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Engineering hydrogels as extracellular matrix mimics

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Abstract

Extracellular matrix (ECM) is a complex cellular environment consisting of proteins, proteoglycans, and other soluble molecules. ECM provides structural support to mammalian cells and a regulatory milieu with a variety of important cell functions, including assembling cells into various tissues and organs, regulating growth and cell–cell communication. Developing a tailored *in vitro* cell culture environment that mimics the intricate and organized nanoscale meshwork of native ECM is desirable. Recent studies have shown the potential of hydrogels to mimic native ECM. Such an engineered native-like ECM is more likely to provide cells with rational cues for diagnostic and therapeutic studies. The research for novel biomaterials has led to an extension of the scope and techniques used to fabricate biomimetic hydrogel scaffolds for tissue engineering and regenerative medicine applications. In this article, we detail the progress of the current state-of-the-art engineering methods to create cell-encapsulating hydrogel tissue constructs as well as their applications in *in vitro* models in biomedicine.

Keywords

biopatterning; cell-encapsulating microfluidic hydrogels; cell microenvironment; extracellular matrix; tissue engineering

Mimicking the extracellular matrix

Cells and tissues are routinely cultured *in vitro* on 2D substrates [1–3]. However, it has been demonstrated that cells or tissues cultured on 2D substrates (e.g., tissue culture plates or flasks) do not mimic cell growth *in vivo*, and fail to express certain tissue-specific genes and proteins at levels comparable to those found *in vivo*. For instance, it has been found that cell–drug interactions in a 2D culture system do not represent the actual working mechanism *in vivo*.

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Thus, 2D culture is not appropriate to be used in *in vitro* drug testing models. This is due to the fact that cells and tissues *in vivo* are immersed within a 3D network constituting a complex extracellular environment with a highly porous nanotopography, while a 2D culture system is too simple to mimic the native environment (Table 1).

From a tissue engineering (TE) standpoint, constructing a culture environment that closely mimicks the native tissue, which is composed of the extracellular matrix (ECM), soluble bioactive factors, and products of homo- and hetero-typical cell–cell interactions, is desirable to replicate tissue functions *in vitro*. However, this remains as one of the major challenges in TE, given the complexity of cell–ECM interactions as well as multicellular architectural features such as repeating tissue units and proper vascular structure. Cells commit to their fate by deriving a vast amount of information from this environment. As a part of the cell environment, ECM has been the most emulated component in TE studies. In native tissue, ECM is mainly a mixture of two classes of macromolecules, glycosaminoglycans and fibrous proteins (e.g., collagen, elastin, fibronectin and laminin), which self-assemble into nanofibrillar supramolecular networks that fill the extracellular space between cells [4]. ECM is a dynamic structure, which provides structural and anchoring support to the cells to improve tissue architecture. It also contributes to signaling, directing cell fate and function through cell–matrix interactions. In addition, the ECM is constantly remodeled by cells during development, homeostasis and wound healing by balancing its synthesis and degradation by a variety of enzymes (e.g., matrix metalloproteinases) [5–6].

Significant advances in the design of artificial matrices have led to an evolution from a simple supporting scaffold to a more complex dynamic biomaterial environment. Ideally, the artificial matrices should: support cell growth and maintenance; provide appropriate mechanical, chemical and biological characteristics mimicking native ECM; and facilitate effective nutrient transfer, gas exchange (i.e., O₂ and CO₂), metabolic waste removal and signal transduction. Scaffolds in various forms, such as, hydrogel and nanofibers, have been studied and employed for different tissue regeneration purposes.

