Chapter 17

Microscale Technologies for Tissue Engineering

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Abstract

Despite the enormous advances in tissue engineering, several challenges still prevent the widespread clinical application of tissue engineering products, such as how to acquire adequate number of cells, how to engineer complex vascularized tissues that mimic the complexity of native tissue architecture and functions. The merger of biomaterials and microscale technologies offer new opportunities to overcome the challenges in tissue engineering to fabricate scaffolds and direct stem cell differentiation. In this review, various applications of microscale technologies have been illustrated in controlling stem cell fate and building complex artificial tissue with well-controlled and vascularized structures. It is envisioned that with the rapid growth of this burgeoning research field, microscale technologies will transform the conventional tissue engineering approaches and greatly contribute to the therapeutic potential of tissue engineering.

Keywords: Microfabrication; Soft Lithography; Stem Cells; Microfluidics; Tissue Engineering.

Outline

1. Introduction

Tissue engineering aims to generate tissues that restore, maintain, or enhance tissue function,[1] with the ultimate goal of providing a permanent solution to the problem of organ failure. Commonly-adopted tissue engineering approaches involve (i) isolated cells or cell substitutes, (ii) biocompatible materials for cellular support and regeneration, or (iii) cell-biomaterial (i.e. scaffold) composites.[2] Cells for tissue engineering have been traditionally derived either as autografts (from the patient), allografts (from a human donor) or xenografts (from a different species). Isolated cells are then cultured on biocompatible scaffolds, which provide physical and chemical support and guide the cell growth and organization into three-dimensional (3D) tissues. Despite the enormous advances in tissue engineering which have resulted in clinically viable products such as skin, several challenges still prevent the widespread clinical application of tissue engineering products. These challenges include a number of business, regulatory and ethical issues as well as scientific barriers. These scientific issues include (a) how to acquire adequate source of cells, (b) how to engineer complex vascularized tissues that mimic the complexity of native tissue architecture, and (c) how to generate tissues with the biomechanical and metabolic functions that mimics normal tissues.

Microscale technologies, all of which interface with engineering, biology and medicine, are emerging as a critical approach in controlling cell microenvironment and generating tissue constructs. Microscale technologies (i.e. microelectromechanical systems, MEMS) are adopted from the micro-fabrication processes in semiconductor and microelectronics industries, which can achieve control of microscale features ranging from 1 μm to 1 cm. Soft lithography is one of the most popular microscale technologies, which can fabricate microscale devices without extensive usage of clean-room and photolithography facilities.[3] In soft lithography, elastomeric stamps (i.e. polydimethylsiloxane, PDMS) are fabricated from patterned silicon wafers to print or mold materials, which can control the topography and

spatial distribution of biomaterials at sub-micron resolution in a convenient, rapid and inexpensive manner. Since many microscale techniques are compatible with cells, they can be readily used to engineer the cell microenvironment. In particular, microscale technologies have been extensively used to control the cell-cell, cell-extracellular matrix (ECM) and cell substrate interactions in tissue culture. Furthermore, microengineering approaches are now being increasingly used to generate tissue scaffolds with enhanced architectural and mechanical properties. Thus microscale technologies, such as soft lithography and photolithography are emerging as promising tools for addressing some of the challenges in tissue engineering. Furthermore, this miniaturization has enabled high throughput testing of the cell behavior in response to various stimuli in an inexpensive, rapid and reproducible manner.

In this chapter, numerous applications of microscale technologies have been introduced, which can potentially solve the challenges in tissue engineering. Although we provide a broad perspective of the field we focus on the applications of the microscale systems in controlling stem cell fate and engineering complex vascularized tissue constructs.

2. Microscale Technologies for Controlling Stem Cell Fate

One of the major challenges to the clinical feasibility of tissue engineering is an adequate number of cells that are immunologically compatible with the patient. Many cell types isolated from the adult tissues are difficult to expand in culture and quickly lose their phenotype. Recently stem cells from the adult and embryonic sources have generated much excitement that a renewable source of cells may be obtainable for tissue engineering applications. Stem cells are pluripotent cells that have the capacity to self-renew and to differentiate into various lineages. Reproducible and directed regulation of the stem cell fate (i.e. self-renewal and differentiation decisions) is critical for the clinical success of stem cell-based therapies. From years of biological research, it is becoming increasingly evident that both intra- and extracellular cues regulate the resulting cell fate decisions. Most stem cells in the body reside in specific niches that signal the cells to behave in response to physiological conditions. Thus signals in the microenvironment that are regulated in space and time direct the cells and result in their subsequent differentiation.[4] Although standard tissue culture techniques have greatly increased our understanding of the stem cell microenvironment, they lack the spatial and temporal regulation of the microenvironment to which stem cells are exposed. Furthermore it is difficult to perform high-throughput studies to examine the complexities of the combinations and concentrations of the various signals on stem cell behavior. In contrast, the ability of the microscale technologies to miniaturize

experiments and increase experimental control can be used to provide new opportunities in studying and directing stem cell fate responses.

2.1. Regulating stem cell fate by controlling cell shape

Extracellular cues are important in regulating stem cell fate decision. In general, the physical properties of the extracellular matrix (ECM) contribute to cell function and behavior. For example, mechanical cues experienced by the cells in its environment regulates its function including cell shape, DNA synthesis, motility and lineage commitment.[5] Several hypotheses have been put forward to explain how the mechanical cues affect the cell physiology. For example, Ingber and colleagues have suggested that shape-dependent control of cell growth and function appears to be mediated by tension-dependent changes in the actin cytoskeleton. They used computer simulations based on dynamic Boolean networks to show that generalized stimuli (e.g. mechanical forces) and specific molecular cues elicit signals which follow different trajectories, but eventually converge onto one of a small set of common end points (growth, quiescence, differentiation, apoptosis, etc.).[6]

Cell shape has been earlier suggested to play a role in differentiation and apoptosis. For example, Watt *et al*. found that the cell shape influences the terminal differentiation in epidermal keratinocytes.[7] When cell spreading is restricted using micropatterned substrates, more round-shaped cells entered apoptosis compared to cells that were allowed to spread on identically fabricated unpatterned substrates, thus demonstrating a role of cell shape as a critical determinant that switches cells between life and apoptosis.[8] Thus cell shape and the cytoskeletal structure play an important role in cellular functions.[9] While changes in the cell shape appear to regulate several cellular processes, its role in the commitment of the multipotential stem cells is not as clearly understood. Chen and colleagues reported that cell shape and cytoskeletal tension determines the lineage commitment of stem cells[10] (Fig. 1). They observed that human mesenchymal stem cells (MSCs) that were allowed to adhere, flatten, and spread, differentiated into osteogenic cells, while unspread, round cells gave rise to adipocytes. Furthermore, they demonstrated that this behavior was mediated through the modulation of the endogeneous RhoA pathway.

