Emerging Applications of Hydrogels and Microscale Technologies in Drug Discovery

a report by
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Cell-based *in vitro* assays are emerging as an integral aspect of today's drug discovery process for screening chemical libraries. These assays can be used to screen molecular libraries for their effects on cell behaviour such as proliferation, viability, differentiation and migration. Currently, many cell-based *in vitro* culture systems are performed by seeding cells within multi-well dishes. These types of systems have become widely used for many screening processes and significant investment has been put on their standardisation for drug discovery (such as the development of liquid-handling robotic systems). However, despite their merits, these assays do not necessarily predict the function of drugs *in vivo*, which has resulted in a large number of failed drug candidates in animal experiments and clinical trials. Therefore, novel methods that can improve the predictability of the performance of drugs in the body can be useful in minimising the high costs associated with finding and validating new drugs.

Cells in the body are exposed to a complex milieu regulated by their interactions with other cells, the surrounding cell matrix and soluble factors. A key element of this microenvironment is the 3-D architecture of the extracellular matrix (ECM). Upon removing cells from this microenvironment many cell types, such as liver cells, quickly lose their function. Therefore, the ability to mimic the *in vivo* microenvironment of cells outside the body could be a potentially powerful tool in increasing the predictive ability of cell-based assays. Here we discuss the emergence of two distinct technologies that, either independently or in combination, promise to improve cell-based assays for drug discovery and diagnostics applications.

Microscale Technologies

The ability to control the cellular microenvironment with microscale resolution is a powerful tool in fabricating cell-based assays, directing stem cell fate and constructing tissue engineered constructs. Microscale technologies, which were traditionally used in the microelectronics industry to fabricate computer chips, have recently emerged as a useful approach to control the various aspects of the cellular microenvironment (see *Figure 1*). In particular, the development of techniques, such as soft lithography, that can be cheaply and easily used to fabricate micro- and nano-devices without the need for microfabrication facilities, has greatly enhanced the widespread application of microscale technologies in drug discovery.

Microscale technologies have emerged as a powerful tool to pattern cells on substrates. In this approach, techniques such as microcontact printing and micromoulding are used to generate adhesive micropatterns on substrates. These techniques have been used to create arrays of cells as well as to control the shape of individual cells. Furthermore, by using these techniques novel biological insights have been gained regarding the effects of cell shape on apoptosis and differentiation. Micropatterning techniques are a powerful method in standardising *in vitro* drug discovery assays since they can be easily incorporated within the microwell systems used currently for drug screening.

To control the degree of cell–cell contact between different cell types, patterned co-culture techniques have been developed. In these techniques the degree of homotypic and heterotypic cell–cell interactions can be controlled on 2-D substrates by generating micropatterns of multiple cells on a substrate. Patterned co-cultures have been used to control the degree of cell–cell interactions between hepatocytes and non-parenchymal cells. These cultures have been shown to maintain hepatocyte function in culture at elevated levels in comparison with other methods. Furthermore, patterned co-cultures of human embryonic stem cells and feeder cells have been shown to maintain phenotype of embryonic stem cells and prevent their differentiation. The ease of integration of these technologies into currently applied screening assays makes them highly useful for drug discovery.

Cell–fluid phase interactions can be also be regulated using microscale technologies. For example, by using microfluidic arrays it is possible to miniaturise experiments to enable high-throughput

experimentation. In addition, by generating gradients of molecules within microchannels it is possible to simultaneously test the effects of different concentrations of molecules on the cells. Although current microfluidic-based approaches for generating 3-D cultures have not been standardised for the rigorous and robust methods required for high-throughput screening, the promise of these technologies is immense.

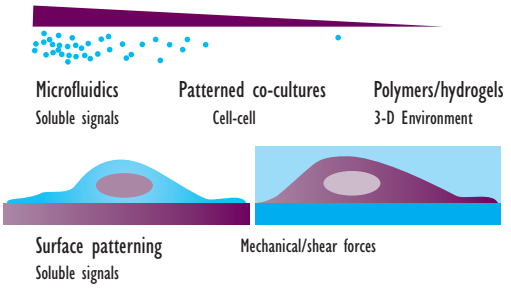
3-D Matrices

The development of novel 3-D matrices is a powerful addition to the existing cell-based assays. Materials such as hydrogels, i.e. crosslinked networks of hydrophilic polymers, can be used to mimic the 3-D architecture of the cells, and provide key signalling molecules. A number of studies have demonstrated that cells in 3-D matrices have an improved function relative to their culture on 2-D substrates. Ideally the 3-D matrices should mimic the structure and biological function of native ECM as much as possible, both in terms of chemical composition and physical structure. It is known that in addition to providing a physical support for the cells, native ECM also provides a substrate with specific ligands for cell adhesion and migration, and regulates cellular proliferation and function by providing various growth factors. It is reasonable to expect that an ECM-mimicking 3-D structure will play a similar role to promote tissue regeneration *in vitro* as native ECM does *in vivo*.

