CHAPTER 3

Emerging Technology of Hydrogels in Drug Discovery

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Summary

Material design for cell proliferation and differentiation is one of the key technologies for tissue engineering. Three dimensional (3D) structures have been investigated to enhance cells proliferation because they have larger surface for cell attachment and proliferation. As the design of culture method, it is important to create the local environment suitable for cells cultured, such as oxygen, nutrient, and the metabolic environment as close as possible to natural ones. The conventional static culture method cannot sufficiently supply oxygen and nutrition to cells in the 3D structures and exclude cell wastes for their proliferation and differentiation. Therefore, it is important to design an environment and culture method to facilitate and enhance the viability and functions of cells while it promotes matrix synthesis by cells cultured in 3D structures. One approach is design of highly hydrated networks (3D hydrogel) which are sufficient for survival of large numbers of cells for extended periods of time through diffusion of nutrients, bioactive factors, and oxygen. The objective of this chapter is to provide information about the very recent developmental technologies of 3D hydrogels to control the cellular microenvironment for tissue engineering and drug delivery applications.

KEYWORDS: 3D Culture, Hydrogel, Extracellular Matrix (ECM), Microenvironment; Drug Discovery

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INTRODUCTION

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 behavior 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 standardization 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 minimizing 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 three dimensional (3D) architecture of the extracellular matrix (ECM). By removing the cells from this microenvironment many cell types, such as liver cells, quickly loose 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 producing 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[1] (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 (Figure 2)[2].

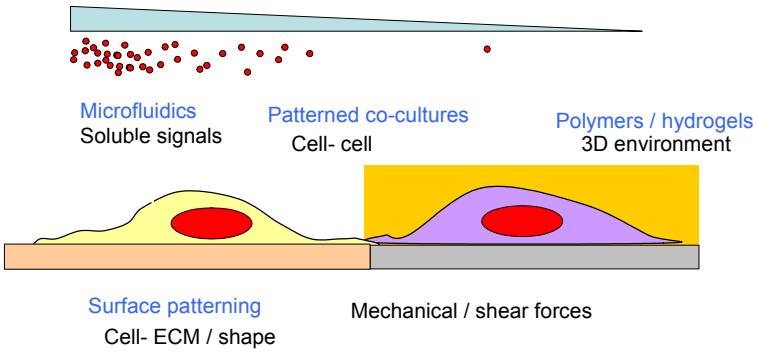

Figure 1. A schematic of various microengineering approaches used to control the cellular microenvironment.

Microscale technologies have emerged as a powerful tool to pattern cells on substrates. In this approach, techniques such as microcontact printing and micromolding 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[3] and differentiation[4]. Micropatterning techniques are a powerful method in standardizing *in vitro* drug discovery assays since they can be easily incorporated within the microwell systems used currently for drug screening.

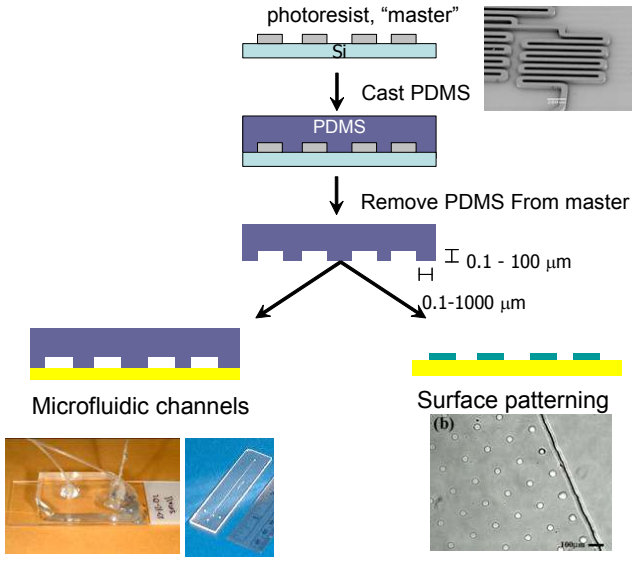

Figure 2. Soft lithographic approaches to fabricate microfluidic channels and to pattern substrates.

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 2D 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[5]. 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[6]. The ease of integration of these technologies into currently applied screening assays makes them highly useful for drug discovery.

Cell-fluid phase interactions can also be regulated using microscale technologies. For example, by using microfluidic arrays it is possible to miniaturize 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[7]. Although, current microfluidic based approaches for generating 3D cultures have not been standardized for the rigorous and robust methods required for high-throughput screening, the promise of these technologies is immense

3D MATRICES

The development of novel 3D 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 3D architecture of the extrecellular matrix (ECM), and provide key signaling molecules. A number of studies have demonstrated that cells in 3D matrices have an improved function relative to their culture on 2D substrates[8,9]. Ideally the 3D 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 3D structure will play a similar role to promote tissue regeneration *in vitro* as native ECM does *in vivo*.

