Overview

Hydrogels and microtechnologies for engineering the cellular microenvironment

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Hydrogels represent a class of materials suitable for numerous biomedical applications such as tissue engineering and drug delivery. Hydrogels are by definition capable of absorbing large amount of fluid, making them adequate for cell seeding and encapsulation as well as for implantation because of their biocompatibility and excellent diffusion properties. They also possess other desirable properties for fundamental research as they have the ability to mimic the basic three-dimensional (3D) biological, chemical, and mechanical properties of native tissues. Furthermore, their biological interactions with cells can be modified through the numerous side groups of the polymeric chains. Thus, the biological, chemical, and mechanical properties, as well as the degradation kinetics of hydrogels can be tailored depending on the application. In addition, their fabrication process can be combined with microtechnologies to enable precise control of cell-scale features such as surface topography and the presence of adhesion motifs on the hydrogel material. This ability to control the microscale structure of hydrogels has been used to engineer tissue models and to study cell behavior mechanisms *in vitro*. New approaches such as bottom-up and directed assembly of microscale hydrogels (microgels) are currently emerging as powerful methods to enable the fabrication of 3D constructs replicating the microenvironment found *in vivo*.

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INTRODUCTION

Hydrogels are suitable for numerous biomedical and pharmacological applications ranging from contact lenses to carriers for drug delivery. They are hydrophilic and possess mechanical properties similar to those of native tissues and organs, which also make them attractive for tissue engineering and regenerative medicine applications.[1] A wide variety of natural and synthetic polymer compositions and crosslinking techniques have been used to fabricate and functionalize hydrogels with biological and biochemical cues.[2,3] Hydrogels can sustain cell encapsulation and induce cell–cell and cell–extracellular matrix (ECM) interactions within the bulk of the material. Moreover, they can be combined with microfabrication technologies to precisely engineer the cell microenvironment and to direct cell behavior *in vitro*.[4] The engineering of tissues and organs requires a scaffold that provides the cells with an adequate microenvironment promoting cell adhesion and function, and allowing for the diffusion of soluble factors. The fabrication of tissues *in vitro* is currently moving toward biologically

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inspired approaches to replicate the cell microenvironment and control the spatial localization of active molecules within the structure of the scaffolding material.[5] The ability to generate a biomimetic and physiologically relevant cellular microenvironment *in vitro* will be instrumental in the fabrication of scaffolds promoting the coordination of cellular events found in living tissues.[6]

HYDROGEL SYNTHESIS AND NETWORK FORMATION

Natural and Synthetic Hydrogels

When isolated from their native 3D environment and cultured on two-dimensional (2D) surfaces such as tissue culture polystyrene, cells experience changes in their phenotype and morphology.[7] There is a need for surrogate materials replicating the physiological characteristics of native tissue to adequately study cell behavior and biological phenomenon *in vitro*. Natural ECM proteins such as collagen and fibrin as well as polysaccharides such as hyaluronic acid (HA) and alginate have previously been used to produce hydrogels.[8] These proteins can be readily obtained and purified from multiple tissue sources, which make them a popular choice for hydrogel fabrication. They possess desirable properties for biomedical applications as they have the ability to mimic the basic properties of native ECM proteins and to encapsulate cells in a 3D environment. The biological interactions of hydrogels with encapsulated cells can be facilitated by modifying their chemical side groups to favor cell growth and adhesion. Thus, they are extensively used in fundamental cell biology studies and tissue engineering.[9]

Synthetic polymers have also been used to generate hydrogels using various molecules such as poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA). The main advantage of synthetic hydrogels over natural ECM molecules is the capability to rationally design their properties. The mechanical and chemical properties of these polymers can also be tailored for different applications without the immunogenicity-related concerns of some naturally occurring polymers. They can be synthesized in a robust and reproducible manner, providing a controlled environment that can be modified with specific adhesion ligands to engineer desired cell–matrix interactions.[10] Moreover, a multitude of methods have recently been developed to generate natural and synthetic hydrogel structures with precise geometries, allowing the fabrication of biomimetic scaffolds for medical applications.

