Biological structures are inherently complex in nature. Structural hierarchy, chemical anisotropy, and compositional heterogeneity are ubiquitous in biological systems and play a key role in the functionality of living systems. For decades, methods such as soft lithography have enabled recreation of such arrangements through precise spatial control of molecular patterns in 2D. With technological advances and increasing understanding of molecular and structural biology, there has been an increasing interest in recreating such spatial organizations in 3D. In this review, a comprehensive summary of the latest technologies being used to create 3D patterns of functional molecules within hydrogels for tissue engineering applications is presented. The review is divided into five groups of technologies defined according to the main driving force used to fabricate the patterns including light, precise chemical design, microfluidics, 3D printing, and non-contact forces (i.e. electric, magnetic, or acoustic fields and self-assembly).

1. Introduction

1.1. Patterns in Nature and Biological Systems

Nature is filled with beautiful structures exhibiting repetitive motifs of precise shape and size. However, these patterns are far from merely aesthetic features but rather a necessity for locomotion, reproduction, protection, and alimentation. For example, the beautiful V-shape patterns displayed by flying geese help the flock sense its surroundings and rapidly transmit information throughout the group. The periodicity of melanin spots or patterned stripes present on the skin of animals such as giraffes, leopards, or zebras as well as gradients of colors on the feathers of birds serve as camouflage, signaling or recognition. Trees develop branching patterns to maintain their structural properties in the face of external factors such as wind, light, competitors, and biotic or abiotic stressors with minimal energy consumption. These beautiful and functional patterns are a direct consequence of the precise spatial organization that nature has evolved.

The functionality of patterns is not limited to the macroscopic world. In 1952, Alan Turing’s work in morphogenesis highlighted the relevance of chemical gradients in the generation of patterns and their crucial effect on cell differentiation. Similarly, well-defined patterns play a key role in the development and functionality of tissues such as skin, heart and kidney. For example, osteons, the fundamental functional unit of cortical bones, are multiple adjacent 100–500 μm diameter structures, which themselves are composed of organized collagen fibrils exhibiting a 67 nm periodicity with 40 nm gaps and calcium phosphate crystals. In addition, the human liver, which serves a crucial role in maintaining physiological homeostasis, relies on packed 1 mm thick hexagonal repeating lobule units of spongy tissue surrounding a central hepatic vein interconnected with a regular portal triad. Unfortunately, the effectiveness of patterns can also be observed in pathological conditions. For example, cells within the tumor microenvironment are spatially localized and rely on oxygen concentration gradients to grow and advance the tumor. These examples not only illustrate the functionality of patterns in biological systems but also inspire us to develop patterns in our quest to harness the complexity and functionality of biological structures and tissues.

1.2. The Need to Recreate Structural and Chemical Anisotropy

Modular tissue engineering (TE) strategies have been developed to engineer tissues and organs by the fabrication of living building blocks from the bottom-up. Furthermore, advances in cell manipulation such as induced pluripotent stem cells (iPSCs), the homing capability of mesenchymal stem
cells (MSCs), and genomic editing are enabling the fabrication of higher architectural cellular designs. Organoids and microvesicles are being used to recreate native tissue-like micro-environments by spatially organizing multi-cellular structures into tissue-like constructs. Despite these advances, current approaches still suffer from lack of reproducibility in recreating the structural complexity of in vivo scenarios, poor temporal control over physiological cues, and limited scalability for widespread use. Thus, the development of structures that can truly mimic the molecular diversity, structural complexity, and functionality of living systems will require innovative fabrication strategies.

An ideal tissue model, such as an organ-on-a-chip or biomimetic hydrogel, should recreate the physical and chemical cues of the in vivo environment as well as the complex interactions between cells and their microenvironment. Hydrogels, interchangeably referred to as scaffolds in this review, are particularly attractive materials that resemble the hydrated porous structure of the native extracellular matrix (ECM) given their high water content, tissue-like elasticity, and potential to incorporate biomolecular signals. Pioneering hydrogels have been developed using components such as peptides, proteins, and synthetic or natural polymers enabling well-defined spatial distribution of biochemical and biophysical signals modulating focal adhesion and contact guidance at cellular level. In addition, supramolecular tools such as the use of host-guest complexes, diffusion-reaction processes and multicomponent self-assembly can be incorporated to further introduce biochemical epitopes over the nano- and micro-scale. However, the capacity to recreate the structural and chemical anisotropy observed in tissues and organs, remains underdeveloped.

Realization of the need for and potential to fabricate patterns is not new. Soft-lithographic techniques, developed by Whitesides and colleagues more than two decades ago, have had an enormous impact in the fields of cell biology, biochemistry, biotechnology, and bioengineering. Inspired by this work, techniques such as dip pen nanolithography, nanoimprint lithography and molecular assembly patterning have enabled well-defined patterns of biomolecules on 2D surfaces, which have facilitated studies of cell adhesion, proliferation, differentiation and communication amongst others. Furthermore, topographical patterns (also referred to as 2.5D patterns) have also been explored.

Figure 1. Patterns in nature. Patterns are ubiquitous in nature across size-scales including for example A) animal behaviors such as the V-shape geese flying organization, B) animal skins such as stripes in zebras or spatially-distributed melanin coloration in giraffes, C) branching patterns in leaves and trees, D) spatially organized cells during development (scale bars: 100 µm) (Reproduced with permission. Copyright 2013, Science), E) spatially organized collagen fibres and calcium phosphate crystals generating osteons in bone (Reproduced with permission. Copyright 2017, Springer Nature) or, F) patterns emerging in tumor environments as a result of different cell types and oxygen gradients. Reproduced with permission. Copyright 2014, Frontiers Media.
and used to guide cells and biological processes through physical features at the nano, micro, and nano/micro scales, within 3D structures, or in combination with chemical signals. These examples illustrate the impact that 2D or 2.5D topographical cues can have on our capacity to study or recreate biological processes (Figure 2A). Imagine the possibilities if we were able to easily fabricate similar patterns within complex 3D environments (Figure 2B). Toward this goal, a variety of techniques are being developed including light, chemical design, microfluidics, 3D printing, and non-contact forces involving electric, magnetic, acoustic fields, or self-assembly (Figure 3). We limit our review to studies focused on patterning hydrogels aiming to recreate biological structures for applications in tissue engineering, drug screening, and in vitro models.

### 2. Fabrication of Patterns by Photo-Patterning

Light has been extensively used to create patterns in hydrogels. Photo-patterning requires moieties (photosensitive or...
photoliable) capable of undergoing reactions upon light exposure. We divide this section into different multilayer patterning techniques, which use light to both create the patterns and gel simultaneously (Figure 4A) and post-gelation patterning techniques, which create the patterns after gelation (Figure 5A). Other techniques that do not require a photosensitive moiety but still employ light for patterning are also discussed. For techniques based on photo-induced click chemistry, see ‘Fabrication of Patterns by Precise Chemical Design’ (Section 3 in this article).

2.1. Multilayer Patterning

These techniques aim to elicit photo-polymerization with spatio-temporal control. In photo-polymerization, a chain reaction is triggered by light using a photo-sensitive molecule, a photo-initiator that is not part of the polymer backbone (Figure 4A). Based on this principle, approaches such as stereolithography (SLA), projection stereolithography (pSLA), and photo-grafting lithography (PGL) have been developed. These methods are based on the layer-by-layer principle of additive manufacturing and rely on a controlled photo-polymerization reaction on the x-y plane while gradually moving into the z-axis (Figure 4).

2.1.1. Stereolithography

Either by direct laser writing or mask-assisted, SLA has enabled the incorporation of functional molecules such as short peptide sequences, ECM-like proteins, and even modified short polymer chains within hydrogels, creating stripes, spots, and hexagonal or triangular patterns down to 80 µm in size. For example, by focusing UV light through a hexagonal mask, Arg-Gly-Asp (RGD) peptide sequences were patterned into a poly-ethylene glycol (PEG) hydrogel to develop a hepatic-like tissue construct with ≈500 µm resolution, which exhibited high cell viability and metabolic functionality (Figure 4C).

2.1.2. Projection Stereolithography

This technique enables fabrication at higher resolutions (20–200 µm in lateral dimension) in some cases down to 5 µm compared to SLA due to the addition of a digital mirror array that reflects the pattern template structure into the pre-polymer solution (Figure 4D). Computer aided design (CAD) can also be adjusted in pSLA, allowing the fabrication of micropatterned structures such as interconnected columns, pillars, and cubes of ≈100 µm in size and separations in the range of 1 to 10 µm. Precisely selecting the hydrogel material and photomasks enables pattern fabrication of variable shapes and sizes. However, the maximum feasible pattern resolution is ≈80 µm, given the need for a photo-mask and pattern thicknesses beyond a few microns are difficult to obtain due to the light scattering effects on the formed hydrogel.

Figure 3. Methodologies for fabricating 3D patterns. Schematic representation of the main methods presented and corresponding to the sections of the review. E = electric field, M = magnetic field, S = acoustic field.
The presence of RGD within the gelatin backbone in combination with the CaCO₃ microparticle-induced microporosity enabled full invasion of human umbilical vein endothelial cells (HUVECs) into the patterned scaffold. Similarly, GelMa doped with poly(ethylene oxide) was used to fabricate porous hydrogels with patterns of stars, pyramids, and spirals exhibiting resolutions of 20–30 µm. PSLA has also been used to easily create a perfusable hepatocyte bioreactor attached to commercially available filters by patterning low molecular weight (Mₘ) polyethylene glycol diacrylate (PEGDA) as aligned rectangular or staggered ellipses measuring ≈300 µm in width and 700 to 900 µm in length. Furthermore, pSLA enables the use of more than one projected mask in a single lithographic process, which has been used to produce a 3 × 3 mm hepatic 3D model by sequentially patterning lobule shapes mixing hepatic cells, GelMA, and glycidal methacrylate–hyaluronic acid (Figure 4E). The model exhibited physical and functional resemblance to the native hepatic tissue. Patterns of stiffness within hydrogels were also produced employing pSLA. Norris et al. developed PEGDA hydrogels exhibiting gradients of stiffness between 12.9–220.8 kPa by a direct correlation of the grey scale pixel intensity of the CAD and the output gel stiffness. These stiffness patterns were shown to affect human mesenchymal stem cell (hMSC) adhesion, migration, and cross-talk communication. In addition, pSLA enables the fabrication of gradient channel widths ranging from 50 to 250 µm by easily combining three projected masks. In summary, pSLA has improved resolution compared to SLA but its need for a reflected mask and the lack of focused light limits its capacity to fabricate complex and inter-connected pattern shapes.