Micropatterning enables the confinement of the individual or group of cells within defined spatially controlled spaces limiting the body extension. Cell shape can be easily controlled by means of using an elastomeric stamps.[11] Singhvi *et al*. used an elastomeric stamp to imprint gold surfaces with specific patterns of self-assembled monolayers of alkanethiols and, thereby, to create islands of defined shape and size that support ECM protein adsorption and cell attachment. Using

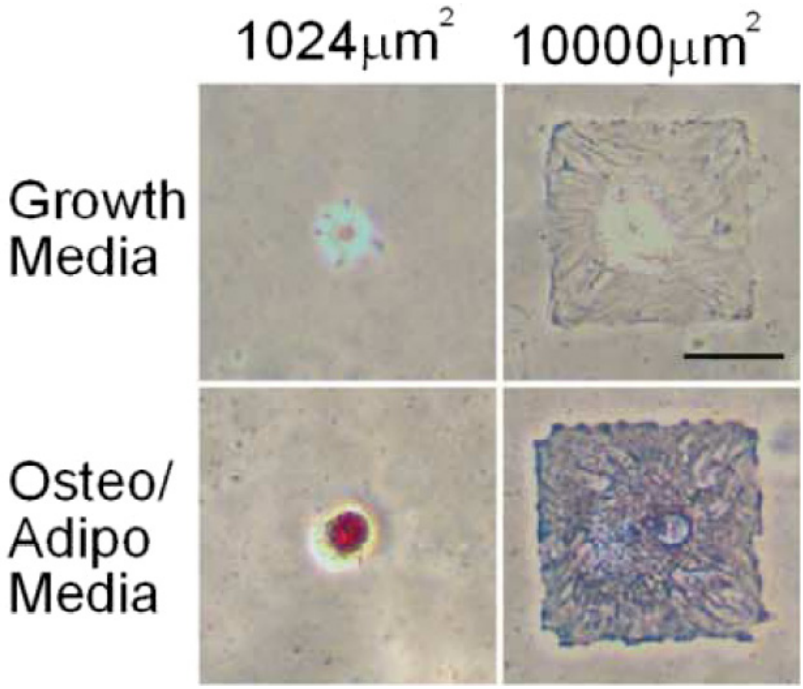

Fig. 1. Cell shape regulates commitment of human mesenchymal stem cells to adipocyte or osteoblast fate.[10] When cells are exposed to a mixture of Osteo/Adipo differentiated media, the cells cultured on the small islands stained for lipids (red), indicating differentiation into adipogenic fates; whereas cells on large islands stained for alkaline phosphatase (blue) indicating differentiation into osteoblasts.

this technique cells were arrayed in predetermined locations in defined shape. It was demonstrated that by limiting the degree of cell extension, cell growth and protein secretion could be controlled.[11]

2.2. Microwells for uniform embryoid body culture and control of cell-cell contact

Embryonic stem cells in suspension culture form multicellular aggregates called embryoid bodies (EBs) that contain the three germ layers. EBs recapitulate the early stages of the embryonic development and give rise to a wide spectrum of cell types. EBs are usually generated by means of the traditional hanging drop method or by suspension cultures in non-tissue culture treated plates.[12] Other methods to fabricate EBs include culture in methylcellulose semisolid media, in round-bottomed 96-well plates, in stirred-suspension cultures using spinner flasks and in

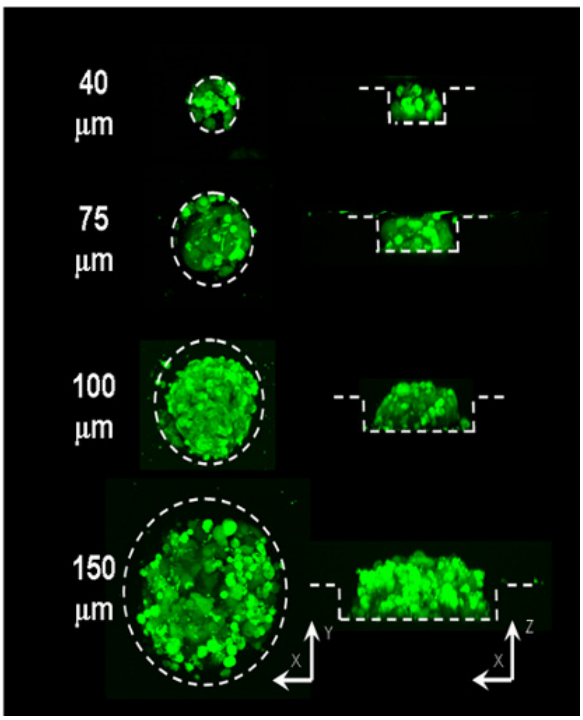

Fig. 2. Microwells for culturing EBs:[14] Confocal laser light microscopy images of cell aggregates within 40, 75, 100, and 150 μm microwells after five days stained with CFSE. Cells formed 3D embryoid bodies with diameters closely corresponding to those of their respective microwells. The first column of images shows aerial views of the cell aggregates whereas the second column shows vertical cross-sections of cell aggregates within PEG microwells.

rotary flasks.[12,13] These methods essentially prevent cell adhesion to the surface of the vessel and promote cell aggregation. The major limitations of the above mentioned methods are the large size and shape distribution of the EBs as well as cumbersome procedures involved in making more homogeneous EBs. To overcome these limitations, Karp *et al*. used non-adhesive microwells made from poly(ethylene glycol) (PEG) to culture homogeneous EBs with controllable sizes and shapes in a rapid and reproducible manner[14] (Fig. 2). PEG inhibits protein adsorption and cell adhesion which results in cell aggregation and the formation of EBs. Moreover they demonstrated that EBs can be retrieved from microwells with a greater than 95% viability. Also it is hypothesized that the size of the EBs influences the subsequent differentiation and hence this technique can be useful for inducing more directed differentiation without addition of exogenous growth factors. The microfabrication-based generation of the EBs is scalable, cost-effective

and simple to perform. Furthermore it can be used for performing high throughput screening and toxicity studies. For example, it is known that microwells can be used to immobilize cells within microfluidic arrays to generate devices that can be used to perform high-throughput experiments.[15]