HYDROGEL FORMATION AND CROSSLINKING MECHANISMS

Hydrogels can be generated by crosslinking hydrophilic polymers into a 3D solid structure. The gelation process requires the polymeric chains to be assembled by physical or chemical reactions. The parameters regulating the crosslinking mechanism, such as the amount of crosslinker and the thermodynamic properties of the chemical reaction, can be used to control the properties of hydrogels.[11] For cell encapsulation, specific characteristics such as macromer hydrophilicity, diffusivity, and water content need to be considered to avoid a loss of cell viability (Figure 1). The crosslinking density also needs to be optimized to provide adequate mechanical properties to the network. This parameter is essential to facilitate oxygen and nutrients diffusion within the hydrogel. More recently, natural and synthetic polymers have been combined with microtechnologies to introduce complexity into the structure of hydrogels. Techniques such as micromolding and photopolymerization have been used to engineer the properties of scaffolds and have proven to be suitable mechanisms to polymerize hydrogels containing encapsulated cells.[4]

ENGINEERING THE CELLULAR MICROENVIRONMENT

Tailoring the Physical, Chemical, and Biological Properties of Hydrogels

The physical properties of hydrogels can be regulated by the chemistry of the polymeric backbone, its hydrophilicity, polymer concentration, and crosslinking density. Increasing the crosslinking density and monomer concentration generally result in increased stiffness and reduced degradation rates because of a larger number of bonds that need to be cleaved during degradation of the material. The stiffness of hydrogels can be tuned by varying the percentage of polymer used in the solution before the crosslinking procedure[12] and can be controlled by adjusting the crosslinking agent and the crosslinking density during hydrogel formation. Increasing the number of bonds in the polymer also limits the ability of water molecules to diffuse in and out of the material. Thus, the degree of crosslinking of polymer networks can be used to tailor both the structural stability and the porosity of the material. Therefore, the degree of crosslinking is an important aspect in regulating the transport of solutes through hydrogel structures.

As the materials used to generate hydrogel structures can be made from both synthetic and

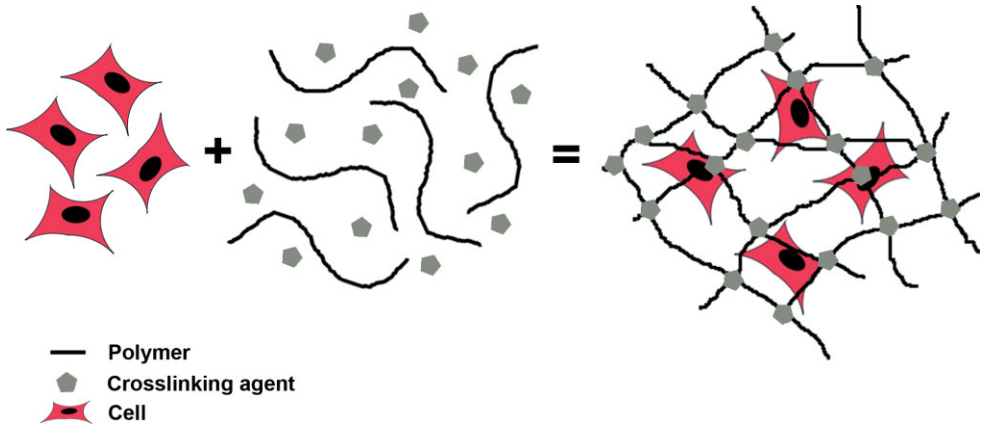

FIGURE 1 | Hydrogel materials have the ability to recapitulate the basic properties of the extracellular matrix (ECM) found *in vivo*. They are particularly suitable for cell encapsulation, making them an attractive material for biomedical applications.

natural polymers, their degradation process can result from a range of mechanisms such as the presence of light, enzymes, water, or other triggers. The degradation of natural polymers is mostly a combination of enzymatic reactions. In the case of synthetic hydrogels, readily hydrolyzable groups and proteolitically degradable peptides can be engineered into the backbone of the polymeric chain.[13] Therefore, the properties of water- and enzyme-sensitive hydrogels can be modified to control the degradation kinetics and cell-mediated remodeling, which are required for the generation of 3D tissues.[14]

Hydrogels can be functionalized to regulate their interactions with cells. They can be chemically or biologically engineered to achieve a certain bioactivity providing longer and more efficient cell support.[15] They can also be modified to include adhesion ligands and other molecules such as cytokines, chemokines, and growth factors inducing specific cell response.[10,16] For example, hydrogel materials such as PEG and agarose are suitable for cell encapsulation; however, their surfaces resist protein adsorption and are not suitable for cell adhesion. Therefore, they were modified with adhesive motifs such as adhesive peptides, which are short fragments of bioactive molecules known to enhance cell binding.[17] These molecules interact with specific cell surface receptors and initiate the signaling cascade resulting in improved cell adhesion. These functionalization approaches have been used to engineer the properties of the material and the release of growth factors inside hydrogels to facilitate cell adhesion, migration, and differentiation.[16]

Microfabrication Technologies

The cellular microenvironment is known to regulate cell behavior.[18] This microenvironment consists of an ensemble of biological, chemical, and mechanical cues derived from the ECM, the soluble factors, and the multiple surrounding cell types. It plays a central role starting from the early stages of development and continuously influences cell and tissue morphogenesis throughout life. However, cell adhesion, organization, and differentiation depend on the nature and the characteristics of the substrate on which they adhere. As a result, traditional cell and tissue culture systems in 2D have been replaced with scaffolds aiming to better reproduce the 3D physiological environment. Microfabrication techniques, such as soft lithography, micromolding, and photolithography, emerging from the microelectronics industries have been increasingly used to generate precisely engineered materials for biomedical applications (Figure 2). These techniques have provided a broad set of tools capable of probing and controlling cell behavior by including cell-scale features in materials and allowing for precise control of the cellular microenvironment.