2.1.3. Photo-Grafting Lithography

In an effort to increase pattern resolution, focalized laser light involving multiphoton processes has been employed (Figure 4F). One such approach is PGL, which is capable of precisely localizing (bio)functional molecules within hydrogels by confining the photo-reaction within the focal area of the laser beam. Two- and three-photon PGL techniques (also known as two- and three-photon SLA, respectively) have been used to obtain complex geometries (Figure 4G) with features down to
3 \mu m in size and 1.5 \mu m separations within gelatin, hyaluronic acid (HA), and PEG hydrogels, even in the presence of cells.[103,129] In a less conventional manner, two-photon PGL was used to produce cubic 100 \times 100 \times 500 \mu m RGD patterns in PEG hydrogels to guide cellular growth.[105] This strategy relied on a pre-crosslinking process of the hydrogel to hold in place human dermal fibroblast clusters in a supporting fibrin hydrogel. RGD sequences were then photo-patterned, triggering fibroblast migration along the RGD patterns (Figure 4H). Similar guiding structures were achieved by laser-ablation of dorsal root ganglion (DRG) encapsulated within fibrinogen, albumin, and gelatin PEG-conjugated hydrogels.[130]

In another example, PGL was used to produce 250–300 \mu m diameter alginate beads that were spatially patterned into a variety of shapes.[131] PGL has effectively combined the advantages of focusing a laser beam with additive manufacturing to produce high resolution (≈3 \mu m) patterns within hydrogels, offering a noticeable improvement compared to SLA and pSLA.

2.2. Post-Gelation Patterning

Post-gelation patterning requires a synthetic hydrogel containing a reactive light-sensitive moiety (Figure 5A).[112] For biological applications, caged probes, photosensitive compounds that mask the biomolecule of interest in a quiescent form, are widely used.[133,134] Among caged probes, o-nitrobenzyl, cumarin-4-ylmethyl and their derivatives are widely employed due to their good pre-photolysis stability with long wavelength absorption and high absorption coefficients, rapid photo-release rate, and non-toxic photo-released by-products.[135] For patterning purposes, further reaction with light produces the rupture of the selected caged probe, exposing either the desired patterning molecule or another functional group which is able to undergo a second reaction with the desired patterning molecule. Here, we divide post-gelation patterning techniques according to the use of a templating mask (i.e., mask photolithography (MP) or by direct laser photolithography (focused laser irradiation (FLI) and two-photon laser scanning photolithography (TPLSP)) (Figure 5B–F).

2.2.1. Mask Photolithography

The concept of using a master fabricated by soft-lithography[141] was extended to 3D patterning in hydrogels in MP.[142] By using a mask to cover the surface of a hydrogel under exposure to
light, the selectively irradiated unmasked regions can undergo photo-reaction, resulting in the formation of structural and biochemical patterns (Figure 5B). The resolution of this approach is limited by the non-monochromatic nature of light from a UV–vis lamp and light diffraction into the hydrogel system, limiting as well the depth of the generated patterns. A non-covalent photo-patterning method of gelatin-based hydrogels was produced using MP to physically pattern caged collagen mimetic peptides with sequential stripes of 50 and 100 μm in width. To overcome the low resolution resulting from the non-monochromatic light sources, MP was combined with an enzymatic reaction in a multi-step process enabling to pattern well-defined geometries of peptides, proteins, and growth factors within PEG- and HA-based hydrogels. Similarly, MP was employed to selectively release epidermal growth factor (EGF) in 400 μm wide stripe patterns within PEG hydrogels encapsulated with human cervical cancer (HeLa) cells in vitro and to create functional 900 μm diameter RGD patterns within PEGDA hydrogels directly and safely in a mouse model. In addition, MP can be combined with other fabrication techniques. For example, hydrogels produced from 220 μm diameter electrosprun HA nanofibres were successfully patterned with RGD peptides spatially organized in straight lines or circles with resolutions between 100 and 200 μm (Figure 5C). It is well established that cell behavior is affected by the mechanics of the extracellular environment. In this context, MP offers an opportunity to investigate with higher precision, the effects of matrix mechanics on cell behavior in vitro. For example, high Mw PEGDA hydrogels were fabricated with distinct linear regions measuring 20 to 100 μm in width exhibiting tensile elastic moduli that were 4–7 times greater in the direction of the patterns compared to the perpendicular orientation. Other examples include patterns within PEGDA hydrogels exhibiting nonlinear mechanical responses, patterns in gelatin-PG hydrogels to enable spatially controlled degradation, and patterns within alginate hydrogels enabling spatially controlled stiffness capable of guiding cell morphology. However, in spite of the great range of potential applications, MP remains limited by the use of UV–vis as a light source, low resolution resulting from light scattering, and some degree of size and geometry dependency on the photo-mask employed.

2.2.2. Laser Photolithography: Focused Laser Irradiation and Two-Photon Laser Scanning Photolithography

Pioneering work by Shoichet and co-workers demonstrated the possibility to use FLI to pattern an agarose hydrogel modified with an o-nitrobenzyl photolabile derivative with 160 μm diameter and 1 mm deep RGD column patterns capable of directing neurite growth. The work inspired a variety of other FLI-based approaches using fluorescent biomolecules, proteins, and DNA to pattern hydrogels with features ranging between 5 and 50 μm. Nonetheless, FLI suffers from being limited in resolution as a result of the linear-nature of the single excited photon used. Thus, taking advantage of the use of both scattered and unscattered photons, TPLSP has produced patterns with superior resolution and spatial localization, including for example the generation of single or sequential gradients. Furthermore, TPLSP enables the use of a secondary high affinity intermediate reaction, such as a Diels Alder cycloaddition between the hydrogel and patterning molecules to enhance pattern fidelity and resolution. Other methods, such as cryogelation, can be incorporated to enhance structural complexity while enabling functional patterning of EGF, ciliary neurotrophic factor, RGD, and (Ile–Lys–Val–Ala–Val) IKVAV peptides into 200 μm diameter channels, facilitating spatially-controlled growth of epithelial breast cancer spheroids. TPLSP can also be used as a subtractive manufacturing technique. For example, fully endothelialised perfusable 10 μm diameter vascularized constructs were fabricated by photo-degradation of a PEG hydrogel functionalized with o-nitrobenzyl moieties. Through the same subtractive concept, horseshoe-shaped micro-channels were selectively eroded around spheroids of human adipose derived stem cells (hADSCs) suspended in a PEG-HA hydrogel, promoting cell spreading in the patterned hydrogel. In another example, a single TPLSP process was used to fabricate 50 μm diameter collagen channels and selectively pattern P-selectin using a simultaneous photo-degradation/patterning process. In summary, FLI and TPLSP offer the possibility to pattern short peptides, proteins, and growth factors within the volume of a hydrogel, while MP requires patterning from the surface of a material. However, these techniques require chemical modifications to prepare the materials and are limited to either a hydrogel backbone with caged probes or gels with minimal light scattering.

2.3. Other Techniques Employing Light without a Photosensitive Moiety

Both multilayer patterning or post-gelation patterning employ photo-sensitive moieties to fabricate hierarchical micrometer structures within soft materials (Figures 4A and 5A). However, materials with no photo-reactive moieties can still be used in patterning processes. Applegate et al. have developed a multiphoton absorption technique to fabricate patterns with resolutions down to 5 μm within silk fibroin hydrogels. The technique has proved to be suitable for generating branch-like channels for cell invasion assays. In another study, a near-infrared femtosecond laser focusing process was used to photothermally pattern collagen hydrogels with channels of 10–250 μm in width, promoting endothelial migration and vasculature formation. Furthermore, laser ablation was similarly used to create hollow patterns within PEG hydrogels, enabling the fabrication of perfusable microfluidic networks with geometrical features ranging between 5 and 100 μm. Amongst

3. Fabrication of Patterns by Precise Chemical Design

Chemical (bio)conjugation reactions such as biotin–streptadavin or tyramine–peroxides conjugation, PEGylatig, peptides conjugation, and coupling–click reactions have been widely employed to produce cell-responsive materials. Amongst
them, click chemistry has proved to be a powerful tool to combine functional molecules under mild conditions, with great efficiency and selectivity, high yields, and harmless by-products. The orthogonality of click chemistry precursors positions it as a viable candidate for the synthesis of hydrogels (Figure 6A) and applications such as cell transplantation, drug delivery, in vitro models, and therapeutics. This approach has been used with both simple (e.g., PEG) and more complex (e.g., glycosaminoglycans, proteins) hydrogels. Given this versatility, this section will mainly focus on photo-induced click chemistry reactions but others will be discussed too.