A significant growth of interest in hydrogels started around the 1990s, partly due to the rapid emergence of the TE field, as hydrogels possess characteristics of native ECM [6–16], paving the way for functional tissues (Figure 1) [10,17–19]. The biocompatibility of various hydrogels (e.g., collagen, agarose and polyethylene glycol) is well characterized and the state-of-the-art nano- and microfabrication technologies (e.g., lithography, nano- and micro-fluidics, micromolding and biopatterning) provide the techniques to engineer scaffolds with intricate structures [11,20–25]. However, challenges remain when it comes to engineering functional tissues. Hydrogel-based cell-encapsulating constructs with embedded microchannels have recently been investigated, and became a promising tool to generate active tissue mimics by improving nutrient and gas transport [7,8]. Such cell-encapsulating hydrogel platforms could be employed for other applications, such as *in vitro* models for drug testing and toxicological assays. Given the intricate nature of the problem, the ultimate success of all these applications requires an interdisciplinary approach involving engineering, chemistry, materials science and cell biology.

In this article, we present hydrogels as scaffolds to mimic native ECM. Then, we provide a comprehensive description of state-of-the-art technologies by addressing the existing challenges with a focus on cell-encapsulating microfluidic hydrogels. Furthermore, the potential applications of such engineered cell microenvironments are discussed.

Engineered hydrogel scaffolds as ECM mimics

The efforts to engineer a cell microenvironment that mimics the dynamic native ECM have been driven by the clinical demand for tissue (or organ) repair and replacement [18,26].

Construction of functional tissues relies on the structural environment, cell–biomaterial interactions and incorporated biological signals (e.g., growth factors encapsulated in hydrogels) [27]. Thus, the scaffolds must offer properties (i.e., mechanical and chemical) that lead to cellular function in a native manner. In this sense, hydrogels have advantages when utilized as scaffolds for TE as one can easily adjust their physico-chemical (electrical charge and pore size) [28–32], and mechanical (stiffness, tensile strength) [33–34] properties to levels that are desirable for tissue scaffolds [7–9,35–36], cell encapsulation [37–39,227], immobilization [40] and drug delivery [41–44].

Hydrogels are 3D cross-linked insoluble, hydrophilic networks of polymers that partially resemble the physical characteristics of native ECM [16]. Polymers in hydrogel format can absorb a large amount of water or biological fluid (up to 99%) due to the presence of interconnected microscopic pores. Some hydrogels possess features of fluid transport and stimulus responsive characteristics (e.g., pH, temperature and light) [45]. Another appealing feature of hydrogels as scaffolds for TE is their biomechanical similarity to native ECM. The limitation of hydrogel mechanical properties is well known [46]. A hydrogel with the desired mechanical properties (in terms of stiffness and tensile strength [33–34]) can be achieved by adjusting various parameters including the type of polymers used, their concentrations and the crosslinking density [34]. Biocompatible hydrogel scaffolds can be obtained by selecting biocompatible synthetic or natural polymers and crosslinkers [47].

A variety of natural and synthetic polymers have been used to fabricate hydrogels. Collagen [48], hyaluronic acid [49], chondroitin sulfate [50], fibrin [51], fibronectin [52], alginate [53], agarose [8], chitosan [54] and silk [55] have been the most commonly used natural polymers for TE and regenerative medicine applications. Among all these natural polymers, collagen has been the most widely investigated since it is the most abundant structural protein of ECM in multiple tissues [56], including bladder [57], heart valve [58], blood vessel [59], skin [60] and the liver [61]. Synthetic biodegradable polymers, such as poly(ethylene glycol) [7,62], poly(lactic acid) [36], poly(glycolic acid) [63], and a copolymer poly(lactic-glycolic) acid [64] have also been used for engineered scaffolds. To increase the biological (e.g., hydrophilicity, cell-adhesiveness, degradability), biophysical (e.g., porosity, branched vasculature) and mechanical (e.g., stiffness, viscoelasticity) properties of tissue scaffolds, combinations of natural or synthetic hydrogels (i.e., hybrid hydrogels) have also been utilized [65]. Such ‘bioartificial’ scaffolds possess desirable mechanical properties and biocompatibility due to the coexistence of both synthetic and biological components. The biological properties of such scaffolds can further be improved by surface chemistry as the biomaterial composition makes them amenable to surface modification and biomimetic coatings [66–68].