Direct cell-cell contact is known to affect many stem cell fates decisions. Therefore, methods that can be used to control direct cell-cell contact between the same cell types and different cell types will be beneficial for stem cell differentiation studies. To effectively control heterotypic cell-cell interactions, Bhatia and colleagues have developed a number of methods to control the interaction of hepatocytes with non-parenchymal cell types.[16,17] Furthermore, Khademhosseini and colleagues have generated several techniques by using layer-by-layer assembly of electrostatic polymers to engineer surface properties. In their studies they used layer-by-layer deposition of ECM components such as hyaluronic acid (HA), fibronectin and collagen to generate co-cultures by sequentially patterning adhesive and non-adhesive regions.[18]

In addition to static cell-cell contact, dynamic studies of cellular interactions are important in understanding many biological processes such as stem cell homing and embryonic development. To engineer cell-cell contact dynamically, Hui and Bhatia have generated a device based on interdigitating silicon plates that could be seeded with different cell types and brought together in close proximity.[19] Furthermore, a technique based on micropatterned stencils can also be used to generate patterned co-cultures with dynamic control. In this approach stencils made of reversibly sealable parylene-C membranes, a biocompatible material were sealed and peeled off from a variety of substrates such as PDMS, polystyrene and standard cell culture plates. Using parylene-C and the layer-by-layer technique, a system was developed in which one cell type could be sequentially exposed to various cell types.[20]

2.3. Microarrays for directing stem cell fates

Microarrays have been used extensively in basic biology research, screening diseases, drug discovery and toxicology for molecular profiling of samples at the DNA, RNA, and protein level.[18,19] Due to the complexity of the factors affecting stem cell differentiation, it is essential to analyze the stem cell microenvironment in a high throughput manner. The use of microarrays technology would make such analysis faster and cheaper since miniaturized experiments can be done in a rapid manner without the use of extensive reagents. Microarrays can be used for the identification of lineage specific markers expressed after the differentiation of stem cells into a particular lineage. Microarrays have been applied for identifying the regulatory and cell-fate signaling pathways of stem cell differentiation.[21]

Microarrays have also been used to control cell microenvironment. For example, Karp *et al*. used PEG microwells to control the shape and size of EBs by controlling the features of the microwells.[14] They found that the size of the EBs influences the eventual differentiation of the cells. In a similar experiment, Park *et al*. used PDMS stencils to control the size of ES cell aggregates. They found that aggregates with 100 μm diameter showed predominance of ectodermal markers while the aggregates with 500 μm diameter displayed increased expression of mesodermal and endodermal markers.[22] Thus initial size of the ES cell aggregate may play a role in ES cell differentiation.

Recently, robotic spotters that can be used to dispense nanoliters of fluid have been used to fabricate microarrays, in which cell–matrix interactions can be screened in a high-throughput manner. For example, synthetic biomaterial arrays have been fabricated to test the interaction of stem cells with various extracellular signals.[23] In this approach, thousands of polymeric materials were synthesized, and their effect on differentiation of human embryonic stem cells and human MSC was evaluated.[22] These interactions have led to unexpected cell–material interactions. This technology may be widely applicable in cell–microenvironment studies and in the identification of cues that induce desired cell responses. In addition to analyzing synthetic material libraries, the effect of natural ECM molecules on cell fate can be evaluated in a high-throughput manner. In one example, combinatorial matrices of various natural ECM proteins were tested for their ability to maintain the function of differentiated hepatocytes and to induce hepatic differentiation from murine embryonic stem cells.[24]

2.4. Microfluidic system for controlling stem cell fate

Microfluidic systems are becoming increasingly used in biological applications for manipulating small quantities of samples in a fast and low cost manner.[25] Microfluidic systems can provide a powerful tool to investigate the extracellular signals that regulate cell fate, because they can control cell-soluble factor interactions and be merged with high throughput technologies to test many environmental factors simultaneously. The advantages include reduced consumption of samples and reagents, shorter analysis times, greater sensitivity, portability that allows *in situ* and real-time analysis, and disposability.[26–29] These characteristics make microfluidic systems beneficial for the analysis of the stem cell microenvironment and for directing the stem cell fate. An example of the use of microfluidic systems in studying stem cell behavior was performed by Chung *et al*.[30] In their studies a gradient generating microfluidic device was used to test the effects of various concentrations of growth factors on the response of neural stem cell differentiation. Although the long term culture of cells in microchannels remains a

challenge, a number of researchers are working on various problems associated with seeding, culturing and analyzing cells within microfluidic channels. Thus the use of these systems in analyzing stem cell behavior has the potential to contribute to developing optimized conditions for directed differentiation of stem cells.

3. Microscale Technologies for Engineering Complex Tissue with Different Cell Type and Vascularized Structure

Living tissues are ensembles of different cell types embedded in complex and well-defined geometries and within a defined matrix that is unique to each tissue type. In tissue engineering, cells are cultured on degradable scaffolds that provide the physical and chemical cues to guide cellular differentiation and assembly into 3D tissues. The assembly of cells into tissues is a highly orchestrated set of events that requires time scales ranging from seconds to weeks and dimensions ranging from 0.0001 to 10 cm.[2] Conventional methods to fabricate artificial tissues rely on cell assembly which in some cases does not proceed to the degree that mimics native tissues, resulting in 3D constructs that lack the complexity associated with the architecture of tissues *in vivo*. Another major challenge of engineering tissues *in vitro* is lack of proper vascularization. Oxygen and other nutrients can only diffuse through a short distance before being consumed (a few hundred micrometers at most), which constraints the size of the engineered tissue. Several approaches resulting from microscale technologies provide new hope to overcome these challenges to build tissue with vascularized structures in a reproducible manner.

