Integrating self-assembly and biofabrication for the development of structures with enhanced complexity and hierarchical control

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Abstract

Nature has evolved to grow and regenerate tissues and organs using self-assembling processes capable of organizing a wide variety of molecular building-blocks at multiple size scales. As the field of biofabrication progresses, it is essential to develop innovative ways that can enhance our capacity to build more complex macroscopic structures using molecular and nanoscale components in a rational manner. In this review, we highlight the emerging opportunities, advantages, and challenges of incorporating self-assembly with biofabrication for the development of more biologically relevant, active, and functional structures. The review is organized in four sections. First, to better appreciate the benefits of this integrated approach, we summarize recent advances in self-assembly and biofabrication aimed at improving hierarchical control. Then, we discuss work focused on combining self-assembly with biofabrication along three areas including a) conventional bioprinting techniques using self-assembling bioinks; b) new methods where self-assembly drives the fabrication process, and c) techniques based on cellular self-assembly. The ultimate goal of this review is to emphasize the importance of structural hierarchy in biological systems and to highlight the potential behind the integration of biofabrication and self-assembly towards the development of more functional structures for tissue engineering and regenerative medicine.

Keywords: Bioprinting; Self-Assembly; Hierarchy; Tissue Engineering

1. Introduction

Nature has evolved in a hierarchical manner to achieve outstanding material properties and complex organismal behaviours (**Figure 1**). From the efficient nutrient flow exhibited by a plant's stem as a result of its multiscale structure [1] to the adhesive and locomotive properties of the gecko's feet due to the hierarchical organization of its spatulae and setae [2] (**Figure 1 (a)**), hierarchy is a ubiquitous organizing and functional principle of natural systems [3]. Equivalently, the human body relies on levels of structural organization, where each level builds on the next, to achieve complexity and functionality. For example, tendons are multi-level structures with aligned cells embedded between fibrils made from smaller fascicles, which in turn consist of smaller crimp fibres made from even smaller microfibrils of aligned collagen proteins (**Figure 1 (b)**). This strong hierarchical organization gives tendons their remarkable time-dependent viscoelastic properties [4]. Similarly, dental enamel is made of a complex, yet ordered, organization of apatitic calcium phosphate nanocrystals bundled-up into meandering and intertwined prismatic structures that grow over large uneven areas [5] (**Figure 1 (c)**). This hierarchical inorganic structure gives rise to the hardest tissue in our body, dissipating masticatory forces and protecting our teeth with outstanding durability throughout most of our life.

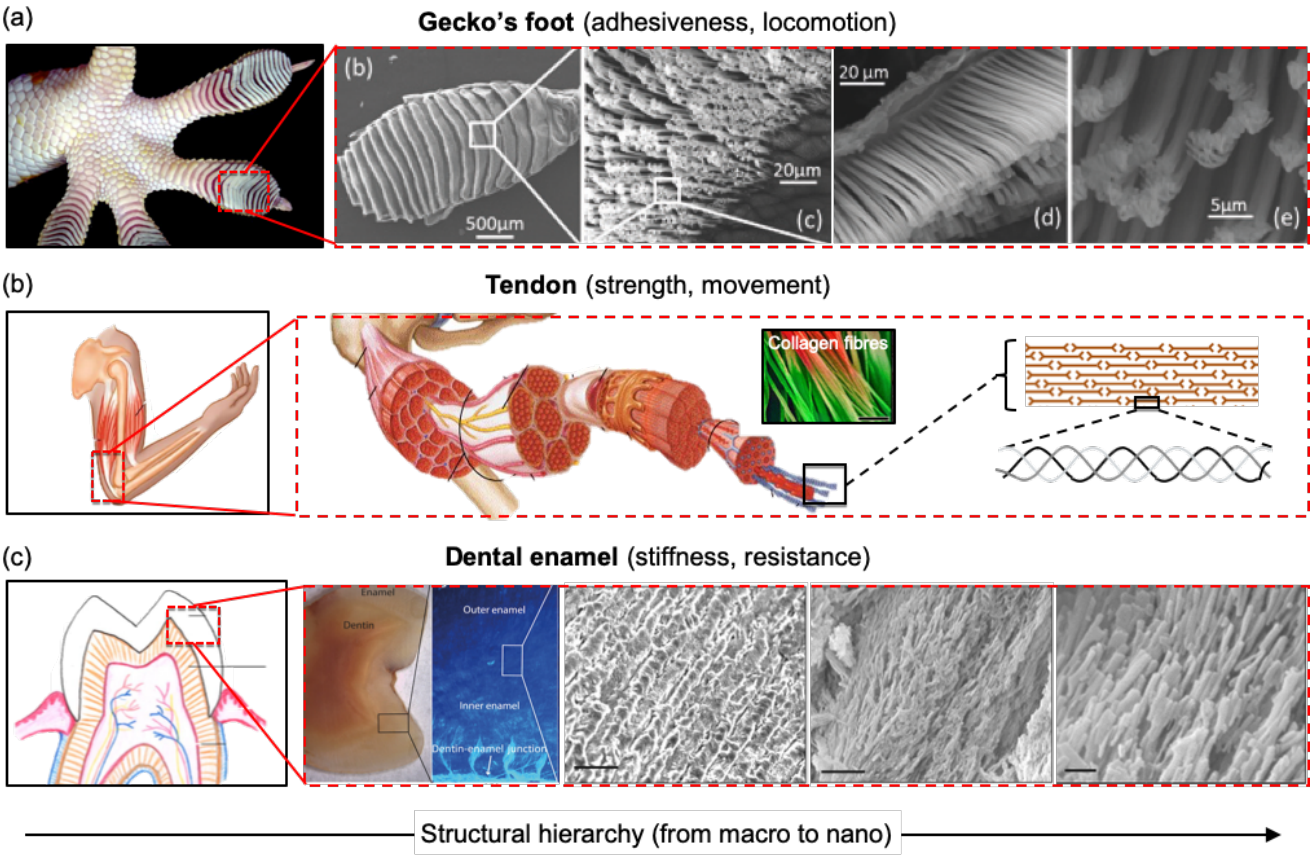

Figure 1 | Structural hierarchy in nature. Illustrations of (**a**) the structure of a gecko's foot consisting of multiple sized fibrils responsible for the adhesiveness and locomotion of the animal (SEM images: [166] ©2012 Professor Zhenhai Xia, Photograph: quertesy of Prof. Kellar Autumn ©2006), (**b**) the hierarchical organization of collagen in tendons providing strength and movement to the tissue (Movement of muscle: [167] ©2011 Pearson, Tendon hierarchy: [168] ©2007 McGraw-Hill Ryerson, SEM image: [169] ©2013 OMICS International), and (**c**) dental enamel consisting of apatite nanocrystals organized in well-defined microprisms leading to the remarkable stiffness and acid resistance of the native tissue ([57,170,171], ©2007 Elsevier, ©2016 AAAS, and ©2018 Springer Nature Publishing AG).