3.1. PEG-Based Hydrogel Backbone

Anseth and co-workers first proposed the use of click hydrogels for cell encapsulation and fabrication of patterns using a
bi-orthogonal Huisgen cycloaddition between four-arm PEG tetra-azole and bis(di-fluorinated cyclooctyne) di-functionalized polypeptides.\textsuperscript{186} Then, using a second photo-aided click reaction, an alkene moiety presented in the polypeptide backbone was micropatterned with a thiol-functionalised RGD sequence, signaling spreading of encapsulated fibroblasts.\textsuperscript{192}

Similarly, other bio-molecules such as vascular endothelial growth factor\textsuperscript{187} and ovalbumin\textsuperscript{188} have been patterned in PEG hydrogels into overlapped squares\textsuperscript{186} stripes\textsuperscript{189} or more complex shapes\textsuperscript{192} with the use of a photomask (Figure 6B). The technique facilitates control of pattern depth by modulating the focal point of a pulsed laser light with a multiphoton technique (e.g., confocal microscope). In addition, varying the ratio between alkene and thiol groups,\textsuperscript{190} RGD stripe-patterns ranging between 200–250 µm were fabricated using a double photo-click reaction.\textsuperscript{191}

Additional levels of sophistication can be added by introducing an o-nitrobenzyl ether photocleavable moiety on the polymer hydrogel backbone\textsuperscript{196} or employing three bio-orthogonal click reactions to remove functional peptides (e.g., RGD) and enable temporal control of signaling.\textsuperscript{192} In this case, the pattern resolution still depends on the photomask used during the photolithographic step, which can generate resolutions down to ~1 µm in the x-y plane and ~3–5 µm in the z axis (Figure 6C). An alternative way to use three bio-orthogonal click reactions to provide spatio-temporal presentation of functional molecules, is to specifically choose orthogonal pairs that are capable of being triggered on demand. For example, biochemical and biomechanical patterns of 10–400 µm in size were patterned in a PEG hydrogel by combining UV and visible light-triggered click reactions.\textsuperscript{197} The use of three bio-orthogonal reactions was taken a step further when strain-promoted azide–alkyne cycloaddition, photodeprotection–oxime-ligation, and ortho–nitrobenzyl ester photoscission were combined to reversibly tether proteins within 3D hydrogels (Figure 6D).\textsuperscript{193}

A major opportunity of thiol-ene click chemistry is the possibility to generate dynamic hydrogels that can begin to emulgate the critical adaptive and reversible nature of the native ECM. As an example, allyl sulfide moieties in hydrogels can act as on-off switches to trigger the sequential exchange of biochemical cues employing the chain transfer reaction of a thyl radical.\textsuperscript{198} Other examples have demonstrated the possibility to generate hydrogels with tunable viscoelastic\textsuperscript{199} and cell release\textsuperscript{200} properties on-demand. The chain exchange offered by the allyl sulfide functional group is limited to the consumption of the activating radical spacers.\textsuperscript{201} To overcome this limit in reversibility, “living” hydrogels (i.e. hydrogels where the activating radical species regenerate by themselves) were produced by patterning peptides and proteins, introducing a cycle of two thiolene photo-induced click chemistry reactions.\textsuperscript{202,203} In addition, taking advantage of the ability of aptamers (artificial oligonucleotides) to bind to proteins,\textsuperscript{204} Zhang et al. used thiol-ene click chemistry to produce 250 × 250 µm thrombin patterns capable of being released on demand.\textsuperscript{205}

Alternative reaction pathways to pattern PEG-based hydrogels are hydrazide condensation and oxime ligation reactions, which are both spontaneous and controllable polymerizations without the need of UV light activation or radical generation.\textsuperscript{279} While the latter produces a stable hydrazone adduct by condensation of a hydrazine and an aldehyde,\textsuperscript{206} the former offers a spontaneous reaction between an alkoxyamine and an aldehyde.\textsuperscript{207} In a two-step process, a PEG network is first formed by mixing a stoichiometric excess of 4-arm-PEG-hydrazine with 4-arm-PEG-nitrobenzyl, which is then patterned with RGD-nitrobenzyl through a second reaction with the unreacted hydrazines present in the hydrogel backbone (Figure 6E).\textsuperscript{194} In a similar manner, off-stoichiometric hydrogels of 8-arm-PEG-aldehyde and 8-arm-PEG-alkoxyamine were formed, and alkoxyamine modified proteins were photo-patterned by oxime ligation.\textsuperscript{208} Most recently, sortagged proteins were employed to pattern growth factors via sequential oxime ligations.\textsuperscript{209} This strategy showed to effectively fabricate dynamic hydrogels with pattern resolution of 20 µm and bioactive epitopes. Click chemistry reactions have provided an approach to impart PEG hydrogels with diverse types of patterns using a wide variety of biomolecules and to do so in controllable and mild reaction conditions with high yields. However, these reactions can suffer from exhaustive synthetic pathways and high costs.

3.2. Glycosaminoglycan and Protein-Based Hydrogel Backbone

Other hydrogels have also been explored as polymer backbones for patterning functional molecules using click chemistry.\textsuperscript{195,210–212} For example, norbornene-functionalized HA hydrogels made through a two-step click photo-induced reaction have been patterned with 100 µm wide stripes of thiol functionalized peptides (Figure 6F).\textsuperscript{195} The same system was employed to fabricate complex gradients of His–Ala–Val (HAV) and RGD peptides and study their synergistic effect on chondrogenesis.\textsuperscript{213} A similar approach was used on stimuli-responsive HA hydrogels patterned with dithiol-functionalized poly(N-isopropylacrylamide) and RGD sequences, promoting anisotropic hMSC growth and control over the thermo-responsive properties of the hydrogel.\textsuperscript{214} In another study, thiol-ene click chemistry was combined with Diels Alder addition to pattern 300 µm wide stripes of RGD peptides within HA-based hydrogels.\textsuperscript{215} Tamura et al. designed a composite PEG, gelatin, and Matrigel hydrogel with a photocleavable nitrobenzyl moiety.\textsuperscript{212} In this case, the azide–alkyne click reaction was used to form the hydrogel network and a photo-patterning process to cleave the nitrobenzyl moiety and release encapsulated HeLa cells from 10 µm wide patterns. Nonetheless, patterning of glycosaminoglycan- and protein-based hydrogels in click chemistry remain limited as a result of their need for complex chemical precursor synthesis and exhaustive purification.

4. Fabrication of Patterns through Microfluidics

The biochemical and biomechanical properties of the in vivo ECM directly affect cellular behaviour.\textsuperscript{44,216} However, a variety of signals (e.g., growth factors, hormones, toxins) produced by distant or neighboring cells impact cellular plasticity, migration, gene expression, and communication.\textsuperscript{64} Therefore, being able to reproduce and study cellular communications within 3D environments is of broad interest. Microfluidics has emerged as a suitable technology to aid in the design and fabrication of
such environments to either fabricate patterns into a hydrogel (microfluidics-to) or to induce physical patterns to create a perfusable systems (microfluidics-into).[89,217]

### 4.1. Microfluidics-to

Over the past 20 years, a variety of approaches have been proposed to pattern hydrogels and cells using microfluidics.[218,219] For example, Matrigel was patterned into 200–500 µm width slabs under laminar flow in a micro-chamber device by combining up to five inlet channels enabling co-cultures and gradients of biomolecules.[217] Other examples include the generation of collagen gradients through a convective mixing microfluidic device,[220,221] patterning of primary hematopoietic stem cells,[220] and patterning of ECM components and multiple cells to recreate a tumor niche (Figure 7A).[221] Patterning with microfluidics permits combination with supportive methodologies such as soft-lithography to pattern 200 µm collagen beads,[222] capillarity forces for rapid 100 µm-wide patterning of cells within PEGDA hydrogels,[223] and extrusion used for fabrication of millimetric 3D structures[224] or multi-layered microfibers.[225] In a sophisticated approach, functional 20 µm hydrogel microfibres were spatially patterned with tunable structural and chemical features by adding pneumatic valves to a multiple inlet microfluidic chip, enabling controlled co-culture organization of hepatocytes and fibroblasts within the fibres (Figure 7B).[226] Microfluidics-to has emerged as a versatile and cell-friendly patterning technique permitting the use of multiple types of hydrogels,[227] while enabling the use of flow to manipulate cells (Figure 7C).[228]

### 4.2. Microfluidics-into

Microfluidics-into platforms are advantageous over other techniques as they can mimic in vivo chemotactic environments with high reproducibility.[89] Recently, a perfusable gelatin hydrogel loaded with hMSCs was shown to controllably induce both osteogenic and chondrogenic differentiation based on the generation of chemical gradients (Figure 7D).[229] Moreover, stripes of aligned Matrigel fibers were patterned between 1 mm-wide channels employing hydrodynamic forces produced by a steady flow of cell media.[230] Furthermore, diverse geometrical features down to 100 µm in diameter were patterned in collagen, Matrigel, and fibrin hydrogels in order to create chemotactic environments for neutrophil migration.[231]

![Figure 7](image-url) Fabrication of patterns through microfluidics. Microfluidics is used to generate patterns within hydrogels (microfluidics-to). A) Microfluidic mixer that combines two inlet chambers (inlets A,B) to fabricate hydrogels with overlapping opposed gradients. Reproduced with permission.[226] Copyright 2011, Springer Nature. B) A system of pressurized valves and chambers was adapted to fabricate red, green, and blue patterns within fibrillar structures. Reproduced with permission.[226] Copyright 2013, Wiley-VCH. B) A system of pressurized valves and chambers was adapted to fabricate red, green, and blue patterns within fibrillar structures. Reproduced with permission.[226] Copyright 2011, Springer Nature. C) A compartmentalised microfluidic system was developed to pattern single or mixed cellular environments (scale bar: 500 µm). Adapted with permission.[228] Copyright 2009, Oxford University Press. Furthermore, microfluidic devices can be used to fabricate gradients into hydrogels (microfluidics-into). D) A closed-microfluidic system allowed for dual delivery of chondrogenic and osteogenic media into a cell-laden hydrogel. Diffusion profiles show the capabilities of the platform to generate gradients. Reproduced with permission.[229] Copyright 2013, Royal Society of Chemistry.
The use of microfluidic platforms has been extensively employed in the biomedical field as an easy and cost-effective tool to develop in vitro therapies for drug-screening and in-depth cellular biology studies. These benefits are promoting the use of microfluidics to improve 3D printing approaches to enhance manufacturing of complex and anisotropic architectural designs.

