# Emerging Technologies for Assembly of Microscale Hydrogels

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**Assembly of cell encapsulating building blocks (*i.e.*, microscale hydrogels) has significant applications in areas including regenerative medicine, tissue engineering, and cell-based *in vitro* assays for pharmaceutical research and drug discovery. Inspired by the repeating functional units observed in native tissues and biological systems (*e.g.*, the lobule in liver, the nephron in kidney), assembly technologies aim to generate complex tissue structures by organizing microscale building blocks. Novel assembly technologies enable fabrication of engineered tissue constructs with controlled properties including tunable microarchitectural and predefined compositional features. Recent advances in micro- and nano-scale technologies have enabled engineering of microgel based three dimensional (3D) constructs. There is a need for high-throughput and scalable methods to assemble microscale units with a complex 3D micro-architecture. Emerging assembly methods include novel technologies based on microfluidics, acoustic and magnetic fields, nanotextured surfaces, and surface tension. In this review, we survey emerging microscale hydrogel assembly methods offering rapid, scalable microgel assembly in 3D, and provide future perspectives and discuss potential applications.**

## 1. Introduction

Tissue engineering is an interdisciplinary field that applies the principals of science and engineering to medicine to regenerate functional tissues.[1–15,206] Growing human tissues and organs is highly important for regenerative medicine which holds great promise to enable improved therapies for multiple degenerative diseases such as diabetes, kidney, liver, and heart failure. Another emerging application of tissue engineering is the development of *in vitro* testing platforms that mimic human physiological systems and their complex interactions with vaccines, drugs or other biologics.[16] In these microphysiological systems, different cell types have to function together and achieve physiologically-relevant crosstalk mimicking native tissues. These platforms should accurately predict the safety and effectiveness of drugs and vaccines, which will eventually accelerate the drug development cycle by enabling early rejection of ineffective or toxic drug candidates.

Tissue engineering approaches aim to recreate the native 3D architecture *in vitro*. Conventional tissue engineering methods employ a "top-down" approach, where cells are seeded onto pre-shaped porous polymer scaffolds with a combination of molecular cues[17–26] (**Table 1**). Scaffolding techniques have traditionally been used to form degradable, porous, polymer scaffolds (*e.g.*, collagen hydrogels and agarose) that are seeded with cells forming 3D environments.[25–37,207,208] Porous scaffolds are artificial structures that support tissue formation.[38] Depending on cell type, natural tissue stiffness and compositional properties (*e.g.* extracellular matrix components such as fibronectin, collagen) can be engineered using scaffolding methods. These techniques usually rely on proliferation of cells after seeding to attain higher cell density. Scaffold-based methods are commonly used in tissue engineering and these approaches have been extensively reviewed in the literature.[39,40] Challenges in current tissue engineering approaches include: 1) achieving complex 3D cellular architecture and organization,[4,40–44] and 2) control over cellular proximity, density and microscale resolution.[7,42–44] Therefore, precise patterning of cells in a specific architecture to form highly organized tissue constructs with high cell density remains as a challenge (Table 1).

Assembly and patterning of multiple cell types encapsulated in hydrogels with control over a biologically relevant length scale has great potential for regenerative medicine,[2,5–7,42–44,49–51] cell-based biochips[52,53] and organ on a chip devices[54,55] for pharmaceutical research and drug discovery.[16,56] However, current bottom-up tissue engineering approaches face challenges in assembling multiple cell types and extracellular matrix

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**DOI: 10.1002/adhm.201200011**

(ECM) components with spatial and temporal control[40,57] (Table 1). Therefore, technologies that allow control over gel assembly in 3D are needed.[14,19,40,42–44,57–60] Here, we reviewed the recent assembly approaches based on microfluidics, acoustic and magnetic fields, nanotextured surfaces, and surface tension (**Figure 1**) to address these challenges.