Multiscale organization is essential even at the most fundamental levels of biological systems. Within the cell, organelles act as organized molecular machines, which in turn depend on the precise organization of the molecular building-blocks that form them. These molecules rely on specific sequences of amino acids, phospholipids, or nucleic acids to acquire precise conformations and perform their functions. In a similar manner, outside the cell, molecular and macromolecular components come together to form the extracellular matrix (ECM), a hierarchical framework of nano and microfibers, pores, membranes, chemical gradients, and anisotropic landscapes of varying stiffnesses, which plays a key role in biological systems. The building-blocks of the ECM also rely on their multiscale organization to collectively signal cells, enable cell-cell communication, and overall facilitate proper cell and tissue function. As we move down in size-scale, hierarchy continues to be fundamental. Proteins depend on both the order of their amino acids at the molecular scale as well as the coordinated manner in which they organize at higher sizescales [6]. Different types of collagens, for example, possess the characteristic triple helix, but distinct tertiary and quaternary structures result in specific functionalities performing as fibrils, networks, anchoring molecules, or transmembrane or basement membrane collagens. These examples illustrate not only the importance of multiscale organization in functionality but the versatility and diversity that it generates.

In tissue engineering (TE) and regenerative medicine (RM), it is essential to design materials, structures, and processes with hierarchy as a central functional principle in mind. Traditional TE and RM strategies have been mostly based on either “top-down” (etching down of bulk material) or “bottom-up” (arrangement of smaller components into larger assemblies) methods. However, while each one of these approaches carries unique advantages, they also suffer from disadvantages that have limited their ability to recreate the hierarchy and function of biological systems. For example, in three-dimensional (3D) bioprinting, a layer-by-layer deposition approach is used to create macroscale structures [7]. While this method enables fabrication of precise microscale features (*e.g.* porosity and topography) down to a few tens of microns, the method does not allow for control over the key nano and molecular scales. On the other hand, self-assembling systems are able to build a wide variety of precise nanostructures from specific molecular building-blocks but suffer from a limited capacity to organize them beyond the high nanoscale. However, these two approaches have emerged from fundamentally different areas of expertise and consequently are based on fundamentally different mechanistic principles, which have delayed their integration.

In this review, we argue that unifying top-down (*e.g*. bioprinting) with bottom-up (*e.g.* self-assembly) represents a new approach to biofabrication with the potential to create structures with an unprecedented level of hierarchy, complexity, and functionality. We divide the review into four sections. In the ***first section****,* we highlight recent bioinks and self-assembling materials that are being developed as part of either top-down or bottom-up strategies to engineer hierarchical materials for TE and RM. In the ***second section***, we discuss emerging platforms based on self-assembling bioinks (SABs), and conventional biofabrication focused on extrusion, inkjet, and electrospinning techniques. In the ***third section****,* we present novel self-assembly-driven fabrication platforms which we term *“supramolecular biofabrication”* and in the ***fourth section****,* we finalize with a summary of biofabrication techniques based on cellular self-assembly. We highlight distinct advantages, current challenges, and opportunities that are likely to emerge as our capacity to biofabricate with molecular and multiscale control continues to increase (**Figure 2**).

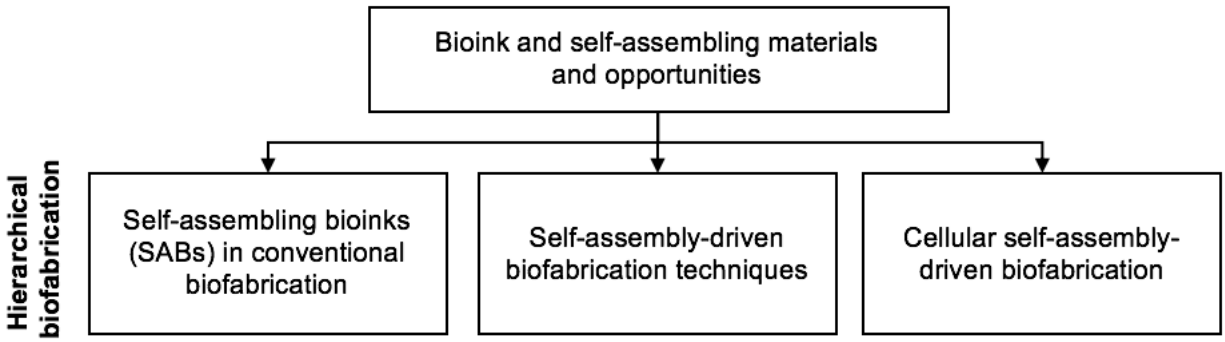

Figure 2 | Review structure. Schematic illustrating the four main sections of this review article.

2. Top-down and bottom-up strategies to achieve hierarchy

Biofabrication is *"the automated generation of biologically functional products…through bioprinting or bioassembly and subsequent tissue maturation processes"* [8]. Bioprinting is defined as the controlled two-dimensional (2D) or 3D positioning of materials (and cells) in a defined spatial organization using dispensing mechanisms and computer-aided designs [8,9]. In the context of TE and RM, bioprinting includes both a technique that defines the process of creation and a bioink that materializes into the desired structure or tissue. In this section, we highlight key material considerations of bioinks, describe recent work focused on improving their capacity to create more complex and hierarchical structures, and summarize state of the art in self-assembling materials with potential use as bioinks.

2.1 Bioink materials and opportunities

2.1.1 Single component inks

Bioinks are composed of cells or cells plus biomaterials [10]. Here, we focus on bioinks that contain a mixture of cells and biomaterials. Several criteria are considered when selecting the optimal material. On the one hand, the bioinks should be biocompatible, preferably pre-exist in native tissue, enable interaction with cells, have low stiffness, and exhibit high porosity to facilitate cell migration and flow of nutrients [7,11]. On the other, bioinks should possess rapid gelation, mechanical stability, sufficient stiffness to retain shape, and behave as a simple fluid to predict flow/droplet behaviour for maximum print resolution [12,13,14]. These are directly opposing criteria, which have traditionally resulted in a trade-off between the bioink's biological performance and resolution. Commonly used bioink materials can be synthetic (*e.g.* polymers) or natural (*e.g.* proteins, polysaccharides, and decellularized tissues) [15]. Synthetic polymers such as polyethylene glycol (PEG) [16,17], polycaprolactone (PCL) [18], and gelatin methcrylamide (GelMA) [19] tend to be FDA approved, exhibit fluid behaviour, and can be used with most bioprinting techniques but have limited chemical complexity or bioactivity. Conversely, natural materials such as collagen [20,21], fibrinogen and fibrin [22,23], hyaluronic acid (HA) [24,25], silk [26,27], alginate [25,28,29], agarose [30,31], or chitosan [32,33] offer biological activity but often require reinforcement using synthetic polymers to improve resolution and mechanical stability.

