Nano technique allows precise injection of living cells

June 14 2007

Specialized pulsed lasers have been used to inject individual cells with a variety of materials, but little is known about how this type of injection might affect living cells. For the first time, researchers at Rensselaer Polytechnic Institute have analyzed this nanoscale injection process on living cells and discovered that minor changes in the intensity of the laser could mark the difference between a healthy cell and a dead one.

The findings will be presented by lead researcher Ingrid Wilke, assistant professor of physics at Rensselaer, at the World of Photonics Congress in Munich, Germany on June 20, 2007. The research originally appeared in the April 2007 edition of *Physical Review E.*

Human illness begins and advances at the cellular level. Understanding how materials like proteins or drug ingredients affect an individual cell can give researchers important insight into how that material might impact the entire human body, according to Wilke. This makes discoveries at the cellular level extremely important.

The new findings could serve as a set of guidelines for future research that requires precise microinjection of live single cells. Such research ranges from testing drugs for toxicity to targeting tumor cells with chemotherapy.

“The technique will allow researchers to use unprecedented precision to microinject cells or even perform nanosurgery on cells,” Wilke said.

“The problem with previous methods of single-cell injection was low cell viability and low efficacy,” Wilke said. Other physical microinjection methods are greatly hindered in living cells by the natural protective shield encasing mammalian cells. Breaking through this strong, microscopic fortress while still keeping the cell alive and undamaged has proven extremely difficult.

The researchers used tightly focused femtosecond laser beam pulses that created a pore or opening in the cellular wall of living cells and encouraged the cell to take in different molecules. The laser beam serves as a “needle” that punctures the protective skin around the cell, encouraging the cell to take up the material surrounding it. In this case, the researchers used a yellow iodine dye as their nanoscale “vaccine” so the injection results could be easily viewed in microscopic images.

A femtosecond is one billionth of one millionth of a second. The pulse from a femtosecond laser is so fast that it appears as a constant beam of light to the naked eye. The lasers emit radiation in the near-infrared (NIR) portion of the spectrum, meaning that the wavelength is too long to be seen by human eyes.

Upon analysis, the femtosecond NIR lasers were found to preserve the integrity of the cells, Wilke said. But only up to a certain intensity.

“The connections between laser intensity and the rate of injection had not been previously explored in-depth,” Wilke said. “We found that the size of the pores was highly dependent on the intensity of the laser. By modifying the strength of the laser, we could encourage the cell to uptake as little or as much of the materials as we desired. We also determined the intensity at which the cell could first be permeated and the level at which to would be disintegrated.”

The researchers first microinjected living bovine aortic cells. They were

able to create different sized pores within the cells that would remain open while the laser continued to pulse and close after the laser beam was stopped.

They later expanded the experiment to include clam eggs (Spisula solidissima oocytes). This form of microinjection is particularly important for cells that are resistant to any other forms of physical microinjection due to an extremely tough cellular membrane, Wilke said. The team also was able to microinject the clam eggs using the femtosecond NIR pulses.

The research discovered that cells were permeated at laser intensities of 4 terawatts per square centimeter. The pore size grew larger as the intensity increased. When the intensity reached more than 35 terawatts per square centimeter, the cellular structure disintegrated and the cell was no longer viable.

“For the first time, we have shown a relationship between pore characteristics and laser beam intensity,” Wilke said. This level of control has not been previously quantified and Wilke says it will allow better regulation of the concentrations of molecules injected into cells.

Source: Rensselaer Polytechnic Institute