3.1. Microscale technologies for template-based cell assembly into 3D micro-tissue

Microscale tissues that mimic the *in vivo* tissue architecture and function can be obtained by inducing controlled cell aggregation. Self-assembled spheroids from single or multi-type of cells have been fabricated by several approaches, such as hanging drop,[31] spinner culture,[32] on less-adhesive substrates.[33] These approaches lack the ability to build tissues with the well-defined architectures. Microscale technologies can provide a solution to this challenge by enabling the fabrication of micro-templates for tissue formation. For instance, by using an approach combining microcontact printing and micromachining, hepatocyte spheroids have been formed.[34] In other examples, microtissues with prescribed microscale geometries have been achieved by directed self-assembly of cells in micro-molded non-adhesive agarose gel.[35] Cells were self-assembled into complex structures such as rods and honeycombs (Fig. 3). This study indicates self-assembled microtissues can be generated from cell suspensions, is not limited to

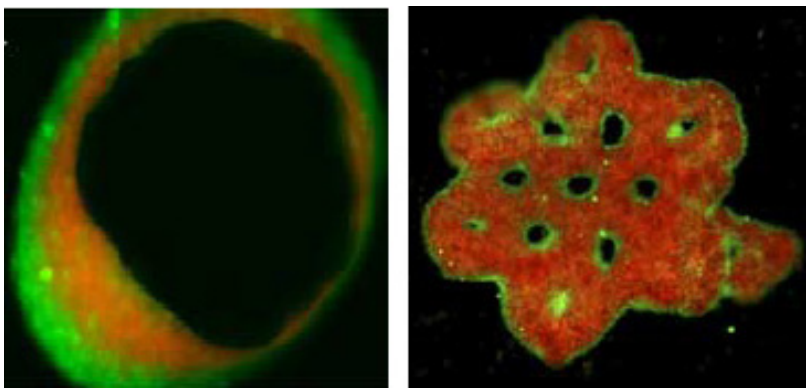

Fig. 3. Template-based self-assembly of cells into microtissues with prescribed structures (i.e. tori and honeycombs).[35]

the conventional spheroidal structure, but with more complex and diverse shapes, such as branched tissue. In addition, template-based assembly of cells could be used to build microtissues containing multiple cell types, which are organized with specific geometries relative to each other. It is envisioned that the integration of template-based microscale technologies with biomaterials and microfluidic will allow construction of more complex artificial tissue for therapeutic applications.

3.2. Scaffolds with micro- and nano-topography

It is known that topography induces change in morphology and motility of many cell types[36] by a process called "contact guidance." Contact guidance refers to the reactions of cells with the topography of their substratum.[37] Microfabrication approaches have been used to control cell morphology and alignment.[38] In natural tissues the ECM provides the nanotopography for the cell. Mimicking the original tissue environment may provide avenues for generating optimal tissue engineered constructs. Scaffolds with micro and nanotopography can be generated by various means including photolithography,[39] electrospinning,[40] chemical etching[41] and other means.[42] Electrospinning is a relatively inexpensive technique used to generate highly porous nanofibrous scaffolds from natural or synthetic polymers such as collagen,[43] poly(lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and poly(L-lactic acid) (PLLA). Cellular functions like the adhesion, growth and proliferation are enhanced when cultured on the nanoscale electrospun scaffolds.[44] Nanofibrous scaffolds have more surface area and hence offer more binding surfaces for cells. Electrospinning also enables control of various parameters like

fiber diameter, surface topology, porosity, and mechanical properties that can potentially affect the cell behavior.[44–46]

3.3. Microengineered hydrogels for tissue engineering

Hydrogels are networks of hydrophilic polymers that are attractive for tissue engineering since their physical (i.e. mechanical strength and biodegradability) and biological properties (i.e. the biocompatibility and resemblance to the natural ECM matrix) can be tailored to mimic tissues. Commonly-used hydrogels include natural hydrogels (i.e. collagen, hyaluronic acid, alginate), synthetic hydrogels (i.e. Poly(ethylene glycol)-diacrylate (PEGDA), poly(vinyl alcohol) (PVA), poly(lactic acid),[47] poly(lactic-co-glycolic acid) (PLGA)) and hybrid natural-synthetic hydrogels.[48] Photocrosslinkable hydrogels have been used for the encapsulation of various cells[49–51] as the building scaffolds for tissue engineering.[52–54] The merger of microengineered hydrogels and microfabrication techniques has significant potential to generate tissue constructs. Both "top-down" and "bottom-up" approaches have been used in using microengineered hydrogels for tissue engineering.[55] Top-down tissue engineering approach control the microscale features (i.e. shape and size) of relatively large pieces of hydrogels. Bottom-up approach refers to fabrication of tissue engineered constructs by the assembly of smaller building blocks.[55] In bottom-up approaches, functional units can be assembled in a modular approach to generate larger tissue structures. Small tissue building blocks are usually formed from microgels or cell aggregates. Patterning of the hydrogels in 2D can be done by soft lithography[56] and photopatterning.[57,58] An example of bottom-up tissue engineering is to pack rod-shaped collagen microgels seeded with HepG2 hepatocyte inside and endothelial cells on the surface within a bioreactor. The microgel-packed bioreactor exhibited interconnected channels between the microgel modules, where medium or blood can penetrate without mass transfer barrier.[59] In another example, cell-laden hydrogels with defined shape were generated by micro-molding photocrosslinkable hydrogels.[49,57] Different cell types can be encapsulated within microgels and assembled to generate larger 3D tissue structures with controlled architecture and cell-cell interaction (Fig. 4). Furthermore, to create 3D structures by bottom-up approach, Bhatia and colleagues used a multilayer photopatterning platform by polymerizing two or more overlapping cell laden PEG structures to form a complex 3D structure.[60]

3.4. 3D tissue/organ printing

3D printing of cells and biomaterials has also been used to generate 3D tissue constructs. Tissue printing offers the ability to deposit cells and other biomaterials in

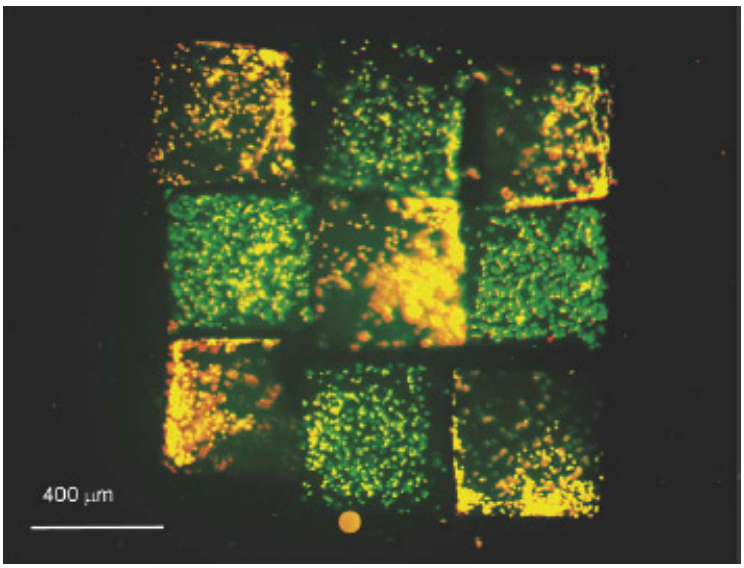

Fig. 4. Microgel arrangement and assembly. Rhodamine (red) and FITC (green) stained cells were encapsulated in separate HA microgels and subsequently arranged in an alternating checkerboard pattern.[57]