Several approaches have been utilized to examine the mechanical (e.g., tension, compression, indentation, swelling) [33,69–70] and physicochemical (e.g., porosity, interconnectivity) [30] properties of both natural and synthetic hydrogels, including extensimetry [71], compression test [72] and bulge test [70]. However, these techniques are invasive and destructive. They are not appropriate to characterize mechanical properties of cell-encapsulating hydrogels during culture [69]. To overcome these specific problems, two techniques involving spherical indentation have been developed [73]; long focal microscopy-based spherical microindentation and optical-coherence tomography-based spherical microindentation techniques. Both monitoring techniques can be utilized to determine the mechanical properties of cell-encapsulating hydrogels for *in vitro* engineering of soft tissues [69]. While the former involves the central indentation of a circumferentially suspended hydrogel using a sphere of a known weight and measurement of the resulting central deformation displacement, the latter is a noninvasive imaging technique based on Hertz contact theory,

where the depth of indentation of a sphere into a hydrogel resting on a substrate can be used to calculate the mechanical properties of the hydrogel.

Challenges associated with hydrogels

The conventional approach in TE involves the process of seeding cells onto a 3D scaffold and inducing them to proliferate, differentiate and eventually to develop into a tissue construct [74]. A combination of chemical cues are also used to improve the outcome [75,76]. There are examples of successful clinical translations of engineered tissues [77–81], such as tissue engineered bladders [78], lung tracheal segments [82] and the lamina propria of human vocal fold developed from highly elastic gels of double-crosslinked hyaluronic acid microparticles [83]. However, challenges still exist with conventional scaffolding methods in mimicking native ECM [84–89]:

- Progress in scaffolding techniques enables the manufacture of scaffolds with complex architecture [90–95]. However, poor cell penetration and noneven cell seeding still exist due to the lack of appropriate spatial and temporal control [95–99];
- Success in emulating artificial matrices [100–102] for relatively simple tissues composed of a single cell type [55,99,103–105] has been demonstrated. Engineering complex tissues with multiple cell types and unique ECM composition has been challenging although successful cases exist;
- Unlike native tissues, most engineered tissues lack a complex microvascular system, which is essential for certain tissues to maintain their viability and function through the transport of nutrients and a plethora of signaling molecules.

Engineering of avascular tissues, such as bone [103], skin [104] and cartilage [64,65] has been successful. Furthermore, the most successful implants are those positioned close to a rich host vascular network [78]. To date, maintaining the viability of the seeded cells at high cell densities (10^7 – 10^9 cells/cm³) within a large *in vitro* construct is a challenge [106]. Thus, replicating the inherent microvascular network of complex tissues represents one of the most fundamental challenges in tissue regeneration [26,29,35,107]. As a future prospect, a functional vascular system incorporated into tissue constructs is critical for the development of thick and complex tissues [108,109].

Construction of an artificial 3D ECM

Microscale engineering is a method that can be used to control the cellular microenvironment [48,110–114] and has the potential to construct matrices mimicking the inhomogeneous and anisotropic properties of native tissues [48,115–117]. In addition, this method can also be applied to modify and control cell–cell and cell–ECM interactions [113]. Using microengineering technologies, hydrogel scaffolds mimicking *in vivo* ECM have been developed to support cell growth, proliferation and to promote tissue generation. These techniques include lithography [31,40,118,119], nano- and microfluidics [48,120–130], micromolding [10,131–133], and biopatterning [134–137], all of which offer the potential to address the aforementioned existing challenges in engineering a predesigned cell microenvironment.

