Microscale Biomaterials for Tissue Engineering 5

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Abstract Microscale and high throughput technologies are powerful tools for addressing many of the challenges in the field of tissue engineering. In this chapter, we present an overview of these technologies and their applications in controlling the cellular microenviroment for tissue engineering applications. We focus on concepts and techniques that can be used to create two- and three-dimensional tissue engineering substrates and scaffolds. Common to these techniques is the ability to control one or more aspects of the cellular microenvironment, including chemical and mechanical cues, cell–cell, cell–matrix,

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and cell-soluble factor interactions. We also discuss recent developments in high throughput techniques that are used to explore the vast number of combinations of factors that comprise the cellular microenvironment.

Keywords Cellular microenvironment • High throughput arrays • Microfabrication • Stem cells

5.1 Introduction

Cellular behavior and the development of tissues and organs occur in response to precise sets of environmental cues. These cues can include interactions with neighbouring cells, ligand binding from molecular signalling, and interactions with the surrounding extracellular matrix (ECM) [1]. These cues and signalling events occur at the micro- and nanometer length scales, the sum of which defines the cellular microenvironment. The successful production of in vitro tissue constructs requires the recapitulation of in vivo microenvironmental factors [2]. It is here that the fields of microscale technologies and tissue engineering intersect [3]. Microscale technologies, some of which have been developed in microelectronics and micro-electro-mechanical systems (MEMS) can control material features from <1 μm to >1 cm, thus providing unprecedented control over the interface between cells and substrate and the surrounding environment [4]. In addition, the biological, chemical, and mechanical properties of biomaterials can also be tuned to further control the cellular miroenvironment.

The cellular microenvironment is comprised of, cell–cell, cell–ECM, and cell-soluble factor interactions. Microscale technologies have been used to control each of these interactions. For example, microengineered wells are used to control cell aggregate size [5, 6]; microcontact printing of cell adhesion proteins is used to control the shape of adhered cells [7–9]; soft lithography techniques can be used to pattern co-cultures [10]; photocrosslinkable hydrogels are used to create cell-laden microgels [11]; microfludic devices are used to create complex soluble factor gradients [12]; and porous scaffolds are used to provide a three-dimensional (3D) environment for cells to reorganize [13].

In tissue engineering, creating an environment with the appropriate biological and mechanical cues in essential. The ideal tissue engineering scaffold must: (1) match the biological and mechanical requirements of the desired tissue construct; (2) be able to support the desired cell types; and (3) be manufacturable. Microscale processing and fabrication impose certain material property criteria on biomaterials, and often the challenge in microscale tissue engineering is balancing the microscale processing criteria with the desired materials properties with respect to the cell culture.

The experimental challenge that tissue engineers face is not only to develop technologies to control the cellular microenvironment but also, to investigate the vast number of possible combinations of different interactions. It is here that technologies such as robotic spotting have begun to make significant advancements [14, 15]. With these methods, researchers

have been able to synthesize and screen biomaterials in a combinatorial manner [16] and investigate combinations of soluble factors and cell–matrix interactions [17, 18].

Over the past decade, the number of fabrication techniques specifically designed for tissue engineering applications has grown considerably. In a number of cases those techniques have been adapted, sometimes with considerable modifications, from the microelectronics industry. Additionally, there has been significant efforts toward the development of microscale fabrication techniques that specifically address challenges in bio- and tissue engineering applications. Microfabrication techniques can be classified into two main groups: (1) techniques for the fabrication of planar, or two-dimensional (2D), surfaces; and (2) techniques for the fabrication of 3D scaffolds. Patterning of surfaces with bioactivity or biological functionalities allows for control over the interface between substrate material and cellular actions, i.e., surfaces are modified to interact with cells in a specific and controllable manner.

In this chapter, we discuss the concepts and techniques that are currently used to fabricate 2D and 3D microscale features for in vitro tissue engineering applications. We focus on those techniques that have been used to control different aspects of the cell microenvironment, including soft lithography, photolithography, and microfluidics. We also discuss robotic spotting techniques that have been used to create microarrays of biomaterials for the combinatorial and high throughput investigation of the cellular microenvironment. Furthermore, we highlight pertinent examples from the scientific literature that demonstrate the success of microscale and high throughput technologies in the field of tissue engineering.

5.2 Microscale Technologies for Engineering Biomaterials and the Cell Microenvironment

Some of the first applications of microscale technologies in biotechnology and tissue engineering were based on surface modification techniques [4, 9]. Since these initial experiments, it has become well established that techniques such as photolithography and soft lithography are able to produce substrates with microscale features that may be useful for biological applications. A great deal of insight into cell biology, bioengineering and tissue engineering has come from investigating the interactions between cells and substrates; however, the complexities of biological systems often extend to a third spatial dimension [19–21]. Fidelity in mimicking the in vivo microenvironment has been the driving force behind microengineering and fabrication of microscale systems with 3D structures. Mimicking complex microarchitectures of native tissues also poses a significant challange. With techniques such as photolithography, micromolding and microfluidics, researchers have begun to study cell biology in ways that were previously not possible. Robotic printing technologies have also recently been used to synthesize and screen biomaterials [16, 17].

Here, we discuss some of the most common techniques that are used to microengineer biomaterials and engineer the cell microenvironment, including photolithography, soft lithography, and microfluidics. We also describe technologies used to create microscale systems for the high throughput and combinatorial investigation of the cell microenvironment, such as

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robotic printing, and microfluidics. Many of these techniques are not mutally exclusive, and often two or more techniques can be used together to produce the desired result.

5.2.1 Photolithography

Initially developed for the microelectronics industry, photolithography is used in biological applications and tissue engineering to pattern substrates with chemical and biological functionalities. Photolithography is broadly defined as the selective modification of a photosensitive surface by illumination through a photomask. The result is selective surface modifications corresponding to the pattern of exposed and masked areas. Subsequent steps can be used to selectively modify the patterned suface.

The resulting microscale patterns of chemical functionalities can be used to pattern cells by selective control of the positioning of adhesion proteins [22]. For example, proteins can be covalently bound to surfaces modified with monolayers of alkanethiol, alkaneamino, or other alkane chains with terminal functional groups. Protein patterns can also be created by selective photoablation of proteins adsorbed on silicon or glass surfaces. It is also possible to create microscale patterns of encapsulating cells in patterns of photocrosslinkable materials.

Similarly, arrays of immoblized DNA have also been created [23]. These patterning and arraying technologies have been used extensively in the early high througput screening technologies, where protein and DNA arrays were created to explore protein ligand and DNA hybridization events in a high throughput manner [24]. While photolithography has been quite successful in biological studies and tissue engineering devices, it is limited to widespread use due to the need for expensive clean room facilites and high equipment costs. An alternate strategy is soft lithography.

5.2.2 Soft Lithography

The microscale fabrication techniques that have been borrowed from the microelectronics industry have been highly successful, but there are several techniques that have been specifically developed for use in chemistry and biology. The most common, and perhaps the most successful, is the family of tools described as soft lithography. The soft lithography techniques stand out as collectively they provide a simple, inexpensive, and reliable option for researchers to produce substrates with microscale chemical, biological, and topographical features [4, 25].

