEO-like coatings and the deposition of Ag-PEO-like ones [1].

Bacteria: The reference *Staphylococcus epidermidis* ATCC35984 slime producing strain was used. Bacteria in the mid-exponential phase were suspended in 0.9 % NaCl at a concentration of $3x10^9$ bacteria/ml.

Parallel Flow Chamber: Bacterial adhesion to the substrates and *icaA* expression were examined 2 and 4 hours post adhesion, under shear rates 50 and 2000 s^{-1}.

Techniques: Surface properties were examined by: Atomic Force Microscopy, X-ray Photoelectron Spectroscopy and Contact Angle Measurements. Ag release was assessed by means of ICP-MS in bi-distilled water. Bacterial adhesion was evaluated by the Colony Forming Unit counting method, Scanning Electron Microscopy and Confocal Laser Scanning Microscopy. Total RNA from both planktonic and adherent bacteria was isolated and rRT-PCR towards a 207 bp part of 23S rDNA gene, allowed the detection of the expression levels of *ica*A [2].

Results and Discussion

Bacterial adhesion and viability was much higher on the PEO coated PET than on the Ag/PEO coated one. A silver dissolution rate of 100 ppb/cm^2/day was observed. The initial Ag release though at concentrations lower than the necessary to kill most of the adhering bacteria (subinhibitory) significantly increased *icaA* gene expression for the bacteria interacting with the Ag/PEO coating, 2 hours post adhesion, especially under the higher shear rate. Bacterial adhesion therefore under stress-inducing conditions, subinhibitory concentrations of Ag and high shear rate, increased *icaA* expression. The increase in adhesion time though and the subsequent increase in Ag release decreased both bacterial viability and *icaA* gene expression.

Conclusion

The results suggest that the incorporation of Ag in PEO as a biomaterial coating can significantly decrease bacterial adhesion and viability with time, in comparison to PEO alone. Its release though at the minimum inhibitory concentration, during the initial adhesion phase, seems to be of vital importance, especially if it is used for biomaterials that are inserted in high shear rate environments; such as aortic grafts and catheters. The combination of both phenotypic and genotypic analysis offers the potential to explore the links between phenotypic responses to bacteria-material interactions and gene expression alterations, and can therefore be used in biocompatibility assessment.

References

Sardella *et al*, Plasma Processes and Polymers, 3:456-469, 2006

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