## 2. Bottom-Up Tissue Engineering and Building Blocks in Assembly

*In vivo*, cells in functional units are embedded in a 3D microenvironment composed of ECM and neighboring cells with a defined spatial distribution.[61] Tissue functionality derives from these components, cells' interactions and relative locations.[2,5,6,25,42,60,63–67] Inspired by the repeating functional units observed *in vivo* (*e.g.*, repeating hexagonal lobules in the liver,[45] and nephrons in kidney), bottom-up tissue engineering aims to generate complex tissue structures by assembling cell encapsulating building blocks, *i.e.*, microgels.[15,40,42–44,46–48,71]

We first briefly review microgel fabrication methods, as they are the essential building blocks for assembly. The commonly used methods for microgel fabrication can be listed as: (1) molding, (2) photolithography, (3) molecular synthesis, (4) folding, and (5) microdroplet generation. Various techniques have been adopted to create molds for polymeric materials and hydrogels. Some of these techniques have recently been used for tissue engineering.[61] For example, molding of polyurethane into individual components is demonstrated[209] in **Figure 2**A.

A recent review highlighted methods to fabricate soft materials using lithography.[69] Particularly, we focused on lithography methods for microgels. For example, continuous-flow lithography is a technique that can generate various shapes of microgels at a throughput of ~100 gels per second.[70] Another technique is the stop-flow photolithography, which is based on creating microgels using a simple, and inexpensive photomask.[42,43,71,72] In this method, microgels are prepared by mixing pre-polymer solution and photoinitiator,[42,71,43] which is then UV crosslinked with a pre-designed mask that defines the microgel geometries (Figure 2B).

Hydrogels and their use in biomedical research have been reviewed earlier.[73] Previously, amphiphilic molecules were synthetized to generate aligned monodomain gels[74] (Figure 2C). The mechanism of the alignment of these gels was due to favorable enthalpic interaction of the fibers that aligns peptides to alkyl ends.[74] These aligned gels have potential applications in regenerative therapies for neural tissues. Silk proteins form supramolecular structures in the form of hydrogels under certain ambient conditions.[75] Silk gels received attention due to their biocompatible, biodegradable, versatile properties and unique mechanical characteristics.[76]

Various mechanisms drive folding in nature[77] (Figure 2D). Folding into precise three dimensional shapes depends on a number of factors for thin films, including the bending modulus and the material geometry. Two-dimensional sheets will fold into three-dimensional structures as a function of the number and direction of folds. For example, two hydrogel layers may be placed on top of one another, each with a different swelling ratio. When hydrated, differences in swelling ratios induce

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**Table 1.** Comparison of assembly and 3D tissue engineering technologies.

| Performance metric | Microfluidic Assembly[115–117] | Ratchet assembly[71] | Acoustic Assembly[42] | Magnetic Assembly[43] | Surface tension driven assembly[205] | Scaffold based engineering[25–27,40] | Laser Bioprinting[64,91] | Inkjet Bioprinting[88–90] |
|---|---|---|---|---|---|---|---|---|
| Throughput | Low | High | High | High | Medium | Medium | Medium | High |
| Microgel Size | 50 μm and smaller | 50–1000 μm | 50–1000 μm | 50–1000 μm | 200–1000 μm | N/A | N/A | 100–1000 μm |
| Complexity | High | Low | Low | Medium | Low | Low | High | High |
| Adverse Effects on Cells | Low | Low | Low | Low | Medium | Low | Medium | Medium |
| Spatial Resolution | High | Medium | Medium | High | Low | Low | High | High |
| Cell density | High | High | High | High | High | High | Medium | Medium |

different levels of stress in each layer, causing the hydrogel to bend.[210] Another example involves pre-straining two metals to induce different levels of stress in an object, such as bimetallic layers in a thermocouple. Folding a 2D surface into a 3D structure using the surface tension and wetting properties of thin films is motivated by small scale assembly.[211] Polymeric (*e.g.,* phema hydrogel and PDMS), metallic (*e.g.,* gold), and semiconducting (*e.g.,* silicon) thin films have been used in folding applications in response to surface tension.[211]

Microdroplet technologies based on bioprinting can be used to generate cell encapsulating microscale hydrogel droplets that can be assembled to form 3D complex constructs.[14,19,44,78–87,102,106] Bioprinting technologies have been developed to spatially control organization of cells in combination with scaffolding materials.[44,58,102,103,106] Today's bioprinting technologies, inkjet,[88–90] laser,[64,91] and bio-electrosprays[92,93] were not specifically designed to generate droplets encapsulating living cells.[14] For instance, initial technologies utilized modified inkjet printers filled with cell suspensions.[14,19,22,94] Recent nozzle-free acoustic and valve-based methods have opened a vista towards cell printing technologies with high viability and functionality.[40,44,95–108] Current technologies are advancing to address challenges of bioprinting (Table 1) such as: (1) frequent clogging, (2) high shear stress on cells, (3) control in the number of printed cells, (4) unreliability associated with ejection of drops containing no cells, (5) repeatability and low throughput function, and (6) heating by light absorption during laser printing.[14,19,95,109,110–113] These challenges will be addressed by emerging technologies that enhance the ability to assemble and pattern cells and microscale hydrogels using technologies, which are essential in forming complex 3D tissues.