a rapid layer-by-layer fashion to create tissue structures. The most common conventional printing methods are laser printing and inkjet printing. Ringeisen *et al.* used laser printing to print pluripotent embryonal carcinoma cells. They found that cells printed onto a layer of hydrogel had greater than 95% viability and minimal single-strand DNA damage. Also the cells expressed microtubular associated protein 2 and myosin heavy chain protein after appropriate stimulus indicating successful neural and muscular pathway differentiation.[61] Barron *et al.* used biological laser printing (BioLP) to print human osteosarcoma cells into a biopolymer matrix, and after six days of incubation, the printed cells showed 100% viability.[62] To print complicated structures like the vasculature, laser-guided direct writing (LGDW) has been used. For example, Nahmias *et al.* utilized LGDW to pattern human umbilical vein endothelial cells (HUVEC) in two and three dimensions with micrometer accuracy. Furthermore, they co-cultured the vascular structures with hepatocytes to generate tubular structures similar to hepatic sinusoid.[63,64] The main drawbacks of the laser printing are the heat generated in the process which may affect the cells and furthermore laser printing is unsuitable for larger structures involving thousands of cells.

Boland and colleagues created complex cellular patterns and structures by automated and direct inkjet printing of primary embryonic hippocampal and cortical neurons. They also generated 3D cellular structure by alternate inkjet

printing of NT2 cells and fibrin gels in a layer-by-layer fashion. Various analyses showed that the printed cells were healthy and displayed normal cellular functions. Piezoelectric-based droplet ejectors have been used to achieve continuous or drop-on-demand ejection of the fluid. The ejector is harmless to sensitive fluids and biological samples can be ejected.[65,66] The non-contact piezoelectric-based ejector technology is applied to fabricate protein microarrays,[67–69] biosensors, and cell-based assays.[70] Acoustic-based non-contact printing is an emerging technology that can be used for 3D cell printing that overcomes some of the shortcomings of the previous technologies. The advantages of the acoustics-based printing are that it is non-contact and is devoid of heat, pressure and shear. Furthermore, Demirci *et al*. used acoustic-based printers to print picoliter droplets with single to few cells in each droplet. The ejected cells using their device, which can control the rate of the droplets and its directionality, to print cells with >90% viability across various cell types.[71]

The scope of the 3D tissue printing has widened to organ printing in recent years due to the advancement in the printing technology. 3D organ printing is an emerging science that tries to overcome the main challenges in tissue engineering, replicating the complexity of the tissues and providing vascular supply. Mironov *et al*. have shown that closely placed cell aggregates and embryonic heart mesenchymal (cushion tissue) fragments could fuse into ring and tube-like structures in 3D gel[72] (Fig. 5). Despite these impressive results which show the scope of organ printing, several challenges need to be overcome before the full potential can be achieved.

3.5. Microfluidics for engineering the vasculature

In vivo, cells reside in close proximity to blood vessels that supply tissues with nutrients and oxygen and remove waste products and carbon dioxide. The ability to create thick tissues is a major tissue engineering challenge, requiring the development of a vascular network for a suitable vascular supply. Although conventional techniques for scaffold fabrication, such as solvent casting and particulate leaching, cannot be used to fabricate scaffolds with controllable pore geometry, size, and interconnectivity, the ability to engineer more complex features such as a vasculature in the scaffolds is of interests. Microscale technologies have been used to construct tissue engineering scaffolds with desired microvasculature structures. Engineering vascularized tissue scaffolds have been realized by micromaching on silicon wafer[73] and soft lithography. For soft lithography, biocompatible polymers, such as PDMS, PLGA[74] and PGS[75] have been used respectively in replica molding to fabricate capillary networks. After being coated with fibronectin, these capillary networks can be used to culture endothelial cells

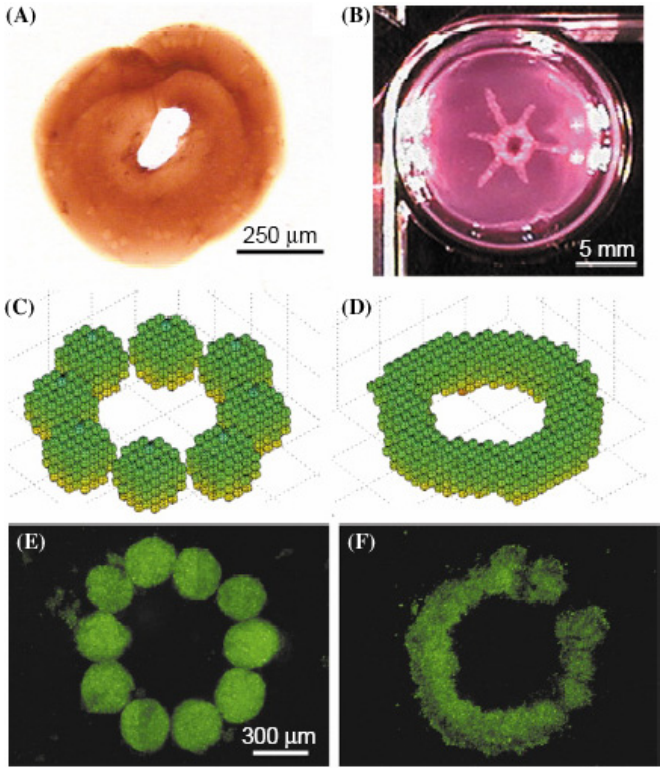

Fig. 5. Organ printing.[72] **(A)** Sequentially printed layers of collagen Type I gel. **(B)** Manually printed living tube with radial branches from the chick 27 stage HH embryonic heart cushion tissue placed in 3D collagen Type 1 gel. Tube was formed as a result of fusion of three sequential rings. Every ring consists of 16–18 closed placed and fused embryonic cushion tissue explants. **(C** and **D)** Mathematical model of cell aggregate behavior when implanted in a 3D model gel. **(E** and **F)** Fusion of ten aggregates of Chinese Hamster Ovary 60 cells implanted into RGD containing thermo-reversible gel and genetically labeled with green fluorescent protein: **(E)** before fusion and **(F)** final disc-like configuration after fusion.