Soft lithography is a microfabrication technique that uses an elastomeric stamp molded from patterned silicon wafers to print or make materials with micro- and nanoscale resolution.[18–20] Variations of soft lithography, such as microcontact printing can be used to engineer cell-scale surface topographies and spatial distribution of molecules and ligands on a substrate.[21,22] Micromolding is another technique that can be used to fabricate micropatterned thermoplastics, elastomers, and hydrogels. This approach was shown to be useful in shaping precursor polymers into specific geometries and sizes, before gelation and crosslinking.[4] It can also be used to produce microfluidic channels and scaffolds in a rapid and cost-effective manner. Photolithography can also be used to engineer hydrogels and other light-sensitive biomaterials. In this process, a thin film of photocrosslinkable polymer

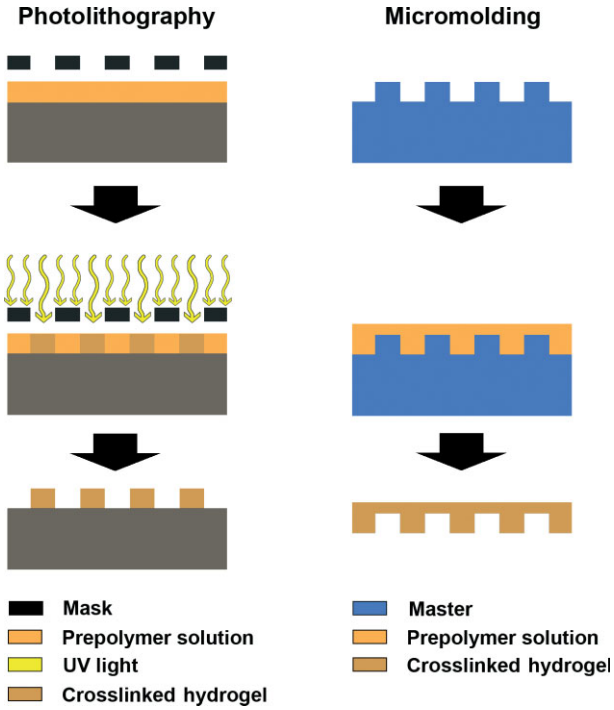

FIGURE 2 | Microtechnologies such as photolithography and micromolding can be used to fabricate cell-scale features into hydrogels, enabling the control of the cellular microenvironment.

is exposed to light through a mask. The mask protects certain areas of the polymer, whereas the exposed areas get crosslinked by the photoreaction. Different types of materials can be readily microfabricated in a precise and reliable fashion. Microfabrication capabilities and biocompatibility of synthetic materials (alkanethiols, PEG), naturally occurring proteins (collagen, fibronectin), and polysaccharides (agarose, hyaluronan) among others have been investigated for biomedical applications.[18,23]

Although photolithography and micromolding have allowed the production of scaffolds with patterned features and controlled architectures,[24] most microfabricated hydrogels have precise planar geometries but still lack control over their thickness. New techniques enabling the fabrication of features in both *x*, *y*, and *z* directions are currently seen as promising and versatile methods to produce biomimetic scaffolds. For example, photopatterned hydrogel materials can be made using multiple applications of photomasks to build 3D microstructures in a layer-by-layer fashion.[25] In addition to photolithography, other 3D polymerization systems, such as laser polymerization combined with automated stages, have been used to fabricate structures with improved resolution in the *z* direction.[26,27] Crosslinking techniques utilizing digital-micromirror-device (DMD)-based patterning and stereolithography are currently being investigated to provide control over the deposition of cells and proteins in spatially defined structures inside scaffolds.[28,29] Microcontact printing can also be used to transfer features with high spatial resolution onto a material, resulting in surfaces with defined chemical micropatterns.[30] These advances in microtechnologies could provide significant insights on cell behavior *in vitro* and could result in great technological advances *in vivo* to enhance the biological function of polymeric materials.

