An eye-opening method for imaging probe delivery

After a severe stroke or head injury, patients hardly benefit from surgical procedures involving their brains. Yet to assess the extent of post-trauma neuron repair or blood vessel proliferation, medical practitioners usually have to extract a brain tissue sample. Although important for planning correct treatment, the process may slow recovery and could lead to complications. In response to this problem, Philip K. Liu and colleagues at Massachusetts General Hospital and at Harvard Medical School, both in Charlestown, have created a fully non-invasive diagnostic technique that could be modified to report a wide range of disorders.

Inspiration for the scientists’ work came from a series of observations, starting with the fact that conditions causing blood-brain barrier leakage allow neuronal magnetic resonance probes to penetrate otherwise sealed-off brain cells. Delivery of the probes, they found, could be achieved invasively through infusion into the cerebral ventricles. Ventricular fluid, however, flows into the lymphatic system — such as the lymph vessels under the conjunctival sac of the eye. Thus, the team reasoned, the probes could be delivered painlessly to patients’ brains via eyedrops.

Before testing their idea using an animal model, the researchers created a pair of composite probes. The first component of each comprised superparamagnetic iron oxide nanoparticles that appeared clearly in magnetic resonance images and that showed good retention in brain cells. To lend the particles binding specificity for intracellular targets of interest, the scientists linked them to short DNA sequences. For detection of gliosis — fibrous outgrowth of cells called glia or astrocytes in a damaged brain — the target for binding was the messenger RNA for glial fibrillary acidic protein. For comparison, the researchers chose to target the messenger RNA of β-actin, a protein found in several nonglial cell types whose production changes little immediately after injury.

Because the brain probe will not reach healthy brains, the investigators had to artificially induce blood-brain barrier rupture in mice prior to delivery to simulate clinical conditions. Reducing blood supply in the cerebral cortex, inflicting puncture wounds or blocking the carotid arteries promoted leakage in experimental mice and mimicked minimal injury, significant trauma and cardiac arrest, respectively. Corresponding control animals underwent mock procedures. The researchers later instilled a solution containing one of the composite probes into the rodents’ eyes.

With a Bruker MRI device, the scientists next generated T2 images and subtraction R2 maps. Using the eyedrops containing gliosis-specific composite on mice that had experienced cortex disruption, the researchers verified barrier leakage and probe effectiveness. Proceeding to brain maps of animals with healing puncture wounds and artery blockage, they found that the probe clustered in regions where subsequent histological examination revealed actual pathology. The β-actin probe also showed expected accumulation patterns in experimental mice, while control animals had normal MRI results in all cases.

The investigators’ findings show not only the possibility of noninvasive delivery but also the potential for probe-linked DNA sequences to reveal the effects of conditions ranging from mental illness to spinal cord injury. They hope to apply their technique to these contexts in the future. Nonetheless, before the method reaches hospitals, researchers must conduct clinical trials on humans to address issues such as dosage, window of detection and toxicity of iron particles. Once perfected, Liu explained, the probe could prove comparatively user-friendly: Patients could use the drops at home and continue their daily routine before the magnetic resonance procedure.

Michael J. Lander

Trapping organelles in droplets helps keep track of them

There are several ways to study particles smaller than the diffraction limit if you pin them down to a substrate — atomic force microscopy is but one possible technique to use. Counting or measuring such tiny particles while they are moving about freely in solution, however, does not work so well. Light-scattering methods are used but lack high sensitivity. Fluorescence correlation spectroscopy and particle tracking with video imaging and software analysis are feasible, but these techniques are not as effective as they could be because particles can move in and out of the imaging area.

Now researchers at the University of Washington in Seattle, led by Daniel T.
Chiu, have developed a technique to study nanometer-scale particles by first trapping them in a very small volume of solution — droplets only about 10 µm in diameter.

The investigators placed several aqueous droplets with a scattering of their target particles — rat synaptic vesicles or nanobeads — onto a coverslip, then identified those droplets that had between one and five particles to measure. They excited the bead or the dye-tagged vesicles with a 488-nm beam from a Coherent laser or with a 633-nm beam from a Coherent HeNe laser. The researchers designed the system to split and recombine the beams to provide both confocal and epifluorescence illumination. They used a Nikon microscope with a 1.45-NA
objective to direct the beam and to collect the emissions.

For particle tracking, the scientists used a CCD camera made by Roper Scientific of Tucson, Ariz.; for fluorescence correlation spectroscopy, they used an avalanche photodiode from PerkinElmer Optoelectronics of Fremont, Calif., along with an autocorrelator from Correlator.com of Bridgewater, N.J.

According to the researchers, the particle-tracking technique works best with bright particles and with particles larger than about 100 nm, which are less susceptible to photobleaching or to damage caused by the constant illumination of the imaging area.

On the other hand, correlation spectroscopy is better suited to smaller, dimmer particles because a smaller region of the droplet is illuminated and, thus, the sampling rate can be higher.

Images acquired with the particle-tracking technique showed the path of a single bead as it moved across the droplet—not the size and shape. Therefore, the investigators had to calculate the optimum exposure time and frame rate to maximize detection sensitivity and to minimize blurring.

In the fluorescence correlation spectroscopy experiments, they found that several factors affected accurate sizing. One was the proximity of the particles to the inside wall of the droplet: The wake caused by the particle in motion increases the friction between the particle and the wall, slowing the particle and, therefore, affecting measurements.

Other factors that affect sizing and counting include surface charges of the particles being studied, the surface properties of the substrate, the ionic and buffer strength of the solution, and the properties of any surfactants that might be used. The investigators noted, however, that it was not critical to eliminate these and other factors as long as only the particle's trajectories in the center of the droplet were used—a condition that they satisfied by “parking” the laser probe volume in the center of the droplet.

According to Chiu, the investigators will use the droplet technique to study synapses from other animals as well as mitochondria and other subcellular organelles.

“We want to integrate this capability with other droplet microfluidic techniques we developed as well as with other single-molecule separation and analysis techniques that we have been working on,” he said.

Lynn M. Savage

To err is human, but study shows mistakes may be predicted using MRI

People performing routine, monotonous tasks occasionally are prone to making mistakes. According to Tom Eichele from the University of Bergen in Norway, examining the trial-by-trial dynamics in functional MRI activation patterns before, during and after an error could provide a better understanding of how performance monitoring works compared with just looking at the average activation after errors are made, as has been done in previous studies.

In functional MRI, stimulus-related activity is delayed and convoluted by the hemodynamic response, and the data typically are analyzed with a time series model. To examine the trial-by-trial dynamics, the scientists needed to recover

A study shows that MRI may be used to predict mistakes made while performing routine, monotonous tasks. Data analysis identified four independent components that predicted errors. The activation maps for these components are shown rendered onto transverse slices. Activation areas are depicted in red, deactivations in blue. Reprinted with permission of PNAS.