Soft lithography involves the use of an elastomeric material to transfer and replicate patterns of topography and chemical surface modifications with features down to several hundreds of nanometers to a surface of interest. Patterns are first generated on a silicon wafer and then replicated onto an elastomeric mold or stamp. Molds can be used to create microscale topographies or microscale structures made of a moldable biomaterial. Stamps

can be used to transfer patterns of different solutions or "inks", and elastomeric structures can be used to make microfluidic devices.

Poly(dimethyl siloxane) (PDMS) is often the material of choice in soft lithography as it is soft and flexible, transparent to visible and UV light, permeable to oxygen, inexpensive, unreactive, suitable for cell cultures, and the prepolymer is easy to handle and prepare. A solid PDMS mold or stamp is prepared by mixing the prepolymer solution with a curing agent. The mixture must be well mixed and air bubbles removed before pouring the unset mixture onto the template, or into the master mold.

Replica molding and microcontact printing are techniques within the soft lithography family that have become well established in the biological and tissue engineering fields. Both use an elastomeric mold, usually PDMS, with a topography and shape replicated from a patterned template. The template is often a silicon wafer, but can also be other templating materials. The elastomer is peeled away from the template after crosslinking resulting in a negative replica of the template.

In microcontact printing a solution or "ink" is transfered from an elasomeric mold to a substrate. The solution is often a mixture of protein(s) or small molecules, and is only transferred at the interface(s) that make contact [4, 9]. Thus, a replica pattern of the mold of ink is produced on the target substrate. Microcontact printing has been used to create microscale patterns of biocompatible polymers including PEG, and cell adhesion proteins including collagen and fibronection, among others. Replica molding is mainly used for the fabrication of microcontact printing stamps and the production of microscale structures for microfluidic devices, but, it can also be used to create structures to impose geometric constraints in tissue cultures.

5.2.3 Microfluidics

PDMS molds fabricated by replica molding can be used to create microfluidic channels by placing the mold onto a glass substrate. Such microfluidic devices are used in many different applications, and are discussed here as they have been used to create microscale patterns of surface modification in a similar manner as described in the above section on soft lithography [26, 27]. PDMS microfluidic channels are also used to selectively deliver cells and small molecules to substrates or to form topographies at the interface between two reactive fluids [28]. Microfluidic devices can also be used to generate gradients either on surfaces or with soluble factors [29, 30].

In addition to creating soluble factor gradients and surface patterns, microfluidic systems have emerged as a useful option to provide controlled fluid flow to microscale cell cultures. There are a number of examples of microfluidic bioreactors, these types of applications are reviewed elsewhere [31, 32]. Microfluidic bioreactors have been used to investigate cell responses to shear stress and pulsitile flows, as well as being used to mimic vasculature [3, 33]. Generally, these applications are limited to planar systems, as thick 3D structures with intricate microchannels made from biocompatible materials have proven difficult to produce.

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5.2.4 Robotic Spotting and Printing

Robotic spotting technologies have been widely used to produce DNA and protein microarrays. DNA arrays are commonly used for genomic analysis and quantitative gene-expression measurement [34], and microarrayed proteins are used to screen protein–ligand interactions in a high throughput manner [35]. Protein and DNA microarrays are reviewed in detail elsewhere [23, 36–38]. Here, we will describe spotting technologies that have been used to create biomaterial microarrays, and protein arrays for investigating cell–ECM interactions [16, 17]. Additionally, there have been attempts to use robotic spotting techniques to create arrays of 3D cell-laden microgels [39].

Microarray technologies print nano-liter sized spots by dipping pins into the solutions of interest and spotting the solution onto a suitably modified substrate. To fabricate DNA microarrays the solutions contain probe DNA, whereas in the case of synthetic biomaterial microarrays the solutions are prepolymer solutions. Slots in the pins are filled by capillary action, and spots are printed as the filled pins come in contact with the substrate. Spot size is dependent on the diameter of the pins as well as the solution viscosity and composition. With robotic manipulators, microarrays of thousands of individual spots can be produced on a single glass slide. With pin heads containing as many as 96 pins, tens of thousands of spots can be generated quickly. The time required to create microarrays is, in part, dependent on the complexity of microarrayer, with some models capable of printing 60 slides each with 10,000 individual spots in a few hours.

5.2.5 Other Microscale Techniques

There are several other microscale technologies that have been used to fabricate tissue engineering constructs and to create tools for investigating the cellular microenvironment. For example, bioprinting, the freeform fabrication of synthetic and natural polymers with incorporated growth factors, proteins, and living cells, has been used to create 3D scaffolds with complex architectures [40, 41]. There has also been much effort focused on the development of microfibrous and microporous scaffolds [42, 43]. Additionally, advancements to the basic set of soft lithography techniques has led to the development of new microscale patterning techniques such as simultaneous chemical and topography transfer [44, 45]. Finally, control of chemical and physical properties and features is possible with layer-by-layer assembly [46].

5.3 Microscale Engineering of the Cell Microenvironment

Microscale technologies are potentially powerful tools in solving a number of challenges in the field of tissue engineering. This section highlights some recent advances in microscale technologies for creating and controlling the cellular microenvironment, and for

investigating and directing cellular behavior. Many of the examples discussed here use a combination of the microscale fabrication concepts and technologies described in Sect. 5.2. In doing so, these studies produce new cell-materials composites, as well as techniques to control cell-microenvironment interactions.

5.3.1 Cell Patterning and Controlling Cell–Cell Contacts

Surface patterning of cell adhesion molecules is a highly successful means of investigating cell patterns and controlling cell–ECM interactions, cell shape, and cell–cell contacts. Often, cell adhesion molecules are patterned by microcontact printing and photolithography. These patterns are used to control the spatial organization and positioning of a single or multiple cell types.

Cells adhere onto micropatterned substrates and align to the shape of the underlying adhesive region. For example, Chen et al. demonstrated that the change of cell shapes influences proliferation and apoptosis [47]. It has also been demonstrated that cell shape can control stem cell differentiation. In one example, mesenchymal stem cells were shown to differentiate into osteoblasts when cultured on large adhesive islands and adipocytes when cultured on small islands [48]. In another example, micropatterns of fibronectin and laminin were used to pattern cardiovascular smooth and striated muscle cells, and measure the contractility of the engineered tissue-like constructs [49]. By using specific micropatterns and statistical analysis of cell compartment positions, Thery et al. demonstrated that ECM geometry determines the orientation of cell polarity axes [50]. For more examples of micropatterning technologies for regulating cell behavior the reader is directed to other reviews [51–53].

Microscale technologies have also been used to create micropatterned co-cultures. For example, micropatterning and layer-by-layer techniques have been combined to create co-cultures of hepatocytes and fibroblasts with ECM proteins [54, 55]. Chien et al. created co-cultures on multilayer micropatterned films of poly(acrylic acid) (PAA) conjugated with 4-azidoaniline and PAA/polyacrylamide (PAM) multilayer films [56]. Microfabricated stencils have also been used to control cell–cell interactions. To this end, Jinno et al. used microscale stencils made from paralyene-C to create dynamic co-cultures [10]. This technique was used to investigate temporal changes in co-cultures of murine embryonic cells with other cell types.