Hydrogels can be synthesized using either natural or synthetic polymers (reviewed elsewhere[10]). 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 functionalize. Alternatively, synthetic matrices can be highly controlled to signal the cells, however, 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 can not be remodeled 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 fibers such as collagen and elastin have diameters ranging from several tens to hundreds of nanometers. The nano-scaled protein fibers entangle with each other to form a nonwoven mesh that provides tensile strength and elasticity. Three different approaches toward the formation of nano-fibrous materials have emerged: self-assembly, electrospinning, and phase separation. Each of these approaches has a unique set of characteristics, which lends to its development as a scaffolding system. For instance, self-assembly can generate small diameter nanofibers in the lowest end of the range of natural ECM, while electrospinning can generate large diameter nanofibers on the upper end of the range of natural ECM. Phase separation, on the other hand, has generated nanofibers in the same range as natural ECM collagen and allows for the design of macroporous structures. These artificial ECMs have the potential to accommodate cells, to guide their growth and to encourage subsequent tissue regeneration. Self-assembly, that is, the autonomous organization of molecules into patterns or substrates without human intervention, are common throughout nature and technology. Self-assembly of natural or synthetic macromolecules produces nano-scaled supramolecular structures and nanofibers. Specifically designed amphiphilic peptides that contain a carbon alkyl tail and several other functional peptide regions have been synthesized and shown to form nanofibers through self-assembly process by mixing cell suspensions in media with dilute aqueous solutions of the peptide amphiphile (PA)[11]. These self-assembled nanofibers have been used recently to study selective differentiation of neural progenitor cells[12]. Therefore, self-assembled nanofibers may have great applications in recreating the 3D microenvironment of cells for cell-based assays.

MICROENGINEERED 3D MATRICES

The merge of microscale technologies with 3D matrices promises to provide a powerful method for controlling the cellular microenvironment. By using micromolding approaches to mold biomaterials into desired structures various approaches have been developed to generate 3D tissue culture systems (Figure 3). In some of these approaches, micromolded 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.

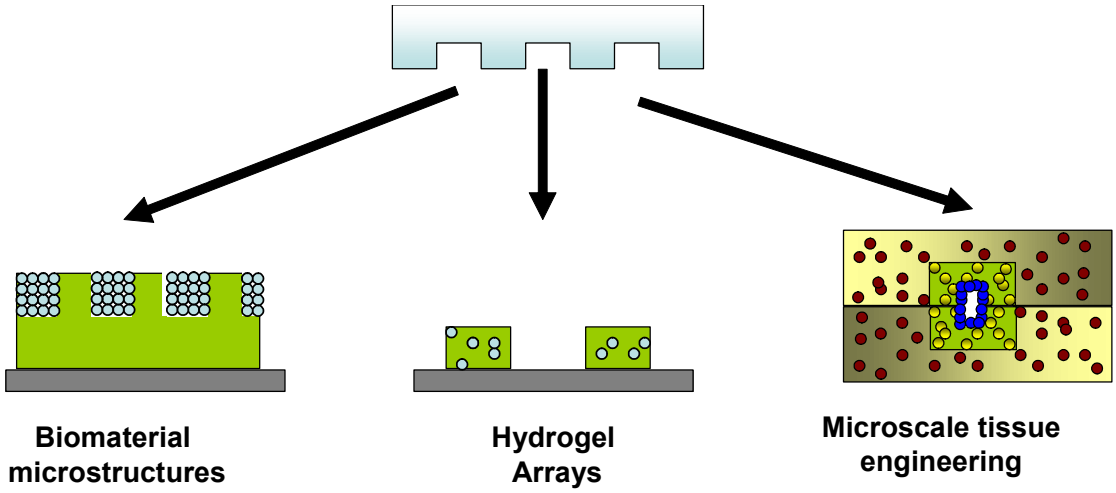

Figure 3. Micromolding of biomaterials for drug discovery. Microscale hydrogels can be used as templates for generating 3D cellular aggregates (Left), to fabricate cell-laden hydrogel microarrays (Center) or to fabricate microscale tissue engineered constructs that mimic native tissue architecture (Right).