2.1.2 Multicomponent inks

To improve the complexity and resolution of bioprinting beyond that provided by the printer device, multicomponent bioinks are being explored. These materials combine two or more building-blocks either through mixing or simultaneous deposition. For example, by mixing

gelatine with natural biopolymers such as fibrinogen and alginate facilitated extrusion of microfilaments down to 500 μm in diameter while increasing the biological relevance by including ECM components [34]. This approach also facilitates integration of synthetic and natural materials within a single ink. For example, using simultaneous extrusion of PCL, gelatine, and cell-laden fibrin, it was possible to recreate sections of skeletal muscle [35], or by combining PCL and alginate with cells and growth factors, osteochondral constructs were fabricated [36]. An elegant approach was developed by Highley *et al.*, who devised an ink made up of microgels made from multiple types of polymer microgels jammed together to form an extrudable filament [37]. By using different polymers, the authors were able to modulate the bioink's viscosity, printability, and cell viability. An increasingly popular approach is the use of decellularized ECM (dECM) [38]. This approach offers the advantage of being patient-specific and the possibility to be combined with synthetic materials [39] but has raised concerns of tissue sterilization and patient-compatibility [38,40]. A comparable patient-specific ink used platelet-rich plasma with alginate [41]. Collectively, these approaches have improved the chemical diversity of bioinks, but the capacity to spatially control the location and distribution of biological cues [42] remains elusive. In this context, supramolecular bioinks could not only enable molecular design and diversity but also offer the ability to organize these cues spatially and hierarchically, taking us a step closer to the way biological systems operate.

2.2 Self-assembling materials and opportunities

Self-assembly is the automated aggregation of individual molecules into well-defined and reproducible higher-ordered structures using non-covalent interactions such as van der Walls, hydrogen, hydrophobic, and electrostatic forces. From the precise folding of individual proteins (**Figure 3 (a1)**) or DNA molecules (**Figure 3 (a2)**) to higher-order assemblies of phospholipids into cell membranes or proteins (**Figure 3 (a3)-(a4)**) into the tobacco mosaic virus, self-assembly is nature's primary way to fabricate, turning small molecules into coordinated hierarchical structures with functionality [43] (**Figure 3a**). As we continue to devise ways to better recreate the complexity of biological scenarios, tissues, and organs, we must take into account the fundamental role that self-assembly plays in them.

In the context of TE and RM, self-assembly offers an unparalleled opportunity to not only build with unprecedented programmability but also to build structures with innovative properties and the capacity to interact with cells with high selectivity [44] (**Figure 3 (b)**). Through this approach, functional nanomaterials have been developed using peptides [45–48] (**Figure 3 (b1)**), proteins [49], DNA [50,51] (**Figure 3 (b2)**), and polymers [52] among others [53,54].

2.2.1 DNA- and protein-based self-assembling hydrogels

DNA- and protein-based self-assembling hydrogels have been developed. For example, collagen-based hydrogels have shown how chondrocytes preferentially reside on fibres [55] and fibrillar hydrogels mimicking the ECM structure can be made from self-assembling cellulose [56]. Furthermore, using a recombinant elastin protein, sophisticated supramolecular structures can be generated, which can be used to guide mineralization at multiple scales [57–59] (**Figure 3 (b4)**). Other functional macromolecules such as DNA have also been used to assemble into macroscopic materials with functions such as programmable mechanosensing [60] (**Figure 3 (b2)**). However, the inherent complexity of larger biomolecules limits the capacity to manipulate them and control their assembly. A way to overcome these restrictions is to modify the ink material. For example, Mooney and co-workers have modified alginate with biotin/streptavidin to allow for enhanced controlled assembly [61]. Another example is to use materials that promote

cellular self-assembly. Examples of this approach include the use of a polymer hydrogel (PEG) combined with a flow bioreactor to promote cellular self-assembly of a vascular network [62] and the use of patterned substrates to drive self-assembly of cells into a controllable sized aggregate [63].

2.2.2 Peptide-based self-assembling hydrogels

On the other hand, peptides, shorter chains of amino acids, offer the possibility to design self-assembling systems with a higher level of control and reproducibility. These systems take advantage of both the properties of the individual building-blocks as well as their chemical makeup to direct their assembly. For example, the H-bonding forces that enable α-helix, β-sheet, or β-hairpin conformations in proteins are exploited to direct the assembly of the individual components into the specific higher-ordered structure. For example, ground-breaking work from Stupp and co-workers has demonstrated the possibility to use peptide amphiphiles (PAs) (**Figure 3 (b1)**) to build well-defined nanofibres capable of stimulating cartilage [64], bone [65], and spinal cord regeneration [66]. Other leading work includes that of Zhang and colleagues who have developed ECM-like matrices with broad impact in cell culture [67] or Gazit and co-workers who have pioneered minimalistic self-assembling material platforms based on dipeptides [68].

Inspired by these systems, peptide hydrogel materials with exciting properties have been developed such as the capacity to adapt to environmental conditions [69], stimulate immune responses [70], exhibit antimicrobial properties [71], possess self-healing properties [72], and even recreate protein structures such as collagen [73]. Another advantage of self-assembling peptides is the possibility to generate well-defined microstructures by manipulating their self-assembled nanostructure. For example, different strategies have been used to manipulate PAs into hydrogels with aligned nanofibres [74], surface microtopographies [75] or hollow hierarchical gels [76]. The ability to assemble peptides into aligned nanostructures at multiple scales within a printed construct is highly advantageous towards mimicking anisotropic tissues such as muscle, nerve, cartilage, or cornea. This approach has also opened the possibility to co-assemble and integrate different types of building blocks, further enhancing the complexity of the generated materials [77]. Nonetheless, these materials have traditionally suffered from two key characteristics that have restricted their use in bioprinting, including a limited capacity to control their assembly beyond the nanoscale and lack of suitable mechanical properties.