Microwell arrays are also emerging as important tools for controlling cell microenvironments in vitro. Microwell arrays have been fabricated from many types of materials including glass, polymers or silicon and have been shown to be useful for various cell culture applications. Microwell arrays have also been used to investigate the dynamics of individual stem cell fate decisions in a high-throughput manner. In one study, an automated microscope was used to track the proliferation and death of individual neural progenitor cells cultured within microwells over several days, to generate clonally-derived information of cellular kinetics and cell fate decisions [57].

Microwell arrays can also be readily used to produce cell aggregates, such as embryonic bodies (EBs), of controlled sizes and shapes. These cultures have shown that the size of EBs influences stem cell self-renewal and differentiation (Fig. 5.1). For instance, it has been demonstrated that larger EBs preferentially undergo cardiac differentiation, whereas smaller

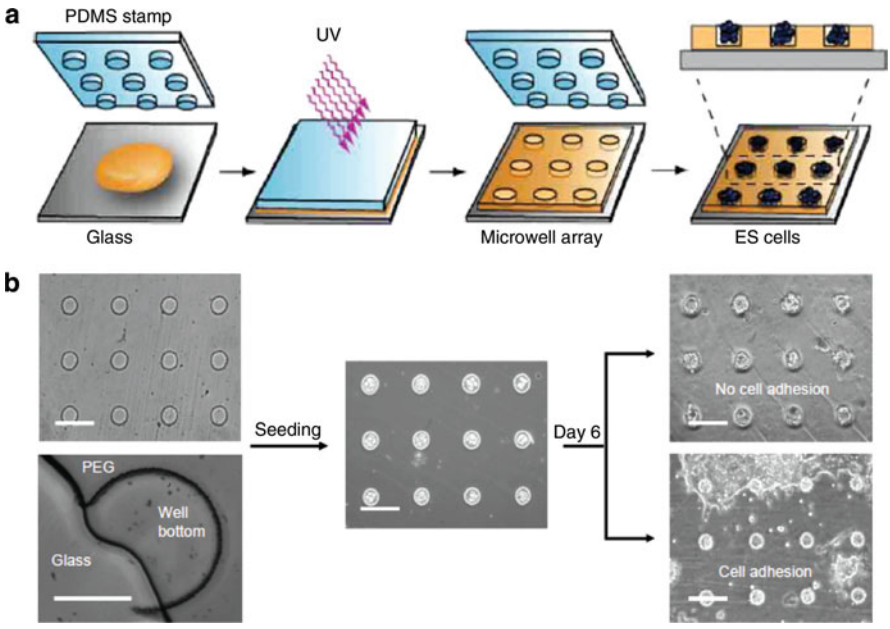


Fig. 5.1 Soft lithography approach to creating hydrogel microstructures for culturing cell aggregates of controlled sizes. (**a**) Schematic representation of the micromolding process to generate a PEG microwell array from a photocrosslinkable PEG-DA prepolymer solution. PEG was molded using a PDMS stamp followed by UV photocrosslinking. The cross-sectional image shows a microwell array loaded with ES cells. (**b**) Phase contrast images show a 50 μm microwell before and after seeding. Higher magnification of a 175 μm microwell that was cut vertically shows that the entire microwell surface was made of PEG. Embryonic stem cell aggregates grew until constrained by the size of the well, yielding a culture of homogeneously size aggregates. Reproduced with permission [5]

ones result in endothelial-like cells [58]. In addition, microwell cell culture provides quasi-3D single cell microenvironments that mimic essential features of the native 3D extracellular milieu by functionalizing the inner surface of microwells with ECM molecules [59, 60]. These studies will help to understand differences in cell behavior between 2- and 3D cultures.

5.3.2 Controlling Tissue Microarchitecture

The fabrication of biomimetic tissue microarchitecture may be an important factor for engineering functionalized tissues, and scaffolds and hydrogels that provide structural support and biochemical cues for cell attachment, spreading, proliferation, and differentiation are essential to the success of these technologies. A promising technique for the construction of 3D engineered tissues is the encapsulation of cells in hydrogels. In

comparison to prefabricated scaffolds, cells can be entrapped or encapsulated inside a hydrogel scaffold during fabrication. Hydrogels are ideal for encapsulating cells as they are highly hydrated and can provide an environment similar to that of native tissues.

In combination with soft lithography or photolithography techniques, it is possible to create microscale hydrogels with controlled shapes and sizes. Control of the size and shape of hydrogels and cell-laden hydrogels opens many possible applications for the construction of tissue-like structures with controlled tissue architecture and control over the cell-micro-environment interactions.