5. Fabrication of Patterns Through 3D Printing

In the last five years, interest in additive manufacturing for tissue engineering and bioengineering has exponentially increased. Versatility and tuneability of ink materials, scalability, microscale precision, and robustness of fabricated structures are enabling the growth of 3D printing. At its core, a 3D printer is a computer-controlled pattern-generating device using either a laser optic or an ink-based print-head to additively deposit and solidify an ink material into a precise architecture. In this section, we discuss work focused on creating functional heterogeneities within the volume of 3D printed hydrogels (i.e., patterns fabricated within the 3D printed scaffold, independently of the convoluted structures achievable with a 3D printer platform). This section is divided into vascularized scaffolds, large scale constructs, non-conventional approaches, and suspended manufacturing. We refer the reader to other comprehensive reviews highlighting printing materials, inks, and methodologies.

5.1. 3D Printing Patterns to Mimic Vasculature

The functionality of artificial tissues depends on the assembly of cells and ECM within an appropriate perfusable vasculature. Toward this goal, 3D printing has enabled the fabrication of vascular-like constructs within hydrogels using sacrificial strategies to generate hollow structures. Taking advantage of the thermosensitive nature of gelatin, this approach has been used to print 1 mm-wide sacrificial gelatin patterns within collagen and fibrin hydrogels, giving rise to endothelialised perfusable vascular-like networks. Similarly, thermosensitive Pluronic F127 has been employed as a sacrificial material within GelMA to engineer vascular systems and printed agarose fibres have been used as sacrificial features to produce vascular-like patterns into various hydrogels. Vascular-like structures can also be fabricated without a sacrificial strategy. For example, in a non-sacrificial approach, a GelMA vascular grid was patterned with single or double biomolecules by adapting a microfluidic device to the 3D printer.

5.2. 3D Printing Patterns in Large Scale

A functional vasculature is particularly critical to enable 3D printing of large scale structures. Tackling this challenge, Atala and colleagues have devised perfusable human-scale tissue constructs using an ingenious integrated tissue-organ...

Figure 8. Fabrication of patterns through 3D printing. A) A multthead 3D printer was adapted to combine three types of materials, producing an engineered tissue construct of red HUVEC-containing channels and green HDNF cell laden within GelMA, generating lumen formation after two days in culture (scale bars: 300 μm). Adapted with permission. Copyright 2014, Wiley-VCH. B) Vascular-like patterns were fabricated by 3D printing agarose fibres within photo crosslinkable hydrogels (scale bar: 3 mm). Reproduced with permission. Copyright 2001, Royal Society of Chemistry. C) A low viscosity bioink was explored to form vascularized tissue constructs by patterning two inks with the same printer extruder through simultaneous deposition with resolutions of ~100 μm. Reproduced with permission. Copyright 2015, Wiley-VCH. D) Human-scale and clinically relevant hydrogel scaffolds were printed with resolutions of 200 μm by combining up to seven ink reservoirs and a pneumatic valve system. Reproduced with permission. Copyright 2016, Wiley-VCH. E) A Kenics static mixer was adapted into an extrusion printer, allowing to obtain woodpile structures with internal micro-patterns. Reproduced with permission. Copyright 2020, IOP Publishing. F) A guest-host reaction was explored with 3D printing to produce complex patterns allowing fabrication of variable sizes due to the adaptation of several needles (scale bar: 200 μm). Reproduced with permission. Copyright 2015, Wiley-VCH. G) An in situ crosslinking approach was employed with an extrusion 3D printer to obtain core-shell patterns within fibrillary structures (scale bar: 500 μm). Reproduced with permission. Copyright 2016, Wiley-VCH.
5.3. 3D Printing Combined with Host–Guest and Click Chemistry Interactions

High-efficiency orthogonal chemical reactions offer an attractive avenue for creating patterns within 3D printed structures with higher levels of spatial control. One approach exploits the use of non-covalent molecular recognition between high affinity motifs.[257] For example, using an adamantane/β-cyclodextrin guest/host pair, an HA-based ink was used to “ghost write” HA structures with encapsulated cells within 50 to 400 μm wide patterns (Figure 8F).[252] By adding a covalently UV cross-linkable moiety, this approach enabled the fabrication of scaffolds with enhanced mechanical stability.[258] Another strategy exploits Michael addition reactions to simultaneously inject bioprint thiol-star-PEG and a maleimide-heparin structure comprising a diverse range of patterns down to 50 μm in size.[259] Alternatively, by adapting core-shell-like structures to 3D printers, liquid droplets containing horseradish peroxidase (acting as the core) were patterned with 100 μm spacing in a thermally-degradable poly(lactic-co-glycolic) acid hydrogel doped with gold nanorods (acting as the shell) allowing on-demand thermal release of the core enzymes.[260] Using a similar approach, filaments of 100 to 250 μm width were 3D printed exposing two types of cells in a core-shell or truncated fashion by modulating the in situ crosslinking of methacrylated hyaluronic acid (Figure 8G).[261]

The development of 3D patterns via printing techniques has also been explored using bioinks for example, containing bacterial cells[260] and creating macroscopic structures with resolutions down to 30 μm for applications ranging from bio-sensing to drug production. A self-healing material was 3D printed in woodpile designs with ≈100 μm to 1 mm diameter features while controlling the orientation of the cellulose fibrils, enabling the fabrication of constructs with anisotropic elastic and swelling properties.[272] The variable orientation of cellulose fibrils lead to swelling behaviors either in the longitudinal or transverse direction, conferring the scaffold with tunable properties in 4D. Hydrogels comprising volumes with different swelling ratios have been explored to fabricate pyramids, tubes, spheres, and other geometrical shapes embedded with cells[273,274] and tissue modules.[275] While more work needs to be done to demonstrate the utility of these structures in the field of TE, these examples exemplify the potential of 4D printing to enhance functionality and complexity.[276]

5.4. Patterning Through Suspended Manufacturing

Recently, the fabrication of anisotropic structures in a colloidal suspended media has emerged as a patterning technology.[265–267] Briefly, a shear thinning hydrogel (highly viscoelastic fluid with self-healing capabilities) is used as media, known as sacrificial support-bath, while a liquid or gelling material is printed within it. The so-called “writing in the granular gel media” approach has explored the numerous advantages of this process.[266] Suspended manufacturing is rapidly gaining interest due to the possibility to use different kinds of hydrogel materials such as polyvinyl alcohol (PVA), PDMS, PEG, HA, and alginate.[267] For example, a functional cardiac ventricle was fabricated with chemically unmodified collagen by simple extrusion of collagen within a support gelatin microparticle slurry bath.[268] Furthermore, the suspended manufacturing approach was used to fabricate a composite hydrogel of HA, gelan, and osteoblast cells in a suspended self-healing fluid gel, producing mechanically anisotropic osteocondral plugs for implantation into bone defects.[269]

5.5. 4D Printing

Recently, increasing interest in controlling the time dimension within 3D printing has given rise to what is known as 4D printing. For example, by printing patterns of hydrogels with different swelling ratios,[270] it is possible to fabricate shape morphing materials or structures that can adapt as a result of external stimuli.[271] Through this approach, and inspired by natural processes such as a blooming flower, a composite ink of stiff cellulose fibrils embedded in a soft acrylamide hydrogel was 3D printed in woodpile designs with ≈100 μm to 1 mm diameter features while controlling the orientation of the cellulose fibrils, enabling the fabrication of constructs with anisotropic elastic and swelling properties.[272] The variable orientation of cellulose fibrils lead to swelling behaviors either in the longitudinal or transverse direction, conferring the scaffold with tunable properties in 4D. Hydrogels comprising volumes with different swelling ratios have been explored to fabricate pyramids, tubes, spheres, and other geometrical shapes embedded with cells[273,274] and tissue modules.[275] While more work needs to be done to demonstrate the utility of these structures in the field of TE, these examples exemplify the potential of 4D printing to enhance functionality and complexity.[276]

6. Fabrication of Patterns Through Non-Contact Forces

Electric, magnetic, and acoustic fields as well as self-assembly interactions can also be used as manipulating forces to engineer patterns of specific bio-functional cues within 3D environments.[277]

6.1. Electropatterning

Electric fields have been traditionally used to stimulate cell populations.[286,287] Nevertheless, non-uniform (dielectrophoresis)[288]
Figure 9. Non-contact forces for patterning. Magnetic, electric, and acoustic fields as well as self-assembly are used in 3D patterning within hydrogels. A) A positive-dielectrophoresis (pDEP) process was employed to pattern alginate microgels within an agarose gel (Reproduced with permission.\cite{90} Copyright 2001, Royal Society of Chemistry) and B) green-labeled cells within red-labeled cells. Reproduced with permission.\cite{278} Copyright 2001, Royal Society of Chemistry. C) Cells can be patterned into 500 \( \mu \)m diameter traps by means of negative-dielectrophoretic (nDEP) patterning (scale bars: 200 \( \mu \)m). Reproduced with permission.\cite{279} Copyright 2011, RSC Publishing. D) Anisotropic and complex environments can be obtained by direct electric field electro-patterning in a process named 3D electrophoresis-assisted lithography (3DEAL). The process enables deep patterns of multiple molecules and with different (e.g., gradients) molecular distribution. Reproduced with permission.\cite{280} Copyright 2017, Wiley-VCH. E) Myoblast populations can be organised within GelMA hydrogels by means of acoustic waves, modifying their agglomeration by modulating the time under the wave applied (scale bars: 200 \( \mu \)m). Reproduced with permission.\cite{281} Copyright 2019, Wiley-VCH. F) Complex patterns can be generated by phase shift and transducer switching of an acoustic wave, allowing to pattern up to three cell types (scale bar: 100 \( \mu \)m). Reproduced with permission.\cite{282} Copyright 2014, Royal Society of Chemistry. G) Directed assembly of cell laden lock-and-key shaped microgels allowing the combination of up to four cell types (scale bars: 200 \( \mu \)m). Reproduced with permission.\cite{283} Copyright 2008, National Academy of Sciences. H) Peptide amphiphile (PA)-protein interfacial self-assembly based on a diffusion–reaction process used to create graphene oxide patterns.\cite{284} Copyright 2020, Frontiers Media. I) PA-protein hydrodynamically guided co-assembly to form patterned hydrogels with distinct geometries. Reproduced with permission.\cite{285} Copyright 2018, Wiley-VCH.

