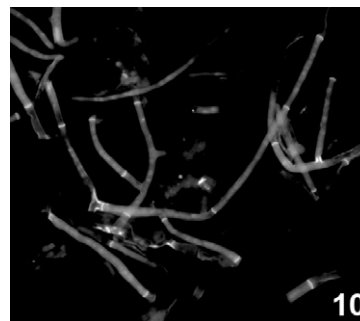


BioSpotlight

Fungal Homokaryons

Delivering plasmid DNA into cells to create mutant lines is a common and well-established procedure for bacteria, plant, and animal cells. However, this procedure becomes complicated when it comes to filamentous fungi because the long branching hyphae cells that form the mycelia are multinucleate. In these fungi, the introduced DNA plasmid will often recombine into a single nucleus by either homologous or ectopic recombination, resulting in heterokaryons that are not suitable for genetic analyses. In some species, homokaryotic clones can be created by multiple rounds of single spore isolation; in others, hyphal tip transfer is required, which depends on extensive technical skills as well as several culturing rounds before a pure genetic clone is obtained. Using the plant pathogen *Sclerotinia sclerotiorum*, Z. Dallal Bashi, G. Kahachatourians, and D. Hegedus (University of Saskatchewan, Saskatoon, Canada) developed a technique for isolating homokaryons following *Agrobacterium tumefaciens* transformation using ultrasound pulses to generate biseptate unicellular structures. Mycelial fragments with a single nucleus containing the desired sequence can then be identified by colony PCR. To demonstrate their new technique, the authors transformed *S. sclerotiorum* mycelial fragments with a construct wherein the *smk3* gene, which encodes a protein involved in cell wall formation and function, was interrupted by a hygromycin resistance cassette. Starting from colonies grown in selective media, the authors identified those with the highest ratio of transformed nuclei to wild-type nuclei, as demonstrated by PCR, and used them to develop homokaryotic lines. The mycelia were then sonicated and samples with the



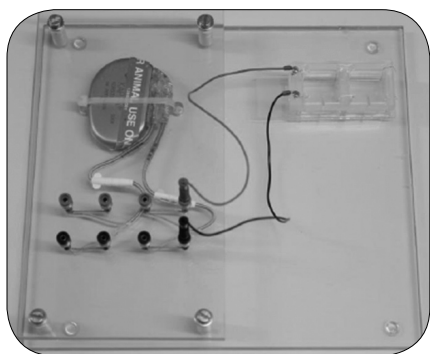
Fluorescence microscopy of *S. sclerotiorum* mycelia fragmented by sonication.

highest number of biseptate fragments and lowest number of multicellular hyphae were plated on selection medium. Reverse transcription PCR and sequencing of *smk3* alleles revealed that mycelia were homokaryotic, contained the introduced mutation, and that the hygromycin transformation cassette had integrated by homologous recombination. This study demonstrates that sonic disruption of mycelia can generate unicellular structures more quickly and simply than traditional hyphal tip transfer, creating a viable new alternative for homokaryon selection.

(See “Isolation of fungal homokaryotic lines from heterokaryotic transformants by sonic disruption of mycelia” on page 41.)

Keeping Pace

Researchers often use primary cultures of neonatal rat ventricular cardiomyocytes (NRVCMs) to study the transcription of specific cardiac genes. Electric pulses continuously stimulate heart contractions in vivo, and lead to morphological and functional changes in cardiomyocyte populations as well as changes in the expression of cardiac-



Pacemaker fixed to a polycarbonate platform.

specific genes. However, it is difficult to mimic these events in the lab. While systems exist for in vitro stimulation of cardiomyocytes or engineered cardiac tissue, they are cumbersome and are not designed to work within standard tissue culture incubators. Even when modified to accommodate the high humidity and temperatures present within incubators, the pulsing systems must be plugged into power outlets, requiring researchers to run cords through the doors or other openings in the incubators, potentially compromising sterility. So, in spite of the potential benefits of electric stimulation when studying cardiomyocyte biology, many researchers still work on unstimulated cultures. To address this, R. Martherus, V. Zeijlemaker, and T. Ayoubi at Maastricht University (Maastricht, The Netherlands) describe their design of a portable culture system for cardiomyocyte stimulation that delivers electric pulses using standard medical cardiac pacemakers, which are battery-operated, sterilizable, and small enough to easily fit

within the confines of most incubators. In their system, cells are cultured inside commercially available chamber slides, which are amenable to live-cell imaging, immunohistochemistry, and molecular analysis. The authors designed reusable polycarbonate lids for the chamber slides with platinum electrodes running along opposite sides of each chamber. The pacemakers were then connected to the electrodes and programmed to deliver timed pulses in patterns resembling the required excitation levels for normal ventricular tissue. Using an optimized NRVCM culture protocol, the authors obtained reproducible expression from luciferase reporter constructs that were electric pulse-dependent. This culture protocol and pacemaker-controlled system allows the isolation and stimulation of pure cardiomyocyte cultures with excellent contractile properties.

(See “Electrical stimulation of primary neonatal rat ventricular cardiomyocytes using pacemakers” on page 65.)