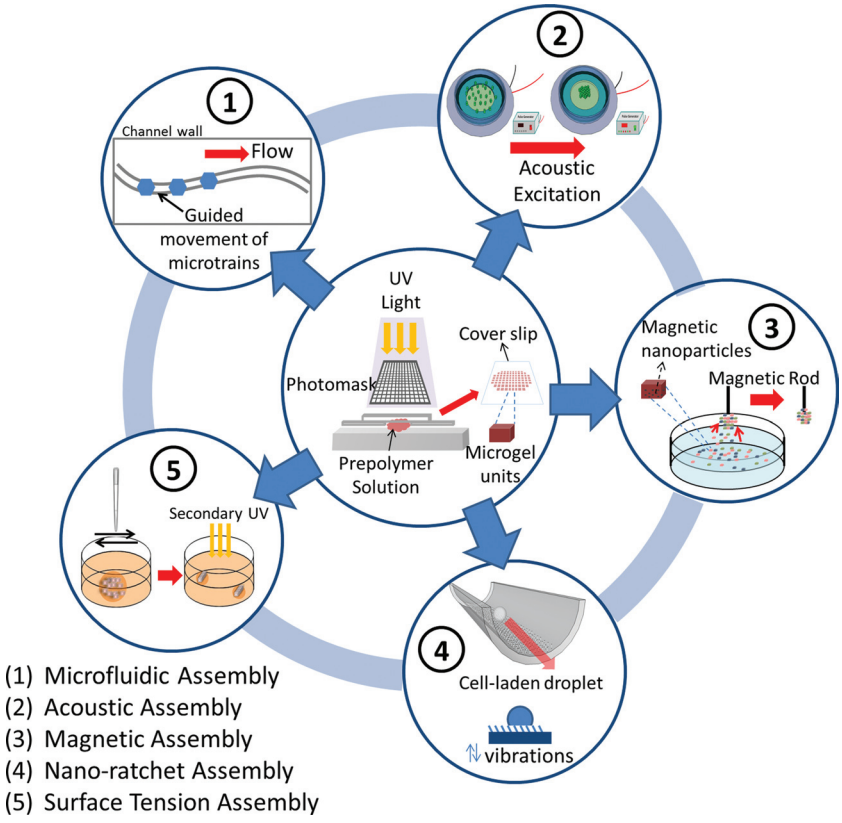


**Figure 1.** Methods for directed assembly of microscale hydrogels for microphysiological 3D systems: microfluidic assembly, nano-ratchet assembly, acoustic assembly, magnetic assembly, and surface tension driven assembly.

## 3. 3D Microgel Assembly Approaches

When cells are cultured in two-dimensional (2D) monolayers, they display significant phenotypic and genotypic difference compared to cells in native tissues as well as in 3D culture conditions.[114] Hence, 2D systems do not effectively represent the complex 3D tissue environment.[27,40,67,114] Assembly approaches need to enable rapid and scalable fabrication of engineered tissue constructs with predefined geometrical and biological features with multiple material types and intricate architecture.[42,43] Here, we review emerging strategies (Figure 1) to address these challenges to create 2D and 3D tissue structures with applications in tissue/organ regeneration, pharmaceutical research and drug discovery.