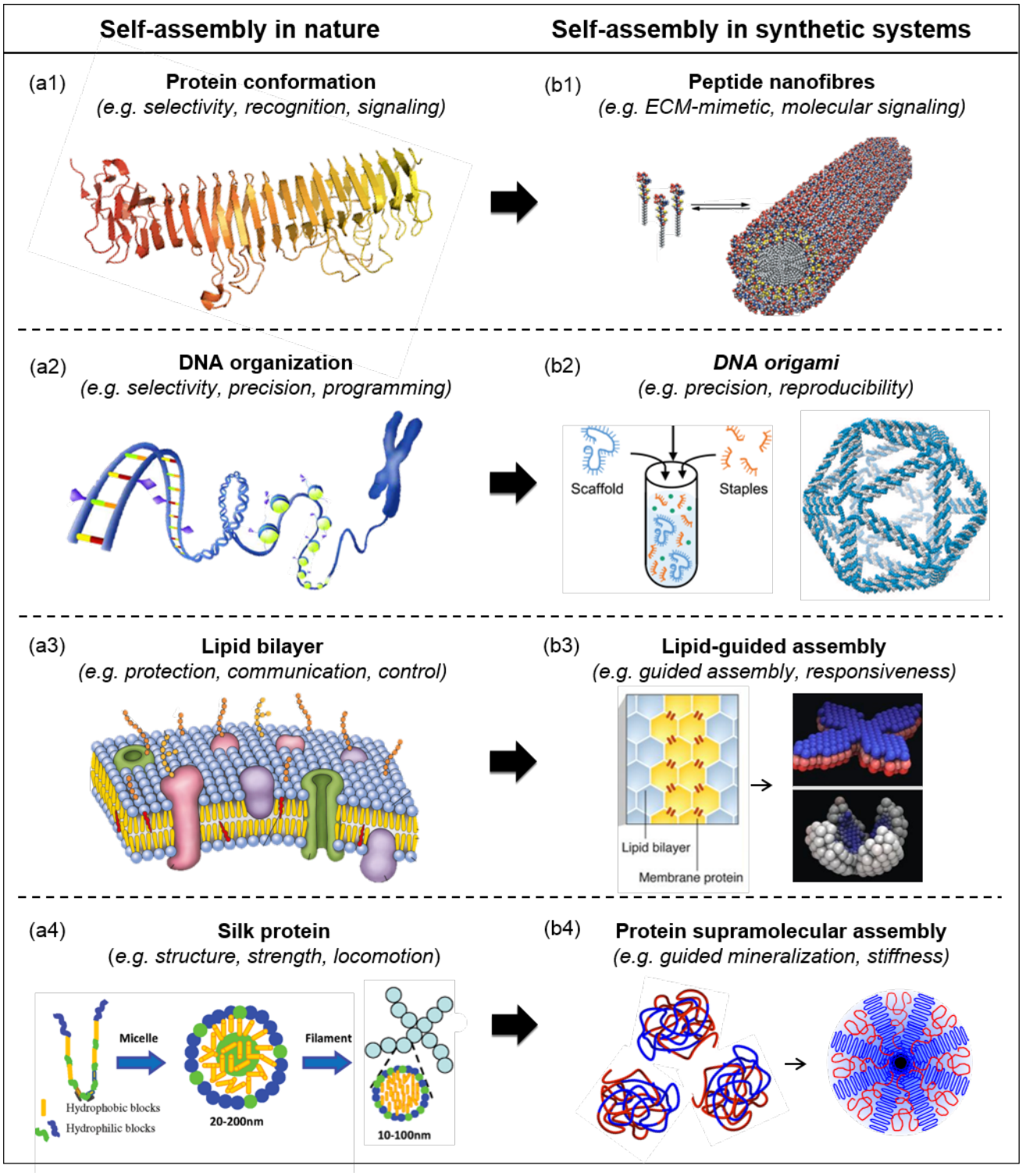

Figure 3 | Self-assembly in nature and synthetic systems. Illustrations of (**a**) four examples of self-assembling systems found in nature including (**a1**) protein conformation [172] ©1996 Springer Nature Publishing AG, (**a2**) DNA double helical organisation [173], (**a3**) cell lipid bilayer membrane [174] ©2011 Elsevier, and (**a4**) silk protein folding [49] ©2012 ACS Publications, and (**b**) four examples of how these systems inspire synthetic self-assembling ones including (**b1**) self-assembling peptide nanofibres [45] ©2001 AAAS, (**b2**) DNA origami [50] ©2016 AAAS, (**b3**) lipid-guided assembly [139] ©2013 AAAS, and (**b4**) protein supramolecular assembly [57] ©2018 Springer US.

3. Self-assembling bioinks (SABs) in conventional biofabrication

It is exciting to think of the possibilities that would emerge from combining biofabrication and self-assembly [78]. Both of these approaches have tackled TE and RM challenges from completely different angles, which consequently has forged them into technologies dominated by fundamentally different underlying principles and with distinct sets of advantages and disadvantages. However, there is an untapped opportunity to develop novel methods that integrate biofabrication and self-assembly. In many ways, the advantages of one approach tend to overcome the disadvantages of the other (**Table 1**). Imagine the ability to bioprint with multiple types of biomolecules that immediately assemble into a milieu of defined nanostructures that selectively stimulate cells while organizing them into precise anatomical geometries with hierarchical order (**Figure 4**). However, reaching this goal will not only require integration of traditionally unrelated fields but also new ways of thinking about biofabrication that surpass established conceptual boundaries. In this section, we highlight studies that use either extrusion, inkjet, or electrospinning techniques with self-assembling bioinks (SABs). We define SABs as those that comprise smaller components such as peptides, proteins, polymers, or DNA and that assemble into well-defined higher-ordered structures in a reproducible manner. Thus, we will focus exclusively on examples that create higher-ordered structures using a combination of self-assembly and biofabrication.

Table 1 | Overview of the advantages and challenges within biofabrication (top-down) and self-assembly (bottom-up) strategies.

	Biofabrication	**Self-assembly**	
Advantages	+ Precise micro-to-macro scale control + Precise porosity, shape, geometry + Control of surface topographies + High reproducibility + Easy replication of structure from CAD scans + Tends to be inexpensive + High scalability	- Limited micro-to-macro control - Limited control of shape beyond nanoscale - Limited control of surface topography - Limited reproducibility beyond nanoscale - Low synthesis yield and high variability - Tends to require expensive materials - Low scalability	Challenges
Challenges	- Limited molecular-to-nano control - Material compatibility restrictions - Limited communication with cells - Limited recreation of biological nanostructures - Limited capacity for precise bioactivity - Limited to superficial/external features - Fabrication time tends to be slow	+ Precise molecular-to-nano control + Use of functional bio- and macro-molecules + Selective communication with cells + Capacity to recreate biological nanostructures + Capacity for precise bioactivity + Physical/chemical features within the bulk + Rapid material assembly	Advantages