Among all the microscale TE methods, ‘top-down’ or ‘bottom-up’ approaches have recently emerged as forefront technologies to create tissue-like constructs of a higher complexity (e.g., microarchitecture made of multiple cell types and ECM components) [10,96,138]. Top-down approach emphasizes control of microscale features (i.e., shape and size) of relatively large hydrogel constructs [8,139]. Despite significant advances, there are several challenges with top-down approaches (e.g., the intricate microstructural features of native tissues such as cell density and microarchitecture) [84–89]. On the other hand, the modular bottom-up approach

aims to generate larger tissue constructs with controllable and repeatable patterns by assembling smaller building blocks (e.g., cell-encapsulating microgels). Bottom-up approaches are exciting since they mimic native tissues composed of repeating functional units [10] (e.g., the lobule in the liver [9]). In this article, we will focus on bottom-up approaches where the newest technologies and methods are emerging. The bottom-up approach requires methods to fabricate functional units and assemble them together at high throughput. Some nano- and microscale technologies have been employed to assemble cell-encapsulating microgels including manual handling [108,131,140,141], microfluidics [8,142] or directed assembly [7] to build layers of microgels. Furthermore, enhanced perfusion has also been observed in hydrogels created through a random assembly of micro-modules encapsulating endothelial cells. [106,143,144]. However, diffusion through hydrogels is limited to relatively small constructs due to inefficient mass transport [145,146]. To sustain cell growth, a microenvironment must provide effective nutrient transfer, gas exchange (i.e., O₂ and CO₂) and metabolic waste removal. To address this issue, the microfluidic channels formed within cell-encapsulating hydrogels may be used to maximize the perfusion capacity of the constructs [8,35,147–149,179].

Microengineering of cell-encapsulating hydrogels

Micromolding and photolithography are two cutting-edge technologies that have been used to generate 3D cell-laden hydrogel microstructures with controlled features (e.g., shape and size) [131,150]. In generating micromolded hydrogels, precursors are first molded and then gelled to generate structures of a variety of shapes and sizes (Figure 2). Using fluoro-based materials with higher surface energies and enhanced fabrication resolution it is possible to micromold nano-scale particles that have applications in drug delivery. However, the ability to engineer the nanoscale topography of hydrogels will also be important in generating improved 3D TE scaffolds with microstructures [10]. Micromolding has become particularly appealing due to soft lithography, which has enabled easy fabrication of poly(dimethyl siloxane) (PDMS) molds from prefabricated silicon wafers. Photolithography provides reliable shape definition, typically by using photomasks for patterning multiple cells with materials to facilitate the selective adhesion of one cell type to a specific regions (Figure 3). Using this technology, photo-crosslinkable hydrogels are placed underneath a mask that controls the exposure of light to particular regions of a film of hydrogel precursors. Where the light is exposed, the photo-crosslinkable hydrogel will crosslink to generate structures that are in the shape of the mask. Advances in soft lithography have enabled [8,151–152] and microfabrication of features as small as a few micrometers. In particular, silicon-based elastomers have been widely used in microfluidics due to their simple fabrication and material properties, such as gas permeability, optical transparency and flexibility [153]. However, the hydrophobic nature of PDMS requires surface treatments, such as plasma treatment, to increase cell adhesion [154,155].

A combination of photopatterning and electropatterning offers the capability to encapsulate cells with a high spatial resolution (< 10 μm) within a microscale hydrogel (microgel). These microgels can later be organized into specific geometries to generate larger structures [156, 157]. Several other microengineering techniques have also been utilized to pattern cells in 3D gels, such as laser scanning lithography [158–159] and dielectrophoresis [160]. A study reported the construction of a functional 3D hepatic tissue using poly-ethylene glycol, in which nutrient delivery was achieved by convective flow. This demonstrates that lithographic arraying methods can be efficiently used to construct 3D cell-encapsulating hydrogel scaffolds with complex internal architecture [9]. A flow photolithographic method, which combines the advantages of both microscope projection photolithography and microfluidics, has been used to continuously synthesize microgels while controlling particle size and shape [142]. However, there are several limitations with this method, such as the cytotoxicity of the photoinitiator, cell damage induced by UV light exposure and low throughput. Furthermore, this technique

requires an additional system to assemble microscale building blocks into a desired 3D architecture through self-assembly [10,26], or by other means such as manual handling [131, 141], and microfluidics [8,142]. The three most frequently used methods to generate cell encapsulating microgels are compared in Table 2. Novel methods to assemble these microgels into 3D constructs need to be further developed.

