Real-time Detection of Nanoparticle Toxicity Using Electric Cell-Impedance Sensing

Bhavana Mohanraj[1*], Nathan R. Schiele[1*], Cerasela Z. Dinu[2***], Douglas B. Chrisey[1**], David T. Corr[1*]
(1) Rensselaer Polytechnic Institute, (2) West Virginia University
*Biomedical Engineering, **Materials Science and Engineering, ***Chemical Engineering

Statement of Purpose: Electric Cell-Impedance Sensing (Applied Biophysics, Troy, NY) is a real-time system which applies *in situ* an oscillating electric field to cells in culture, and measures the impedance as they attach and spread on the electrode surface [1]. Previous work has explored the use of ECIS to quantify the cytotoxicity of chemical agents and viruses [2, 3]. Here we present a novel application of ECIS for the detection of cytotoxicity in mammalian cells after exposure to nanoparticles. Nanotechnology is a rapidly expanding field with potential applications in tissue engineering and materials development. However, the toxicity of nanomaterials must be investigated prior to their use in new and advancing technologies. Cell-impedance sensing has the potential to measure the changes in cell morphology and behavior due to nanoparticle (NPs) exposure, and thus provide insight into cytotoxicity with high sensitivity.

Methods: The Electric Cell-Impedance Sensing system was used to measure the changes in the impedance of a cellular monolayer. Interdigitated (8W10E+) gold electrode well plates were seeded with Madine-Derby Canine Kidney (MDCK) cells at a concentration of ~$2.5x10^5$ cells/mL, and cultured with Dulbecco's Modified Eagles Medium (DMEM), 10% fetal bovine serum, and 1% pen/strep until confluent. The impedance of the cell monolayer was continually measured at multiple frequencies, with an interval of 80 seconds. Once the cells were confluent, the medium was removed and replaced with DMEM, then allowed to stabilize prior to treatment with the silver nanoparticles (~0.65 nm). The barrier function of the cell monolayer was analyzed using RbA modeling software in the ECIS software package. The barrier function (Rb) is proportional to the number of tight junctions formed between cells in the monolayer and correlates to any cell morphological changes. Changes in cell morphology were verified visually using optical microscopy.

Results: The resistance of the MDCK cell monolayer dropped drastically when exposed to silver nanoparticles in a dose dependent manner (Figure 1).

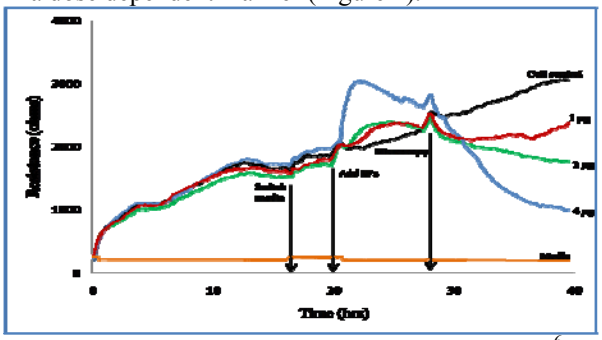

Figure 1. Resistance of the MDCK monolayer (~10^6 cells) measured at 4 kHz decreased after exposure to ~0.65 nm silver nanoparticles; I_{RMS} = 0.71 µA.

A change in cell morphology was detected within 2 hours of exposure for a dose of 4 µg of silver nanoparticles. Exposure to silver nanoparticles caused cell rounding, which resulted in a decrease in surface coverage of the electrode, and drop in impedance. Visual inspection of the cells confirmed that there was a distinct change in morphology after exposure to the nanoparticles, compared to that of the control. As a more direct measure of cell behavior and morphology, the changes in barrier function were measured over the course of the experiment (Figure 2). The barrier function indicates the formation of tight junctions between the cells in the monolayer. At 2 hours after initial cell seeding, an Rb value of zero indicated the cells had not yet formed a monolayer with tight junctions. At 12 hours after seeding, an Rb between 2.22 and 3.23 indicated that the cells had formed tight junctions, which were not disturbed when the media was replaced. However, at 2 hours after the addition of silver nanoparticles, the Rb values begin to decrease for all doses of silver nanoparticles. For the highest dose of Ag nanoparticles, the Rb equals zero after 14 hours of exposure. In constrast, the Rb of the cell control (media only) continued to increase to 14.39.

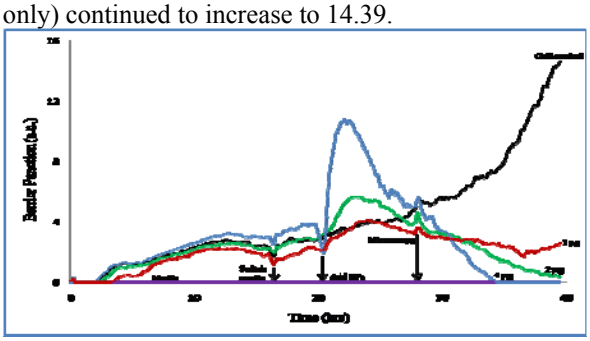

Figure 2. Barrier function of the monolayer measured at 4 Hz decreased in a dose-dependent manner after exposure to silver NPs.

Conclusions: Our results support the conclusion that the cytotoxicity of silver nanoparticles can be measured with high sensitivity using the ECIS system. We were able to detect as low as 1 µg of silver nanoparticles per 10^6 cells using cell-impedance sensing. The barrier function was also a direct quantitative measure of the loss of tight junction proteins due to nanoparticle exposure. Tight junctions facilitate intercellular communication, and the destruction of these proteins indicates a decrease in cell viability. Further work would include the use of idealized constructs, fabricated by laser direct writing, to optimize the magnitude of the signal and the response time. We conclude that ECIS can be used to accurately quantify changes in cell morphology and behavior in real time in response to toxin exposure.

References:

1. Giaever I. Proc. Natl. Acad. Sci. 1984; 81:3761-3764.
2. McCoy M.H. J. Virol. 2005; 130: 157–161.
3. Curtis T.M. J. Appl. Toxicol. 2009; 29:374-380.