(Figure 9A–C) or uniform (electrophoresis)\cite{286} (Figure 9D) electric fields have also been used to create 3D patterns. Pioneering work by Albrecht et al. reported on a dielectrophoretic process to produce 250 \( \mu \)m wide stripe patterns of cells within microgels.\cite{90} In this study, either fibroblasts or mouse embryonic liver progenitor cells were first encapsulated into 30–70 \( \mu \)m diameter alginate microspheres and dispersed into liquid agarose. Then, a positive dielectrophoretic process was used to align the cell-loaded microspheres in the agarose hydrogels into precise 100 \( \mu \)m stripes separated by 400 \( \mu \)m spaces (Figure 9A). This approach has been combined with photo-patterning to fabricate more complex co-culture arrays (Figure 9B)\cite{278} as well as patterned gradients of drug-containing gold nanoparticles into GelMA to test myoblast responses for drug-screening.\cite{290} Similarly, a negative dielectrophoresis technique has also been used to pattern human embryonic kidney (HEK) cells as part of hydrogel free systems\cite{291} as well as HEK cells within GelMA (Figure 9C).\cite{279} Techniques based on electrodeposition\cite{292} and light induced electrodeposition\cite{293} have used uniform electric fields to fabricate patterns of variable geometries within alginate hydrogels with resolutions down to \( \approx 250 \mu \)m and 1 mm, respectively. Electrodeposition enables tuning of pattern shape\cite{294} but is generally limited to alginate-based systems and patterns of relatively low resolution. Improving on this, an electrogelation method has been developed to successfully produce patterns down to 100 \( \mu \)m in size, but is mainly suitable for poly(3,4-ethylene-dioxythiophene):polystyrene–sulfonate (PEDOT:PSS) hydrogels.\cite{295}
Inspired by these studies, we have recently reported on a 3D electrophoresis-assisted lithography (3DEAL) method, which takes advantage of a uniform electric field to pattern multiple proteins into readily available hydrogels. Through this approach, cylindrical patterns that can go down to 30 µm in diameter and up to 2 cm in depth were produced in agarose hydrogels preserving their bio-functionalities. The technique enables patterning of multiple molecules including for example fibronectin, which was shown to maintain its cell-binding properties. Furthermore, 3DEAL is suitable to localize multiple proteins arranged in a variety of different geometrical patterns including high aspect ratio parallel and perpendicular columns, curved lines, and gradients of molecular composition (Figure 9D).

### 6.2. Magnetopatterning

Magnetic forces can also be used to mobilize and localize cues and cells within 3D environments. For instance, magnetically labeled human melanoma M1 and fibroblast cells were cultured together and specially localized by a pin-holder and a magnet to mimic a tumor environment. In another approach, glycosylated superparamagnetic iron oxide nanoparticles containing the growth factor BMP-2 were patterned into hydrogels and used to spatially control mineralization. Similarly, RGD-functionalized magnetic silica rods were also used to produce aligned patterns onto a composite hydrogel, promoting precise orientation of the encapsulated cells. Nonetheless, the use of magnetic forces to pattern hydrogels has been less common due to the need for synthetic modifications required to add a magnetic responsive moiety to the desired patterning molecule.

### 6.3. Acoustopatterning

Acoustopatterning has been recently employed as a clean (i.e., remote controlled) technique to generate complex cell patterns. This technique takes advantage of density differences between cells and the surrounding fluid, promoting cell migration toward the node planes of the acoustic wave where pressure is minimal. Through this approach, a bio-acoustic levitational assembly method was used to fabricate 250 µm wide multi-layered patterns of neuro-progenitor cells within fibrin hydrogels, facilitating neuro-differentiation investigations. Similarly, a Faraday wave applied to a fibrin hydrogel promoted iPSC-derived cardiomyocytes to form multiple 20 µm thick layers and ultrasound standing waves spatially organized populations of myoblasts in 120–150 µm wide columns within hydrogels. The later study also demonstrated the possibility to produce mechanically anisotropic scaffolds (Figure 9E). Similarly, an ultrasonic bulk acoustic wave was employed to preferentially align hADSC in stripe patterns within alginate hydrogels. Higher control over the localization, direction, and patterning geometry was achieved by employing a hexagonal acoustic tweezer in the process, which was used to create multiple types of patterns of Schwann cells (Figure 9F) within a DRG co-culture in a free-hydrogel media. Acoustic waves have also been used to recapitulate the 3D collateral vessel structure in the hindlimb muscle by patterning 50 µm wide stripes of hMSCs and endothelial cells within HA hydrogels.

An elegant approach named Sound Induced Morphogenesis has been developed by Serra and colleagues enabling multilayer patterns for the rapid fabrication of vascular networks.

Overall, electro-, magneto-, and acousto-patterning represent clean methodologies to fabricate patterns within hydrogels. Despite the requirement of a specific stimuli-responsive moiety, they have been shown effective for generating patterns of multiple types of cells. Particularly, electropatterning has opened a wide range of applications in the fields of bioengineering, drug discovery, and TE. Its versatility in terms of patterning molecules (ranging from small growth factors to bulkier proteins) and hydrogels (both natural and synthetic) provides important advantages over its magneto- and acousto-counterparts.

### 6.4. Patterning Through Self-Assembly

Self-assembly offers the possibility to grow from the bottom up, 3D hydrogels comprising patterns using non-covalent forces (i.e., hydrogen bonding, hydrophobic interactions, π-π stacking). This approach is particularly attractive because it opens opportunities to fabricate and create structural and chemical patterns at cellular and sub-cellular size scales, facilitating optimization of material–cell interactions. Furthermore, self-assembly also offers the possibility to spontaneously create patterns with nanoscale precision and controlled molecular presentation, without the need for extrinsic forces. For example, hydrophobic forces arising at liquid–liquid interfaces have been used to guide the assembly of PEG microgels into linear, branched, or offset aggregates, supporting fibroblast encapsulation (Figure 9G). In a similar approach, micro gels with several shapes were mixed in a pre-polymer PEG solution and spread on a PDMS surface developing a construct with biological relevance supporting cell encapsulation on their intricate patterned architectures.

More recently, the inherent affinity of the four nucleotide bases of DNA was used in combination with the assembly strategy of DNA strands (e.g., single, double, multistranded) to assemble anisotropic architectures upon environmental cues, enzymatic reactions, ion content, target binding, and B/Z-isofrom transitions. Multicomponent self-assembly can also be exploited to generate supramolecular events such as compartmentalization and diffusion–reaction processes that can be guided to create patterns within growing hydrogel constructs. This approach has been used to generate hydrogel structures with intricate geometries with internal patterns such as peptide–protein layers or be used to incorporate the components such as graphene oxide (Figure 9H). Furthermore, hydrodynamic fluid forces can also be used as extrinsic forces to guide self-assembly to further enhance geometrical and pattern complexity (Figure 9I).

### 7. Conclusions and Outlook

#### 7.1. Applications, Advantages, and Disadvantages

Inspired by the inherent complexity and functionality of living systems, there is increasing interest to recreate the ubiquitous...
Table 1. Overall presentation of all the technologies discussed, stressing the patterns resolution and molecule, hydrogel material and their application.

