Cytotoxicity of Gold Nanoparticles in Mast Cells

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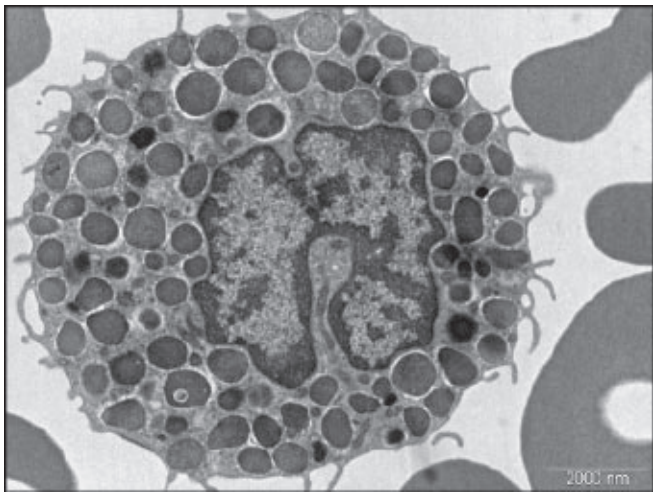

Figure 1: TEM image of a mast cell from a mouse.

Abstract:

Mast cells, shown in Figure 1, are a type of immune cell found in connective tissue of the body. When the cells are stimulated they release chemicals such as serotonin from their cellular granules into the extracellular matrix.

Mast cells were cultured with various concentrations of gold nanoparticles. Amperometric techniques indicate that the nanoparticles do have a negative effect on the mast cells' release of chemicals. Transmission electron microscopy (TEM) was used to determine the location of the nanoparticles in the cells. The nanoparticles appear to cluster in the granules near the cellular membrane of the cells.

Introduction:

Gold nanoparticles are currently being used in a wide variety of applications, from use in photodynamic therapy to use as a contrast agent. One of the unique properties of gold nanoparticles is that many of their characteristics—size, shape, and surface charge, for example—can easily be varied. Previous nanoparticle cytotoxicity studies have tended to deal primarily with live/dead assays, which offer only a limited means of analysis of nanoparticle's effects. A type of electrochemical analysis method called amperometry was used to study the effects of the nanoparticles on cells. Amperometry investigates the release of chemicals (such as the aforementioned serotonin) from the cell. Amperometry can be used to improve on live/dead assays and gather more detailed information about the effect of nanoparticles on cellular function. Mast cells were chosen for study since they are a model immune system cell and some of these released chemicals, such as serotonin, are electroactive and can be investigated using amperometry.

Experimental Procedure:

First, cellular function was studied via amperometry. Cells were harvested from the abdominal cavities of mice and then cultured for about 24 hours with nanoparticles of diameter 26.5 ± 6.0 nm. These cells were then stimulated with a calcium ionphore, causing degranulation of the cell and release of chemicals. The released electroactive chemicals were oxidized, causing the release of electrons to the electrode where the resultant current was analyzed to find that the addition of nanoparticles does affect cellular function. Figure 2 shows the current versus time graph for the chemical release from a mast cell that had been treated with nanoparticles. The current spikes have a much lower amplitude than that of the control group, and other key data such as half-width are also statistically different. To determine more specifically

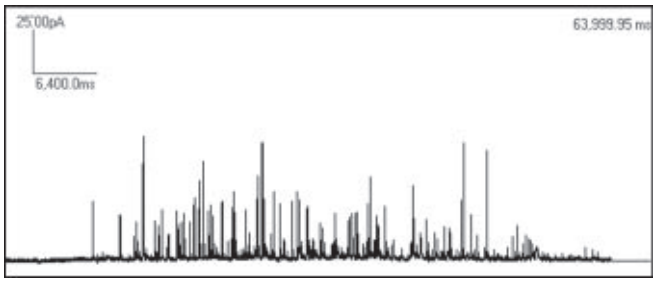

Figure 2: Amperometry data for mast cell with gold nanoparticles.

how the nanoparticles were affecting the cells, the cells were then prepared for viewing using a TEM.