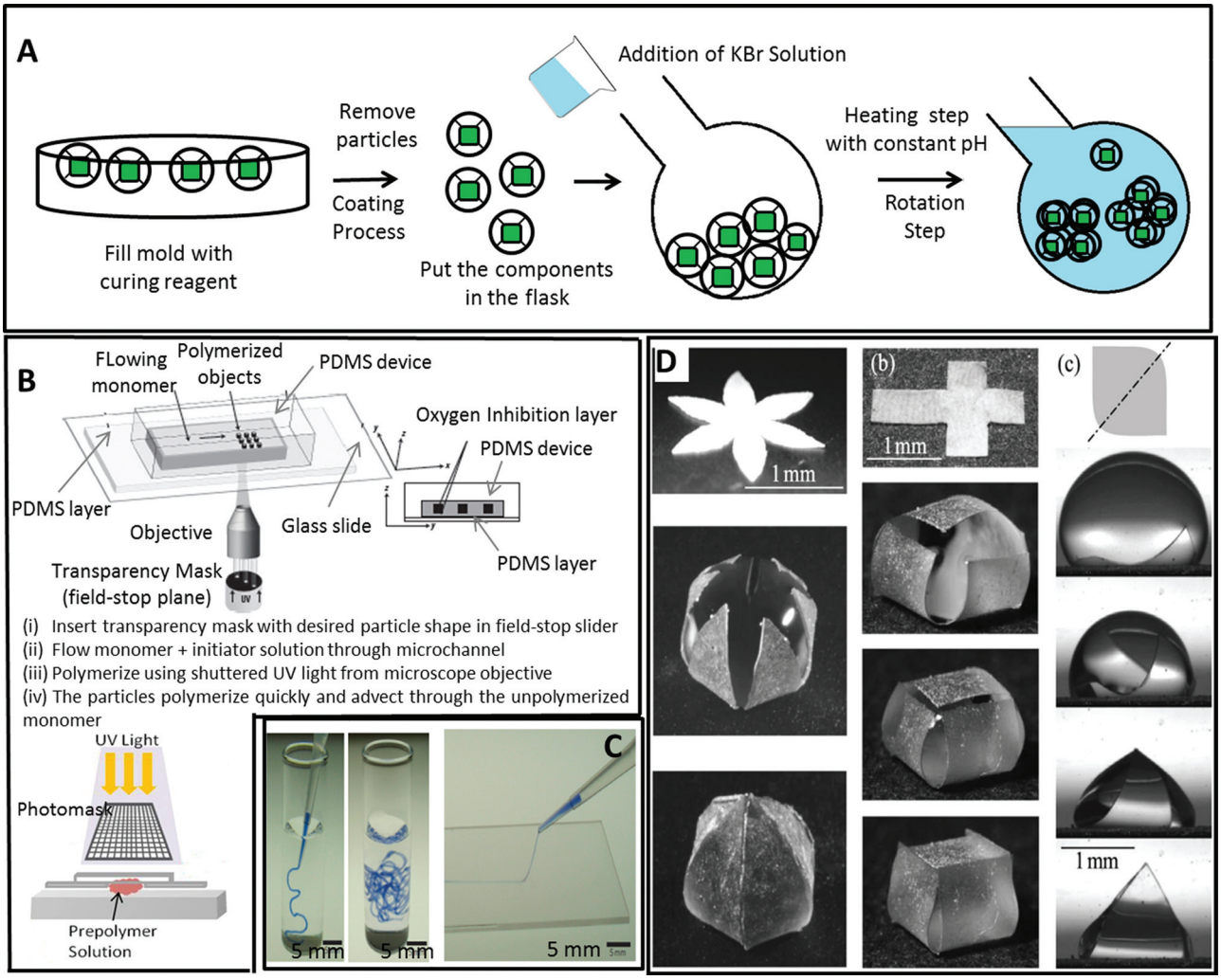


**Figure 2.** Fabrication of microscale units for assembly: (A) Molding, (B) Continuous-flow lithography (top) and Photolithography (bottom), (C) Molecular synthesis, and (D) Folding. Reproduced with permission; A: from [209] Copyright 1999, AAAS; B: from[70] Copyright 2006, NPG; C: from [74] Copyright 2010, NPG;D: from [77] Copyright 2007, AIP.

### 3.1. Railed Microfluidic Assembly

Microstructures were assembled for parallel fabrication of devices using microfluidic channels.[115–118] As an immediate application of this method, assembly of hydrogels has been performed to create 2D complex geometries (**Figure 3**A) such as Eiffel Tower shape.[115] For the assembly system, grooves were fabricated on channels and supplemented with polymeric microstructures. In this study, microstructures were guided through different solutions, *e.g.,* photocurable oligomer and water, in 1D and 2D microfluidic channels. Microtrains made of different polymeric materials were assembled heterogeneously via cross-solution movement. This approach eliminated multiple alignment and separate material patterning steps, which have to be performed in conventional lithography.