| Patterning methodology | Pattern | Hydrogel$^c$ | Application |
|-------------------------|---------|--------------|-------------|
| Photo-patterning        | SLA     | Hydrogel + Cells | Ch-NIPAM$^{[109]}$ | - Hepatic tissue construct |
|                         |         | RGD-sequence$^{[102]}$ | Collagen$^{[112]}$ | - Multilayer hydrogel microstructures |
|                         |         | Flavin mononucleotide$^{[112]}$ | Fibrinogen$^{[111]}$ | - In vitro models (cartilage, angiogenic and osteogenic niches, morphogenesis and tissue repair, cell-matrix and cell–cell interactions) |
|                         |         | 50–500 µm (12 µm)$^b$ | GelMA$^{[102,114,113]}$ |   |
|                         |         |                             | PEGDA$^{[101,108,109,114,113]}$ |   |
|                         |         |                             | Pluronic F127$^{[111]}$ |   |
|                         | pSLA    | Hydrogel + Cells | Acrylic acid$^{[115]}$ | - In vitro models (liver, hybrid tissue morphogenesis, tissue structure–function relationship, multicellular microenvironments, stimuli gradients, prevascularized structures) |
|                         |         | Proteins$^{[115]}$ | PEGDA$^{[115–119,121–123]}$ | - Tissue engineered scaffold (heterogeneous ECM composition, ischemia treatment) |
|                         |         | Polydiacetylene nanoparticles$^{[119]}$ | GelMA$^{[120,121,124]}$ | - Topographical studies |
|                         |         | CaCO₃ microparticles$^{[120]}$ | g-MAHA$^{[102,124]}$ | - 3D detoxification device |
|                         |         | RGD-sequence$^{[123]}$ | - Drug toxicity evaluation |
|                         | 5–300 µm | Alginate$^{[135]}$ | - In vitro models (cell locomotion, wound healing, inflammation, embryogenesis and tumor cell metastasis, neuronal morphogenesis, nanoscale surface topography |
|                         |         | Aryl azide PEG$^{[104]}$ | Fibrin$^{[105]}$ | - Medical cargo carriers and biohybrid actuators |
|                         |         | Poly(di)acrylates$^{[125,129]}$ | HAMA$^{[103]}$ | - Tissue engineered scaffold allowing osteogenic differentiation |
|                         |         | Methacrylamide-Gelatin$^{[125,129]}$ | GelMA$^{[142]}$ | - Drug discovery platform |
|                         | PGL     | Hydrogel + Cells$^{[129]}$ | 1.5–300 µm |   |
|                         |         | Hydrogel (ablation)$^{[30]}$ | Alginate$^{[135]}$ |   |
|                         |         | RGD sequence$^{[103]}$ | PEGDA$^{[101,120,121,124]}$ |   |
|                         |         | Monocarboxylate-derivatized molecules$^{[120]}$ | GelMA$^{[102,120,121,124]}$ |   |
|                         |         | Azides$^{[128]}$ | - Tissue engineered scaffold (heterogeneous ECM composition, ischemia treatment) |
|                         |         | Alginic acid$^{[135]}$ | Norbornene-HA$^{[134]}$ | - Topographical studies |
|                         | 10 µm–1 mm | Calcium$^{[151]}$ | PEG$^{[150]}$ | - 3D detoxification device |
|                         |         | Liposomes chelator$^{[151]}$ | Urethane diacrylate$^{[127]}$ | - Drug discovery platform |
|                         |         | Peptide sequence$^{[136,138,145]}$ | - In vitro models (cell fate studies, cardiomyocytes alignment, matrix mechanics, |
|                         |         | Proteins$^{[144]}$ | - In vivo tissue repair guidance and patterning |
|                         |         | Calcium$^{[151]}$ | - Drugs immobilization |
|                         |         | Liposomes chelator$^{[151]}$ | HA-Acrylate$^{[136]}$ | - Therapeutic protein delivery |
|                         | FRI & TPLSP | Peptide sequence$^{[152,153,159,163,165]}$ | Agarose$^{[139,152,160,162]}$ | - Tissue engineered scaffolds (heart valve, transdermal |
|                         |         | P2CK$^{[40]}$ | GelMA$^{[156]}$ | - In vitro models (axonal growth, cellular migration, cell matrix interaction, mechanically tunable, differentiation and cytoskeletal integrity via prenylation reactions, endothelial cells, cancer) |
|                         |         | Barnase$^{[139]}$ | HA$^{[153,18]}$ |   |
|                         |         | Streptavidin$^{[39]}$ | HA-Peptide$^{[164,165]}$ |   |
|                         |         | DNA$^{[15]}$ | HA-SH$^{[161]}$ |   |
|                         |         | Vinyl$^{[166]}$ maleimide$^{[160]}$ moieties | VEGF$^{[166]}$ |   |
|                         |         | PEG(gradient)$^{[166]}$ | EGFl$^{[166]}$ |   |
|                         | 5–10 µm | VEGF (gradient)$^{[162]}$ | VEGF$^{[166]}$ |   |
|                         | Other (FRI & TPLSP) | Degradation voids | Collagen$^{[130]}$ | - In vitro model for cell alignment, migration, and proliferation. |
|                         |         | 1–200 µm | PEG-Peptide$^{[166]}$ | - Biomimetic, multicellular, and clinically relevant engineered tissue scaffold |
|                         |         | 10–500 µm | Silk fibroin$^{[160]}$ |   |
|                         | Chemical design | (PEG-N$_2$/Peptide-III)$^{[52,186,203]}$ | (PEG-N$_2$/Peptide-$\equiv$)$^{[52,186,203]}$ | - In vitro models (cell–matrix interactions, dynamic biophysical and biochemical properties, vasculature with modular stiffness) |
|                         |          | (PEG-SH/PEG- =)$^{[182,205]}$ | (PEG-SH/PEG- =)$^{[182,205]}$ | - In situ modification of assembling peptides |
|                         |          | (PEG-SH/MMP- =)$^{[182]}$ | (PEG-SH/MMP- =)$^{[182]}$ | - Engineered scaffold for smart protein carrier and controlled release |
|                         |          | (PEG-II/Peptide-$\equiv$)$^{[152,197,202]}$ | (PEG-II/Peptide-$\equiv$)$^{[152,197,202]}$ |   |
### Table 1. Continued.

| Patterning methodology | Pattern | Hydrogel<sup>)</sup> | Application |
|------------------------|---------|----------------------|-------------|
| **Patterning methodology** | **Pattern** | **Resolution** | **Application** |
| **Oxime ligation** | Antibodies<sup>253</sup> | 10–50 µm | (PEG-ONH<sub>2</sub>/Peptide-N<sub>3</sub>)<sup>193</sup> | - In vitro model for regulation of complex biological functions and cellular pathways employing a library of near-native bioactive proteins |
| | BSA<sup>193</sup> | | (PEG-ONH<sub>2</sub>/PEG-CHO)<sup>208,209</sup> | |
| | Sortagged proteins<sup>209</sup> | | | |
| | Vitronectin<sup>193</sup> | | | |
| **Hydrazone ligation** | RGD sequence<sup>206</sup> | 200 µm | PEG-NH2 | - In vitro model with on-demand control of mechanical and biological functionalities |
| **Glycosaminoglycan** | RGD sequence | 100–300 µm | (HA−/NIPAM-SH)<sup>214</sup> | - In vitro models (dynamic mechanical and biological functionalities, chondrogenesis, analysis of stimuli-responsive crosslinkers) |
| | Peptide sequences (gradient)<sup>217</sup> | | (HA−-furan)/PEG-maleimide<sup>215</sup> | |
| | NIPAM/DTPEO<sup>214</sup> | | | |
| **Azide–alkyne** | Gelatin<sup>212</sup> | 20 µm | Gelatin-N<sub>3</sub> | - Tissue engineered scaffold |
| **Microfluidics** | Hydrogel + Cells | 3–300 µm | Alginates<sup>225,226</sup> | - In vitro models (cellular communication and behavior, paracrine signaling, tumor, dynamic events) |
| | CSF-1<sup>217</sup> | | Collagen<sup>220,222,227</sup> | - Engineered scaffold for drug-releasing |
| | Glass beads<sup>222</sup> | | GelMA<sup>221</sup> | - Soft-actuators for medical use |
| | Cells (spheroids)<sup>228</sup> | | Matrigel<sup>217,224</sup> | |
| | | | NIPAM-co-AAc<sup>223</sup> | |
| | | | PEGDA<sup>219,223</sup> | |
| | | | Pluronic F108<sup>228</sup> | |
| | | | | | |
| **3D printing** | Hydrogel + Cells | 100–500 µm | Agarose<sup>244</sup> | - Tissue engineered scaffold with micrometer chemical environment control |
| | Hydrogel | 100 – 200 µm | Alginates<sup>217</sup> | - In vitro models (morphogen delivery, aligned ECM components, hemodynamics and cellular interactions) |
| | | | Collagen<sup>221</sup> | |
| | | | Fibrin<sup>213</sup> | |
| | | | Gelatin<sup>219</sup> | |
| | | | Matrigel<sup>210,213</sup> | |
| **Vascular mimetic** | Hydrogel | 10–200 µm | Alginates<sup>209,213</sup> | - In vitro models (freestanding vasculature, vascular channels with perfused open lumen, bone tissue construct with functional vasculature, cardiac |
| | Hydrogel + Cells | | Collagen<sup>235,236</sup> | - Engineered perfusable large-scale vascularized tissue constructs |
| | | | Gelatin<sup>235</sup> | - Drug discovery |
| | | | Matrigel<sup>215</sup> | |
| | | | | | |
| **Large scale** | Hydrogel | 10–200 µm | Alginates<sup>209,213</sup> | - 3D vascularized cellular construct of clinically relevant size, shape, and structural integrity |
| | Hydrogel + Cells | | Collagen<sup>235,236</sup> | - Biomimetic scaffold modeling the CNS tissue architecture |
| | | | Gelatin<sup>235</sup> | |
| | | | Matrigel<sup>215</sup> | |
| | | | | | |
| **Guest–Host-Click-core-shell-interactions** | Hydrogel | 30–800 µm | Alginates<sup>211</sup> | - In vitro models (chemical and cellular controllable compositions, directing cell-fate) |
| | Hydrogel + Cells | | Collagen<sup>211</sup> | - Engineered tissue constructs stimuli responsive |
| | | | GelMA<sup>215</sup> | - Living responsive construct and actuators |
| | | | HA<sup>215</sup> | |
| | | | Heparin<sup>211</sup> | |
| | | | PC<sup>151</sup> | |
| | | | PEG<sup>219</sup> | |
| | | | PEGDA<sup>213</sup> | |
| | | | Pluronic F127-DA<sup>261</sup> | |
Table 1. Continued.

| Patterning methodology  | Pattern                  | Hydrogel(2) | Application                                      |
|-------------------------|--------------------------|-------------|--------------------------------------------------|
|                        | Functional molecule(1)   | Resolution  |                                                   |
| Suspended manufacture   | Hydrogel(26)             | 100–250 μm  | - Dynamic and biomimetic tissue engineered scaffold |
|                        | Hydrogel + Cells         |             | - Cardiac tissue engineered with contractile function |
|                        |                          |             |                                                   |
| Non-contact Electro-patterning | Hydrogel(26)        | 50–400 μm   | - Tissue engineered constructs (local microenvironment modulation, controlled physical and chemical cues) |
|                        | Hydrogel + Cells         |             | - In vitro model for high throughput drug screening and gene expression |
|                        | Cells in alginate microspheres(26) |             |                                                   |
|                        | Cells(29)                | 1 μm–2 mm   | - In vitro models (tumor invasion, bioactive signals gradient) |
|                        | Drugs(29)                |             | - Multi-cellular tissue engineered flexible scaffolds |
|                        | Microspheres(29)         |             |                                                   |
|                        | Microspheres            | <1–500 μm   | - In vitro models (user-defined cell positions, multicellular architecture, vascular morphogenesis, neurodifferentiated multi-layered model, disease treatment) |
|                        |                         |             | - Organoids development                           |
|                        |                         |             | - Bioelectronics                                  |
| Magneto-patterning      | Cells(91)                |             |                                                   |
|                        | BMP-2 (gradient)(28)     |             |                                                   |
|                        | Silica rods-RGD          |             |                                                   |
|                        | functionalized(29)       |             |                                                   |
| Acoustic-patterning     | Cells(1)                 | 1 μm (few cells) | - In vitro models (tunable architecture and complexity, hierarchical multi-layered structures, complex bioactive structure) |
|                        |                          |             |                                                   |
| Self-assembly           | Microgels(284)           | <1–500 μm   | - In vitro models (tunable architecture and complexity, hierarchical multi-layered structures, complex bioactive structure) |
|                        | Microgels + Cells        |             |                                                   |
|                        | Graphene oxide(284)      |             |                                                   |
|                        | Proteins(285)            |             |                                                   |
|                        |                          |             |                                                   |