HYDROGELS IN TISSUE ENGINEERING AND HIGH CONTENT SCREENING

Hydrogel based materials have been used for a variety of biomedical applications ranging from drug delivery vehicles to wound dressings. They are capable of absorbing large amount of fluids, their properties can be tailored, and they are suitable for cell encapsulation as well as for implantation. For example, PEG is FDA approved for certain medical applications and is widely used in drug delivery and tissue engineering applications because it is biocompatible, can be engineered with a range of bioactive groups or degradable linkers, and is permeable to nutrients and soluble factors.[31] Moreover, it is adaptable to microfabrication technologies and can be engineered into cell-laden 3D structures. Similarly, PVA has been used in a variety of biomedical applications ranging from contact lenses to drug carriers and tissue engineering scaffolds. Hydrogels made of PVA are found to be biocompatible and have elasticity properties similar to native tissues. They are easy to synthesize and can sustain traditional sterilization procedures required prior implantation.[32] Recent advances in synthetic chemistry have also resulted in the synthesis of new polymers that can be injected as liquids and can polymerize into a gel *in vivo* by free radical crosslinking to be used as fillers.[33] This demonstrates the potential and versatility of hydrogels in their use for biomedical applications.

Tissue Engineering

Tissue engineering is a field that aims to develop new strategies to produce a variety of tissues both *in vitro* and *in vivo* to restore, maintain, or enhance tissue function (Figure 3). Hydrogels represent one of the most common scaffolding materials used to support cell adhesion, proliferation, and tissue growth.[34] Unlike most scaffolds that are seeded with cells using gravity, centrifugal force, vacuum, or flow to achieve uniform distribution, hydrogels can encapsulate cells

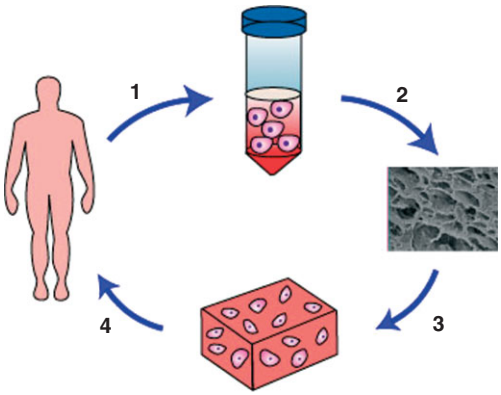

FIGURE 3 | Tissue engineering and regenerative medicine. Cells are harvested from the patient, expanded in culture (1) and seeded into a porous scaffolding material (2). The cell-seeded scaffold (3) can then be implanted into the patient to restore tissue function (4).

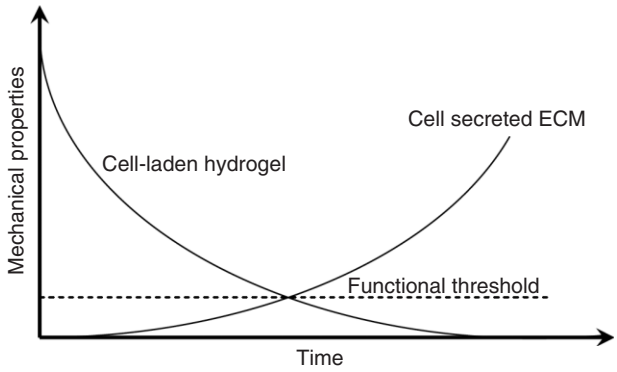

FIGURE 4 | Evolution of the mechanical properties of a cell-laden hydrogel as a function of time. The cell-seeded scaffolding material is degraded by the cells, which reduce its mechanical properties. In parallel, cells produce extracellular matrix (ECM) resulting in tissue regeneration and an increase in the mechanical properties of the engineered tissue. The intersection between the curves representing the degradation kinetics of the biomaterial and the ECM synthesis by the cells need to remain over the threshold required for adequate tissue function throughout the regeneration process.

during the fabrication process. This is a significant advantage to generate physiologically relevant 3D scaffold for cell and tissue growth. In addition, the physical and biological properties of hydrogels can be engineered to mimic the tissue it is intended to replace.[35,36] For example, PEG hydrogels have been used to produce tissues such as cartilage and bone, encapsulating chondrocytes,[37,38] and osteoblasts.[17,39] For these load bearing applications, it is of the utmost importance for the material to possess sufficient mechanical strength throughout the regeneration process (Figure 4). Thus, hydrogel biomechanics represent an essential part of their design. Beyond the restoration of the original architecture for tissue engineering applications, these substrates also need to provide sufficient permeability to allow for adequate transport of large molecules and to support cellular metabolic activities normally present in native tissue. The optimization of these 3D tissue structures can be used as powerful platforms for fundamental *in vitro* studies and represent a benefit for various fields such as developmental biology, pharmacology, immunology, pathology, and regenerative medicine.[40]

Hydrogels can also be engineered to be semipermeable, suitable to keep cells and fluid separate while allowing diffusion of soluble factors within their structure.[41] This property can be used to encapsulate cells in micron to millimeter size capsules that can serve as delivery vehicles for cell-based therapies. PEG hydrogels have been used to encapsulate islet cells[42,43] for the delivery of cell secreted factors such as insulin.[44] These microcapsules can also be engineered to allow for the diffusion of nutrients and removal of metabolites, while prohibiting interaction of encapsulated cells with the immune system, therefore avoiding the rejection of the implant by the host.