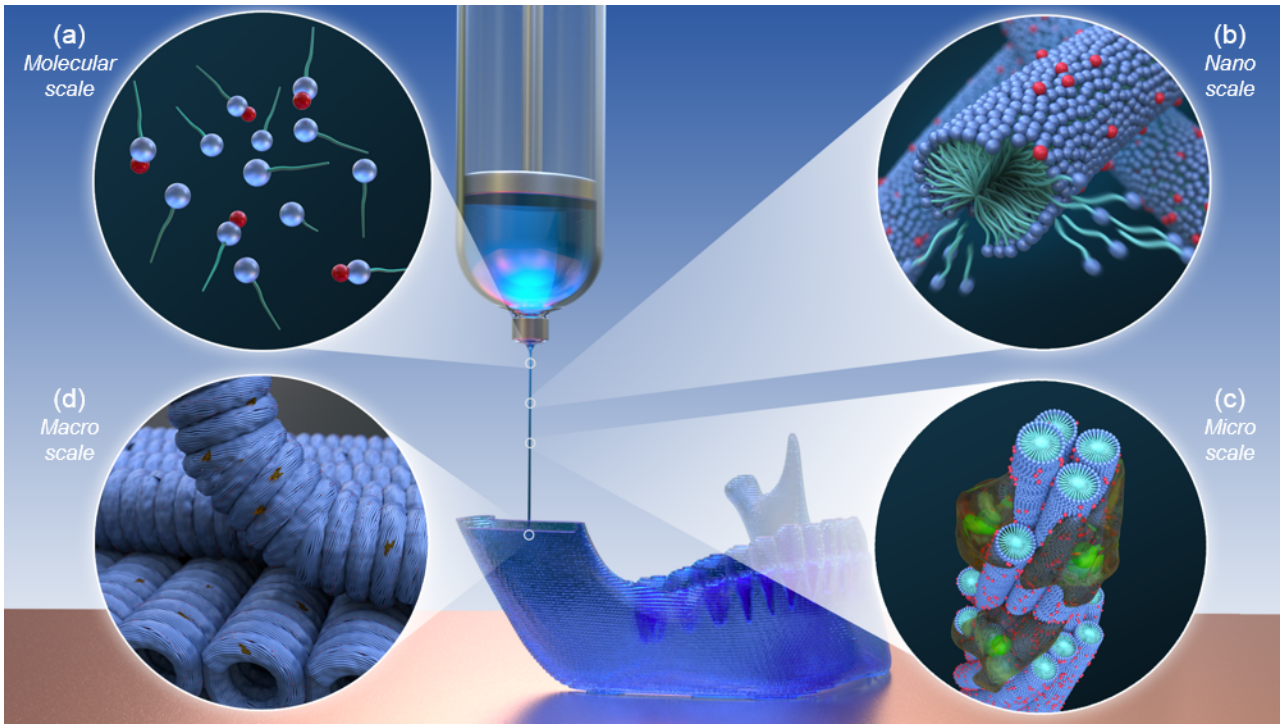

Figure 4 | Hierarchical biofabrication. Schematic representation of the unification of bioprinting (biofabrication) with self-assembly leading to hierarchical control of both biomolecular signals and physical structures across length-scales. (**a**) Bioink mixture of self-assembling components with (red spheres) and without bioactive epitopes and cells, (**b**) assembly into nanofibres with the capacity to precisely display the bioactive epitopes on their surface, (**c**) assembly of the nanofibres into microscopic bundles capable of directing cell growth, and (**d**) printing into precise anatomical macroscopic structures.

3.1 Extrusion

Extrusion, also known as 'direct ink writing', uses pneumatic or mechanical pressure to extrude a continuous filament of ink. The ink must either gel at the nozzle opening or exhibit a shear-thinning behaviour whereby a solid gel temporarily behaves like a liquid and flows under pressure.

3.1.1 Adapting self-assembling materials to extrusion printing

Self-assembling materials are particularly attractive for extrusion systems, as well as general injection, given their shear-thinning behaviour as a result of reversible non-covalent interactions [79–83]. The adaptation of peptides for extrusion has directly translated into a variety of commercial SABs. For example, the company Biogelx™ sells a SAB based on Fmoc-diphenylalanine and Fmoc-serine, which assemble into nanofibers [84,85] while BIOGEL™ uses short chain oligo-peptides that assemble into nanofibres, which has been used to print filaments down to ~ 300 μm diameter [86]. Conversely, the adaptation of natural self-assembling building blocks such as proteins and polysaccharides is restricted by their inherent complexity, which also makes them more difficult to control and manipulate. As such, their use in extrusion requires modification to enhance flowability and mechanical properties, often done by combining them with a polymer. For example, while silk is prone to clogging the extrusion nozzle due to shear-induced β-sheet crystallisation, Das *et al.* combined silk with gelatine to prevent crystallisation

and enable the formation of sub 90 μm diameter filaments [87]. In addition, SABs offer the possibility to avoid the use of post-processing steps, which can often be cytotoxic. For example, extruded structures using a silk/gelatine bioink can be stabilized by simply using sonication to promote β-sheet assemblies between the two components [88]. In another example, recombinant silk was mixed with fibroblasts and gelled at physiological temperature before extruding [89]. These studies exemplify the possibility of extruding self-assembling materials.

3.1.2 Opportunities, advantages, and limitations

SABs offer the unique advantage of not only providing a rich landscape of ECM-like nanoscale structures, networks, and pores but also a precise presentation of biological signals. For example, self-assembling nanofibers can display bioactive epitopes only on their surface [90,91]. This feature offers opportunities to improve the functionality of extrusion SABs. For example, Yan *et al.* used PAs with the laminin mimetic head group sequence IKVAV conjugated to a thiolated-gelatine bioink, which selectively presented the IKVAV on the surface of the nanofibers to promote bile-duct formation [92] (**Figure 5 (a)**). The SAB was used to extrude ~ 250 μm diameter filaments forming controllably spaced pores. While increasing the PA concentration led to increased nanofiber density in the bioink, the printability was reduced. Thus, there are competing advantages between optimal peptide concentration and printability. Another advantage of SABs is the possibility to optimize network density to control nanoscale porosity and consequently, parameters such as nutrient diffusion and cell-cell communication. For example, using a silk/PEG material, Zheng *et al.* developed an extrusion-based SAB where gelation occurs as a function of the β-sheet-driven assembly of silk molecules [26]. By modulating the concentration of the molecules, the extruded structures exhibited different levels of permeability. These examples demonstrate the opportunities that emerge when SABs are incorporated within extrusion printing integrating physical and chemical features at the nanoscale with macroscale pores and structures.

Self-assembly also facilitates the design of self-healing materials, which have enabled extrusion within supporting hydrogels. In this review, we focus exclusively on self-healing properties arising from self-assembling mechanisms and not through the reversibility of covalent bonds. Taking advantage of the transient non-covalent interactions of self-assembling materials, Burdick and colleagues developed a guest-host modified HA that, as a result of its shear thinning behaviour, can be extruded into a supporting self-healing hydrogel [93,94] (**Figure 5 (b)**). The system is capable of fabricating filamentous structures down to ~ 35 μm in diameter and exhibiting twists and turns. Moreover, the supramolecular nature of the SAB enables incorporation of cell-interactive peptides or UV cross-linkable sequences to create perfusable paths [93] that can be used for example as *in vitro* models for angiogenesis [94]. Similarly, O'Bryan *et al*. used a self-assembling block co-polymer combining diblock (polystyrene-block-ethylene/propylene) and triblock (polystyrene-block-ethylene/butylene-block-polystyrene) polymers capable of self-assembling into ~ 1 - 2 nm structures with polystyrene cores surrounded by ethylene-based coronas [95]. In pure triblock polymer mixtures, the intermolecular bridges between the ethylene/bytlene blocks lead to an unprintable solid macroscopic network. However, the addition of diblock polymers disrupts the bridges, resulting in self-assembling micro-organogels with tuneable rheological properties within which silicone elastomer structures down to ~ 250 μm in size can be printed [95].