to form blood vessels. Microfluidic devices have also been shown as a promising tool to facilitate the exchange of nutrients and soluble factors in 3D tissue constructs. In microfluidics system, the controlled flow of fluids with minimal reagent consumption can be achieved within microscale channels in high-throughput manner. In a recent example, microfluidic channels from cell-laden hydrogels have been developed by using a soft lithographic technique.[51] Only those cells near the microfluidic channels remained viable after three days, demonstrating the importance of a perfused network of microchannels for delivering nutrients and

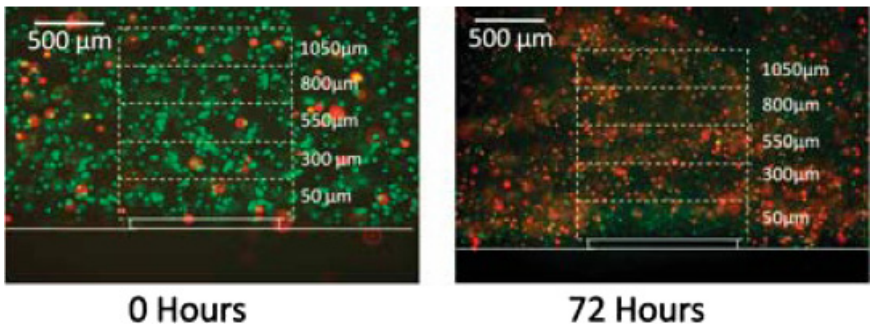

Fig. 6. Microfluidics for supplying nutrients to tissues.[51] The majority of cells were viable (as stained red) upon initial device fabrication; after 72-hour culture, only those cells near the microfluidic channels remained viable after three days.

oxygen to maintain cell viability in large hydrogels (Fig. 6). Cell-laden microfluidic hydrogels can also be scaled up by stacking the biomimetic vascular patterns to generate multi-layer vascularization in multiple discrete planes.

4. Conclusion

The merger of biomaterials and microscale technologies and their application to tissue engineering offer new opportunities to overcome the challenges faced by existing technologies to fabricate scaffolds and direct stem cell differentiation. In this review, the various applications of microscale technologies have been illustrated in controlling the stem cell fate and building complex artificial tissue with well-controlled and vascularized structures. It is believed that with the rapid growth of this burgeoning research field, microscale technologies will transform the conventional tissue engineering approaches and greatly contribute to the therapeutic potential of tissue engineering.

References

1. Langer, R. and Vacanti, J.P. Tissue engineering. *Science* **260**, 920–926 (1993).
2. Griffith, L.G. and Naughton, G. Tissue engineering — current challenges and expanding opportunities. *Science* **295**, 1009–1014 (2002).
3. Whitesides, G.M., Ostuni, E., Takayama, S., Jiang, X. and Ingber, D.E. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng*. **3**, 335–373 (2001).
4. Czyz, J. and Wobus, A. Embryonic stem cell differentiation: the role of extracellular factors. *Differentiation* **68**, 167–174 (2001).

5. Bhadriraju, K. and Hansen, L.K. Extracellular matrix- and cytoskeleton-dependent changes in cell shape and stiffness. *Exp. Cell. Res.* **278**, 92–100(2002).
6. Huang, S. and Ingber, D.E. Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Exp. Cell Res.* **261**, 91–103 (2000).
7. Watt, F.M., Jordan, P.W. and O'Neill, C.H. Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc. Natl. Acad. Sci. USA* **85**, 5576–5580 (1988).
8. Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M. and Ingber, D.E. Geometric control of cell life and death. *Science* **276**, 1425–1428 (1997).
9. Le Beyec, J., Xu, R., Lee, S.Y., Nelson, C.M., Rizki, A., Alcaraz, J. and Bissell, M.J. Cell shape regulates global histone acetylation in human mammary epithelial cells. *Exp. Cell Res.* **313**, 3066–3075 (2007).
10. McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K. and Chen, C.S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483–495 (2004).
11. Singhvi, R., Kumar, A., Lopez, G.P., Stephanopoulos, G.N., Wang, D.I., Whitesides, G.M. and Ingber, D.E. Engineering cell shape and function. *Science* **264**, 696–698 (1994).
12. Kurosawa, H. Methods for inducing embryoid body formation: *in vitro* differentiation system of embryonic stem cells. *J. Biosci. Bioeng.* **103**, 389–398 (2007).
13. Wang, X.L., Wang, C.Y., Yu, X.J., Zhao, Y.S., Li, J., Duan, C.M. and Guo, X.M. Scalable production of embryoid bodies with the rotay cell culture system. *Sheng Li Xue Bao* **57**, 486–492 (2005).
14. Karp, J.M., Yeh, J., Eng, G., Fukuda, J., Blumling, J., Suh, K.Y., Cheng, J., Mahdavi, A., Borenstein, J., Langer, R. and Khademhosseini, A. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab Chip* **7**, 786–794 (2007).
15. Khademhosseini, A., Yeh, J., Eng, G., Karp, J., Kaji, H., Borenstein, J., Farokhzad, O.C. and Langer, R. Cell docking inside microwells within reversibly sealed microfluidic channels for fabricating multiphenotype cell arrays. *Lab Chip* **5**, 1380–1386 (2005).
16. Bhatia, S.N., Balis, U.J., Yarmush, M.L. and Toner, M. Effect of cell–cell interactions in preservation of cellular phenotype: co-cultivation of hepatocytes and non-parenchymal cells. *FASEB J.* **13**, 1883–1900 (1999).
17. Bhatia, S.N., Yarmush, M.L. and Toner, M. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. *J. Biomed. Mater. Res.* **34**, 189–199 (1997).