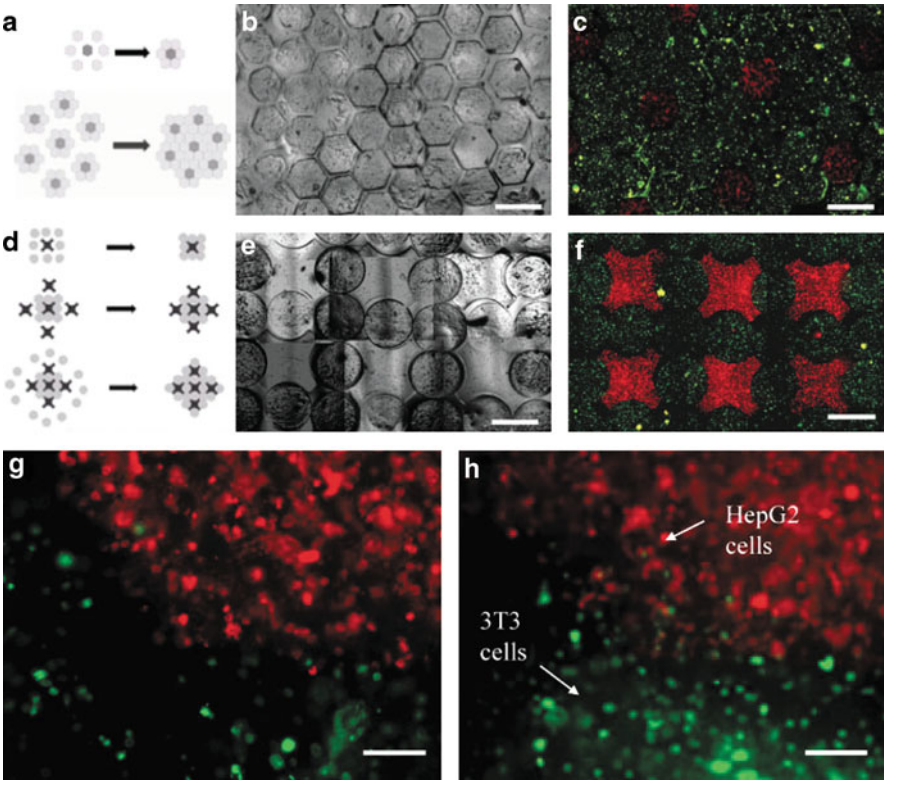


Fig. 5.2 Assemblies of cell-laden microgel structures. Microgels are produced by photolithography and assembled at an air-liquid interface. Schematic representations of co-culture microgels (**a**) and assemblies of microgels (**d**). Phase contrast (**b**) and fluorescence (**c**) images of assemblies of microgels. Phase contrast (**e**) and fluorescence (**f**) images of lock-and-key complex microgel building blocks with controlled co-culture conditions. The *scale bar* is 1 mm. The interface between microgels of containing 3T3 fibroblasts (*green*) and HepG2 cells (*red*) after 1 day (**g**) and 7 days (**h**). The *scale bar* in (**g**), (**h**) is 100 μm. and fluorescence (**b**) images of the microgel assemblies with the red-labeled cells (encapsulated in microgels) and green-labeled cells (encapsulated in bulk hydrogel). Morphology and viability staining of the cell-laden microgel before assembly (**c**, **d**) and after assembly in patterned bulk hydrogel (**e**, **f**). *Scale bars* for (**b**, **c**, **e**, **f**) are 400 μm. Reproduced with permission [65]

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A number of studies have demonstrated microgels made from various types of synthetic polymers, such as poly(ethylene glycol) (PEG), and their applicability for cell encapsulation [61–63]. Shape controlled cell-laden microgels can be created by various means such as micromolding and photolithography to mimic tissue units. These tissue units may be assembled in an emerging technique called "*bottom-up*" tissue assembly and may be a promising approach for creating tissues from smaller building blocks [64–66]. These microgels, that are on the order of the few tens to hundreds of micrometers, can be assembled to form large surfaces and 3D structures with high precision in the geometry and the position of the sub-units (Fig. 5.2).

Multi-layered photolithography can also be used to create 3D tissue-like structures [62, 67]. An interesting example of this technique is the fabrication of an artificial 3D network of hepatocytes encapsulated in PEG. Hepatocytes in these structures remain metabolically active cells for days, demonstrating the possibility of long term applications of the encapsulated cells.

5.3.3 Cell-Soluble Factor Interactions

Microfluidic technologies have been used in a number of different applications for studying different aspects of cell biology such as the effects of shear flow and concentration gradients on cells, as well as high throughput screening. Microfluidic systems have also been used to control the spatial and temporal presentation of soluble factors to cellular and subcellular structures, thus allowing for the investigation of activated biochemical signalling pathways in response to soluble stimuli [68, 69]. An active area for using microfluidics in regenerative medicine is to direct the differentiation of stem cells. For example, microfluidic systems can be used to study embryonic stem (ES) cells within microbioreactors [70]. Microreactors enable biological processes to be carried out under tightly controlled (oxygen, nutrients, or other molecular and physical regulatory factors) conditions and minimize batch to batch variability [71].

Gradients of soluble factors play important roles in a number of physiological processes, and a number of microfluidic designs have been developed to create stable gradients to study these processes. In one example, microfluidic devices were used to create and dynamically control chemical gradients to investigate the biological mechanisms responsible for the recruitent of neutrophils to injured, and infected areas [72]. Oxygen gradients have been created for the study of oxygen-induced migration of olfactory sensing in *Caenorhabditis elegans* [73]. Microfludic systems have also been used to create in vitro models of hepatic zones. In this example, a perfusable microfluidic bioreactor was used to create physiological oxygen gradients in hepatocyte and non-parenchymal cell co-cultures to study drug induced hepatotocity [74].

Multilayer soft lithography, an extension of the soft lithography paradigm, has been used to fabricate 3D microfluidic structures. These structures are fabricated by assembling separate layers of PDMS membranes produced by replica molding [75]. The chips fabricated by multilayer soft lithography can be used to fabricate micromechanical valves that prevent cross contamination or leakage between steps of the processes [76] and has been used for protein crystallization [77], nanoliter-volume PCR [78], microfabricated fluorescence

activated cell sorting (μFACS) [79] and single-cell enzyme screening [80]. Moreover, multilayer soft lithographic methods can also be used to fabricate microfluidic channels and scaffolds for tissue engineering in a convenient, rapid and inexpensive manner [4, 81].

5.4 High Thoughput and Combinatorial Investigations of the Cell Microenvironment

Microscale technologies can also be used to miniaturize traditional experimentation and enable high throughput microarrays and microfluidic systems. These techniques can be used to perform thousands of tests in parallel and have become important tools in drug discovery and other relevant fields [82, 83]. Microarray technologies can also be used to investigate combinatorial microenvironments, and the high throuphput screening of cell–ECM, cell–cell, and cell-soluble factor interactions. In this section, we review examples of microscale technologies used to investigate combinatorial microenvironments, and the high throughput synthesis and screening of biomaterials for tissue engineering applications.

5.4.1 High Throughput Synthesis and Screening of Cell Microenvironments

High throughput arrays are promising tools to test the effect of large combinatorial libraries of biomaterials, environmental stimuli and chemicals on cell behavior. A broad range of different molecules including small molecules, polymers, antibodies and other proteins can be arrayed using robotic spotting technology or soft lithography [84]. In general, to perform an assay, cells are seeded uniformly across the entire array and analyzed collectively using appropriate detection methods. For example, a microarray-based gene expression system in which cells are cultured on a glass slide with different DNAs printed in defined locations has been developed [85]. This work demonstrates that thousands of different nucleic acid sequences can be transfected into cells in parallel, and that the outcomes of these transfections can be analyzed in a high throughput manner. The platform has also been extended to RNA-mediated interference (RNAi) [86].

In another example, a microarray composed of different synthetic polymers was developed to investigate the response of ES cells to various extracellular signals [16]. In this approach, thousands of synthesized polymeric materials were printed on a slide and the effect on the differentiation of human ES cells was evaluated (Fig. 5.3).

Microarrays have also been used to investigate the effects of ECM components on stem cell differentiation. In one approach, arrays of micropatterned combinations of ECM proteins were used to investigate ES cell differentiation and to study the maintenance of primary rat hepatocytes during in vitro cell culture [17, 18]. Using a similar method, arrays of ECM proteins and Wnt signalling molecules were created to study the role of modulating the ECM environment on hepatic stellate cells [87]. Technologies for investigating 3D cell cultures in microarray format have also been developed. Arrays of murine ES cells encapsulated in alginate hydrogels have been created to study stem cell-soluble factor interactions in a 3D environment [88].

