COMMENTARY

New ways to print living cells promise breakthroughs for engineering complex tissues *in vitro*

Ginger S. WITHERS¹

Department of Biology, Whitman College, 345 Boyer Ave., Walla Walla, WA 99362, U.S.A.

The ability to control the placement of cells and the assembly of networks *in vitro* has tremendous potential for understanding the regulation of development as well as for generating artificial tissues. To date, most engineering tools that can place materials with precision are not compatible with the requirements of living cells, and so approaches to tissue engineering have focused on patterning substrates as a way of controlling cell growth rather than patterning cells directly. In this issue of *Biochemical Journal*, however, Eagles et al. adapt electrohydrodynamic printing tech-

nology to 'print' living cells from a neuronal cell line on to a substrate. The importance of this approach is that it has the potential for unprecedented control over the position of cells in culture by directly placing them, thus allowing for the systematic assembly of cell networks.

Key words: electrospraying, inkjet printing, microfabrication, neural engineering, neuronal culture, tissue engineering.

INTRODUCTION

A major challenge in the field of bioengineering is to develop methods that will allow biologists to model the spatial complexity of multicellular networks as they assemble into the specialized tissues of the body. If spatial patterning of cells can be modelled *in vitro*, then artificial circuits can be constructed in an environment that is accessible to manipulation, facilitating the study of cell–cell and cell–environment interactions. Ultimately, the ability to orchestrate cell assembly into a tissue-like organization will lead to new strategies for repairing tissues when they are diseased or damaged, and could provide a potential source of replacement tissue. In addition, it will enable the construction of biosensors that can be used to facilitate the design and testing of new drugs, or can serve as sentinels to detect the presence of compounds in the environment.

Although the concept of tissue engineering, and its therapeutic promise, is relatively simple to describe, the reality of modelling biological tissues is far more complex. One basic problem that must be solved is how to place cells with precision in a culture system. In many tissues, the position of cells is critical to their function. For example, in some organs, cells of a single type may be arranged in a sheet, or positioned in complex arrays with multiple other cell types interspersed. In other words, location matters. In cultures made from dissociated cells, some, if not all, of this anatomical order is lost. These cells are grown under conditions that facilitate survival and ideally promote their assembly into a rudimentary network of cells, but the initial dispersion of cells is random and uncontrolled. Thus the location of individual cells is left largely to chance. To begin to model a complex tissue in three dimensions, or even to create a more 'simple' network of cells in only two dimensions, requires that cells be positioned in such a way as to mimic the intercellular relationships that occur naturally *in situ*.

BIOENGINEERING APPROACHES THAT SEEK TO IMPOSE BIOLOGICAL ORDER *IN VITRO*

Many technological advances in engineering are currently being adapted to applications in biology [1]. For example, nano- and micro-fabrication tools offer the ability to create and control patterns of materials with submicrometre precision. Using micro-fabrication to pattern adhesive molecules, although indirect, is one strategy that has been frequently used to organize cells *in vitro* [2,3]. With this approach, the size and shape of an adhesive substrate can be varied to control the distribution of cells and to influence the possible directions of cell migration or growth. Although selective patterning of a substrate can be used to influence where cells attach along a surface, these approaches are indirect and still rely on chance for a cell to 'fall' on the pattern, rather than putting the desired cell at a specific predetermined location. Why not use these approaches to selectively place cells? Unfortunately, many of the tools used for fabrication cannot be used to place cells directly on to a surface, because they are not compatible with the requirements of living cells and tissues. Either the patterning procedure uses harsh chemicals, as is the case with photolithography, or drying steps, as in microcontact printing (i.e. elastomer stamping) (see [4] for a review of these methods as they apply to cell patterning). In addition, microfabrication equipment is highly specialized and not readily available to many biologists.

NEW APPROACHES CONTROL THE PLACEMENT OF LIVING CELLS

Just as advances in microfabrication have spurred technical developments in bioengineering, advances in printing technology are translating into biological applications [5,6]. Inkjet nozzles can eject a drop of ink on to a surface with relatively high (20–30 μm) accuracy. Considering the fine patterns that can be printed with inkjet printers, the notion of printing similarly precise

¹ email withergs@whitman.edu

patterns of biological material is not a stretch of the imagination. And, if these printers can be modified to print substrates or cells, their availability and affordability would make them routine tools of the research laboratory.

Recent reports have shown that an inkjet printer can be modified to put live cells on to a substrate [7,8]. Xu et al. [8] printed two different kinds of mammalian cells, an epithelial cell line [CHO (Chinese-hamster ovary) cells] and rat embryonic motoneurons, with good viability through the printing process. Survival rates beyond several days, however, appeared to be compromised, indicating potential limitations in this approach. The inkjet printing process is harsh, with very high temperatures (in excess of 300°C) and shear force (so high it is difficult to measure), generated in the nozzle. Most cells, especially delicate ones like neurons, are likely to be compromised during this process. In addition, because the tip of the nozzle is not much bigger than a cell body (20–30 µm), cells can clog the tip.