Additionally, micromolding approaches can also be used to microencapsulate cells within 3D microgels. These microgels minimize oxygen and nutrient diffusion limitations that are associated with larger hydrogels. For example, by using micromolding and photolithographic approaches, 3D cell laden hydrogel microarrays have been fabricated (Figure 4) [13,14]. Furthermore, such techniques have been used to fabricate microscale tissue-like structures using a modular approach in which collagen micromolded structures were laden with endothelial cells and packed densely to create a tissue like structures[15].

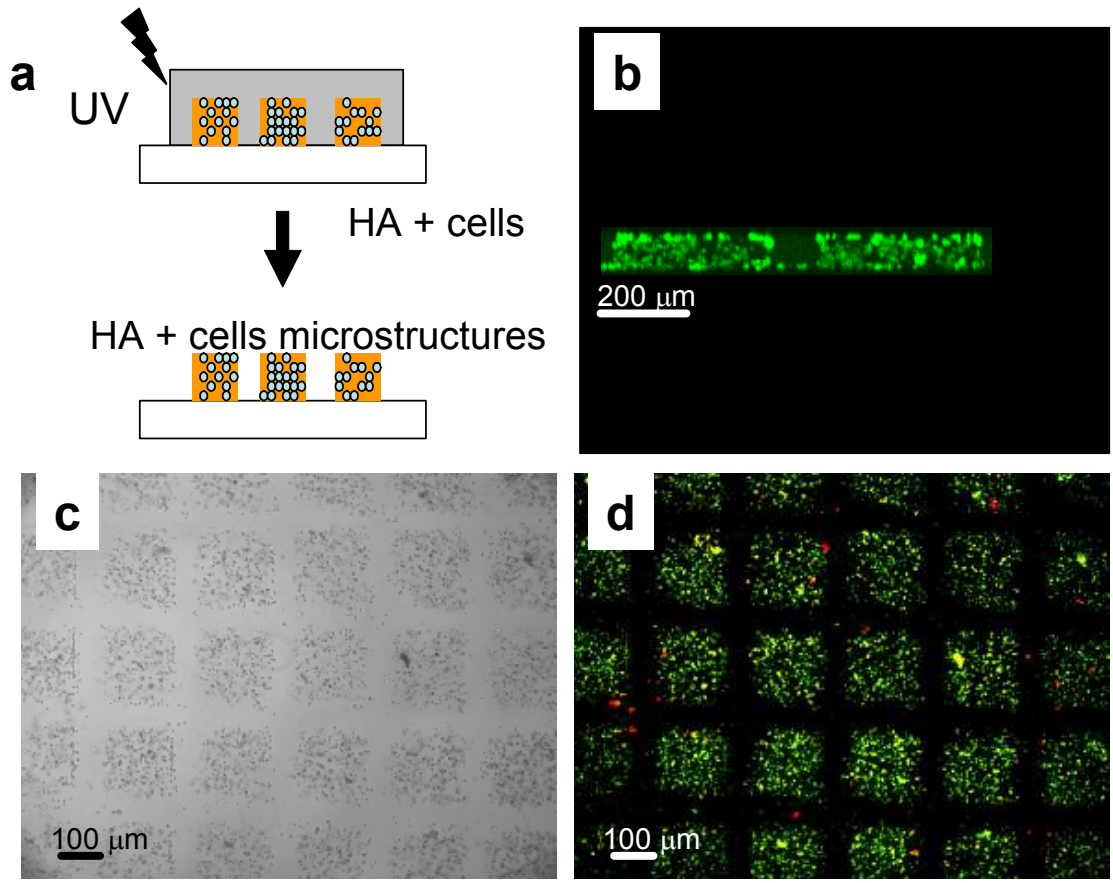

Figure 4: Cell laden hydrogel microarrays. (a) Cell laden hydrogel microarrays were fabricated by molding photocrosslinkable hydrogel precursors containing cells and then exposing the substrate to UV light. (b) Confocal microscopy image of cells encapsulated within HA hydrogels. (c) light and (d) fluorescent images the hydrogel microarrays containing cells that were stained with live (green) /dead (red) assay. As it can be seen, most cells stay alive in the hydrogels.

An alternative method to using hydrogels for 3D fabrication of microscale tissues for cell-based assays is to fabricate 3D hydrogel structures with macropores. Macropores aid to deliver 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 recent emergence of technologies to microfabricate microfluidic channels within hydrogels[16,17], can be used to generate microscale tissues with controlled hydrogels. Although presently, such technologies have not been used for tissue culture technologies, their potential applications for fabricating 3D engineered systems is of great potential for drug discovery (Figure 5).