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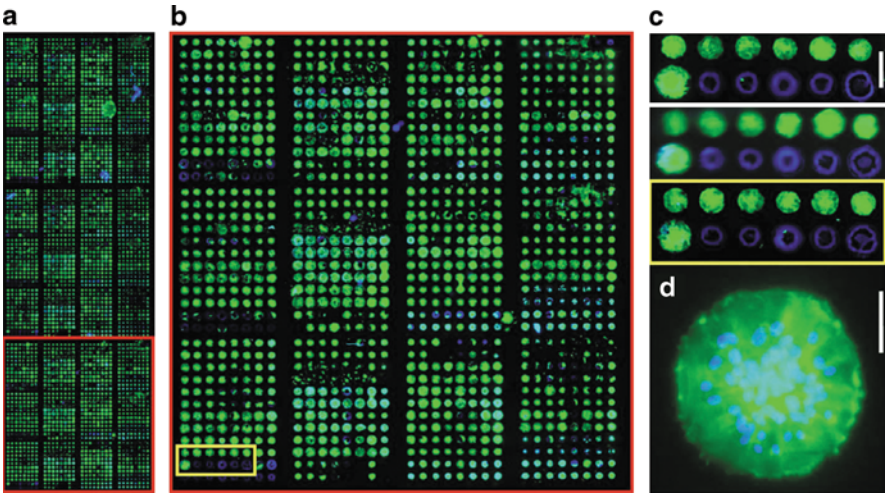


Fig. 5.3 Human mesenchymal stem cells (hMSCs) grown on polymer microarrays. (**a**–**c**) hMSCs were cultured on a polymer microarray and stained for actin (*green*; 500 μm *scale bar*). (**d**) High magnification images of a polymer spot with hMSC cells, stained with actin (*green*) and DNA/nucleus (*blue*; 100 μm *scale bar*). Reproduced with permission [103]

Elucidation of the mechanisms responsible for the observed cellular behavior in combinatorial ECM and biomaterials arrays requires rigorous characterization of the arrayed systems. To this end, there have been many efforts in creating high throughput chemical and physical analyses including, high throughput chemical surface characterizations, and nanomechanical measurements. In one example, Tweedie et al. developed a nanomechanical-profiling approach to analyze biomaterial arrays. An array of 1,700 individual photopolymerizable polymers was synthesized and the mechanical properties of each polymer was investigated by an automated nanomechanical screening system [89]. A similar library of photocrosslinkable polymer was characterized by time-of-flight secondary ion mass spectrometry, X-ray photoelectron spectroscopy, and water contact angle in a high throughput manner [90].

5.4.2 Microfluidics for High Throughput Investigations of the Cell Microenvironment

Microfluidics is a versatile technology that has been used to precisely control spatiotemporal aspects of the soluble cellular microenvironment. With simple microfluidic devices researchers have been able to create chemical gradients, control transient stimuli, and create changes in the soluble environment over time. Cell arrays are powerful platforms for high throughput screening of cell–matrix and cell–cell interactions, and in combination with microfluidics, cell arrays can also be used to investigate cell-soluble interactions in a high throughput manner.

Microfluidics can also be used to create cell arrays by manipulating cell location with controlled fluid flows. For example, high-density arrays of single cells isolated in a purely

hydrodynamic fashion have been developed [91], and multiple cell types have been selectively docked in microwells within microchannels [28]. In the latter approach, reversible sealing of a PDMS mold was used to immobilize a series of microchannel patterns on the microwells to enable sequential delivery of fluids to each microwell.

Gradient-generating microfluidic devices have been used for real time monitoring of cell behavior including migration, proliferation, differentiation and apoptosis [92]. For example, a cell array, with an integrated concentration gradient generator has been developed for long term monitoring of cell growth and proliferation [93]. In another example, a microfluidic device with a gradient generation component was developed for pharmacological gradient profiling [94]. Using this device, drug streams were held at different concentrations and voltage-gate K^+ ion channels were screened using scanning probe measurements. Gradient generation permits many soluble factor conditions to be analyzed in a combinatorial fashion and is well suited for high throughput assays in which a large number of conditions need to be screened in parallel.

High throughput microfluidic systems can significantly increase the efficiency of drug target selection, lead compound generation and identification by offering parallel experimentation and reduced reagent consumption. For example, Thorsen et al. developed a high throughput microfluidic chip which integrated 1,000 valves with 256 individual chambers [80]. Using a similar device, different cell types were screened against a number of different toxins in parallel [95].

The merging of cell microarrays and microfluidics has led to the creation of screening methods that can systematically vary one or more parameters, temporally and spatially, across the cell microarray. In one example, the activation of transcription factor NF-kappaβ across a microarray of HeLa S3 cells was monitored in real-time in response to varying concentrations of the cytokine TNFα [96]. In another example, a strategy was developed to simultaneously create different dynamic soluble microenvironments across a cell array [97]. Microfluidic channels upstream of the cell array were used to generate different temporal profiles of soluble factors, the effects of which were monitored by fluorescence in cell lines with specific gene reporters.

Microfluidic strategies have also proven useful in optimizing stem cell culture conditions. In a fully automated cell culture screening system, 96 independent culture chambers were provided unique culture media compositions and feeding schedules (Fig. 5.4) [98]. The device was used to quantify effects of transient stimulation schedules on the osteogenic differentiation of human mesenchymal stem cells. A similar strategy has been developed for studying stem cell cultures in 3D microenvironments [99]. In this approach, individually addressable cultures of murine embryonic fibroblasts embedded in 3D Matrigel were subject to dynamic soluble microenvironments. This system also allows for the study of culture-to-culture communication of diffusable factors.

5.5 Future Directions

The development of new microscale techniques has led to dramatic enhancements in the ability to study biological systems at the micrometer length scale. In particular, there has been much success in using microscale technologies to control the cellular microenvironment,

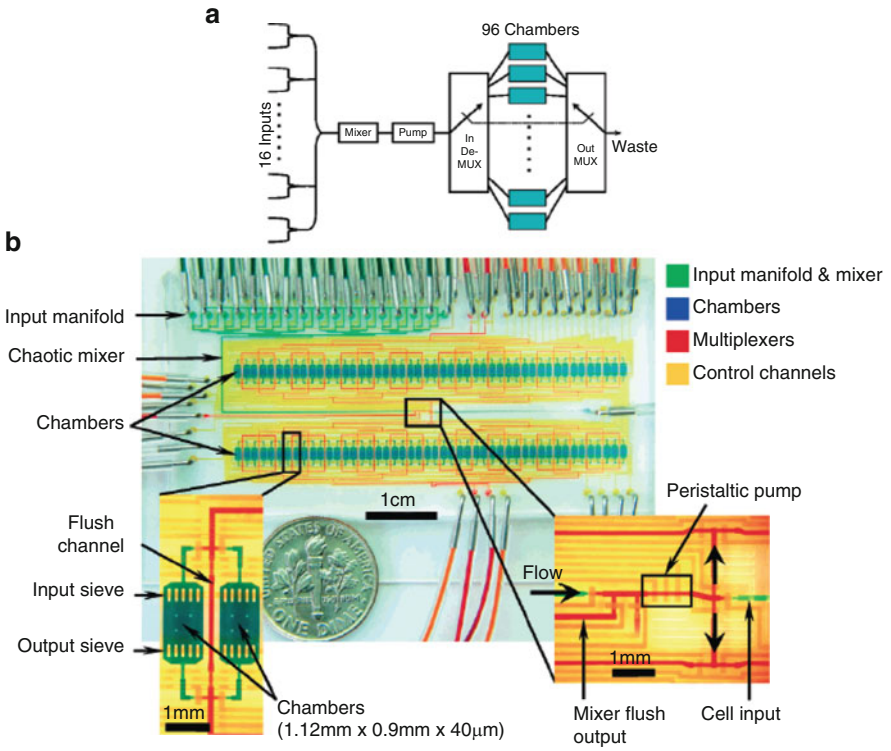


Fig. 5.4 A Microfluidic device for screening cell culture conditions. (**a**) A simplified schematic diagram of the fluidic path in the device (MUX, multiplexer). (**b**) Photograph of a microfludic culture device with the channels filled with colored water so that different parts of the device can be seen. *Left inset*: higher magnification images of two culture chambers. *Right inset*: higher magnification image of the input multiplexer [98]

and to investigate cellular responses to changes in the cellular microenvironment. However, there is still much work to be accomplished at the intersection of microfabrication and cell biology. The field of tissue engineering will benefit from new microscale technologies that can control different aspects of 3D microenviroments. The main challenge that microscale technologies can help address is the recapitulation of the in vivo microenvironment, in vitro.