This railed microfluidic method has been developed to create polymeric microarchitectures.[117] 2D photopatterned microstructures were assembled using axis-translation process in microfluidic channels. This method is highly deterministic compared to other self-assembly technologies such as surface tension driven assembly. However, the railed microfluidics require production of a large number of complex polymeric microcarriers. There are also other fluidic techniques for assembling, transporting, and reconfiguring microstructures such as laser activated bubble latching[119] and dynamically programmable fluidic assembly.[120,121] These techniques haven't been applied to microgel assembly with the purpose of tissue engineering.

### 3.2. Acoustic Assembly

Acoustic fields have traditionally been employed for *in vivo* imaging in medicine.[122–124] Recently, acoustic actuation technologies integrated with microfluidics, and acoustic based droplet generation methods have been developed to manipulate microscale particles and cells.[95–97,101,125–129] Example applications of acoustic technologies include particle microcentrifugation,[130,131] aggregation and trapping,[132–135] continuous flow manipulation and separation of cells,[136–140] cell

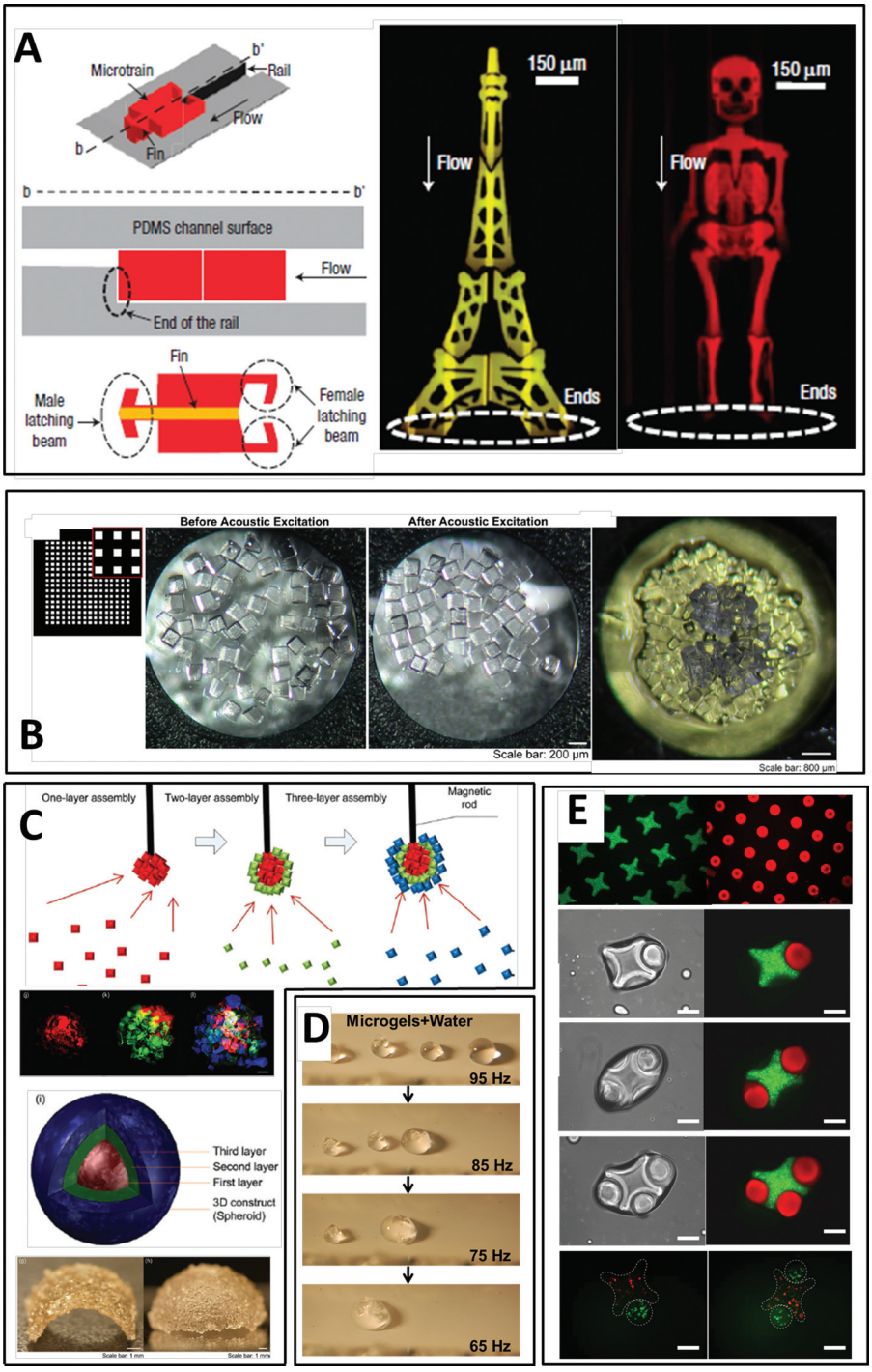


**Figure 3.** Directed assembly methods in literature for microscale hydrogels. (A) Microfluidic assembly. Reproduced with permission from[115]. Copyright 2008, NPG. (left) A schematic diagram for a microtrain (top), its cross-sectional view (middle), and design of a single microlatch component (bottom). (middle) Eiffel assembly, and (right) Skeleton assembly. **(B)** Assembly of microgels by acoustic fields in 2D and in 3D. Reproduced with permission from [42]. Copyright 2011, Elsevier. (left) Before and after acoustic excitation: single-layer formation (200 µm × 200 µm microgels). (right) New microgels were introduced onto a single layer to create a double-layer structure. **(C)** Assembly of microgels in 2D and 3D by magnetic fields. Reproduced with permission from[43]. (top) M-gels are collected from 3 different gel batches via magnetic forces to fabricate three-layer spheroids. First layer gels are stained with rhodamine-B; second layer gels are stained with FITC-dextran; third layer gels are stained with TPB (1,1,4,4-tetraphenyl-1,3-butadiene). (middle) Magnified image of the assembled single-layer 3D construct. (bottom) Images of arc- and dome-shaped constructs using a flexible surface and magnetic assembly. **(D)** Transport and assembly of microgels on ratchets. Reproduced with permission from [71]. Copyright 2011, AIP. **(E)** Surface tension driven self-assembly of