These technical challenges may now have been overcome through the use of a modified version of the inkjet. In this issue of *Biochemical Journal*, Eagles et al. [9] demonstrate that EHDJ (electrohydrodynamic jet) printing technology can be modified to deposit droplets of living cells from a nervous-system-derived cell line. Applying a voltage of approx. 30 kV between an electrode and the print nozzle causes the contents of the nozzle to be expelled and disperse into droplets. Although the tip of the nozzle in inkjet printing is restricted in size, an EHDJ nozzle can be much larger, reducing the shear force and the chance of clogging. Two exciting findings from this paper are that: (1) CAD cells, a neuron-like cell line, can withstand the applied voltages used in electrospraying, attach to the substrate and remain viable, suggesting that neurons could withstand the printing procedure as well, and (2) 1 month after printing, at least some cells that survived the printing procedure could be induced to extend processes and take on a neuron-like phenotype. Now that living cells from a nervous-system-derived cell line have been printed on to a substrate successfully, the answers to two key questions will test the potential of this new EHDJ technique. The first question addresses the precision with which cell ejection can be controlled. For example, can cells be printed to a specific predetermined spot on a surface? The second question addresses the robustness of the method. Will neurons that have been dissociated directly from nervous system tissue withstand the EHDJ printing procedure? If fragile primary neurons can be printed accurately, then this new method will open the door to high-precision neural-circuit building.

FUTURE PROSPECTS FOR PRINTING CELLS

If individual neurons can be placed with precision, then it should be possible to combine printing techniques to control cell placement with methods that can pattern substrate molecules. The combination of these two approaches will give both spatial and temporal control over the formation of neuronal circuits, allowing scientists to tease apart essential elements of how these networks form and determine what kinds of communication take place within them. For example, microfabrication has already been used to align a substrate pattern with an electrode array, increasing the probability that cells will contact a recording electrode [10]. If cells can be placed on to electrodes with precision, then the patterned substrate could be a molecule that promotes a specific

aspect of development (e.g. synapse formation), rather than simply facilitating adhesion. Less elegant, but effective and readily available, methods of substrate placement could also be combined with cell printing to engineer cellular networks that control the spatial and temporal interactions between cells and the microenvironment in which they grow. For example, an atomizer could spray dots of a growth-promoting molecule on top of a general cell adhesive. This 'micro-island' approach has been used previously to study synapse formation [11]. Alternatively, stripes of a molecule can be made to create lanes of attractive or repulsive cues that alter cell growth [12].

A more ambitious goal is to use this technology to produce three-dimensional cultures of neural or other cell tissues. Although EHDJ has not yet been tested for this purpose, other printing technologies have been used to develop new methods in three-dimensional tissue engineering [5]. These kinds of 'organ printing' and three-dimensional tissue cultures are still in their infancy in terms of technical development and proof of concept, but they hold promise for understanding mechanisms that control migration of cells, outgrowth of neuronal processes and regeneration. Ultimately, engineered tissues can be used to construct devices for therapeutic purposes or to serve as biosensors. Regardless of when these more ambitious projects will be realized, the ability to place living cells selectively on to substrates alone has tremendous potential for testing the basic rules of cell development and for building controlled cell networks.

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REFERENCES

- 1 Stevens, M. M. and George, J. H. (2005) Exploring and engineering the cell surface interface. *Science* **310**, 1135–1138
- 2 Oliva, A. A., James, C. D., Kingman, C. E., Craighead, H. G. and Banker, G. A. (2003) Patterning axonal guidance molecules using a novel strategy for microcontact printing. *Neurochem. Res.* **28**, 1639–1648
- 3 Kane, R. S., Takayama, S., Ostuni, E., Ingber, D. E. and Whitesides, G. M. (1999) Patterning proteins and cells using soft lithography. *Biomaterials* **20**, 2363–2376
- 4 Park, T. H. and Shuler, M. L. (2003) Integration of cell culture and microfabrication technology. *Biotechnol. Prog.* **19**, 243–253
- 5 Mironov, V., Boland, T., Trusk, T., Forgacs, G. and Markwald, R. R. (2003) Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* **21**, 157–161
- 6 Sanjana, N. E. and Fuller, S. B. (2004) A fast flexible ink-jet printing method for patterning dissociated neurons in culture. *J. Neurosci. Methods* **136**, 151–163
- 7 Roth, E. A., Xu, T., Das, M., Gregory, C., Hickman, J. J. and Boland, T. (2004) Inkjet printing for high-throughput cell patterning. *Biomaterials* **25**, 3707–3715
- 8 Xu, T., Jin, J., Gregory, C., Hickman, J. J. and Boland, T. (2005) Inkjet printing of viable mammalian cells. *Biomaterials* **26**, 93–99
- 9 Eagles, P. A. M., Qureshi, A. N. and Jayasinghe, S. N. (2006) Electrohydrodynamic jetting of mouse neuronal cells. *Biochem. J.* **394**, 375–378
- 10 James, C. D., Davis, R. C., Meyer, M., Perez, A., Turner, S., Withers, G., Kam, L., Banker, G., Craighead, H. G., Isaacson, M. et al. (2000) Aligned microcontact printing of micrometer-scale poly-L-lysine structures for controlled growth of cultured neurons on planar microelectrode arrays. *IEEE Trans. Biomed. Eng.* **47**, 17–21
- 11 Rao, A., Cha, E. M. and Craig, A. M. (2000) Mismatched appositions of presynaptic and postsynaptic components in isolated hippocampal neurons. *J. Neurosci.* **20**, 8344–8353
- 12 Vielmetter, J., Stolze, B., Bonhoeffer, F. and Stuermer, C. A. (1990) *In vitro* assay to test differential substrate affinities of growing axons and migratory cells. *Exp. Brain Res.* **81**, 283–287

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