BIOMEMS

Building with cells

New ways to interact with biological cells *in vitro* offer greater levels of control over their location and milieu — much as they would experience in real tissues *in vivo*. Such microscale control reveals new insights into their biology and may lead to new technologies.

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The function of cells is intimately tied to their three-dimensional organization, which would be fairly easy to control if cells were the size of marbles. But they're not. Because cells typically are micrometres in size, researchers can't simply pick them up with their fingers and position them as they wish. They need to use technology to interface with cells. A major theme at a symposium* at the Materials Research Society (MRS) Spring Meeting in San Francisco was the progress made in this quest. Essentially, if we can't make cells bigger, then we'll just have to make our tools smaller.

One approach to organizing multicellular structures uses BioMEMS. BioMEMS is perhaps the least imprecise term to describe the use of nanometre- or micrometre-scale 'features' to interface with bioscience, be it basic biology, medicine or industrial biology. BioMEMS gets its name from MEMS, which are micro-electromechanical systems, though most BioMEMS have very little in common with traditional MEMS. BioMEMS rarely involve silicon — cheap materials and optical transparency are often needed — or moving parts, a hallmark of electromechanical systems. For biotechnology, moving parts represent weak links, and given all the other unknowns in biology, avoiding extra hassle is a good thing.

Although the early applications of BioMEMS were in analytical (labs-on-a-chip) and biomedical instrumentation, the scope has expanded dramatically in the past few years. The stimulus was the development of technologies, such as soft lithography[1], that enabled the micropatterning of 'soft' materials (such as proteins and cells) with semiconductor precision. Several pioneering studies elegantly showed how positioning cells could enhance both cellular understanding and control.

For the former, scientists micropatterned arrays of micrometre-sized spots of extracellular matrix (ECM) proteins (onto which certain cells attach) surrounded by non-adhesive regions. They created either single cell-sized spots or multiple subcellular spots, both with the same projected area. Because the cells could only attach to the substrate where there was ECM, this allowed them to vary the cell–ECM contact area independently of the projected size of the cell, leading to the discovery that the cell size and not the cell–ECM contact area determines whether a cell dies or divides[2].

On the latter front, growing appreciation that tissue function is highly dependent on the microscale three-dimensional arrangement of its constituent cells inspired researchers to replicate this arrangement *in vitro*. Early work micropatterning liver cells in defined two-dimensional arrangements with support cells led to the discovery that liver-cell function was enhanced at the interface between the two cell types, and thus that defined positioning of cells could enhance the function of artificial tissues[3].

In both of these cases, the ability to position cells at the micrometre scale enabled further discoveries. Several reports at the MRS symposium expanded on these themes. Moving beyond two-dimensional cell arrangements is necessary for recreating *in vitro* the tissue complexity — and thus function — found *in vivo*. But after micropatterning one layer of cells on top of their ECM, researchers found that repeating another micropatterning step would squash the cells. One clever approach to creating three-dimensional

* *Biomicroelectromechanical Systems (BioMEMS) Symposium*, Materials Research Society Spring Meeting, San Francisco, USA, April 21–25, 2003.

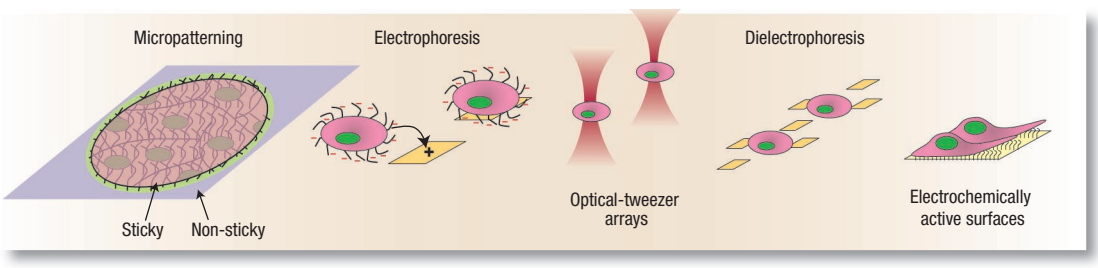

Figure 1 Conventional cell micropatterning is being refined to allow researchers greater influence over cells *in vitro*. From left to right: conventional cell micropatterning using 'sticky' proteins, 'cellular' electrophoresis, optical tweezer arrays, dielectrophoresis, and electrochemically active layers, provide various degrees of control over cell position and environment.

patterns uses microfluidic channels to layer cells on top of each other (T. Desai, Boston Univ.). By repeatedly flowing media containing cells and ECM in the microchannels, they can build up three-dimensional structures on the substrate. The key is to ensure that the deposited layers of cells can endure the fluid shear involved in subsequent patterning steps. Initial work has focused on building artificial arteries *in vitro*, starting with the layering of endothelial cells on top of smooth muscle cells.

Approaches to positioning cells that rely on micropatterning of ECM molecules are quite powerful. However, they fail when one needs to place cells independently of that ECM: because its role is to create 'sticky' regions, cells will attach everywhere within those regions, rather than in desired subregions. In addition, the static nature of ECM micropatterning precludes the formation of dynamic cell patterns. Alternative approaches thus seek to actively create patterns of cells, adding in functionality at the cost of increased complexity and lower throughput. Just as charged molecules can be moved (and separated) in electric fields using electrophoresis, cells — which have negatively charged membranes — can be guided to specific locations using 'cellular' electrophoresis (M. Ozkan, University of California, Riverside). Once in position, they can be actively held in place by using fibre-optic bundles to create arrays of optical tweezers (J. Tam, Tufts University, Medford, Massachusetts) — or their electrical analogue — dielectrophoretic systems (J. Voldman, Massachusetts Institute of Technology). Using self-assembled monolayers that can electrochemically release ligands — and the cells attached to them — is yet another approach that turns the passive ECM into an active player (M. Mrksich, Univ. Chicago).

Instead of repositioning the cells, sometimes much can be gained by keeping cells fixed and circulating the liquid in which they sit. By taking advantage of the relatively slow mixing times of microfluidic flows, researchers have been able to create spatial gradients in reagent concentrations that are sharp enough to go from 100% on one side of an individual biological cell to 0% on the other[4]. This enables stimulation of one side of a cell but not the other, in contrast to conventional reagent flows that bathe the whole cell in the molecule of interest.

This has led to the observation of unexpected glucocorticoid receptor (GR) signalling dynamics (P. Leduc, Carnegie Mellon Univ.). The GR is an intracellular receptor that on binding to a ligand translocates to the nucleus and activates transcription. By stimulating only one side of a cell with the ligand and watching fluorescently tagged GR, researchers discovered that instead of directly entering the nucleus along the shortest path, activated GR actually shuttles around the nucleus from the stimulated side of the cell to the unstimulated side of the cell before entering the nucleus. The cause of this dynamic behaviour remains unclear, but this example and the others described above, highlight the power derived from placing cells at the micrometre scale; new ways to manipulate cells lead to new discoveries.

References

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