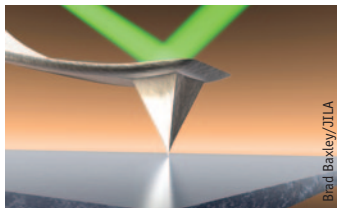
BIOPHYSICS

Upgrades for the AFM

Researchers continue to improve the performance of the atomic force microscope.



Changes in the composition and use of AFM cantilevers improve performance.

The atomic force microscope (AFM) was a technological wonder when it was first developed. Performance limits have prompted some researchers to use alternative techniques such as the optical trap, but improvements to AFM ensure that it remains a relevant and important technology for measuring forces on single biomolecules with picoNewton-force precision and imaging molecules with subnanometer resolution. Three groups describe additional improvements to this versatile workhorse.

Thomas Perkins and his group use both optical traps and AFMs, but they are continuously working to enhance the AFM. They now describe an intriguing, and perhaps counterintuitive, finding that uncovers a simple way to improve the force stability and precision of the AFM for force spectroscopy (Churnside *et al.*, 2012). Most commercial AFM tips are coated in gold to improve the optical signal of the measurement laser reflected off the AFM cantilever. They show that removing the gold reduced force drift during force spectroscopy measurements performed in liquid over the course of 2 hours by 10- to 60-fold. As expected, gold removal also reduced reflectivity by about tenfold, but the lower positional noise was sufficient to obtain subpicoNewton-force precision superior to the precision of gold-coated cantilevers.

Although force spectroscopy requires soft cantilevers, stiffer cantilevers are used for imaging. These can easily perturb a biological specimen. A tapping-mode AFM, whose tip is vertically oscillated over the sample, allows tracking of the tip-sample interaction and thus surface imaging with minimal perturbation. The use of smaller cantilevers and faster oscillation has improved imaging resolution, but when using photothermal control of very small cantilevers

CELL BIOLOGY

A BLOOD-BRAIN BARRIER IN A DISH

Codifferentiating human pluripotent stem cells along neural and endothelial lineages provides cues to efficiently generate blood-brain barrier endothelial cells.

Human pluripotent stem cells (hPSCs) are exciting for many reasons. Perhaps the most talked about is their potential for cell therapy. Equally compelling, however, is that they are a source of human cell types that are otherwise difficult to obtain and hence to study in the lab. The recent derivation of blood-brain barrier (BBB) endothelial cells from hPSCs by Eric Shusta and Sean Palecek at the University of Wisconsin–Madison is a case in point.

Shusta and colleagues have been developing animal and human BBB models in the culture dish for years. But animals, rodents in particular, do not yield substantial numbers of brain endothelial cells; furthermore, there are functional differences between animal and human BBB. Primary cells of the human BBB can be acquired from autopsied tissues or from resected brain samples of patients with epilepsy or tumors, but these specimens are not easily obtainable. Shusta hoped to solve all these problems by teaming up with Palecek and his group, who have expertise with human pluripotent stem cells.

The collaboration has proven fruitful. The researchers knew that signals from developing neural cells in the early embryonic brain are important for promoting the development of the specialized endothelial cells that form the BBB, the brain microvascular endothelial cells (BMECs). They reasoned that codifferentiation of hPSCs into neural and endothelial cells could mimic conditions in the embryonic brain and yield BMECs.

The researchers cultured hPSC lines under standard culture conditions, on Matrigel and in mTESR1 medium, and subsequently induced differentiation. Within a week or so, they observed a mixture of mainly neural progenitors, immature neurons and endothelial cells in the cultures. A subsequent switch to culture medium containing growth factors known

in liquid, the hydrodynamic forces cause the resonant frequency to change dramatically as the cantilever approaches the surface. With conventional frequency tracking of the cantilever, this can result in tip-mediated sample damage. Hoogenboom and colleagues show that continuous adjustment of the reference frequency during the cantilever approach to the sample limits unwanted interactions and enhances imaging resolution (Leung *et al*., 2012). Using the method to image DNA in an aqueous environment, they distinguished different structural conformations and what appeared to be the major and minor grooves.

Reproducibility of force spectroscopy experiments relies on accuracy, and this typically is achieved by determining the unique spring constant of the AFM cantilever. Unfortunately, measuring this spring constant is difficult and often neglected. One established technique relies on adding a defined mass to the cantilever, but this is challenging and destructive. Gibson and colleagues take the opposite tactic and use a focused ion beam to remove a defined mass from the cantilever (Slattery *et al*., 2012). Perhaps surprisingly, this had a negligible effect on the cantilever's mechanical properties, and the modified cantilever could continue to be used. Unfortunately, the technique is less accurate for many popular softer cantilevers and those coated in a dense material such as gold. But for researchers with suitable cantilevers and access to a focused ion beam, the method will make accurate AFM measurements easier.

"Since all three methods consider different aspects of improving the force precision and accuracy of AFM, they could all be applied in parallel," says Perkins. "Each method is addressing different instrumental issues, but collectively they will continue to improve the AFM's ability to image, pull and manipulate material on the nanoscale."

Daniel Evanko

RESEARCH PAPERS

Churnside, A.B. *et al.* Routine and timely sub-picoNewton force stability and precision for biological applications of atomic force microscopy. *Nano Lett.* **12**, 3557–3561 (2012).

Leung, C. *et al.* Atomic force microscopy with nanoscale cantilevers resolves different structural conformations of the DNA double helix. *Nano Lett.* **12**, 3846–3850 (2012).

Slattery, A.D. *et al.* Atomic force microscope cantilever calibration using a focused ion beam. *Nanotechnology* **23**, 285704 (2012).

to promote endothelial differentiation yielded up to 60% endothelial cells with a marker profile suggesting they were BMECs.

To test the function of these cells and establish their BMEC subtype beyond doubt, it was necessary to purify them. Shusta, Palecek and colleagues found that this was easily accomplished by passaging the cells onto a collagen-fibronectin extracellular matrix routinely used for culturing primary BMECs. The resulting pure population of cells formed tight endothelial monolayers with the expected marker profiles, high electrical resistance that increased upon exposure to astrocytes, and substrate transport properties typical of BMECs.

These combined differentiation and purification methods will yield sufficiently pure populations of functional endothelial cells for use in screens for drug permeability or modulators of BBB function. But that is not the only application of this system. "We hope to use this for blood-brain barrier science," says Shusta. "One could model aspects of neurological disease or investigate blood-brain barrier development." Indeed, the researchers used the coculture system to demonstrate that Wnts are a component of the cues with which neural cells promote differentiation of BMECs, in keeping with previous work.

Will this type of coculture model be useful for differentiation of hPSCs to other cell types? Sean Palecek thinks so. "This is a strategy that's fairly promising when you don't know the specific cues that are required to generate a certain cell type. You can create an environment in the culture dish similar to what they would see during development and systematically identify factors important for lineage specification," he says. He cautions, however, that extending this strategy to cellular subtypes from organs that develop later than the brain may prove to be more of a challenge.

Natalie de Souza

RESEARCH PAPERS

Lippmann, E.S. *et al.* Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. *Nat. Biotechnol.* advance online publication (24 June 2012).