Hydrogels can be synthesised using either natural or synthetic polymers (reviewed elsewhere). Natural hydrogels such as collagen and hyaluronic acids are natural components of the cellular microenvironment and provide a natural matrix for cells; however, they are difficult to modify and functionalise. Alternatively, synthetic matrices can be highly controlled to signal the cells, but these matrices require the addition of specific ligands and molecules to make them functional. Furthermore, some of the matrices such as poly(ethylene glycol) (PEG) hydrogels are not biodegradable and cannot be remodelled by cells.

A well-known feature of native ECM structures is the nano-scaled dimensions of their physical structure. In a typical connective tissue, structural protein fibres such as collagen and elastin have diameters ranging from several tens to hundreds of nanometres. The nano-scaled protein fibres entangle with each other to form a non-woven mesh that provides tensile strength and elasticity. Three different approaches to the formation of nano-fibrous materials have emerged: self-assembly, electrospinning and phase separation. Each of these approaches has a unique set of characteristics that lends to its development as a scaffolding system. For instance,

Figure 1: Schematic of Various Microengineering Approaches Used to Control the Cellular Microenvironment



self-assembly can generate small diameter nanofibres in the lowest end of the range of natural ECM collagen, while electrospinning can generate large diameter nanofibres on the upper end of the range of natural ECM collagen. Phase separation, on the other hand, has generated nanofibres in the same range as natural ECM collagen and allows for the design of macroporous structures. These attempts at an artificial ECM have the potential to accommodate cells, to guide their growth and to encourage subsequent tissue regeneration. Self-assembly, that is, the autonomous organisation of molecules into patterns or substrates without human intervention, is common throughout nature and technology. Self-assembly of natural or synthetic macromolecules produces nano-scaled supramolecular structures and nanofibres. Specifically designed amphiphilic peptides that contain a carbon alkyl tail and several other functional peptide regions have been synthesised and shown to form nanofibres through a self-assembly process by mixing cell suspensions in media with dilute aqueous solutions of the peptide amphiphile (PA). These self-assembled nanofibres have been used recently to study selective differentiation of neural progenitor cells. Therefore, self-assembled nanofibres may have great applications in recreating the 3-D microenvironment of cells for cell-based assays.

Microengineered 3-D Matrices

The merger of microscale technologies with 3-D matrices promises to provide a powerful method for controlling the cellular microenvironment. By using micromoulding approaches to mould biomaterials into desired structures various approaches have been developed to generate 3-D tissue culture systems. In some of these approaches, micromoulded biomaterials have been used to generate templates for creating cellular aggregates. For example, non-adhesive PEG microwells can be used as templates to fabricate controlled arrays of tissue microstructures. Therefore, using this technology, cell spheroids that better maintain cell function can be uniformly generated and used for screening.

Additionally, micromoulding approaches can be used to microencapsulate cells within 3-D microgels. These microgels minimise the oxygen and nutrient diffusion limitations that are associated with larger hydrogels. For example, by using micromoulding and photolithographic approaches, 3-D cell-laden hydrogel microarrays have been fabricated. Furthermore, such techniques have been used to fabricate microscale tissue-like structures using a modular approach in which collagen micromoulded structures were laden with endothelial cells and packed densely to create tissue-like structures. An alternative method to using hydrogels for 3-D fabrication of microscale tissues for cell-based assays is to fabricate 3-D hydrogel structures with macropores. Macropores aid delivery of reagents and nutrients to cells and therefore can be used to mimic the microvasculature system of tissues by enabling the delivery of nutrients and the removal of waste products. The recently emerged technologies that can microfabricate microfluidic channels within hydrogels can be used to generate microscale tissues with controlled hydrogels. Although such technologies have not been used for tissue culture technologies to date, their possible application for fabricating 3-D engineered systems is of great potential for drug discovery.

Conclusions

The current limitations concerning the functional predictability of *in-vitro* cell-based assays may be improved by combining microengineering approaches and 3-D matrices. These approaches can be used to control various aspects of the cellular microenvironment and enable high-throughput studies. A significant barrier to incorporating microscale technologies and 3-D hydrogels into existing high-throughput assays is the lack of data on the robustness and standardisation of the techniques relative to the already existing assays. To incorporate these assays they must be standardised and optimised for today's existing drug discovery assays. Despite these challenges, the ability of these technologies to increase the predictability of *in vitro* assays makes them useful for improving today's high-throughput cell-based assays. ■

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