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Parafilm Dependant Cell Patterning

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We present a novel yet simple technique to perform cell patterning on glass substrates, compatible with long term cell culture having high stability and reproducibility. Several methods have been described for cell patterning based on microcontact printing and deposition of cellophilic material such as fibronectin or laminin [1-4] or based on creating microchannels from an etched structure physically limiting the area for growth of cells [5]. Unfortunately none of the described methods is readily available or achievable in most cell biological laboratories.

Coverslips, 25 mm in diameter, were used as a substrate. Parafilm M (Pechiney Plastic Packaging Inc, Neenah, WI) was applied to the coverslip by stretching the film and folding the corners around the edges of the coverslip. During stretching the parafilm becomes electro-statically charged which facilitates a tight seal to the glass coverslip. The high surface tension of the hydrophobic parafilm constrains cell suspension to only the exposed glass substrate. In the parafilm the desired patterns were cut using a razorblade and removed with forceps creating a mask for the cell culture. The patterns used here were narrow lines (200 μm wide), with individual lines separated by short distances of approximately 200 μm, or with triangular shapes.

COS 7 ($1x10^5$ cells/ml) kidney epithelial cells in suspension were seeded onto the patterned areas with volumes of 10-20 μl. The cells were allowed to settle on the coverslip and adhere during 1 hour incubation at 37 ºC. Parafilm was then removed and non-adherent cells were washed away by gentle washing in PBS. Coverslips were then cultured in Petri dishes for up to 96 hours before inspection. To verify the viability of the cells after patterning they were stained with the live cell stain calcein AM (1 μM, Molecular Probes, Eugene, OR). To distinguish between cells placed in adjacent patterns, cells in suspension were pre-stained with calcein AM and calcein red-orange AM (Molecular Probes) respectively. Photomicrographs of the resulting cell patterns were recorded using a fluorescence microscope equipped with appropriate filters. Surprisingly, the calcein pre-stain was not stable for the long time cultivation experiments. Therefore, we used COS 7 cells stably expressing GFP and wild type (WT) non-fluorescent COS 7 cells identified using a combination of fluorescence and bright field microscopy.

Cells seeded in parallel line patterns with spacing of 200 microns resulted in an almost perfect line (Fig. 1). However, some cells were found in areas that had been covered by the parafilm. Parallel lines separated by a 200 μm gap and filled with two populations of cells, calcein pre-stained (green) and calcein red-orange (red), showed the same almost perfect line shape with minimal contamination of the parafilm covered area (Fig. 2).

It is well known that cells placed on a coverslip will grow and migrate over time. We placed GFP and WT COS 7 cells in two adjacent lines and inspected the culture 96 hours after removal of parafilm. We observed cell migration into the border area that resulted in a mixed population of GFP and WT cells (Fig. 3). The sharp tip of a triangular pattern (Fig. 4) shows that more complex shapes can be constructed; the limitation of the patterning is clearly in the precision with which the parafilm can be cut.

We have demonstrated a technique for the patterning of cells on glass coverslips using materials and techniques that are readily available in all laboratories doing cell culture work. The limitations of the method are in the precision of the patterning mask. It should be straight forward to construct a punch template for the patterning where the precision of the mask can be improved. Also the process of making the mask would be simplified by punching the pattern instead of manually cutting with a razor blade.

References

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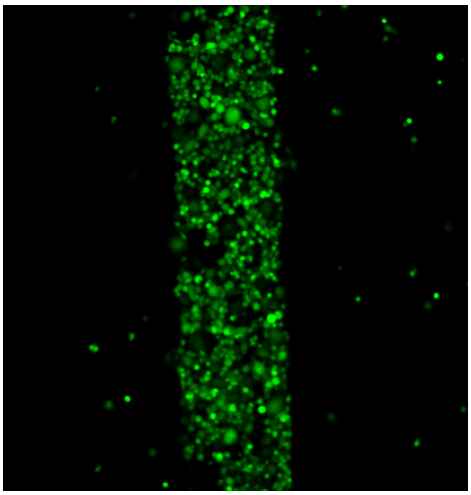

Fig. 1. Cells stained with calcein, seeded in a line pattern width of 200 µm.

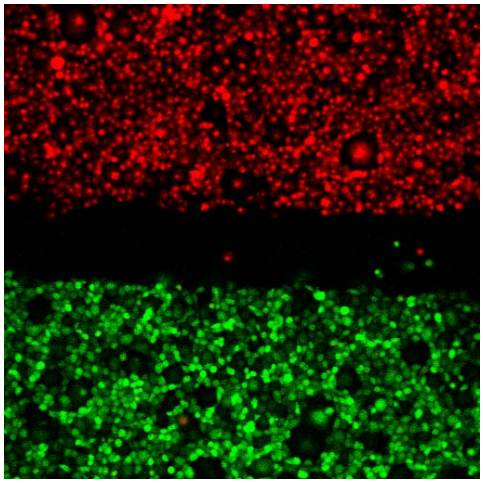

Fig. 2. Cells seeded in two parallel line patterns separated by a 200 µm parafilm gap, stained with calcein (green) and calcein red-orange (red).

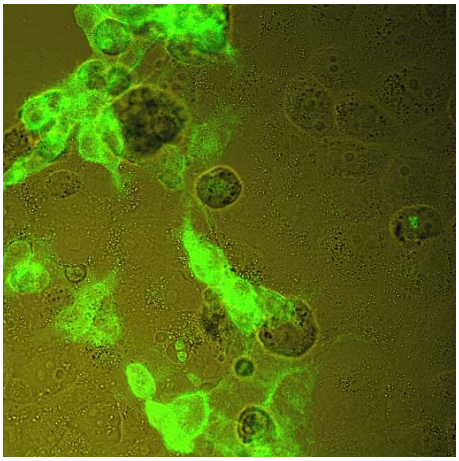

Fig. 3. Cells 96 hours after patterning in two parallel line structures separated by a 200 µm gap. Cells with GFP and WT non-fluorescent cells have migrated and formed a mixed population.

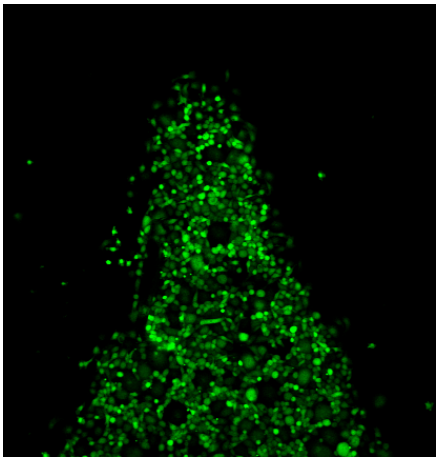

Fig. 4. Cells stained with calcein and seeded in a triangular pattern. The sharp structure in the tip demonstrates that complex shapes of cell cultures can be made, limited by the precision of the patterning mask.

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