Biodegradable hydrogels are commonly used to deliver molecules at a controlled rate both *in vitro* and *in vivo*.[45] The physical and chemical properties of hydrogels designed as drug carriers can be engineered to regulate the rate of release to the surrounding environment. As the polymer degrades in the presence of enzymes or water, the entrapped molecules can be released through erosion mechanisms. Another common delivery method is by diffusion and transport of molecules through the porous structure of the hydrogel. These approaches can be applied to induce specific cell or tissue response in precise locations in the body, depending on the physiologically active molecule utilized and the desired effect for the targeted application. Therefore, the specifications of the vehicle or carrier as well as the method of administration need to be carefully planned to optimize the efficiency of the delivered molecules.[46] Parameters such as polymer composition, size, porosity, molecular weight, and degradation rate can also impact the release kinetics of hydrogels for drug delivery systems. For example, drugs of various molecular weights encapsulated inside PEG hydrogels showed that this parameter combined with the polymer crosslinking density can be used to control the release profile of the system.[47] A similar drug delivery approach was designed to deliver growth factors to stimulate mineralized tissue formation in a bone regeneration model.[48] Microfabrication technologies were used in combination with biodegradable polymers to

control their microscale properties, enabling precise engineering of the carrier used for drug transport.[18,49] These technologies allowed for microscale design of site-specific drug delivery vehicle adapted for oral[50] and intraperitoneal[51] target, requiring tissue specific degradation kinetics and pharmacological properties.

High-throughput Screening

One of the main challenges of hydrogel design is the optimization of a material for a specific application. Conventional material design strategies previously relied on the development of a single polymer on which multiple experiments were conducted to optimize it. More recently, the combination of hydrogel chemistry with microtechnologies has been used to miniaturize assays and enable screening libraries of materials, drugs, and molecules in a high-throughput (HT) fashion. Based on HT studies, a large set of cell–cell and cell–microenvironment interactions can be investigated using materials having various compositions.[52] Hydrogel microarrays allow the rapid synthesis of material libraries and enable the simultaneous assessment of multiple cell culture conditions. This approach provides a general framework for the combinatorial development of synthetic substrates for biomedical applications.[23,53] Results obtained from these assays can be used as a starting point to improve the design of hydrogels and represent a more efficient way to develop and characterize the properties of new materials in a fast and reliable fashion. The quantitative analysis of novel microengineered materials using a large sample size helps to rapidly identify the most efficient design strategies for specific biological purposes such as cell adhesion or differentiation.[52,54] HT assays also enable rapid analysis of material properties such as wettability, surface topography, surface chemistry, and substrate stiffness.[55,56] This approach also contributes to the development of structure–function relationships between material properties and biological performance,[53] this capability proves to be effective in investigating stem cell differentiation.[57,58] In a recent study, a systematic HT study was performed using a library of 50,000 compounds to search for substrates that best promoted self-renewal of mouse embryonic stem cells. As expected, HT methods drastically reduced the amount of time and effort required to perform data an acquisition,[59] increasing the efficiency of the study. HT approaches aiming at the design of new hydrogels will enable the translation of these materials into clinical applications with increased speed and enhanced outcomes at a lower cost.[60]

FUTURE DIRECTIONS

The design of materials intended for biomedical applications has advanced from trial and error selection of readily available materials to the rational design of biomaterials having precise degradation, transport, and mechanical properties as well as specific biological activity. Conventional hydrogel fabrication techniques originally allowed the tuning of only a few parameters like porosity and pore size, whereas new technologies such as microfabrication and HT approaches have significantly improved the design of these materials. In the body, the dynamic interplay between various cell types and their ECM regulate cell and tissue activity. Moreover, cells are hierarchically organized to enable proper function. Although microtechnologies have been shown to improve the biological function of polymeric materials by better mimicking the cellular microenvironment, the difficulty to engineer physiologically relevant interactions in large numbers is a major limitation to the fabrication of functional hydrogels *in vitro*. Many recent advances in biotechnology and microengineering are aiming at developing small scale systems reproducing these interactions with high fidelity.[61] Based on these approaches and on hydrogel materials, precise 2D or 3D objects can be crosslinked and assembled in a layer-by-layer fashion.[24] Patterned cocultures and 3D tissue prototyping represent techniques that will enable physiological mimicking of the organization and complexity of the *in vivo* microenvironment into hydrogel materials.[30]