Microfluidic cell-encapsulating hydrogels

Efficient nutrient and metabolite diffusion has only been observed in relatively small hydrogel-based tissue constructs due to transport limitations [145–146,161–163]. Currently, vascularization remains a challenge and materials strategies that attempt to induce or organize vessel formation, either *de novo* (vasculogenesis) or by sprouting of existing vessels (angiogenesis) will be beneficial [164]. To address this issue, emerging micro- and nanofluidic technologies [165–167] have been utilized to construct microchannels that resemble the vascular system of native tissues [8,127,168–172]. A variety of techniques including lithography [119], micromolding [38,132], microfluidic emulsification [26,120] and hot embossing [121] have been used to build such capillary networks in tissue scaffolds or polymer microchips. More recently, micro- and nano-fluidics have also emerged as powerful technologies to generate microengineered tunable hydrogel scaffolds with tissue-like motifs [45,173–175].

An alternative approach to using cell-encapsulating microfluidic hydrogels to facilitate nutrient and soluble factor exchange within 3D constructs [7–8,35,131,176–182] is to integrate microfluidic channels within 3D cell-encapsulating hydrogel matrices. This could provide *in vitro* tissue constructs with spatial and temporal complexity mimicking native tissues. Biocompatibility of these devices is considered to be an important issue when applied to biomedical and biochemical analysis. The ability to tailor the chemical and structural properties of biomedical devices to control cell attachment, survival, proliferation and differentiation is of crucial importance [183].

Micromolding is another simple approach that has been used to construct microfluidic cell-encapsulating hydrogels, [8]. With this method, cells are first suspended in a gel precursor and then molded on a patterned wafer (generally silicon) to generate microchannels of different sizes and shapes (i.e., square cross-section with different aspect ratios). It has been shown that cells encapsulated within such microfluidic hydrogels remained viable [8] due to the perfused network of microchannels, through which nutrients and oxygen can be efficiently delivered. In another study, microfluidic networks were directly embedded within a cell-encapsulating hydrogel construct (Figure 4) where high cell viability was observed and the distribution of solutes within the 3D matrix was regulated in a spatio-temporal fashion [35]. A related study shows that the cell viability across the hydrogel was highly correlated to the nutrient perfusion profiles (Figure 5) [179].

Cell printing has been used to pattern cells within scaffolding materials (e.g., natural hydrogels such as collagen). 3D printing methods can be employed for microfluidic cell-encapsulating constructs [35,107,134–135,180–181,184–187]. This approach offers the ability to deposit cell-encapsulating microgels at predetermined locations with multiple cell types at high throughput [107,137]. Currently, several printing techniques have been explored to generate cell-encapsulating gel droplets, such as inkjet [136,188–189], laser [190–192] and acoustic printing [193–195]. Furthermore, a scaffold-free approach has been developed to build cell encapsulating hydrogels with customized tubular structures of defined topology (both linear and branched tubular structures) (Figure 6) [187]. In this method, agarose rods were used as a temporary space occupier of the lumen of branched tubes, which was later manually removed [187]. However, this approach is difficult to adapt for more complex structures due to the need for manual handling. In another study, a thermal inkjet printer was used to simultaneously

deposit human microvascular endothelial cells and fibrin (bio-ink) into fibrinogen solution (bio-paper) to form a cell-encapsulating microfluidic hydrogel (Figure 7) [186]. 3D bioprinting is thus capable of manipulating cellular components with precise spatial and temporal control to create 3D constructs [227]. These controls and high-throughput aspects make printing an attractive cell-encapsulating gel deposition technology. However, the viability and function of the printed cells need to be characterized for each printing technology. Factors, such as shear at the nozzle, heat in the ejection reservoirs and impact on the surface need to be optimized separately for each technology for maximal cell viability and function.

Biomedical applications of hydrogels & 3D cell-encapsulating hydrogels

As the cell encapsulating 3D hydrogels provide cells with a tissue-like extracellular environment, they offer potential applications such as *in vitro* model systems for drug screening, diagnostics and toxicological assays.