(1)Where no specific reference is added, the patterning functional molecule is shared throughout all the hydrogels stated; (2)Employing multiphoton light; (3)Ch-NIPAM, chitosan-g-poly(N-isopropylacrylamide); GelMA, gelatin methacryloyl; PEGDA, poly(ethylene glycol) diacrylate; g-MAHA, glycidal methacrylate–hyaluronic acid; HA, hyaluronic acid; HAMA, hyaluronic acid methacrylate; PLL, poly-L-lysine; PEG, poly(ethylene glycol); NIPAN-AAc, poly(N-isopropylacrylamide)-co-acrylic Acid; PCL, poly(ε-caprolactone); PVA, poly-vinyl alcohol; PEDOT:PSS, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate; PEO, poly(ethylene oxide); PDMS, polydimethylsiloxane; PEGMA, poly(ethylene glycol) methacrylate.

Structural and chemical patterns found in biological structures. These materials have a spectrum of applications (Table 1) expanding from addressing fundamental questions to tackling clinical challenges. A plethora of techniques have been developed to facilitate the fabrication of patterns within 3D hydrogels (Figure 3) but these methods also exhibit important limitations that must be improved to emulate the inherent molecular and structural anisotropy of biological systems (Table 2). For example, photo-patterning techniques can be used to precisely localize short peptide sequences, ECM macromolecules, growth factors, and short modified polymers within geometrical spaces of variable shapes and sizes within photo-crosslinkable or photo-caged modified hydrogels. However, reduced pattern thickness due to scattered light, need for photolabile moieties, and low cell survival in some cases has limited applicability. The introduction of click chemistry reactions as part of 3D patterning methods has enabled the generation of more physiological functional patterns of peptides and proteins in a controllable manner. Nonetheless, exhaustive synthetic pathways and purification steps and difficult scalability have limited the broad use of click chemistry in hydrogel patterning. The field of microfluidics, on the other hand, has opened opportunities to recreate chemotactic processes and molecular or cell gradients with high fidelity. However, despite being adaptable to other...
Biofabrication techniques, microfluidic platforms still require the use of soft-lithographic processes and usually a solid support that restricts its use. More recently, the use of 3D printing has enabled a leap forward in the patterning of hydrogels due to its scalability and low processing times to reproducibly fabricate intricate interconnected structures. Be that as it may, pattern fabrication with 3D printing also has limitations such as the need for precise hydrogel requirements and specialized equipment. Other creative approaches have been developed where extrinsic forces based on electric, magnetic, and acoustic fields

Table 2. Advantages and disadvantages of patterning techniques enabling the fabrication of functional 3D patterns within hydrogels.

| Patterning methodology | Advantages | Disadvantages |
|-------------------------|------------|---------------|
| Multilayer patterning  | SLA        | - Hydrogel formation and patterning occurs at the same time |
| (Photo-patterning)      |            | - Variable pattern shapes (mask dependant) |
|                        | pSLA       | - Mask-less |
|                        |            | - High manufacturing speed (mirror array) |
|                        | PCL        | - Beam of light digitally/mechanically controlled |
|                        |            | - High precision on shapes and definition |
| Post-gelation patterning| MP         | - Low cost |
| (Photo-patterning)      |            | - Ease of use and translation to a clinical environment |
|                        | FLI & TPLSP| - Localised photons into the volume of the material |
|                        |            | - Precise shapes |
|                        |            | - Few micrometers patterns definition |
| Click chemistry         |            | - Requires a photolabile moiety |
| Microfluidics           | Microfluidics-to | - Gradients fabrication |
|                        |            | - High cell survival rates |
|                        |            | - Adaptable to many other biofabrication techniques |
|                        | Microfluidics-into | - Mimics chemotactic processes |
|                        |            | - Perfusable systems |
| 3D Printing             |            | - Complex interconnected structures (e.g., vascular structures, perfusable systems) |
|                        |            | - Low fabrication times |
|                        |            | - Excellent scalability |
|                        |            | - Combination with many other technologies |
|                        |            | - Broad range of applications |
| Non-Contact forces      | Electro-patterning | - Clean technique |
|                        |            | - Versatility on the final application |
|                        |            | - Allow practically any hydrogel |
|                        | Magneto-patterning | - Clean technique |
|                        |            | - Gradients generation |
|                        | Acousto-patterning | - Clean technique |
|                        |            | - Directly cell patterning |
|                        | Self-assembly | - Precise nano-scale control |
|                        |            | - Highly hierarchical structures |
|                        |            | - Need of a charged moiety |

- Reduced pattern thickness (light scatter)
- Mask production (soft-lithography)
- Complex apparatus design
- Waste of material (additive manufacturing)
- Starting material dependant (requires a vinyl moiety)
- Employs UV light
- Requires a photolabile moiety
- Pseudo 2D patterning (i.e., from the surface to the depth of the hydrogel)
- Chemical modification
- Not ideal cell survival
- Arduous chemical modification
- Tedium purification steps for the precursors
- Limited size of the final construct
- Poor scalability
- Need of soft-lithography
- Very small scale
- Requirement of a solid support
- Limited in the architectural designs
- Precise requirements for the starting materials (viscosity, processability, etc.)
- Specialised equipment
- Poor size/resolution rate
- Need of a charged moiety
- Exhaustive chemical modification
- Highly standardised procedure
- Exhaustive chemical modification
are used to pattern proteins, small molecules, and cells within hydrogel scaffolds. Amongst them, electropatterning stands out due to the possibility to pattern multiple types of readily available hydrogels through cost-effective procedures. A limitation of these technologies is the need for patterning molecules that are charged, magnetic, or responsive to acoustic fields. Finally, self-assembly has been used to successfully pattern bio- and macro-molecules with highly hierarchical organized structures from the nano- to the micro-scales. Yet, the lack of reproducibility and extended chemical synthesis to obtain the precursors are drawbacks to consider when using self-assembly for patterns fabrication. In summary, there is no ideal patterning technology. All the patterning methods presented here have a set of advantages and disadvantages that should be carefully considered depending on the specific requirements and application (Table 2).

| Parameter | Photo-patterning | Click chemistry | Microfluidics | Other external forces | 3D Printing |
|-----------|------------------|-----------------|---------------|----------------------|-------------|
| Electric field | Red | Orange | Yellow | Lime | Green |
| Magnetic field | Red | Orange | Yellow | Lime | Green |
| Acoustic wave | Red | Orange | Yellow | Lime | Green |
| Self-assembly | Red | Orange | Yellow | Lime | Green |
| What the technology enables | Capacity to pattern w/ embedded cells | Green | Green | Green | Green |
| Ability to model process with CAD | Green | Green | Green | Green | Green |
| Ease to scale up the technology | Green | Green | Orange | Lime | Green |
| Aseptic patterning settings | Green | Green | Green | Green | Green |
| Physiological patterning settings | Green | Green | Green | Green | Green |
| Reversible patterning | Green | Green | Green | Green | Green |
| Can be combined w/ other techniques | Green | Green | Green | Green | Green |
| Resolution of the resulting patterns | Green | Green | Green | Green | Green |
| Shape of the resulting patterns | Green | Green | Green | Green | Green |
| Chemical modification required | Red | Orange | Yellow | Lime | Green |
| Need for a specific hydrogel | Red | Orange | Yellow | Lime | Green |
| Need for a specific patterning molecule | Red | Orange | Yellow | Lime | Green |
| Requires use of soft-lithography | Red | Orange | Yellow | Lime | Green |
| Standardization required | Red | Orange | Yellow | Lime | Green |
| Need for a highly qualified user | Red | Orange | Yellow | Lime | Green |

7.2. Concluding Remarks and Outlook

Overall, 3D patterning technologies offer the opportunity to engineer complex 3D environments exhibiting functional patterns of biomolecules and other components. These technologies enable the precise localization of biochemical signals to mimic biological structures, study fundamental biological processes in a systematic manner, or generate completely new environments that can enhance desired cell behaviors. Nevertheless, there remain challenges to overcome, such as the capacity to recreate more complex
organ-level structures given the increasing knowledge around systems biology. Consequently, we reason that the future of 3D patterning within hydrogels will be shaped by the pressing need for more precision and control of both material and biological properties around the following goals. First, to improve on pattern resolution aiming to expand the multi-scale control (from the molecular to the macroscopic) of molecular composition within 3D environments. Second, to facilitate temporal control of the molecular composition of patterned scaffolds to implement capabilities to further enhance the capacity to recreate the dynamic nature of for example, the natural ECM, cellular ageing, wound healing, or tumor formation. Third, to enhance multi-molecular complexity by enabling patterning of multiple, yet distinct, functional molecules (e.g., structural proteins, growth factors, and vesicles, among others). Fourth, to enhance control over the structural and mechanical properties of the spatio-temporal patterns. This additional level of complexity would enable recreation of extrinsic or intrinsic forces present within biological microenvironments and integration of mechanobiological parameters.