18. Fukuda, J., Khademhosseini, A., Yeh, J., Eng, G., Cheng, J., Farokhzad, O.C. and Langer, R. Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components. *Biomaterials* **27**, 1479–1486 (2006).
19. Hui, E.E. and Bhatia, S.N. Micromechanical control of cell–cell interactions. *Proc. Natl. Acad. Sci. USA* **104**, 5722–5726 (2007).
20. Wright, D., Rajalingam, B., Selvarasah, S., Dokmeci, M.R. and Khademhosseini, A. Generation of static and dynamic patterned co-cultures using microfabricated parylene-C stencils. *Lab Chip* **7**, 1272–1279 (2007).
21. Chang, H.Y., Thomson, J.A. and Chen, X. Microarray analysis of stem cells and differentiation. *Meth. Enzymol.* **420**, 225–254 (2006).
22. Park, J., Cho, C.H., Parashurama, N., Li, Y., Berthiaume, F., Toner, M., Tilles, A.W. and Yarmush, M.L. Microfabrication-based modulation of embryonic stem cell differentiation. *Lab Chip* **7**, 1018–1028 (2007).
23. Anderson, D.G., Levenberg, S. and Langer, R. Nanoliter-scale synthesis of arrayed biomaterials and application to human embryonic stem cells. *Nat. Biotechnol.* **22**, 863–866 (2004).
24. Flaim, C.J., Chien, S. and Bhatia, S.N. An extracellular matrix microarray for probing cellular differentiation. *Nat. Methods* **2**, 119–125 (2005).
25. Haeberle, S. and Zengerle, R. Microfluidic platforms for lab-on-a-chip applications. *Lab Chip* **7**, 1094–1110 (2007).
26. Manz, A., Harrison, D.J., Verpoorte, E.M.J., Fettinger, J.C., Paulus, A., Ludi, H. and Widmer, H.M. Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip. *J. Chromatogr.* **593**, 253–258 (1992).
27. Atencia, J. and Beebe, D.J. Controlled microfluidic interfaces. *Nature* **437**, 648–655 (2005).
28. Ramsey, J.M. The burgeoning power of the shrinking laboratory. *Nat. Biotechnol.* **17**, 1061–1062 (1999).
29. Whitesides, G.M. and Stroock, A.D. Flexible methods for mircofluidics. *Phys. Today* **54**, 42–48 (2001).
30. Chung, B.G., Flanagan, L.A., Rhee, S.W., Schwartz, P.H., Lee, A.P., Monuki, E.S. and Jeon, N.L. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab Chip* **5**, 401–406 (2005).
31. Del Duca, D., Werbowetski, T. and Del Maestro, R.F. Spheroid preparation from hanging drops: characterization of a model of brain tumor invasion. *J. Neurooncol.* **67**, 295–303 (2004).
32. Lee, K.W., Lee, S.K., Joh, J.W., Kim, S.J., Lee, B.B., Kim, K.W. and Lee, K.U. Influence of pancreatic islets on spheroid formation and functions of hepatocytes in hepatocyte-pancreatic islet spheroid culture. *Tissue Eng.* **10**, 965–977 (2004).

33. Du, Y., Chia, S.M., Han, R., Chang, S., Tang, H. and Yu, H. 3D hepatocyte monolayer on hybrid RGD/galactose substratum. *Biomaterials* **27**, 5669–5680 (2006).
34. Fukuda, J., Mizumoto, H., Nakazawa, K., Kajiwara, T. and Funatsu, K. Hepatocyte organoid culture in elliptic hollow fibers to develop a hybrid artificial liver. *Int. J. Artif. Organs* **27**, 1091–1099 (2004).
35. Dean, D.M., Napolitano, A.P., Youssef, J. and Morgan, J.R. Rods, tori, and honeycombs: the directed self-assembly of microtissues with prescribed microscale geometries. *FASEB J.*, **21**, 4005–4012 (2007).
36. Chua, K.N., Chai, C., Lee, P.C., Ramakrishna, S., Leong, K.W. and Mao, H.Q. Functional nanofiber scaffolds with different spacers modulate adhesion and expansion of cryopreserved umbilical cord blood hematopoietic stem/progenitor cells. *Exp. Hematol.* **35**, 771–781 (2007).
37. Oakley, C. and Brunette, D.M. The sequence of alignment of microtubules, focal contacts and actin filaments in fibroblasts spreading on smooth and grooved titanium substrata. *J. Cell Sci.* **106**(Pt 1), 343–354 (1993).
38. Peterson, E.T. and Papautsky, I. Microtextured polydimethylsiloxane substrates for culturing mesenchymal stem cells. *Methods Mol. Biol.* **321**, 179–197 (2006).
39. Wang, G.J., Hsu, Y.F., Hsu, S.H. and Horng, R.H. JSR photolithography based microvessel scaffold fabrication and cell seeding. *Biomed. Microdev.* **8**, 17–23 (2006).
40. Yoshimoto, H., Shin, Y.M., Terai, H. and Vacanti, J.P. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. *Biomaterials* **24**, 2077–2082 (2003).
41. Wang, Z., Hu, H., Wang, Y., Wang, Y., Wu, Q., Liu, L. and Chen, G. Fabrication of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) microstructures using soft lithography for scaffold applications. *Biomaterials* **27**, 2550–2557 (2006).
42. Borenstein, J.T., Weinberg, E.J., Orrick, B.K., Sundback, C., Kaazempur-Mofrad, M.R. and Vacanti, J.P. Microfabrication of three-dimensional engineered scaffolds. *Tissue Eng.* **13**, 1837–1844 (2007).
43. Matthews, J.A., Wnek, G.E., Simpson, D.G. and Bowlin, G.L. Electrospinning of collagen nanofibers. *Biomacromolecules* **3**, 232–238 (2002).
44. Boudriot, U., Dersch, R., Greiner, A. and Wendorff, J.H. Electrospinning approaches toward scaffold engineering — a brief overview. *Artif. Organs* **30**, 785–792 (2006).
45. Nair, L.S., Bhattacharyya, S. and Laurencin, C.T. Development of novel tissue engineering scaffolds via electrospinning. *Expert Opin. Biol. Ther.* **4**, 659–668 (2004).