As drug discovery & delivery devices

Various polymeric drug-delivery systems have been recently introduced and evaluated [196]. Macromolecular drugs, such as proteins or hydrophilic oligonucleotides, are inherently compatible with hydrogels. Various formulations of hydrogel materials with known mechanical, physical and chemical properties can be prepared to construct drug-delivery vehicles with finely tuned drug release kinetics [41,42,44,197,198]. The highly porous structure of hydrogels enables the introduction of a relatively large load of drugs [199]. In addition, the porosity of hydrogels can be tuned by controlling affinity towards the swelling environment and the crosslinking density. The porosity can also be adjusted to facilitate drug release [200,201]. In drug discovery, miniaturized hydrogel platforms can be used to control the fluid flow, enable high-throughput screening and minimize sample and reagent volumes [196].

As cell-based biosensors & diagnostic tools

Ethical considerations, limited portability and robustness for practical large-scale applications preclude the use of living animals as biosensors. Although other methods (e.g., immunochemical or nucleic-acid based methods) are available for pathogen detection, they often take hours or days to provide meaningful results [202]. Thus, the need for reliable, high-throughput *in vitro* human cell-based alternative methods are attractive for basic research in the fields of safety and risk assessment [203]. Given their easily customizable features, hydrogels (e.g., microgels) could be utilized as components of integrated sensors within microdevices [204]. Cell-based biosensors, employing both prokaryotic and eukaryotic cells as primary transducers, have emerged as powerful tools for rapid detection of hazards and threats associated with food, agriculture, the environment and biosecurity [205,206]. Providing cells with an *in vivo*-like physiological environment for interaction and communication, 3D cell-encapsulating hydrogels have attracted interest in the cell-based chem-bio sensing [207]. Such native-like 3D synthetic microenvironments may provide a better understanding of disease and organ failure caused by various biotic or abiotic agents such as chemical toxins, bacterial and viral pathogens [208].

As stem cell niches

It is generally accepted that adult stem cells *in vivo* reside in special microenvironments (i.e., niches) contributed by other cells, ECM components, molecules such as cytokines and chemokines and physicochemical conditions (e.g., the physical environment, pH, O₂) [199]. Once displaced from this native niche, these cells will begin to differentiate into a diverse range of specialized cell types (pluripotency). The major factor determining stem cell fate is the interplay between stem cells and their niches, which creates a dynamic system necessary for

tissue maintenance and function *in vivo* [101,209]. Thus, the control of ECM composition in engineered constructs has proven to be a valuable tool to guide the development and commitment of stem cells during new tissue generation [101].

Clinical trials for stem cell therapies have so far exceeded 2000 cases and there is an urgent need to develop technologies that can control stem cell behavior in culture. Cell therapy alone is a poorly controlled process [164] and a recent study has revealed that when injected into a post-myocardial infarction heart in mice, stem cells mineralized, probably due to the new mechanical environment in the scar tissue [210,211]. Thus, replicating stem cell 'niche' conditions *in vitro* would enable the design of stem-cell therapeutics, treatment of genetic disorders and cancer, and regenerative medicine [101]. Control of cell fate is perhaps the most limiting factor in the translation of embryonic stem-cell therapy. Although, microscale technologies have been developed for TE and a wide range of natural and synthetic materials have been successfully used for stem cell cultures [212–214], the complexity of the stem cell niche is still difficult to reproduce [209,215], and the controlled use of stem cells in TE remains a challenging goal.