microgels. Reproduced with permission from [46]. Copyright 2008, National Academy of Sciences. Directed assembly of lock-and-key-shaped microgels. Fluorescence images of cross-shaped microgels stained with FITC-dextran and rod-shaped microgels stained with Nile red. Phase-contrast and fluorescence images of lock-and-key assemblies with one, two, and three rods per cross. (bottom) Fluorescence images of assembly of microgels containing green- and red-stained cells. (Scale bars: 200 µm).

synchronization and patterning,[141,142] and droplet concentration and mixing.[143–145] In addition, cell encapsulating microscale units that are sensitive to pressure and heat can be used with acoustic technologies.[96,97,129,146]

Recently, acoustic fields[147–149,212] have been developed to assemble microgels of different shapes and sizes.[42] In this study, microgels suspended in a water droplet were acoustically assembled (Figure 3B). When microgels were subjected to acoustic fields, no significant effect on cell viability was observed. The acoustic field assembly was tested using different geometries of microgels (Tetris, lock-and-key, square, and saw-tooth) to form single-layer organized structures. Next, the acoustic assembly method was utilized to fabricate multiple layer constructs made of microgels. In this approach, suspended unorganized microgels were initially assembled into a single layer organized structure, which was stabilized by secondary crosslinking. Additional microgels were then introduced, and the acoustic field was used to assemble a second layer above the first layer. Using this layer-by-layer approach integrated with acoustic fields, a multilayer organized construct was fabricated (Figure 3B).[42]

### 3.3. Magnetic Assembly

Magnetic fields have been used for applications such as cellular manipulation, cell sorting and isolation, 3D cell culture, and clinical *in vivo* imaging.[150–161] Magnetic fields have recently been adapted to achieve 3D tissue culture in magnetic levitation.[157] In this approach, cells were encapsulated in a hydrogel composed of magnetic iron oxide, bacteriophage, and gold nanoparticles. This method is compatible with soft materials, *e.g.,* hydrogels. Magnetic nanoparticles (MNPs) have also been employed to create 2D patterns,[151,163–165] to form 3D cell culture arrays,[166] and to characterize cell-membrane mechanical properties.[167] In these methods, cells were first mixed with ferrofluids or functionalized MNPs, and then exposed to external magnetic fields allowing controlled assembly. Other methods have been developed based on microfluidics to embed MNPs into microgels,[154,168,169] and applied to multiplexed bioassays.[170,171]