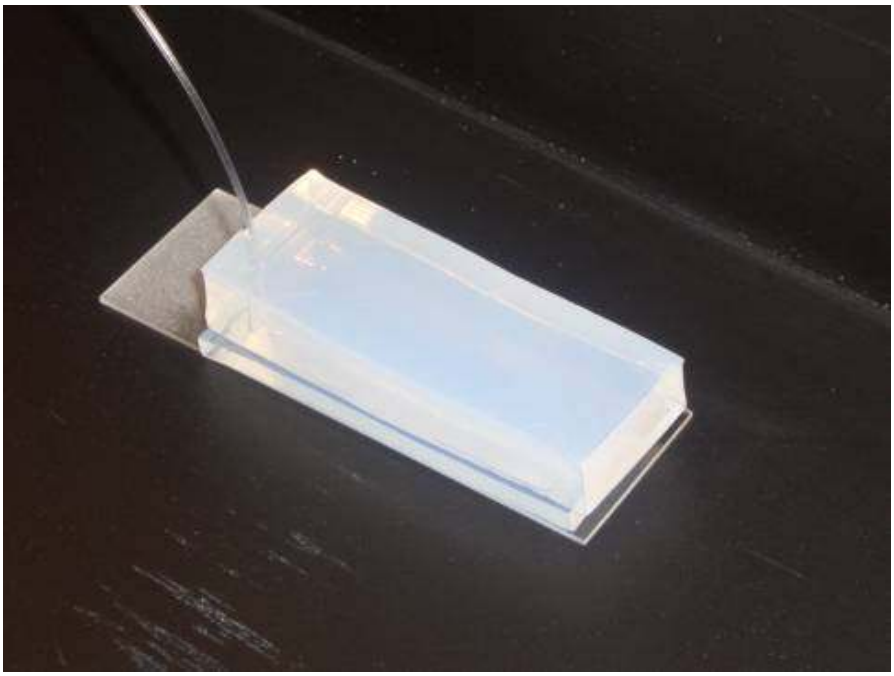

Figure 5: A hydrogel microfluidic device made from agarose.

CONCLUSIONS

The current limitations concerning the functional predictability of *in vitro* cell-based assays may be improved by combining microengineering approaches and 3D matrices. These approaches can be used to control various aspects of the cellular microenvironment and enable high-throughput studies. A significant barrier to incorporate microscale technologies and 3D hydrogels into existing high-throughput assays is the lack of current data on the robustness and standardization of the techniques relative to the already existing assays. To incorporate these assays they must be standardized and optimized 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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References

1. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci U S A 2006;103(8):2480-2487.
2. Whitesides GM, Ostuni E, Takayama S, Jiang XY, Ingber DE. Soft lithography in biology and biochemistry. Annual Review of Biomedical Engineering 2001;3:335-373.
3. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276(5317):1425-8.
4. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 2004;6(4):483-95.
5. Bhatia SN, Yarmush ML, Toner M. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. J Biomed Mater Res 1997;34(2):189-99.
6. Khademhosseini A, Ferreira L, Blumling J, 3rd, Yeh J, Karp JM, Fukuda J, Langer R. Co-culture of human embryonic stem cells with murine embryonic fibroblasts on microwell-patterned substrates. Biomaterials 2006.
7. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. Lab Chip 2005;5(4):401-6.
8. Xie Y, Yang ST, Kniss DA. Three-dimensional cell-scaffold constructs promote efficient gene transfection: implications for cell-based gene therapy. Tissue Eng 2001;7(5):585-98.
9. Mueller-Klieser W. Three-dimensional cell cultures: from molecular mechanisms to clinical applications. Am J Physiol 1997;273(4 Pt 1):C1109-23.
10. Peppas N, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine. Advanced Materials 2006;18:1-17.
11. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. Science 2001;294(5547):1684-8.
12. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science 2004;303(5662):1352-5.
13. Koh WG, Itle LJ, Pishko MV. Molding of hydrogel multiphenotype cell microstructures to create microarrays. Analytical Chemistry 2003;75(21):5783-5789.
14. Khademhosseini A, Eng G, Yeh J, Fukuda J, Blumling J, 3rd, Langer R, Burdick JA. Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment. J Biomed Mater Res A 2006.
15. McGuigan AP, Sefton MV. Vascularized organoid engineered by modular assembly enables blood perfusion. Proc Natl Acad Sci U S A 2006;103(31):11461-6.
16. Cabodi M, Choi NW, Gleghorn JP, Lee CS, Bonassar LJ, Stroock AD. A microfluidic biomaterial. J Am Chem Soc 2005;127(40):13788-9.
17. Paguirigan A, Beebe DJ. Gelatin based microfluidic devices for cell culture. Lab Chip 2006;6(3):407-13.