Another opportunity for SABs is their potential to serve as selective and responsive materials for the controlled and targeted delivery of macromolecules such as drugs and growth factors. This

feature has been demonstrated to be compatible with injectable inks, which are similar in requirements to that of extrusion [46,96,97]. While relatively unexplored in SABs, the function of molecular entrapment through non-covalent interaction with the fibrillar network has been explored. For example, Xia *et al*. reported 3D bioprinting with a SAB based on complementary peptide sequences (KFEFKFEF) designed to reversibly incorporate metal ions to induce fluorescent behaviour [98]. Importantly, the incorporation of these molecules did not affect the shear-thinning or assembling properties of the SAB. Conversely, DNA has been used in extrusion to take advantage of its responsiveness, biodegradability, the permeability of nutrients, and non-swelling/non-shrinking properties. Shin *et al.* developed a conductive SAB by dispersing carbon nanotubes (CNTs) in a mixture of DNA and either GelMA or HA and self-assembling with them through π-π and hydrophobic interactions, respectively [99] (**Figure 5 (c)**). The materials exhibited a high shape fidelity as a direct result of the non-swelling/shrinking properties of DNA and fibrous structures arising from the coated CNTs. Similarly, Gaharwar *et al.* demonstrated that bioactive silicate nanoparticles induce osteogenic differentiation in hMSCs [100].

These examples elucidate the opportunities that SABs offer as a result of their self-assembling nature. However, the properties that give them their versatility and reversibility are also responsible for their limited mechanical strength (< 1 kPa). However, some modulation of their mechanical properties is possible by simply tuning the density of the assembled nanostructures. For example, diphenylalanine based injectable inks can be tuned to assemble into hydrogels ranging in stiffness from ~ 5 - 150 kPa simply by altering the peptide concentration [96]. Within extrusion, aliphatic ultrashort peptides conjugated with a lysine-based ink have been shown to assemble into hydrogels with stiffnesses of up to ~ 40 kPa [101]. Similarly, using a β-hairpin peptide-based bioink, stiffnesses between ~ 400 to 2900 Pa can be achieved simply by modulating the peptide concentration [81]. Interestingly, in this case, not only can these hydrogels regain their stiffness after undergoing shear-thinning during printing, but they can actually become stiffer [81].

It is important to keep in mind that, as most biological structures develop, they begin as soft environments that experience a gradual increase in stiffness. With this in mind, bioinks that offer immediate high stiffness are likely to have limitations in the context of TE and RM. SABs may offer the opportunity to bioprint a soft, dynamic, and highly hydrated environment but at the same time offer sufficient strength, stability, and speed of assembly. Nonetheless, we expect that new supramolecular strategies capable of providing SABs with dramatically enhanced and more versatile mechanical properties will continue to emerge [102–104].

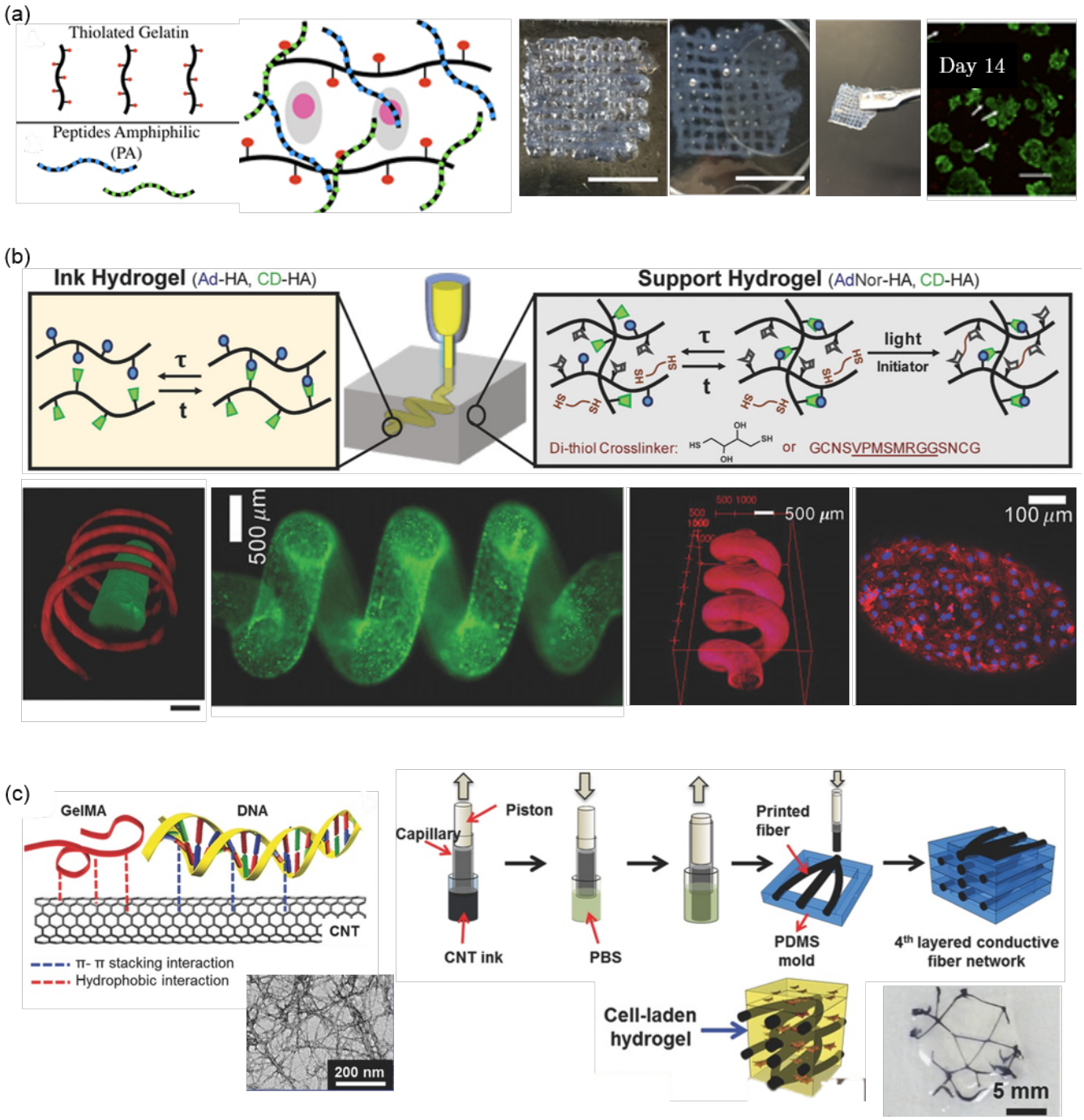

Figure 5 | Self-assembly in extrusion. (**a**) A schematic of a functionalised peptide amphiphile/gelatin-based extrusion ink for bile duct formation with a representative printed matrix before and after crosslinking and a live/dead assay of cholangiocytes at day 14 (Reproduced with permission [92] ©2018 IOP Publishing), (**b**) an example of a supramolecular polymer-based ink and support hydrogel using guest/host chemistry and thus permitting the formation of complex structures which can be used to seed endothelial cells (right, stained with DAPI/blue and CD31/red) (Reproduced with permission [93,94] ©2015, 2018 John Wiley & Sons), and (**c**) an schematic of a self-assembling bioink formed of CNTs decorated with DNA and GelMA permitting the formation of conductive fiber networks in a GelMA hydrogel (Reproduced with permission [99] ©2016 John Wiley & Sons).

