New technique sees into tissue at greater depth, resolution

September 17 2008

By coupling a kicked-up version of microscopy with miniscule particles of gold, Duke University scientists are now able to peer so deep into living tissue that they can see molecules interacting.

If future studies in animal models prove fruitful, the researchers believe that their new approach can have a wide spectrum of clinical applications, from studying the margins of a tumor as it is removed from the body to assessing the effects of anti-cancer agents on the blood vessels that nourish tumors.

The Duke bioengineers combined tightly focused heat with optical coherence tomography (OCT), which has often been called the optical equivalent of ultrasound. OCT is commonly used in medical clinics where imaging at the highest resolution is critical, such as in the retina. These experiments represent the first time the technique has been extended to the functional imaging of cells expressing particular molecular receptors.

"This technique could possibly augment traditional methods of deep-tissue molecular imaging with a relatively high resolution," said Melissa Skala, a postdoctoral fellow working in the laboratory of Joseph Izatt, professor of biomedical engineering in Duke's Pratt School of Engineering. "Not only were we able to get better images, we were able to specifically target the types of cells we were looking for."

The results of the Duke research were posted on line by *Nano Letters,* a

journal published by the American Chemical Society. The research was supported by the National Institutes of Health.

For their experiments, the Duke team attached nanospheres of gold to a targeting molecule known as a monoclonal antibody.

Gold is a metal that not only is an efficient conductor of heat, but whose effects in the body are well known. The antibody they used targets epidermal growth factor receptor (EGFR), a cell-surface receptor implicated in cancer.

These "tagged" antibodies were then applied to the surface of a three-dimensional tissue model composed of human cells – both cancerous and non-cancerous. Skala hoped that these antibodies would home in on cells that were overproducing EGFR on their surfaces, an indicator of cancerous activity. Then the photothermal OCT would be able to detect them by showing where the gold spheres were concentrated.

"When we directed the photothermal OCT at the tissue, we found that the cells that were overexpressing EGFR gave off a signal 300 percent higher than cells with low expressions of EGFR," Skala said.

Adding heat to this form of microscopy technique created a phenomenon much like that seen on very hot days, when portions of the pavement far in the distance seem to float or hover above the road.

"The heat causes a distortion in the way light is reflected off the gold nanospheres in a characteristic way," Skala explained. "As we changed the temperature, the light pathways would change in measurable ways."

In this manner, Skala explained, they were not only able to "see" cells within the tissue, but they were able to capture the molecular function of an antibody attaching to a receptor.

"The use of metal nanoparticles as contrast agents with photothermal OCT technology could lead to a host of potential clinical applications," Izatt said. "Organically-based contrast agents can cause damage or death to the targeted cells, while metal nanospheres are relatively safer."

"Also, given the wide range of nanoparticle shapes and sizes, coupled with the ability to 'tune" the optical wavelength of the OCT, we can customize our approach to many different target types," Izatt said.

Skala plans to expand the use of this approach in animal models to better understand the role of different cancer therapies. Tumors with elevated levels of EGFR are known to have a poor prognosis, and she plans to use photothermal OCT to measure how these tumor types react to different therapies.

Source: Duke University