One of the major experimental challenges in investigating the cell microenvironment is the vast number of possible combinations of different factors and interactions. Many cues and biological signalling events during development are context dependent [100, 101], and basic cell behaviors, such as migration, are drastically different under 2- and 3D environments [102]. Thorough investigation of cell behaviors in vitro, and the ability to determine mechanisms for the observed cell behaviors requires microscale technologies with fine control over the engineered cellular microenvironment.

Techniques to create microarrays of 3D microgels for the investigation of cell–materials interactions will be of great utility in tissue engineering. Such techniques face a number of technical challenges including the creation of combinatorial experiments with cell-laden scaffolds and the high throughput screening of outcomes in 3D. Additionally, the high throughput analysis of arrays of cell aggregates, and the analysis of combinatorial material arrays with cell aggregates will be of great use in studying cell-microenvironment interactions in 3D environments. We also envision that further merging of microfluidics with biomaterials arrays will produce new technologies to investigate combinatorial materials and soluble factor experiments.

ES cells and induced pluripotent stem cells have enormous potential in regenerative medicine and tissue engineering. One of the major limitations to the widespread use of stem cells as a cell source in tissue engineering is the lack of the ability to precisely direct their differentiation. We envision many new microscale technologies to address this challenge. For example, it might be possible to direct cell fate with patterns of signaling molecules. Patterning of cues and developmental signals could direct stem cell cultures to differentiation in a spatially organized manner. In this way, mimics of native tissue structures could be produced.

In the past decade there have been many successes in the development of microscale technologies for controlling the cellular microenvironment. These successes have led to the advancement of tissue engineering, and have helped push the field of tissue engineering closer to clinical therapies. The future holds great promise for the continuing success of the existing and as-of-yet developed microscale technologies.

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References

1. Langer R, Vacanti JP. Tissue engineering. Science 1993;260(5110):920–926.
2. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nature Biotechnology 2005;23(1):47–55.
3. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. Microscale technologies for tissue engineering and biology. Proceedings of the National Academy of Sciences of the United States of America 2006;103(8):2480–2487.
4. 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.
5. Moeller HC, Mian MK, Shrivastava S, Chung BG, Khademhosseini A. A microwell array system for stem cell culture. Biomaterials 2008;29(6):752–763.
6. Karp JM, Yeh J, Eng G, Fukuda J, Blumling J, Suh KY, et al. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. Lab on a Chip 2007;7(6):786–794.
7. Ostuni E, Chen CS, Ingber DE, Whitesides GM. Selective deposition of proteins and cells in arrays of microwells. Langmuir 2001;17(9):2828–2834.

8. Ostuni E, Yan L, Whitesides GM. The interaction of proteins and cells with self-assembled monolayers of alkanethiolates on gold and silver. Colloids and Surfaces. B, Biointerfaces 1999;15(1):3–30.
9. Kane RS, Takayama S, Ostuni E, Ingber DE, Whitesides GM. Patterning proteins and cells using soft lithography. Biomaterials 1999;20(23–24):2363–2376.
10. Jinno S, Moeller HC, Chen CL, Rajalingam B, Chung BG, Dokmeci MR, et al. Macrofabricated multilayer parylene-C stencils for the generation of patterned dynamic co-cultures. Journal of Biomedical Materials Research. Part A 2008;86A(1):278–288.
11. Ling Y, Rubin J, Deng Y, Huang C, Demirci U, Karp JM, et al. A cell-laden microfluidic hydrogel. Lab on a Chip 2007;7(6):756–762.
12. Dertinger SKW, Jiang XY, Li ZY, Murthy VN, Whitesides GM. Gradients of substrate-bound laminin orient axonal specification of neurons. Proceedings of the National Academy of Sciences of the United States of America 2002;99(20):12542–12547.
13. Park JH, Chung BG, Lee WG, Kim J, Brigham MD, Shim J, et al. Microporous cell-laden hydrogels for engineered tissue constructs. Biotechnology and Bioengineering 2010; 106(1): 138–148
14. Hook AL, Anderson DG, Langer R, Williams P, Davies MC, Alexander MR. High throughput methods applied in biomaterial development and discovery. Biomaterials 2010;31(2): 187–198.
15. Peters A, Brey DM, Burdick JA. High-throughput and combinatorial technologies for tissue engineering applications. Tissue Engineering. Part B: Reviews 2009;15(3):225–239.
16. Anderson DG, Levenberg S, Langer R. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. Nature Biotechnology 2004;22(7):863–866.
17. Flaim CJ, Chien S, Bhatia SN. An extracellular matrix microarray for probing cellular differentiation. Nature Methods 2005;2(2):119–125.
18. Flaim CJ, Teng D, Chien S, Bhatia SN. Combinatorial signaling microenvironments for studying stem cell fate. Stem Cells and Development 2008;17(1):29–39.
19. Cukierman E, Pankov R, Stevens DR, Yamada KM. Taking cell–matrix adhesions to the third dimension. Science 2001;294(5547):1708–1712.
20. Doyle AD, Wang FW, Matsumoto K, Yamada KM. One-dimensional topography underlies three-dimensional fibrillar cell migration. The Journal of Cell Biology 2009;184(4):481–490.
21. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science 2005;310(5751):1135–1138.
22. Blawas AS, Reichert WM. Protein patterning. Biomaterials 1998;19(7–9):595–609.
23. Barbulovic-Nad I, Lucente M, Sun Y, Zhang MJ, Wheeler AR, Bussmann M. Bio-microarray fabrication techniques – a review. Critical Reviews in Biotechnology 2006;26(4):237–259.
24. Bertone P, Snyder M. Advances in functional protein microarray technology. 15th Biennial Conference on Methods in Protein Structure Analysis; 2004 Aug 29–Sep 02; Seattle, WA: Blackwell Publishing; 2004. p. 5400–5411.
25. Xia YN, Whitesides GM. Soft lithography. Angewandte Chemie (International edition) 1998; 37(5):551–575.
26. Takayama S, Ostuni E, LeDuc P, Naruse K, Ingber DE, Whitesides GM. Laminar flows – subcellular positioning of small molecules. Nature 2001;411(6841):1016.
27. Takayama S, Ostuni E, Qian XP, McDonald JC, Jiang XY, LeDuc P, et al. Topographical micropatterning of poly(dimethylsiloxane) using laminar flows of liquids in capillaries. Advanced Materials 2001;13(8):570–574.
28. Khademhosseini A, Yeh J, Eng G, Karp J, Kaji H, Borenstein J, et al. Cell docking inside microwells within reversibly sealed microfluidic channels for fabricating multiphenotype cell arrays. Lab on a Chip 2005;5(12):1380–1386.
29. Rhee SW, Taylor AM, Tu CH, Cribbs DH, Cotman CW, Jeon NL. Patterned cell culture inside microfluidic devices. Lab on a Chip 2005;5(1):102–107.

