Nanosilver Surfaces for Improved Understanding of Biocompatibility and Antibacterial Efficacy of Medical Device Coatings

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Statement of Purpose: To reduce infection of medical device surfaces, manufacturers have developed products incorporating nanometer-scale silver antimicrobial[1] coatings (nAg), such as wound dressings and catheters. While silver has a long history of safe medical use, extensive *in vitro* research has identified conditions under which cytotoxicity and genotoxicity from nAg is detected[1]. Inspired by the conditions of intended use of nAg on devices, we have developed well-characterized nAg coatings and have used them to study nAg at the device-host interface through physico-chemical, mammalian cell, and bacterial studies.

Methods: Piranha-cleaned 12 mm glass coverslips, polystyrene (PS) spin-coated coverslips, TEM grids, and QCM chips were nAg-coated by sputtering in a Denton Desktop IV at 50% power in 50 mTorr Ar. TEM was on a JEOL JEM-1400, AFM on an Asylum MFP-3D, QCM on a QSense Auto E4, and ICP-MS on a Thermo XSeries 2. Elutions were overnight in 1 mL water, Hank's Buffered Saline Solution (HBSS), or Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum on an orbital shaker at 25ºC. Alamar Blue (Invitrogen) cytotoxicity assay was performed after 18 h of culture in eluents diluted to 95% with 10,000 RAW 264.7 cells. Direct contact cytotoxicity was performed overnight with 10,000 RAW 264.7 cells in 50 µL droplets. Antibacterial efficacy was determined by incubating 100 µL of water containing 1.5-3x10^4 CFU of E. coli type FDA strain Seattle 1946 for 30 min on samples and then transferring that solution to nutrient agar plates and culturing for 24 h.

Results: Using TEM and AFM imaging, we demonstrate sputter coated nAg coatings with nominal features sizes of 25 nm or less on glass and PS-coated glass (**Fig. 1A** and **1B**). QCM analysis indicates an average sputtering rate of 176±27 ng/cm^2/sec. Glass and PS serve as initial approximations of ceramic and polymer-based device substrates. Visual observation of nAg post-elution (**Fig. 1C**) indicates different Ag(I):Ag(0) ratios (observed as a red shift[2]) due to the eluent and substrate, suggesting variable oxide formation, dissolution, and possible nanoparticle release. ICP-MS indicates that after a 2 day incubation, 63% of Ag from Glass/nAg is found in DMEM while the balance is substrate-bound, prompting on-going studies to understand variables that affect nAg chemistry and release.

While no cytotoxicity of eluted nAg was detected using the Alamar Blue assay (**Fig. 1D**), significant cell death was observed when RAW 264.7 cells were cultured directly on nAg (**Fig. 1E**). A lack of viable, normal-shaped cells was confirmed by Live-Dead staining (not shown), indicating that elution-based studies may not fully capture the response to nAg at an implant-tissue interface. Finally, the antibacterial efficacy of nAg coatings was demonstrated (complete inhibition), further validating them as models of device coatings (**Fig. 1F**).

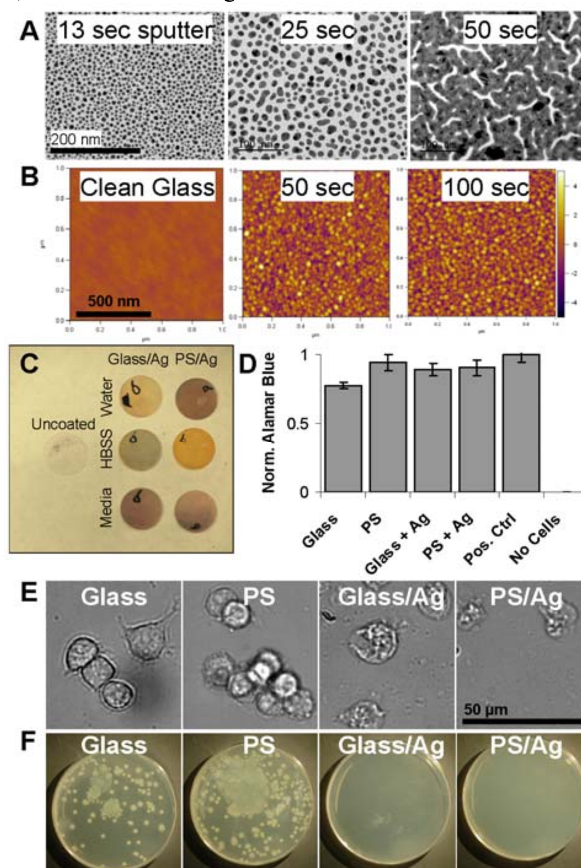

Figure 1. A) TEM shows nAg growth with sputter time. **B)** AFM illustrates consistent surface nano-topography at longer sputter times. **C)** Soaking nAg demonstrates eluent- and surface- based optical changes to coatings. **D)** Alamar blue cytotoxicity assay reveals no toxicity of nAg eluents on RAW264.7 cells, however, **(E)** a direct-contact assay does show nAg-induced toxicity. **F)** nAg exhibits antibacterial efficacy against E. coli.

Conclusions: Through physico-chemical analysis and intended use-based assays, this research is working towards an improved understanding of nAg properties in the context of medical device use.

References: [1]Maillard JY. Crit Rev Microbiol. 2012:Early Online:1-11. [2]Henglein A. Chem Mater. 1998;10:444-450. *The authors acknowledge the FDA Nanotechnology Initiative for funding this research.*

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