Straight Cut

When it comes to dissecting cells from tissue samples, researchers have a couple of options these days. One option is to use laser-capture microdissection technologies that are precise and accurate to slice out their cells of interest. But this approach tends to generate heat and UV light from the laser that is potentially damaging to samples; not to mention that these instruments can be costly. Another approach, called piezo-powered microdissection, relies on the rapid vibration of a microchisel to cut through tissue samples. This approach to microdissection is less expensive than most laser-based approaches, but suffers when it comes to cutting precision and accuracy due to the unwanted motion generated as the microchisel vibrates. To counter this, U. Terpitz and D. Zimmermann at the Max Planck Institute of Biophysics (Frankfurt am Main, Germany) present a simple but elegant modification for microchisels that improves both the precision and accuracy of piezo-powered microdissection, even for more elastic samples such as live plant tissues. Typically, a microchisel operates by oscillating in a backward and forward (vertical) mode to cut—the

faster and stronger the oscillation, the easier it is to cut more elastic tissue samples. The challenge, however, is to find a way to enhance sample cutting in the vertical mode by increasing vibration frequency and amplitude, while minimizing the lateral vibrations of the microchisel, which cause less precise dissection. Terpitz and Zimmermann's solution was to limit those lateral vibrations by encasing the microchisel in a glass capillary that was filled with a UV-hardened adhesive. This modification allowed the authors to increase the frequency and amplitude of vibration, while at the same time reducing the lateral vibrations. This increased the accuracy and precision two-fold. They demonstrated the advantages of their modified microchisel by dissecting guard cells from leaf epidermis of the plant species *Phaseolus vulgaris*. The authors go on to suggest that their modification will find application to tissue types other than plants, and in general make it easier for researchers to isolate specific cell samples in the future.

(See “Isolation of guard cells from fresh epidermis using a piezo-power microdissection system with vibration-attenuated needles” on page 68.)

Realistic Patterning

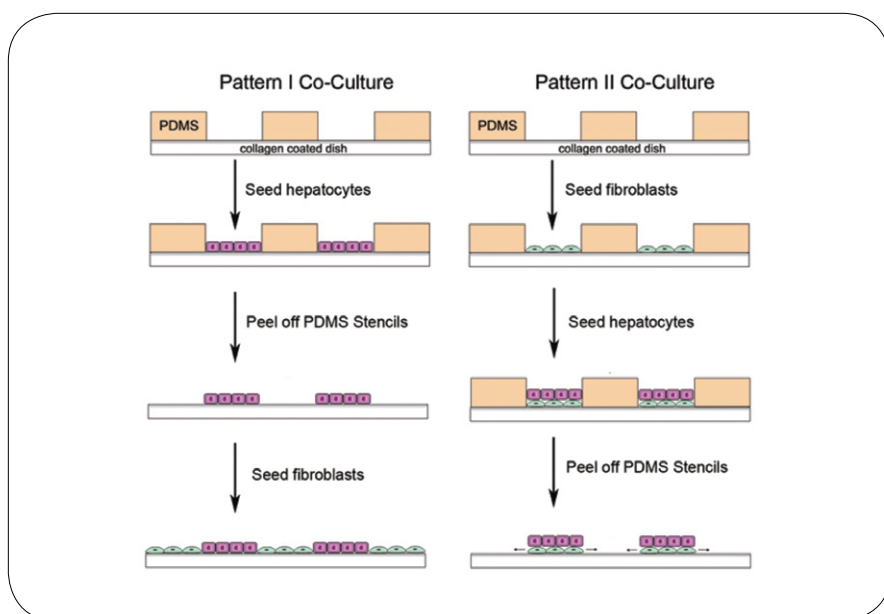
The ability to mimic the *in vivo* world in an *in vitro* setting is critical to a deeper understanding of basic biological function as well as to realizing the clinical potential from fields such as tissue engineering. Culturing cells has traditionally been performed in two dimensions, on plastic plates which lack the structure, signals, and cellular interactions often found in an *in vivo* environment. For tissue engineering to be applied to possible therapeutic purposes,

better methods to culture cells, either alone or in combinations, have to be found. An emerging area of tissue engineering research involves hepatocytes and hepatic tissues for the treatment of liver disease. While researchers have found that co-culturing hepatocytes with non-parenchymal cells can help in long-term culturing of hepatocytes that retain their basic functions, these co-culture approaches still lack the spatial signaling and cellular interactions that have been observed to occur among hepatocytes *in vivo*. Cho and colleagues

at Harvard Medical School (Boston, MA) advance the culturing of these cells through the use of a microfabricated stencil to culture hepatocytes that not only retain basic function, but are actually enhanced in their liver functions and morphology when compared to cells cultured using previous approaches. The authors designed polydimethylsiloxane (PDMS) stencils to create wells where they initially seeded fibroblasts. They then seeded hepatocytes on top of the fibroblasts so that when the stencil was removed, the fibroblasts could proliferate underneath the hepatocytes within islands of co-cultured cells. The authors found that the hepatocytes in these islands displayed well-developed hepatocyte morphology with clearly demarcated cellular borders, and exhibited many of the functional hallmarks of hepatocytes including production and storage of glycogen and urea synthesis. The authors suggest that their new cell micropatterning technique will not only be useful for understanding the basic biology of hepatocytes and their applications to tissue engineering efforts, but should be applicable to other cell types and microenvironments as well.

(See “Layered patterning of hepatocytes in co-culture systems using microfabricated stencils” on page 47.)

Written by Nathan Blow, Ph.D. and Kristie Nybo, Ph.D. 



Schematic diagram of two different hepatocyte/fibroblast co-culture techniques using PDMS stencils.

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