Conclusion

Given the ease of execution, experiments in biosciences have traditionally been carried out in 2D environments [216]. *In vivo*, mammalian cells reside in a 3D microenvironment that provides physical support, chemical growth factors for cell adhesion, survival and growth. These factors are interconnected through a complex network of microvasculature that provides nutrient delivery, gas exchange and signaling cues; all with profound effect on cell behavior, assembly and organization of tissues. This 3D microenvironment plays a critical role in regulating cell fate, ranging from proliferation and migration to apoptosis [217]. Mimicking such a 3D cell microenvironment *in vitro* is crucial for various TE applications (e.g., constructing tissues for repair and replacement). On the other hand, one should consider that heterogeneities in cell behavior would be further exaggerated in richer chemistry and formulation of 3D scaffolds. Furthermore, cells in these synthetic environments must grow at the expense of the scaffold, proliferate, self-assemble, secrete their own ECM and eventually take the shape of scaffold, which resembles the structure of tissue of interest. Thus, engineering tissues with a complex vascular structure and made of many cell types whose organization is crucial for function is an important challenge. Transmission and receipt of complex molecular information involved in cell sorting, boundary formation in tissues and cell movement can be effected through cell–cell and cell–matrix interactions.

Micro- and nano-fluidic technologies have offered the capability to construct hydrogels with complex and delicate structures. Emerging microscale technologies (e.g., micromolding, lithography, biopatterning) have enabled researchers to engineer cell-encapsulate microscale hydrogels with incorporated microchannels that mimic the microvascular system. Multimicrochannel approaches may allow larger tissue constructs to have better diffusion characteristics. Challenges still remain with these technologies, such as:

- Fluid transport limitations;
- Regularity of microchannels, which do not represent the irregular microvascular system and the complexity of the endothelial branched nature of vascularization in native tissues;
- Problems associated with patterning various cell types spatially (i.e., layered tissue motif comprising various cell types).

Although multiple challenges are facing the field, emerging microscale technologies (e.g., micromolding and biopatterning) are exciting and they may enable the engineering of tissue constructs that better mimic native tissues.

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Figure 1. The total number of publications with 'tissue engineering' and 'hydrogel' or 'hydrogels' in the title

The exact numerical values are provided at 5-year intervals (Source: Science Citation Index Expanded [SCI-EXPANDED]). The interest in hydrogels has significantly increased since 1990.

Figure 2. Fabrication of a 3D cell-laden microscale hydrogels using micromolding
 (A) Liquid prepolymer is deposited onto a hydrophilic poly(dimethyl siloxane) (PDMS) pattern. (B) A hydrophobic PDMS cover slip is placed on top of the prepolymer, forming a reversible seal. (C) Polymer liquid is crosslinked using UV light or heat. (D) The PDMS cover slip is removed. (E) Hydrogels are washed from pattern. (F) Hydrogels are free of the pattern. (G) Fluorescent image of microscale tissue constructs comprised of cell-laden hydrogels containing hepatocytes and fibroblasts. Scale bar: 400 μm .
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Figure 3. Fabrication of a 3D cell-laden microscale hydrogel using optofluidic maskless lithography method

(A) The 3D-Optofluidic maskless lithography (OFML). OFML is performed in a two-layered PDMS microfluidic channel with UV curable resin placed in the bottom. The height of the bottom channel is controlled via deformation of the PDMS membrane under pneumatic pressure of the top chamber. Projection patterns are sequentially launched and projected onto the channel. (B) The 3D-OFML system is composed of two parts; the height-tunable PDMS microfluidic channel and the optical system. Pneumatic pressure is applied with a syringe pump and the UV projection for each layer is computer controlled. (C) Microfluidic control methods for exchanging resin materials to generate hybrid microstructures. Solutions can be injected sequentially through the same inlet (top) or multilaminar flow can be exploited (bottom). (D, F) Generation of 3D hydrogel tissue-like microstructures containing different living cells in each part of the structure. HeLa cells stained with red and blue fluorescence (D) are used for heterogeneous cell patterning. (F) Confocal microscope images of the hydrogel block containing cells. Differential interference contrast transmission image shows the circular and the rectangular pattern of hydrogel structure (left). (E) An array of patterned hydrogel with two different fluorescent microspheres. First layer is patterned in cross-shape and the other beads in the square-shape. Images were taken from the bottom of the structure with an inverted optical microscope.