3.2 Inkjet

Inkjet technology, also known as droplet-on-demand, deposits arrays of ink droplets that fuse together to form continuous lines. The ink solution must, therefore, exhibit a viscosity that is both sufficiently low to allow droplet formation and sufficiently high to retain its shape post-printing.

3.2.1 Adapting self-assembling materials to inkjet printing

While SABs are increasingly being used for a broad range of applications in extrusion printing, their use in inkjet bioprinting remains relatively underexplored. Nonetheless, there are emerging examples of inkjet bioprinting with peptides, DNA, or polymers which highlight the opportunities of pursuing this approach. In general, two approaches are being explored for SABs in inkjet printing including overlaying droplets of two or more self-assembling components (*i.e.* A onto B) [101,105,106] or printing of one component into a bath of the other (*i.e.* A into B) [107]. In both cases, self-assembly does not occur in the print-head but rather at the collector site.

3.2.2 Opportunities, advantages, and limitations

Conventional inkjet inks require physical crosslinking post-printing to stabilize the printed structures on a layer-by-layer basis. The crosslinking strategies (*e.g.* UV light, thermal energy) are often not cell compatible, restricting the choice of material. In comparison, inkjet printing with SABs removes the need for crosslinking steps and consequently can result in a more cell-friendly printing process. In an interesting example, Loo *et al.* demonstrated that lysin-containing hexapeptides can be printed sequentially with phosphate-buffered saline (PBS) to induce gelation [101] (**Figure 6 (a)**). These hexapeptides change their secondary structure depending on the concentration used, from random coil to α-helix to β-turn. These shifts dictate the acquired nanostructure. For example, from α-helix to β-turn, there is a reversible formation of nanofibers, which at higher concentrations condense to bundles of nanofibers [108]. The authors used the β-turn stage to form networks of self-assembled nanofibers, which were used to encapsulate small molecules, proteins, and cells. In addition, using inkjet printing, the authors fabricated multidomain scaffolds with spatially organized endothelial cells in the core surrounded by a gel with embedded fibroblasts and keratinocytes on the surface. The study exemplifies the potential of combining self-assembly with inkjet printing by developing tuneable nanostructures that can be organized into higher-ordered constructs able to form anisotropic multicellular environments.

The lack of immediate mechanical strength of self-assembling materials has particularly hampered the use of SABs in inkjet printing. To address this challenge, multicomponent SABs have been used. For example, Li *et al.* grafted single-stranded DNA onto a polypeptide backbone, which in the presence of complementary strands of DNA, resulted in the self-assembly of polypeptide-DNA nanofibrous gels with storage moduli of ~ 5 kPa [105] (**Figure 6 (b)**). In combination with a microvalve-based 3D bioprinter comprising separate cartridges, sequential prints of polypeptide-DNA and DNA linker were used to fabricate easily handled printed millimetre-sized structures of 5 - 20 layers. Given the high affinity provided by the complementary DNA strands, the material exhibited both self-healing properties and degradation via proteases or nucleases. Another approach to enhance the mechanical strength and inkjet printability of SABs relies on the use of supramolecular polymers with the capacity to self-assemble. Hart *et al.* used the π-π-driven assembly of pyrenyl-end groups with chain folding polydiimide to create self-assembling polymer gels [106,109,110] (**Figure 6 (c)**). The two motifs were conjugated onto low molecular weight polymers, which were then printed sequentially to permit supramolecular network formation [106,110]. Using inkjet printing, macro-sized structures were printed using ~ 15 μm diameter drops (~ 15 picolitres), which thanks to the polymer design,

exhibited fluorescent properties. Interestingly, only the individual polymer layers were fluorescent, with the supramolecular network of the two types of polymers exhibiting colour in visible light instead. The authors demonstrated the possibility to change the colour depending on the divalent polymer used [106]. Other advantages that supramolecular polymers offer are that they are inherently cheaper than most peptides, can be designed to be biodegradable, contain multiple binding sites, and can entrap macromolecules in their entangled fibrous network. For example, bioactive silica nanoparticles can be embedded within a polymer SAB without disrupting the printing process and generating macroscopic structures with feature sizes down to 10 - 20 μm [109].