The field of 3D patterning has reached important landmarks, yet key challenges remain to enable a more accurate, robust, and controlled recreation of living structures.

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Conflict of Interest
The authors declare no conflict of interest.

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[1] F. J. Martin-Martinez, K. Jin, D. López Barreiro, M. J. Buehler, ACS Nano 2018, 12, 7425.
[2] Z. Zhang, A. Liu, Z. Huang, S. Fan, X. Zou, X. Deng, Q. Ge, J. Gong, J. Li, W. Gong, Y. Shi, L. Fan, Z. Zhang, X. Jiang, K. Lei, Y. Yuan, A. Xu, H. Shang, Euphytica 2019, 215, 110.
[3] Y. Xiong, R. Cakir, S. M. Phan, A. Ola, K. W. Krauss, C. E. Lovelock, For. Ecol. Manage. 2019, 444, 382.
[4] W. Li, C. Li, Z. Jiang, R. Guo, X. Ping, Biol. Rhythm Res. 2019, 50, 408.
[5] J. E. Edwards, J. Pratt, N. Tress, N. E. Hussey, Deep Sea Res., Part I 2019, 146, 24.
[6] J. Yang, X. Wang, P. Bauer, Appl. Sci. 2018, 8, 2120.
[7] M. Klotzmann, A. Tal, Artif. Life 2012, 18, 91.
[8] H. Liu, X. Wang, Y. Liu, X. Li, Commun. Nonlinear Sci. Numer. Simul. 2019, 75, 280.
[9] M. Kiskowski, T. Glimm, N. Moreno, T. Gamble, Y. Chiari, PLoS Comput. Biol. 2019, 15, e1006943.
[10] I. C. Cuthill, W. L. Allen, K. Arbuckle, B. Caspers, G. Chaplin, M. E. Hauber, G. E. Hill, N. G. Jablonski, C. D. Jiggins, A. Kelber, J. Mappes, J. Marshall, R. Merrill, D. Osorio, R. Prum, N. W. Roberts, A. Roulin, H. M. Rowland, T. N. Sherratt, J. Skelhorn, M. P. Speed, M. Stevens, M. C. Stoddard, D. Stuart-Fox, L. Talas, E. Tibbetts, T. Caro, Science 2017, 357, eaan0221.
[11] J. J. Allen, D. Akkaynak, A. U. Sugden, R. T. Hanlon, Biol. J. Linn. Soc. 2015, 116, 377.
[12] A. Thomas, C. MacDonald, PeerJ 2016, 4, e2035.
[13] A. Romano, R. Séchaud, A. H. Hirzel, A. Roulin, Global Ecol. Biogeogr. 2019, 28, 496.
[14] C. Loehle, Trees 2016, 30, 2061.
[15] T. Jucker, O. Bouriaud, D. A. Coomes, Funct. Ecol. 2015, 29, 1078.
[16] D. Seidel, M. Ebbrecht, Y. Dorji, J. Jambay, C. Ammer, P. Annighöfer, Trees 2019, 33, 911.
[17] J. Juchheim, P. Annighöfer, C. Ammer, K. Calders, P. Raunomon, D. Seidel, Trees 2017, 31, 1723.
[18] C. K. McTavish, M. Catal, D. W. Fulbright, A. M. Jarosz, Plant Dis. 2018, 102, 2330.
[19] R. Beyer, D. Bayer, H. Pretzsch, P. H. Cournède, Ecol. Inf. 2017, 42, 61.
[20] A. M. Turing, Philos. Trans. R. Soc., B 1952, 237, 37.
[21] P. Ball, Philos. Trans. R. Soc., B 2015, 370, 20140212.
[22] C. Dalton, S. Graham, J. Jenner, Toxicol. In Vitro 2015, 30, 454.
[23] D.-H. Kim, E. A. Lipke, P. Kim, R. Cheong, S. Thompson, M. Delannoy, K.-Y. Suh, L. Tung, A. Levchenko, Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 565.
[24] L. Oxburgh, Annu. Rev. Cell Dev. Biol. 2018, 34, 427.
[25] T. Gong, J. Xie, J. Liao, T. Zhang, S. Lin, Y. Lin, Bone Res. 2015, 3, 15029.
[26] S. Ben-Moshe, S. Itzkovitz, Nat. Rev. Gastroenterol. Hepatol. 2019, 16, 395.
[27] E. L. S. Fong, D. A. Harrington, M. C. Farach-Carson, H. Yu, Biomaterials 2016, 108, 197.
[28] N. Peela, D. Truong, H. Saini, H. Chu, S. Masaghi, S. L. Ham, S. Singh, H. Tavana, B. Mosadegh, M. Nikkhah, Biomaterials 2017, 133, 176.
[29] S. Terry, S. Buart, S. Chouaib, Front. Immunol. 2017, 8, 1.
[30] K. Oliver, A. Seddon, R. S. Trask, J. Mater. Sci. 2016, 51, 10663.
[31] P. J. Keller, Science 2013, 340, 1234168.
[32] R. Genthial, E. Bearer, M. C. Schanne-Klein, F. Peyrin, D. Farlay, C. Olivier, Y. Bala, G. Boivin, J. C. Vial, D. Débarre, A. Gourrier, Sci. Rep. 2017, 7, 3419.
[33] M. J. Gerdes, A. Sood, C. Sevinsky, A. D. Pris, M. I. Zavodszyk, F. Ginty, Front. Oncol. 2014, 4, 1.
[34] L. Ouyang, J. P. K. Armstrong, M. Salmeron-Sanchez, M. M. Stevens, Adv. Funct. Mater. 2020, 30, 1909009.
[35] A. Khademhosseini, R. Langer, Nat. Protoc. 2016, 11, 1775.
[36] K. Sabin, N. Kikyo, Transl. Res. 2014, 163, 286.
[37] X. Yin, B. E. Mead, H. Safaei, R. Langer, J. M. Karp, O. Levy, Cell Stem Cell 2016, 18, 25.
[38] F. M. Fumasi, N. Stephanopoulos, J. L. Holloway, J. Appl. Polym. Sci. 2020, 137, 49058.
[39] D. Huh, C. A. Hamilton, D. E. Ingber, Trends Cell Biol. 2011, 21, 745.
[40] Y. S. Zhang, A. Khademhosseini, Nanomedicine 2015, 10, 685.
[41] A. Asthana, W. S. Kisaita, Drug Discovery Today 2013, 18, 533.
[42] J. Malda, J. Visser, F. P. Melchels, T. Jüngst, W. E. Hennink, W. J. A. Dhert, J. Groll, D. W. Hutmacher, Adv. Mater. 2013, 25, 5011.
[43] X. Guan, M. Avci-Adali, E. Alarçin, H. Cheng, S. S. Kashaf, Y. Li, A. Chawla, H. L. Jang, A. Khademhosseini, Biotecnol. J. 2017, 12, 1600394.
[294] Z. Liu, M. Takeuchi, M. Nakajima, T. Fukuda, Y. Hasegawa, Q. Huang, IEEE Robot. Autom. Lett. 2016, 1, 206.
[295] V. R. Feig, H. Tran, M. Lee, K. Liu, Z. Huang, L. Beker, D. C. Mackanic, Z. Bao, Adv. Mater. 2019, 31, 1902869.
[296] C. Li, J. P. Armstrong, I. J. Pence, W. Kit-Anan, J. L. Puetzer, S. Correia Carreira, A. C. Moore, M. M. Stevens, Biomaterials 2018, 176, 24.
[297] F. Zhu, Y. Chen, S. Yang, Q. Wang, F. Liang, X. Qu, Z. Hu, RSC Adv. 2016, 6, 61185.
[298] F. Guo, Z. Mao, Y. Chen, Z. Xie, J. P. Lata, P. Li, L. Ren, J. Liu, J. Yang, M. Dao, S. Suresh, T. J. Huang, Proc. Natl. Acad. Sci. U.S.A. 2016, 113, 1522.
[299] E. S. Comeau, D. C. Hocking, D. Dalecki, J. Cell Sci. 2017, 130, 232.
[300] S. M. Naseer, A. Manbachi, M. Samandari, P. Walch, Y. Gao, Y. S. Zhang, F. Davoudi, W. Wang, K. Abrinia, J. M. Cooper, A. Khademhosseini, S. R. Shin, Biofabrication 2017, 9, 015020.
[301] C. Bouyer, P. Chen, S. Güven, T. T. Demirtaş, T. J. F. Nieland, F. Padilla, U. Demirci, Adv. Mater. 2016, 28, 161.
[302] V. Serpooshan, P. Chen, H. Wu, S. Lee, A. Sharma, D. A. Hu, S. Venkatraman, A. Venkataraman Ganesan, O. B. Usta, M. Yarmush, F. Yang, J. C. Wu, U. Demirci, S. M. Wu, Biomaterials 2017, 131, 47.
[303] P. Chansoria, L. K. Narayanan, K. Schuchard, R. Shirwaiker, Biofabrication 2019, 11, 035015.
[304] B. Kang, J. Shin, H. J. Park, C. Rhyou, D. Kang, S. J. Lee, Y. sup Yoon, S. W. Cho, H. Lee, Nat. Commun. 2018, 9, 9.
[305] D. Petta, V. Basoli, D. Pellicciotta, R. Tognato, J. P. Barcik, C. Arrigoni, E. Della. Bella, A. R. Armiento, C. Candrian, G. R. Richards, M. Alini, M. Moretti, D. Eglin, T. Serra, Biofabrication 2020, 13, 015004.
[306] J. G. Fernandez, A. Khademhosseini, Adv. Mater. 2010, 22, 2538.
[307] Y. Zhang, V. Pan, X. Li, X. Yang, H. Li, P. Wang, Y. Ke, Small 2019, 15, 1900228.

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