Scale bars in (E) and (F) indicate 100 μm .

DMD: Digital micromirror-array device; PDMS: Poly(dimethyl siloxane).
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Figure 4. Fabrication of cellular microfluidic hydrogels using a molding method

(A) The fabrication process. The dotted line indicates PEI coating. (B) Cell viability results using live/dead staining.

H: Height (mm in thickness); PEI: polyethylenimine.

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Figure 5. A schematic perfusion bioreactor

(A) 2D microchannel perfusion/viability model: single- and dual-channeled hydrogel constructs were prepared using cell-encapsulating agarose. (B) Fractional cell viability with single- and dual-channel set-ups taken at day 1, 2 and 3, respectively, after the channel constructs were built.

3T3: Mouse embryonic fibroblast cell line; o.d.: outer diameter;

PDMS: Poly(dimethyl siloxane).

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Figure 6. Printing cellular microfluidic hydrogels

(A) Design template for building branching tubular structures by depositing agarose rods and multicellular spheroids of the same diameter. (B) Fusion patterns of multicellular spheroids (300 μm HSF spheroids) assembled into tubular structures after 6 days of deposition. (C–F) Building a double-layered vascular wall by assembling human umbilical vein smooth muscle cells and HSF multicellular cylinders according to specific patterns (C). (D–F) show the results of H&E (D), smooth muscle α -actin (E) and caspase-3 (F) staining of the structure after 3 days of fusion.

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Figure 7. Fabrication of cellular microfluidic hydrogels using printing

(A) Proliferation-ready endothelial cells aligned inside the fibrin channels using a thermal inkjet printer. The technology may enable building human microvasculature. (B & C) Channel structure of printed microvasculature cultured for 21 days. (B) Printed ring shaped microvasculature cultured for 21 days (C) Integrity of printed structure cultured for 21 days. HMVEC: Human microvascular endothelial cells. Reproduced with permission from [186].

Table 1

A comparison of cell/tissue behavior under 2D and 3D culture conditions.

Feature/function	In 2D	In 3D	Ref.
Tissue-specific architecture	Poor	Rich	[220]
Cell morphology	Flat, extended	Round, contracted	[17,221]
Interactions	Limited	Multiple	[38]
Cell motility	Fast, free	Slow, restricted	[6]
Cell adhesion	Weak	Strong	[222]
Cell growth	Directional	In all directions	[6,223]
Cell proliferation	High	Low	[5,6]
Apoptosis	Induced	Tissue-like	[223,224]
Intracellular stiffness	An order-of-magnitude higher in 3D		[4]
Cell polarization	Partly	Full	[6]
extracellular matrix remodeling	Absent or poor	Present	[5]
Fluid perfusion	1D	3D	[170]
Signaling and diffusion	Asymmetric	Nearly symmetric	[225]
Metabolic rate	High	Low	[223]
Cell survival when exposed to cytotoxic agents	Low	High	[226]

Table 2

A comparison of different methods to fabricate 3D cell-laden hydrogels.

Feature/function	Method			Ref.
	<i>Micromolding</i>	<i>Photolithography</i>	<i>Bioprinting</i>	
Hydrogel type	Multiple	Photo-crosslinkable hydrogel (e.g., PEG)	Injectable hydrogel (e.g., collagen, agarose, alginate)	
Hydrogel shape	Multiple (e.g., cylinder, cubic)	Multiple (limited in thickness)	Spherical	[132]
Hydrogel size	40 μm to mm scale	120 μm to mm scale	37–400 μm	[131,132,142,193,227]
Fabrication throughput	Medium	Low	High	
Cell viability [†]	<50 to >99%	~60 to 100%	~65 to ~100%	[7,87,193,131,132,142,227–231]
Coculture capability	Yes	Yes	Yes	[232]
Spatial deposition control	Not applicable	Not applicable	Yes, with microscale resolution	
Single cell encapsulation	Yes	Yes	Yes	[193]

[†] Cell viability relative to culture.

PEG: Polyethylene glycol.