TEM images show cross-sectional pictures of the cells where image intensity varies according to how a beam of electrons travels through the sample. After being treated with nanoparticles, the cell pellets were fixed using glutaraldehyde and then washed in a buffer. A secondary fixation was completed with osmium tetroxide. The cells were then dehydrated using %50, %70, %95, and finally %100 ethanol concentrations. Next, the cells were placed in a transitional solvent (propylene oxide) before being moved to resin. The cells were infiltrated by resin and cured for at least three days to allow the resin to harden. A microtome was used to section 60-70 nm slices which were then stained with uranyl acetate and a modified Reynolds lead stain and viewed under a TEM at 80 kV accelerating voltage.

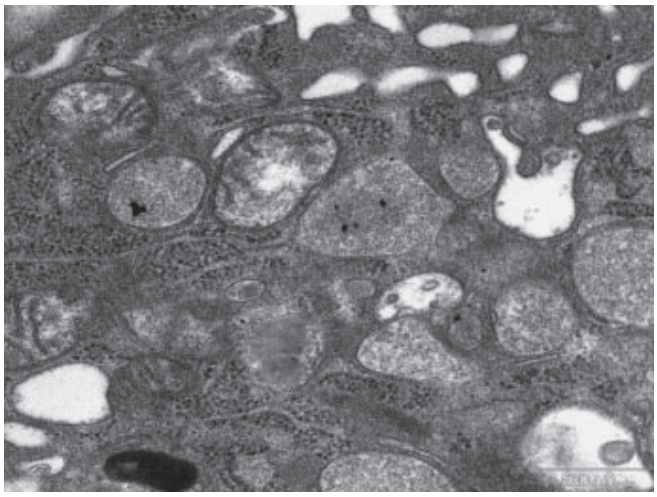

Figure 3: TEM image of gold nanoparticles in a granule of a mast cell.

Results and Conclusions:

Getting good quality TEM images proved to be difficult. There were problems with the hardening of the resin, the premature death of cells, a low cell yield between the cell harvest and the primary fixation of cells, and the difficult differentiation of white blood cells, mature mast cells, and immature mast cells. Once these problems were addressed, characterizing the location of the nanoparticles in the cells was fairly easy. Figures 3 and 4 show TEM images of nanoparticles in the cell.

Nanoparticles tend to cluster in the granules of the mast cells. Since the granules are essential to the primary function of mast cells, this location of nanoparticles could explain the observed negative electrochemical effect of the addition of nanoparticles.

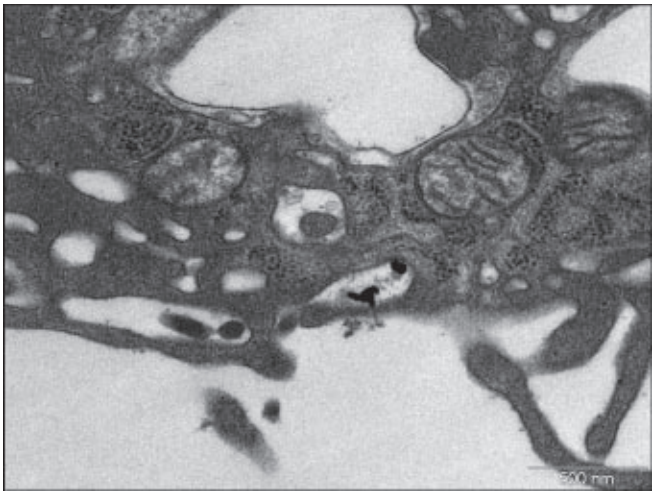

Figure 4: TEM image of gold nanoparticles in a granule near the cellular membrane.

Future Work:

Figure 4 shows nanoparticles that have clustered in a granule of the cell close to the cellular membrane of the cell. Other images have also shown that the nanoparticles have a tendency to be located either on or directly underneath the cellular membrane of the cells. Future work could entail using atomic force microscopy (AFM) to further characterize nanoparticles located near the surface of the cell. Also, nanoparticle characteristics such as size, shape, charge, and mast cell-nanoparticle exposure time could be varied, so as to take advantage of the versatility of gold nanoparticles.

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