Use of nanoparticles comes with cytotoxicity considerations, where nanotoxicity is an emerging field of research.[172] The United States Food and Drug Administration (FDA) has approved the use of MNPs in several applications[174] such as imaging agents depending on the fact that the iron (II/III) will be rapidly discharged from the human body. Tolerability of mammalian cells to MNPs has been demonstrated.[163,164,175] Some of initial results have shown the discharge of MNPs from biodegradable gel constructs.[43] The use of FDA-approved MNPs as building blocks for nanoparticle assisted microgel assembly needs to be further characterized to ensure the safety of these materials for use in humans. MNPs do not need to stay within the assembled constructs for long durations, and they can be released as the gels biodegrade and cells secrete their own ECM.

It has recently been shown that magnetic nanoparticle (MNP) loaded microscale hydrogels (M-gels) can be assembled into 3D multilayer constructs via low-intensity magnetic fields using standard magnets.[43] By spatially controlling the magnetic field, different shapes of 3D constructs were obtained and multilayer assembly of multiple microgel layers and co-cultures were achieved (Figure 3C). In this system, a magnetic field was applied to the assembly chamber by placing magnets in parallel. The resulting constructs assembled in a line geometry of multiple rows and validated by a computational model. It was observed that the assembled constructs retained their shape and remained intact after the magnets were removed. To tune row width, microgels were assembled using different number of magnets placed under the assembler chamber. The assembly process took less than a second indicating a high assembly speed and potential for high throughput. Then, NIH 3T3 cells were encapsulated in M-gels. It was observed that cell viability in M-gels and in controls (without MNPs) were comparable. This magnetic assembly approach was also able to provide precise temporal and/or spatial control in 3D manipulation of microgels. 3D multilayer spherical constructs were fabricated using the assembly system, in which M-gels were collected onto the tip of a magnetic rod. By varying the MNP level in M-gels, single-layer spheroid assemblies of different sizes were achieved. Other complex constructs were also fabricated through the manipulation of M-gels with magnetic fields including flexible 3D surfaces such as arc and dome shapes. The capability to control assembly sizes via varying MNP concentrations allowed fabrication of 3D multilayer spheroids. These results indicated that the developed methodology can potentially become a useful tool to bioengineer 3D tissue constructs.

### 3.4. Nanotextured Surface (Ratchet) Assembly

Nature employs various mechanisms to modify the curvature or wetting properties of free surfaces. Largely inspired by natural systems, many artificial hydrophobic surfaces have been fabricated and analyzed experimentally and theoretically. Numerous techniques for manufacturing robust super-hydrophobic surfaces have been developed, ranging from chemical patterning,[176] photolithography,[177] electro-spinning[178] and vertically grown nano-structures[179] including carbon nanotube forests.[181–183] Unidirectional surfaces offer the opportunity to assemble soft materials (*e.g.,* microgels) via sequential assembly processes that lead to complex architectures with novel properties. Recently, it has been shown that a controlled mechanism of directed spatial manipulation of droplets provides microgel assembly on unidirectional surfaces (Figure 3D). By inducing a vibrational field on the boundary, droplets can be propelled on the unidirectional surface, *i.e.,* in the direction of the asperities.[184] Time-lapse frames of droplet motion with and without microgels were quantified as a function of vibration frequency and it was demonstrated that droplets containing microgels created an automated controllable assembly.[71]