Top-down and Bottom-up Microfabrication Technologies

Microfabrication techniques are emerging as useful tools for cell biology and tissue engineering studies. Two distinct approaches have been used to generate tissue-like structures. These can be classified as either 'top-down' or 'bottom-up' depending on the fabrication process used to engineer the properties of the bulk material.[18] Top-down approaches aim to control the microscale features throughout the structure of somewhat large constructs. Significant advances in scaffold fabrication have been made using top-down techniques, and it has been demonstrated that essential structures such as microvasculature can be engineered in hydrogels using this approach.[62] Bottom-up techniques, on the other hand, aim at generating large-scale tissues by assembling small building blocks into 3D structures.[63] These building blocks or functional units can be produced using HT techniques, allowing the rapid fabrication of multiple microscale hydrogels (i.e., microgels).

The hierarchically assembled microgels result in a mesoscale structure that mimics the characteristics of native tissue. For example, it was shown that cell-laden microgels can be molded into complementary microscale units and assembled into self-organized larger patterns.[63] The main challenge of this fabrication approach is the development of scalable approaches that can enable hierarchical assembly of these building blocks to generate 3D orderly cell-laden architecture.

Modular Assembly of Hydrogels

The fabrication of complex 3D tissues, such as the liver, heart, and kidney, remains a great challenge for tissue engineers as these organs are highly sophisticated and have specialized functions. Composed of multiple cell types, an extensive vasculature and an intricate architecture, these tissues combine the requirement for adequate structure and perfusion to perform their physiologic duty.[64,65] Bottom-up approaches are currently being investigated to reproduce these tissues *in vitro*. Whitesides and coworkers have pioneered mesoscale assembly of millimeter-scale objects into precisely defined 2D and 3D structures using the minimization of interfacial free energy at a liquid–liquid interface.[66,67] Self-assembly processes are triggered by the attempt of a system to minimize its free energy, which results in the aggregation of smaller objects. Therefore, the formation of 3D tissues through self-assembly of small subunits could be used to generate biomimetic and functional tissue structures. Inspired by these findings, cell-laden microgels having lock-and-key shapes have been combined with thermodynamically driven assembly techniques to direct the assembly of these building blocks into 3D tissue constructs with tunable microarchitecture and complexity[63] (Figure 5). In this context, the hydrophilic properties of microgels combined with the hydrophobic properties of the medium are used as the driving force to generate large structures.[68] Mechanical stability of these assemblies can be enhanced by a secondary crosslinking reaction using UV light exposure.

To meet specifications such as adequate physiological architecture, tissue function and vascularization, bottom-up or modular assembly techniques represent potentially scalable approaches to generate biomimetic 3D tissue constructs.[63,69,70] A range of microfabrication approaches can be used to control the shape of microgels and to generate microscale units with precise dimensions in a HT fashion.[71] The main limitation of this self-assembly process is the packing of microgels, which requires hierarchical and organizational driving forces enabling precise microgel placement and assembly. To address this issue, a technique using a solid surface that acts as a template to direct the assembly process has been developed[72] (Figure 6). As a result, microgels were able to densely pack along the surface of the template and were later crosslinked using a second UV exposure generating a 3D structure. From a tissue engineering perspective, the assembly and packing of the microgels needs to be performed following stringent requirements for physiological tissue function. As self-assembly processes rely on energy balances between states or phases, the optimal assembly of the microunits will depend on the material as well as on the nature of the driving force used to assemble the building blocks. Therefore, the control of chemical and physical interactions between the microgels will be essential for the development of self-assembled stable hydrogel structures.[73]