30. Khademhosseini A, Bettinger C, Karp JM, Yeh J, Ling YB, Borenstein J, et al. Interplay of biomaterials and micro-scale technologies for advancing biomedical applications. Journal of Biomaterials Science, Polymer Edition 2006;17(11):1221–1240.
31. Khetani SR, Bhatia SN. Engineering tissues for in vitro applications. Current Opinion in Biotechnology 2006;17(5):524–531.
32. Micheletti M, Lye GJ. Microscalle bioprocess optimisation. Current Opinion in Biotechnology 2006;17(6):611–618.
33. Portner R, Nagel-Heyer S, Goepfert C, Adamietz P, Meenen NM. Bioreactor design for tissue engineering. Journal of Bioscience and Bioengineering 2005;100(3):235–245.
34. Auburn RP, Kreil DP, Meadows LA, Fischer B, Matilla SS, Russell S. Robotic spotting of cDNA and oligonucleotide microarrays. Trends in Biotechnology 2005;23(7):374–379.
35. MacBeath G, Schreiber SL. Printing proteins as microarrays for high-throughput function determination. Science 2000;289(5485):1760–1763.
36. Mirzabekov A, Kolchinsky A. Emerging array-based technologies in proteomics. Current Opinion in Chemical Biology 2002;6(1):70–75.
37. Rusmini F, Zhong ZY, Feijen J. Protein immobilization strategies for protein biochips. Biomacromolecules 2007;8(6):1775–1789.
38. Wilson DS, Nock S. Functional protein microarrays. Current Opinion in Chemical Biology 2002;6(1):81–85.
39. Lee MY, Kumar RA, Sukumaran SM, Hogg MG, Clark DS, Dordick JS. Three-dimensional cellular microarray for high-throughput toxicology assays. Proceedings of the National Academy of Sciences of the United States of America 2008;105(1):59–63.
40. Xu F, et al. A droplet-based building block approach for bladder smooth muscle cell (SMC) proliferation. Biofabrication 2010;2(1):014105.
41. Khalil S, Sun W. Bioprinting endothelial cells with alginate for 3D tissue constructs. Journal of Biomechanical Engineering-Transactions on ASME 2009;131(11):8.
42. Lannutti J, Reneker D, Ma T, Tomasko D, Farson DF. Electrospinning for tissue engineering scaffolds. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 2007;27(3):504–509.
43. Chen GP, Ushida T, Tateishi T. Scaffold design for tissue engineering. Macromolecular Bioscience 2002;2(2):67–77.
44. Quist AP, Pavlovic E, Oscarsson S. Recent advances in microcontact printing. Analytical and Bioanalytical Chemistry 2005;381(3):591–600.
45. Perl A, Reinhoudt DN, Huskens J. Microcontact printing: limitations and achievements. Advanced Materials 2009;21(22):2257–2268.
46. Hammond PT. Form and function in multilayer assembly: new applications at the nanoscale. Advanced Materials 2004;16(15):1271–1293.
47. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276(5317):1425–1428.
48. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Developmental Cell 2004;6(4):483–495.
49. Alford PW, Feinberg AW, Sheehy SP, Parker KK. Biohybrid thin films for measuring contractility in engineered cardiovascular muscle. Biomaterials 2010;31(13):3613–3621.
50. Thery M, Racine V, Piel M, Pepin A, Dimitrov A, Chen Y, et al. Anisotropy of cell adhesive microenvironment governs cell internal organization and orientation of polarity. Proceedings of the National Academy of Sciences of the United States of America 2006;103(52):19771–19776.
51. Falconnet D, Csucs G, Grandin HM, Textor M. Surface engineering approaches to micropattern surfaces for cell-based assays. Biomaterials 2006;27(16):3044–3063.
52. Fink J, Thery M, Azioune A, Dupont R, Chatelain F, Bornens M, et al. Comparative study and improvement of current cell micro-patterning techniques. Lab on a Chip 2007;7(6):672–680.

53. Folch A, Toner M. Microengineering of cellular interactions. Annual Review of Biomedical Engineering 2000;2:227–256.
54. Khademhosseini A, Suh KY, Yang JM, Eng G, Yeh J, Levenberg S, et al. Layer-by-layer deposition of hyaluronic acid and poly-L-lysine for patterned cell co-cultures. Biomaterials 2004;25(17):3583–3592.
55. Fukuda J, Khademhosseini A, Yeh J, Eng G, Cheng JJ, Farokhzad OC, et al. Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components. Biomaterials 2006;27(8):1479–1486.
56. Chien HW, Chang TY, Tsai WB. Spatial control of cellular adhesion using photo-crosslinked micropatterned polyelectrolyte multilayer films. Biomaterials 2009;30(12):2209–2218.
57. Chin VI, Taupin P, Sanga S, Scheel J, Gage FH, Bhatia SN. Microfabricated platform for studying stem cell fates. Biotechnology and Bioengineering 2004;88(3):399–415.
58. Hwang YS, Chung BG, Ortmann D, Hattori N, Moeller HC, Khademhosseini A. Microwell-mediated control of embryoid body size regulates embryonic stem cell fate via differential expression of WNT5a and WNT11. Proceedings of the National Academy of Sciences of the United States of America 2009;106(40):16978–16983.
59. Ochsner M, Dusseiller MR, Grandin HM, Luna-Morris S, Textor M, Vogel V, et al. Microwell arrays for 3D shape control and high resolution analysis of single cells. Lab on a Chip 2007;7(8):1074–1077.
60. Dusseiller MR, Schlaepfer D, Koch M, Kroschewski R, Textor M. An inverted microcontact printing method on topographically structured polystyrene chips for arrayed micro-3-D culturing of single cells. Biomaterials 2005;26(29):5917–5925.
61. Koh WG, Revzin A, Pishko MV. Poly(ethylene glycol) hydrogel microstructures encapsulating living cells. Langmuir 2002;18(7):2459–2462.
62. Liu VA, Bhatia SN. Three-dimensional photopatterning of hydrogels containing living cells. Biomedical Microdevices 2002;4(4):257–266.
63. Peppas NA, Hilt JZ, Khademhosseini A, Langer R. Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Advanced Materials 2006;18(11): 1345–1360.
64. Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR. Organ printing: computer-aided jet-based 3D tissue engineering. Trends in Biotechnology 2003;21(4):157–161.
65. Zamanian B, Masaeli M, Nichol JW, Khabiry M, Hancock MJ, Bae H, et al. Interface-directed self-assembly of cell-laden microgels. Small 2010;6(8):937–944
66. Du Y, Lo E, Vidula MK, Khabiry M, Khademhosseini A. Method of bottom-up directed assembly of cell-laden microgels. Cellular and Molecular Bioengineering 2008;1(2–3):157–162.
67. Tsang VL, Chen AA, Cho LM, Jadin KD, Sah RL, DeLong S, et al. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. The FASEB Journal 2007;21(3): 790–801.
68. Lucchetta EM, Lee JH, Fu LA, Patel NH, Ismagilov RF. Dynamics of Drosophila embryonic patterning network perturbed in space and time using microfluidics. Nature 2005;434(7037): 1134–1138.
69. Sawano A, Takayama S, Matsuda M, Miyawaki A. Lateral propagation of EGF signaling after local stimulation is dependent on receptor density. Developmental Cell 2002;3(2):245–257.
70. Wobus AM, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. Physiological Reviews 2005;85(2):635–678.
71. Burdick JA, Vunjak-Novakovic G. Engineered microenvironments for controlled stem cell differentiation. Tissue Engineering. Part A 2009;15(2):205–219.
72. Irimia D, Liu SY, Tharp WG, Samadani A, Toner M, Poznansky MC. Microfluidic system for measuring neutrophil migratory responses to fast switches of chemical gradients. Lab on a Chip 2006;6(2):191–198.

73. Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, Ellis RE, et al. Oxygen sensation and social feeding mediated by a C-elegans guanylate cyclase homologue. Nature 2004;430(6997): 317–322.
74. Allen JW, Khetani SR, Bhatia SN. In vitro zonation and toxicity in a hepatocyte bioreactor. Toxicological Sciences 2005;84(1):110–119.
75. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. Monolithic microfabricated valves and pumps by multilayer soft lithography. Science 2000;288(5463):113–116.
76. Hong JW, Studer V, Hang G, Anderson WF, Quake SR. A nanoliter-scale nucleic acid processor with parallel architecture. Nature Biotechnology 2004;22(4):435–439.
77. Hansen CL, Skordalakes E, Berger JM, Quake SR. A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. Proceedings of the National Academy of Sciences of the United States of America 2002;99(26):16531–16536.
78. Liu J, Enzelberger M, Quake S. A nanoliter rotary device for polymerase chain reaction. Electrophoresis 2002;23(10):1531–1536.
79. Fu AY, Chou HP, Spence C, Arnold FH, Quake SR. An integrated microfabricated cell sorter. Analytical Chemistry 2002;74(11):2451–2457.
80. Thorsen T, Maerkl SJ, Quake SR. Microfluidic large-scale integration. Science 2002; 298(5593):580–584.
81. Walker GM, Zeringue HC, Beebe DJ. Microenvironment design considerations for cellular scale studies. Lab on a Chip 2004;4(2):91–97.
82. Fernandes TG, Diogo MM, Clark DS, Dordick JS, Cabral JMS. High-throughput cellular microarray platforms: applications in drug discovery, toxicology and stem cell research. Trends in Biotechnology 2009;27(6):342–349.
83. Hong J, Edel JB, de Mello AJ. Micro- and nanofluidic systems for high-throughput biological screening. Drug Discovery Today 2009;14(3–4):134–146.
84. Howbrook DN, van der Valk AM, O'Shaughnessy MC, Sarker DK, Baker SC, Lloyd AW. Developments in microarray technologies. Drug Discovery Today 2003;8(14):642–651.
85. Ziauddin J, Sabatini DM. Microarrays of cells expressing defined cDNAs. Nature 2001;411 (6833):107–110.
86. Sharma S, Rao A. RNAi screening: tips and techniques. Nature Immunology 2009;10(8): 799–804.
87. Brafman DA, de Minicis S, Seki E, Shah KD, Teng DY, Brenner D, et al. Investigating the role of the extracellular environment in modulating hepatic stellate cell biology with arrayed combinatorial microenvironments. Integrative Biology 2009;1(8–9):513–524.
88. Fernandes TG, Kwon S-J, Bale SS, Lee M-Y, Diogo MM, Clark DS, et al. Three-dimensional cell culture microarray for high-throughput studies of stem cell fate. Biotechnology and Bioengineering 2010;106(1):106–118.
89. Tweedie CA, Anderson DG, Langer R, Van Vliet KJ. Combinatorial material mechanics: high-throughput polymer synthesis and nanomechanical screening. Advanced Materials 2005;17(21):2599–2604.
90. Urquhart AJ, Anderson DG, Taylor M, Alexander MR, Langer R, Davies MC. High throughput surface characterisation of a combinatorial material library. Advanced Materials 2007; 19(18):2486–2491.
91. Di Carlo D, Wu LY, Lee LP. Dynamic single cell culture array. Lab on a Chip 2006;6(11): 1445–1449.
92. Singh M, Berkland C, Detamore MS. Strategies and applications for incorporating physical and chemical signal gradients in tissue engineering. Tissue Engineering Part B-Reviews 2008;14(4):341–366.
93. Hung PJ, Lee PJ, Sabounchi P, Aghdam N, Lin R, Lee LP. A novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array. Lab on a Chip 2005;5(1):44–48.

94. Pihl J, Sinclair J, Sahlin E, Karlsson M, Petterson F, Olofsson J, et al. Microfluidic gradient-generating device for pharmacological profiling. Analytical Chemistry 2005;77(13):3897–3903.
95. Wang ZH, Kim MC, Marquez M, Thorsen T. High-density microfluidic arrays for cell cytotoxicity analysis. Lab on a Chip 2007;7(6):740–745.
96. Thompson DM, King KR, Wieder KJ, Toner M, Yarmush ML, Jayaraman A. Dynamic gene expression profiling using a microfabricated living cell array. Analytical Chemistry 2004; 76(14):4098–4103.
97. Kim L, Vahey MD, Lee HY, Voldman J. Microfluidic arrays for logarithmically perfused embryonic stem cell culture. Lab on a Chip 2006;6(3):394–406.
98. Gomez-Sjoberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR. Versatile, fully automated, microfluidic cell culture system. Analytical Chemistry 2007;79(22):8557–8563.
99. Lii J, Hsu W-J, Parsa H, Das A, Rouse R, Sia SK. Real-time microfluidic system for studying mammalian cells in 3D microenvironments. Analytical Chemistry 2008;80(10):3640–3647.
100. Armant DR. Blastocysts don't go it alone. Extrinsic signals fine-tune the intrinsic developmental program of trophoblast cells. Developmental Biology 2005;280(2):260–280.
101. Veeman MT, Axelrod JD, Moon RT. A second canon: functions and mechanisms of beta-catenin-independent wnt signaling. Developmental Cell 2003;5(3):367–377.
102. Dong H, Hartgerink JD. Short homodimeric and heterodimeric coiled coils. Biomacromolecules 2006;7(3):691–695.
103. Anderson DG, Putnam D, Lavik EB, Mahmood TA, Langer R. Biomaterial microarrays: rapid, microscale screening of polymer-cell interaction. Biomaterials 2005;26(23):4892–4897.