Various mechanisms have been proposed to mobilize and direct droplets. Among these are capillary forces,[185] focused acoustics waves,[95,96,186] surface tension,[187] transistor based actuation,[188] electrowetting,[189] and chemical[188,190] or temperature based Leidenfrost ratchet.[100,191] Vibration on asymmetrically textured surfaces[184,192–196] (*i.e.,* surface ratchets) transports small materials (*e.g.,* droplets) without a directed force. This approach eliminates the need for complex fabrication procedures

and controls for applications such as droplet manipulation and movement. Droplet transport or manipulation on a surface by a ratcheting mechanism requires a textured pattern for movement of droplets at low amplitudes of vibration. On the other hand, the nanotextured film provides a directional surface, which is hydrophobic due to its nanoscale texture.[197–200] It was shown that smooth nanotextured film surface, fabricated by oblique angle vapor deposition of poly(p-choloro-xylylene) (PPX), carries microliter droplets along with minimal droplet shape deformations.[184] It was also shown that the tilted fibers on PPX nanofilm resulted in unidirectional wetting and droplet motion.[184]

### 3.5. Surface Tension Driven Assembly

To create 3D self-assembled structures in a sequential manner, tendency of multiphase liquid-liquid systems to minimize the surface area and to reach a lower energy configuration has been used.[201–203] This "hydrophobic effect" based on the tendency of oil and water to segregate is used to assemble hydrogels in a hydrophobic medium.[204] After fabrication of microgels, they were transferred into mineral oil, a hydrophobic medium (Figure 3E). Mechanical agitation was used to aggregate the individual microgels.[46] The effects of agitation rate, time, addition of surfactant, and secondary crosslinking on viability of cells encapsulated in microgels were investigated. By changing the agitation rate, different shapes of assemblies, *e.g.,* random, branched, linear and offset, were observed. The direct alignment of one type of gel next to another was not controlled in this method and the aggregation of hydrogels was unstable outside the mineral oil and needed a secondary crosslinking step for stabilization. Hence, a lock-and-key model was used for assembly. Additionally, at the end of the assembly process, there was a slight amount of residual mineral oil on the surface of hydrogels.

The assembly process was improved by increasing the hydrophilicity of hydrogels and reducing the surface tension of the surrounding solution. The self-assembly of microgels was demonstrated by replacing mineral oil with a more hydrophobic organic solvent (perflourodecalin).[205] By increasing the hydrophobicity of the solution, surface tension led to closely packed accumulation of gels on the surface at the liquid-air interface.[205] Gel assembly was controlled by adjusting stirring speed and time. Although these hydrogels were assembled together due to minimization of surface free energy, a secondary crosslinking step was used to stabilize the assembled hydrogel constructs. These microfabrication techniques use hydrophilic-hydrophobic interactions to assemble hydrogels. These approaches can be further improved by achieving control over guidance of microgels and probabilistic nature of assembly.

## 5. Conclusions and Future Perspectives

Emerging nano- and micro-scale technologies open new avenues to assemble microgels. These technologies bring a fresh approach to bottom-up tissue engineering in terms of the complexity and control that can be achieved especially creating 3D multilayer, complex constructs. The applications of these technologies will cover a broad spectrum of applications including high throughput platforms for testing new drug candidates and tissue engineering for regenerative medicine. For instance, there is a need for *in vitro* testing platforms that mimic the human physiological systems, disease conditions and their complex interactions with vaccines, drugs or other biologic agents. The ability to control the microarchitecture of 3D microphysiological systems is critical. Engineered physiological systems as testing platforms would allow rapid development of new drugs by producing relevant data in a shorter period of time. This approach would also accelerate the development of effective drugs and reduce costs associated with pharmaceutical research by early rejecting the ineffective or toxic candidates. Time and cost reduction in pharmaceutical sciences can be facilitated by engineered microphysiological systems, which would significantly affect the future of healthcare. These engineered systems will also improve our understanding of disease etiology and eventually lead to advances in the quality and number of new therapies that move through the drug development cycle to be used in clinical care.

The emerging strategies for directed assembly of microscale hydrogels and tissue units reviewed here have potential to create spatially organized complex 3D tissues and organs. These engineered tissues can be used to recuperate the lost or deteriorated organ function, which can be used as an alternative or complementary strategy for tissue transplants. The nano- and micro-scale manufacturing technologies will continue to impact science, especially in fields including pharmaceutical, biomedical sciences and clinical medicine. The technologies reviewed here provide solutions and advanced capabilities for precision, repeatability and high throughput processing to develop engineered physiological systems.

## Acknowledgements

We would like to acknowledge NIH grants 1R21HL112114 and R21HL095960.

Received: January 14, 2012
Revised: February 8, 2012
Published online: March 21, 2012

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