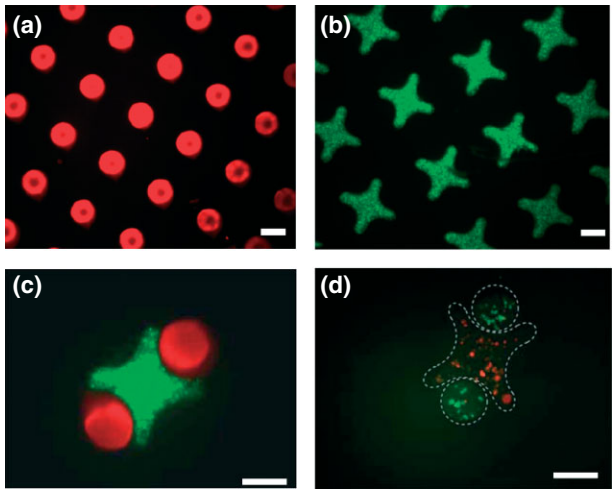

FIGURE 5 | Directed assembly of lock-and-key shaped microgels. Rod-shaped (a) and cross-shaped (b) microgels stained with nile-red and FITC–dextran, respectively. Directed assembly of lock-and-key shaped microgels stained with FITC–dextran and Nile-red (c) and cell-laden microgels stained with calcein AM and PKH26 (d) and (b). Scale bar: 200 μm (Reprinted with permission from Ref 63. Copyright 2008 National Academy of Sciences, USA)

Vascularization of 3D Hydrogels

The main challenge in the translation of tissue engineering technologies to clinical applications is to generate large, functional, and vascularized tissues *in vitro*. The inability to adequately vascularize engineered tissues results in inefficient transport of nutrients and metabolites that are more than a few hundred microns away from a capillary, resulting in cell death and tissue necrosis. The engineering of microcirculation requires endothelial cells to line the

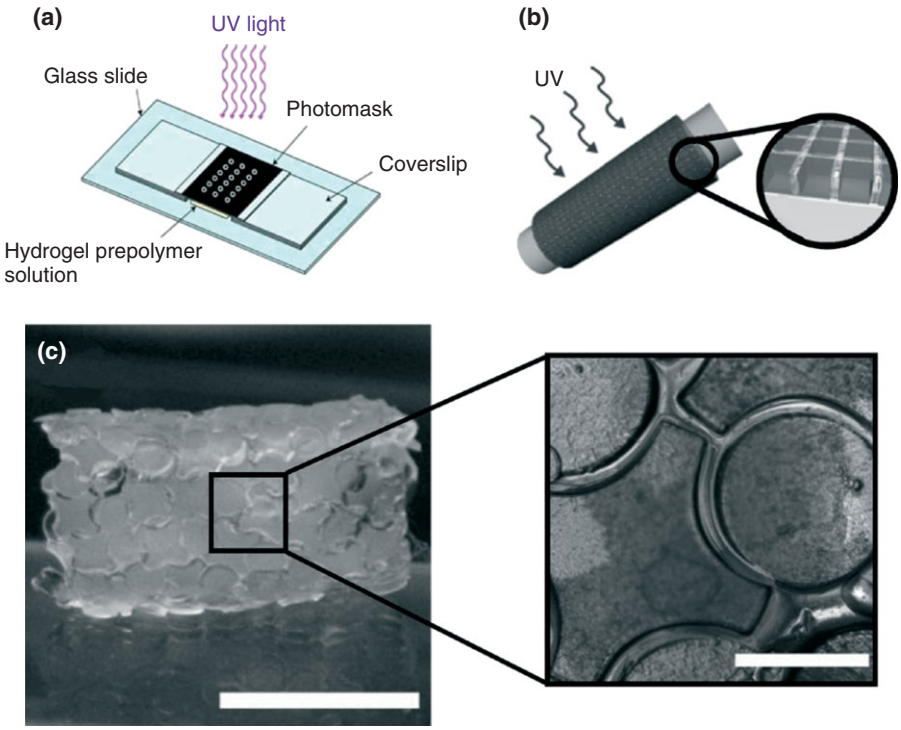

FIGURE 6 | Mesoscale assembly of microgels using a micro-masonry process. Schematic representation of a high-throughput photolithographic approach (a) (Reprinted with permission from Ref 74. Copyright 2011 Wiley-VCH Verlag GmBH&Co. KGaA). Schematic diagram of the micro-masonry assembly process (b). Microgels are assembled on a template before a second crosslinking process, resulting in a 3D structure composed of an assembly of microgels recapitulating the 3D structure of the template used for fabrication (c). Scale bar: 5 mm and 1 mm (magnification) (Reprinted with permission from Ref 72. Copyright 2010 Wiley-VCH Verlag GmBH&Co. KGaA)

interior of every blood vessel in the body and form the endothelium, a dynamic interface between the circulating blood and the surrounding tissue. Previous approaches have relied upon the presence of growth factors or the seeding of endothelial cells in the scaffold to promote angiogenesis.[75,76] However, the time required to generate proper vascularization often results in loss of cell viability, which reduces the efficiency of the technique considerably to produce 3D tissues. On the other hand, strategies such as microfluidic approaches are showing promise as a means to incorporate channel networks inside biodegradable polymers.[77–80]

