Different Biocompatibility Results for Antimicrobial Silver Coatings Based on Assay Format

Eric M. Sussman, Brendan J. Casey, and Benita J. Dair

Division of Chemistry and Materials Science, Office of Science and Engineering Laboratories, Center for Devices and Radiological Health, Office of Medical and Tobacco Products, U.S. Food and Drug Administration

Statement of Purpose: To reduce infection of medical device surfaces, manufacturers have incorporated nanometer-scale silver antimicrobial coatings[1] (nAg) on devices 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 from nAg is detected[1]. We have developed well-characterized nAg coatings and have used them to study nAg at the device-cell interface[2].

Traditionally, *in vitro* testing of the biocompatibility of materials is performed using extract-based assays (e.g. International Standards Organization (ISO) 10993-12) with assumption that if toxicological agents are present in the test article, they will be extracted and detected upon presentation to healthy cell cultures as a reduction in viability. As an alternative, some researchers choose to perform direct-contact cytotoxicity assays because, they reason, cells will become directly adherent to medical device surfaces upon implantation.

In the present work, we demonstrate significant differences in cytotoxicity testing results based on the assay format. This research has implications for understanding the *in vivo* response to nAg coatings.

Methods: 35 mm tissue culture polystyrene Petri dishes were Ag-coated at the settings shown in Fig. 1A in an EMS-150T S sputter coater (Electron Microscopy Sciences, Hatfield, PA) with paper masks to reduce side-wall coatings. As described previously[2], this process results in Ag coatings with nanometer-scale topographical features (nAg). L929 mouse fibroblasts were cultured in DMEM containing 10% fetal bovine serum, HEPES, sodium pyruvate, antibiotics, and fungizone on coated plates, in media plus $AgNO_3$, and in 24 h nAg extracts overnight followed by analyzing aliquots of media for Ag content and performing viability assays using Alamar Blue. Ag concentrations were determined using an XSeries 2 inductively coupled plasma mass spectrometer (ICP-MS) (Thermo, Waltham, MA).

Results: L929 cells grown in direct contact on nAg exhibit reductions in viability with increasing Ag concentrations (Fig. 1B, blue). Compared to the $AgNO_3$ controls (orange), the viability of L929 in similar Ag concentrations is improved but still significant. Cell-free extracts of nAg contain lower amounts of Ag, but still enough to cause toxicity as predicted by the $AgNO_3$ and direct-contact test conditions (red). However, no significant viability reduction is observed (e.g. viability at 7.5 µg/mL Ag is 100%, compared to 25% in direct contact and 0% in $AgNO_3$).

Further experiments were carried out to determine how viability is affected by pre-treating (quenching) of nAg surfaces with media. After 24 h of pre-quenching nAg, up to 25% improvement in viability is observed. Additionally, partial improvements in viability are observed when $AgNO_3$ solutions are pre-quenched for at least 72 h at 4 ºC (data not shown). Thus, we observe assay-specific L929 biocompatibility to nAg.

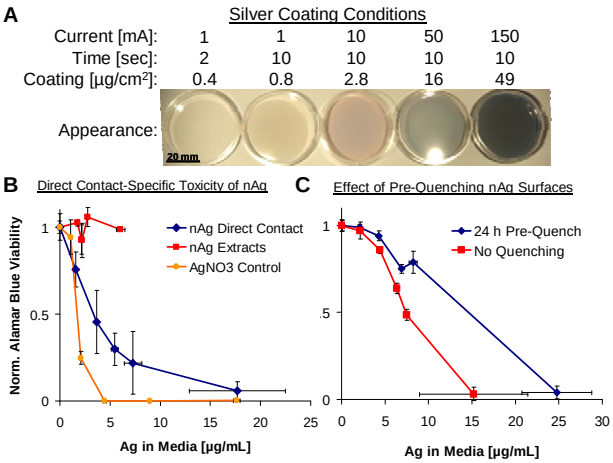

Figure 1. A) Ag sputtering conditions and resulting coating densities and appearances. **B**) Alamar Blue-based viability assays demonstrate toxicity to L929 cells in direct-contact but not extract-based assays. **C**) Pre-quenching nAg reduces but does not eliminate toxicity.

Discussion and Conclusions: This data suggests differential outcomes in cytotoxicity assays depending on the assay format. Specifically, extract-based testing led to no significant toxicity while direct-contact testing showed significant toxicity. Since pre-quenching of the surfaces only partially eliminated direct contact toxicity, it can be hypothesized that at least some of the toxicity of nAg coatings is due to direct contact of cells with nAg. Such a finding is important for predicting the *in vivo* responses to nAg, as some cells will be directly adherent to medical devices, and others will be exposed to Ag in the local milieu. In fact, there is evidence for increased cell death due to nAg *in vitro* and *in vivo*[3].

Since many materials do not support direct contact cell cultures, either due to low levels of cell adhesion or complex geometries, these assays are not often used as part of biocompatibility testing for medical devices. However, such testing may be useful for studying the response to nAg and other novel materials and for generating improved *in vitro-in vivo* correlations. These studies are being extended to extracts of medical devices.

References: [1]Maillard JY. Crit Rev Microbiol. 2012:39:373-83. [2]Sussman EM. SFB Annual Meeting 2013, #849. [3]Nadworny PL. Nanomedicine. 2012:4:241-51. *The authors acknowledge the FDA Nanotechnology Initiative for funding this research and the Oak Ridge Institute for Science and Education.* **Disclaimer**: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.