46. Pham, Q.P., Sharma, U. and Mikos, A.G. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng.* **12**, 1197–1211 (2006).
47. Ivanov, I., Schaab, C., Planitzer, S., Teichmann, U., Machl, A., Theml, S., Meier-Ewert, S., Seizinger, B. and Loferer, H. DNA microarray technology and antimicrobial drug discovery. *Pharmacogenomics* **1**, 169–178 (2000).
48. Flores-Ramirez, N., Elizalde-Pena, E.A., Vasquez-Garcia, S.R., Gonzalez-Hernandez, J., Martinez-Ruvalcaba, A., Sanchez, I.C., Luna-Barcenas, G. and Gupta, R.B. Characterization and degradation of functionalized chitosan with glycidyl methacrylate. *J. Biomater. Sci. Polym. Ed.* **16**, 473–488 (2005).
49. Yeh, J., Ling, Y., Karp, J.M., Gantz, J., Chandawarkar, A., Eng, G., Blumling, J., 3rd, Langer, R. and Khademhosseini, A. Micromolding of shape-controlled, harvestable cell-laden hydrogels. *Biomaterials* **27**, 5391–5398 (2006).
50. Elisseeff, J., McIntosh, W., Anseth, K., Riley, S., Ragan, P. and Langer, R. Photoencapsulation of chondrocytes in poly(ethylene oxide)-based semi-interpenetrating networks. *J. Biomed. Mater. Res.* **51**, 164–171 (2000).
51. Ling, Y., Rubin, J., Deng, Y., Huang, C., Demirci, U., Karp, J.M. and Khademhosseini, A. A cell-laden microfluidic hydrogel. *Lab Chip* **7**, 756–762 (2007).
52. Jager, M., Degistirici, O., Knipper, A., Fischer, J., Sager, M. and Krauspe, R. Bone healing and migration of cord blood derived stem cells into a critical size femoral defect after xenotransplantation. *J. Bone Miner Res.* **22**, 1224–1233 (2007).
53. Hahn, M.S., Teply, B.A., Stevens, M.M., Zeitels, S.M. and Langer, R. Collagen composite hydrogels for vocal fold lamina propria restoration. *Biomaterials* **27**, 1104–1109 (2006).
54. Fedorovich, N.E., Alblas, J., de Wijn, J.R., Hennink, W.E., Verbout, A.J. and Dhert, W.J. Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. *Tissue Eng.* **13**, 1905–1925 (2007).
55. Khademhosseini, A. and Langer, R. Microengineered hydrogels for tissue engineering. *Biomaterials* **28**, 5087–5092 (2007).
56. Suh, K.Y., Seong, J., Khademhosseini, A., Laibinis, P.E. and Langer, R. A simple soft lithographic route to fabrication of poly(ethylene glycol) microstructures for protein and cell patterning. *Biomaterials* **25**, 557–563 (2004).
57. Khademhosseini, A., Eng, G., Yeh, J., Fukuda, J., Blumling, J., 3rd, Langer, R. and Burdick, J.A. Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment. *J. Biomed. Mater. Res. A* **79**, 522–532 (2006).

58. Hahn, M.S., Taite, L.J., Moon, J.J., Rowland, M.C., Ruffino, K.A. and West, J.L. Photolithographic patterning of polyethylene glycol hydrogels. *Biomaterials* **27**, 2519–2524 (2006).
59. Uludag, H., De Vos, P. and Tresco, P.A. Technology of mammalian cell encapsulation. *Adv. Drug Deliv. Rev.* **42**, 29–64 (2000).
60. Liu Tsang, V., Chen, A.A., Cho, L.M., Jadin, K.D., Sah, R.L., DeLong, S., West, J.L. and Bhatia, S.N. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. *FASEB J.* **21**, 790–801 (2007).
61. Ringeisen, B.R., Kim, H., Barron, J.A., Krizman, D.B., Chrisey, D.B., Jackman, S., Auyeung, R.Y. and Spargo, B.J. Laser printing of pluripotent embryonal carcinoma cells. *Tissue Eng.* **10**, 483–491 (2004).
62. Barron, J.A., Krizman, D.B. and Ringeisen, B.R. Laser printing of single cells: statistical analysis, cell viability, and stress. *Ann. Biomed. Eng.* **33**, 121–130 (2005).
63. Nahmias, Y., Schwartz, R.E., Verfaillie, C.M. and Odde, D.J. Laser-guided direct writing for three-dimensional tissue engineering. *Biotechnol. Bioeng.* **92**, 129–136 (2005).
64. Nahmias, Y. and Odde, D.J. Micropatterning of living cells by laser-guided direct writing: application to fabrication of hepatic-endothelial sinusoid-like structures. *Nat. Protoc.* **1**, 2288–2296 (2006).
65. Percin, G. and Khuri-Yakub, B.T. Piezoelectrically actuated flextensional micromachined ultrasound droplet ejectors. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **49**, 756–766 (2002).
66. Percin, G. and Khuri-Yakub, B.T. Piezoelectrically actuated flextensional micromachined ultrasound transducers. *Ultrasonics* **40**, 441–448 (2002).
67. Delehanty, J.B. Printing functional protein microarrays using piezoelectric capillaries. *Methods Mol. Biol*. **264**, 135–143 (2004).
68. Delehanty, J.B. and Ligler, F.S. Method for printing functional protein microarrays. *Biotechniques* **34**, 380–385 (2003).
69. Hsieh, H.B., Fitch, J., White, D., Torres, F., Roy, J., Matusiak, R., Krivacic, B., Kowalski, B., Bruce, R. and Elrod, S. Ultra-high-throughput microarray generation and liquid dispensing using multiple disposable piezoelectric ejectors. *J. Biomol. Screen* **9**, 85–94 (2004).
70. Sumerel, J., Lewis, J., Doraiswamy, A., Deravi, L.F., Sewell, S.L., Gerdon, A.E., Wright, D.W. and Narayan, R.J. Piezoelectric ink jet processing of materials for medical and biological applications. *Biotechnol. J.* **1**, 976–987 (2006).
71. Demirci, U. and Montesano, G. Single cell epitaxy by acoustic picolitre droplets. *Lab Chip* **7**, 1139–1145 (2007).

72. Mironov, V., Boland, T., Trusk, T., Forgacs, G. and Markwald, R.R. Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* **21**, 157–161 (2003).
73. Kaihara, S., Borenstein, J., Koka, R., Lalan, S., Ochoa, E.R., Ravens, M., Pien, H., Cunningham, B. and Vacanti, J.P. Silicon micromachining to tissue engineer branched vascular channels for liver fabrication. *Tissue Eng.* **6**, 105–117 (2000).
74. Vozzi, G., Flaim, C., Ahluwalia, A. and Bhatia, S. Fabrication of PLGA scaffolds using soft lithography and microsyringe deposition. *Biomaterials* **24**, 2533–2540 (2003).
75. King, K., Wang, C., Kaazempur-Mofrad, M., Vacanti, J. and Borenstein, J. Biodegradable microfluidics. *Adv. Mater.* **16**, 2007–2012 (2004).