Most of the vascularized systems built using top-down approaches are found in planar or stacked 2D structures. These are made from stiff polymers such as PDMS or polystyrene, which cannot be integrated with the surrounding tissue *in vivo*.[81] Although previous work has shown that microscale cell-laden channels can be engineered *in vitro*, it is particularly difficult to consecutively branch multidimensional channels inside a 3D structure.[82] Techniques such as direct ink writing and omnidirectional printing have recently been developed to create vascular structures in 3D.[83,84] Despite their enormous potential, these approaches still require further improvements regarding the other specialized cell types required to enable the functionality of the tissue structures surrounding the vascular network. Conversely, modular assembly techniques can be performed in a biphasic reactor using cell-laden microgels produced by photolithography. Photolithography and self-assembled systems were used to build biomimetic vascular-like structures for tissue engineering and *in vitro* models. The directed sequential assembly of cell-laden microgels resulted in a 3D structure with multilevel interconnected branching vasculature[74] (Figure 7) This approach allowed the encapsulation and culture of smooth muscle cells and endothelial cells into a hydrogel for an extended period of time *in vitro*.[74] Compared to previous work, this sequential assembly technique of vascularized units is a step forward in the control over the spatial arrangement of building blocks and the realization of 3D structures.[63,70] The engineering of organs will benefit from the sequential assembly process enabling the fabrication of 3D constructs containing multiple cell types with defined architecture and function.

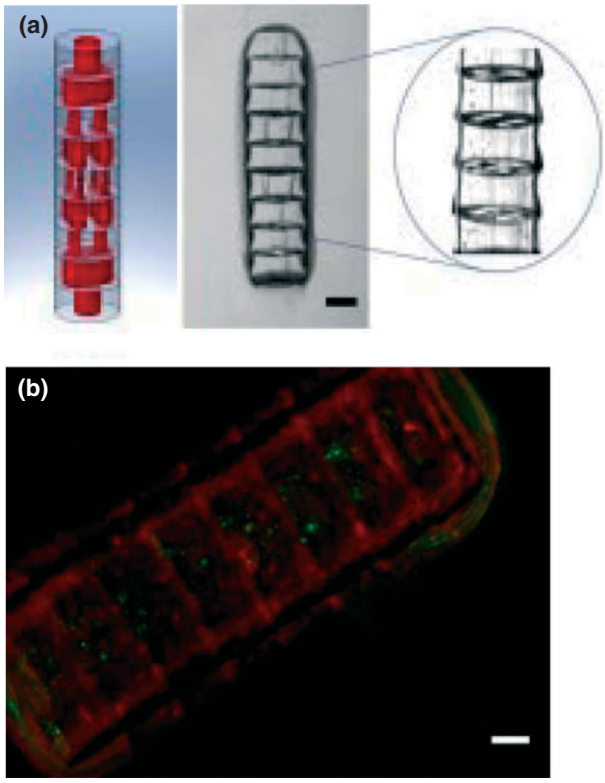

FIGURE 7 | Sequential assembly of microgels using a directed assembly approach. From left to right, design image of a microgel array assembled into tubular structures embedded with 3D branching lumens and phase image of the microgel assembly after secondary crosslinking (a). Scale bar: 500 μm. Fluorescence images of the cell-laden concentric microgel assemblies with endothelial (green) and smooth muscle cells (red) (b). Scale bar: 100 μm (Reprinted with permission from Ref 74. Copyright 2011 John/Wiley & Sons, Inc)

CONCLUSIONS

A wide range of hydrogels can be generated using various natural and synthetic polymers. These materials can be used as drug delivery carriers or as scaffolding materials for tissue engineering applications. The physical, chemical, and biological properties of hydrogels can be tailored by varying the polymer concentration, the crosslinking density, the amount of reagents encapsulated in the bulk material, and the degradation mechanisms of the polymeric chains in the backbone. Advances in polymer chemistry and the development of microfabrication strategies have led to increased complexity in the structure and enhanced biological function of hydrogel constructs. These 'smart' materials are designed to control the cellular microenvironment and to provide the cells with appropriate signals to induce adhesion, migration, proliferation, or differentiation, depending on whether the tissue needs repair, regeneration, or remodeling. Recent advances in cell biology have increased our knowledge of cell–cell and cell–ECM interactions, resulting in the rational design of complex and highly efficient biomaterials. The improvement of scaffold architecture and bioactivity is currently moving toward the design of hydrogels adaptable to specific applications and functionalities. Thus, hydrogels still need to be optimized to produce therapeutic outcomes for clinical applications. It is expected that the integration of interdisciplinary research fields including microtechnologies, cell biology, drug discovery, tissue engineering, and regenerative medicine will result into improved and readily available hydrogels for clinical and therapeutic applications.

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