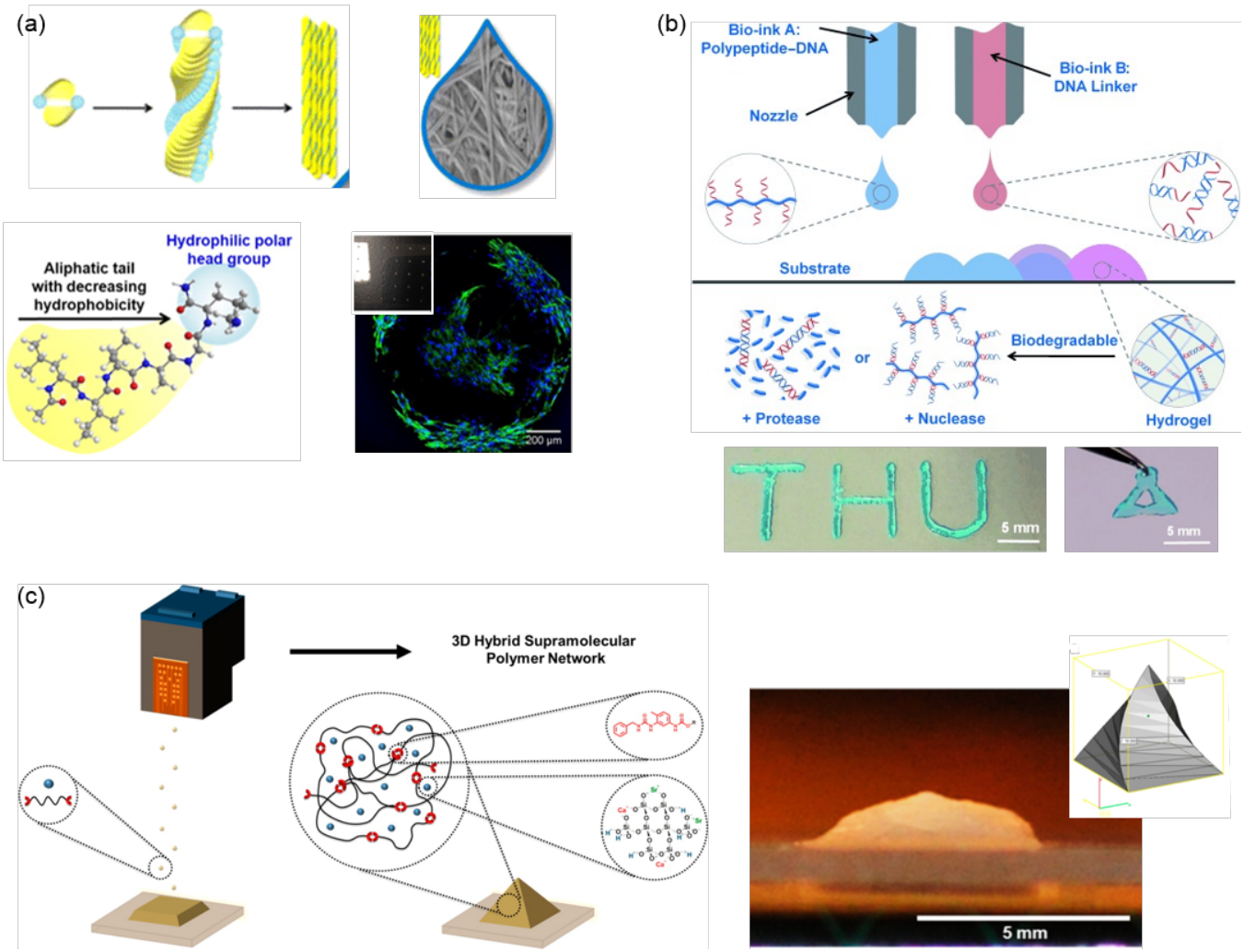

Figure 6 | Self-assembly in inkjet. (**a**) Sequential printing of a PA-based ink and PBS buffer to form microgels with hMSCs aligning along the peptide fibres (Reproduced with permission from [101] ©2015 ACS Publications), (**b**) inkjet printing of a polypeptide-DNA ink overlain with DNA linker permitting the formation of handleable 3D structures consisting of up to 20 layers (Reproduced with permission from [105] ©2015 Wiley-VCH Verlag), and (**c**) sequential printing of a supramolecular polymer-based network and silica particles to create a pyramid structure (Reproduced with permission from [109] ©2016 ACS Publications).

3.3 Electrospinning

Electrospinning relies on the evaporation of an organic solvent or cooling of a polymer to solidify the spun fibres. The use of organic solvents or high temperatures prevents cell encapsulation within the ink. However, compared with extrusion and inkjet, electrospinning has the advantage of creating fibrillar structures ranging from nanometres to microns in diameter with high mechanical tunability.

3.3.1 Adapting self-assembling materials to electrospinning

Conventional electrospinning relies on organic solvents, which represents a disadvantage not only for many biofabrication applications but also for SABs. For example, while self-assembling biopolymers (*e.g.* silk, gelatin, or fibrinogen) have been used extensively in electrospinning [111,112], the organic solvents tend to disrupt their molecular conformation [111]. For instance, the use of fluoroalcohols disrupts the characteristic triple-helix structure of collagen [113], and the rapid evaporation of organic solvents hinder the molecular rearrangement of keratin from an α-helix conformation into stable β-sheets [114]. Due to this compatibility issue, proteins have been spun in combination with synthetic polymers to provide structural stability of the spun fibres through the polymer backbones [114–116]. Despite potential conformational disruptions, the use of proteins within SABs improves the overall viability of cells by providing bioactive epitopes such as cell binding sequences [115]. In one example, the authors propose that the electrospinning process can expose hidden epitopes of fibrinogen and enhance cellular activity [117,118]. However, to take full advantage of the potential of SABs in biofabrication, the process should support the formation of well-defined self-assembled nanostructures in aqueous solvents.

3.3.2 Opportunities, advantages, and limitations

Recombinant proteins have been explored as SABs for electrospinning, for example, a water-soluble silk-elastin-like mimetic protein [119]. Proteins also enable the design of SABs with tuneable secondary structures. For instance, Khadka *et al.* designed an anionic polypeptide, which was spun in water and resulted in a shift from random coil to β-sheet [120] (**Figure 7 (a)**). This shift generated a stable fibre while the collector geometry controlled the fibre orientation (random or aligned). Moreover, the authors argue that although the sequence is not protein mimetic, the modularity of this approach can be used to modulate cell behaviours or introduce functionalities such as aromatic side groups that can act as nucleation points for guiding protein folding. Conversely, self-assembling peptides are still considered broadly unsuitable for electrospinning given their need for aqueous assembly conditions and limited mechanical properties. However, recently, a method of electrospinning self-assembling peptides was reported by Pugliese *et al*. [121] (**Figure 7 (b)**). Here, the peptides were combined with low concentrations of the crosslinker genipin in the organic solvent Hexafluoroisopropanol (HFIP) to produce partially crosslinked spun nanofibers. However, further crosslinking by immersion in a genipin bath was required for stability. The resulting fibres contained randomly orientated nanofibers with an average diameter of 294 nm. These studies demonstrate the feasibility and potential of aqueous electrospinning with synthetic self-assembly-based materials, thus, introducing a potential new route for presenting bioactivity in electrospun scaffolds.

An alternative method to introduce bioactivity into electrospun synthetic polymer scaffolds is to functionalize the spun fibre surfaces with self-assembling materials. For example, Viswanathan *et al*. used an amphiphilic diblock copolymer (poly-oligo(ethylene glycol)methacrylate) with RGDS to functionalize poly(D,L-lactide) spun scaffolds [122]. In another example, PCL fibres with diameters between 300 - 400 nm were coated with 8 - 10 nm diameter PA nanofibers to

precisely display cell-binding and enzymatically-cleavable sequences on the fibre surfaces [123] (**Figure 7 (c)**). Interestingly, the PA nanofibers preferentially coated the PCL fibres as thin 60 nm thick layers as opposed to filling the scaffold pores, thereby increasing the level of hierarchy and spatial control. Moreover, the selective presentation of bioactive sequences transformed the passive PCL into bioactive scaffolds generating a significant increase in cell adhesion and spreading. Similarly, electrospun composite fibres from premixed PCL and self-assembling peptides based on repeats of the amino acid sequence EAK similarly resulted in surface-enriched fibres with embedded peptides as well as enhanced hydrophilicity, more uniform surface topography, and decreased ductility [124]. Interestingly, the use of self-assembling peptides resulted in higher levels of mRNA transcription for bone matrix factors, with higher osteoblast vitality and calcium deposition.

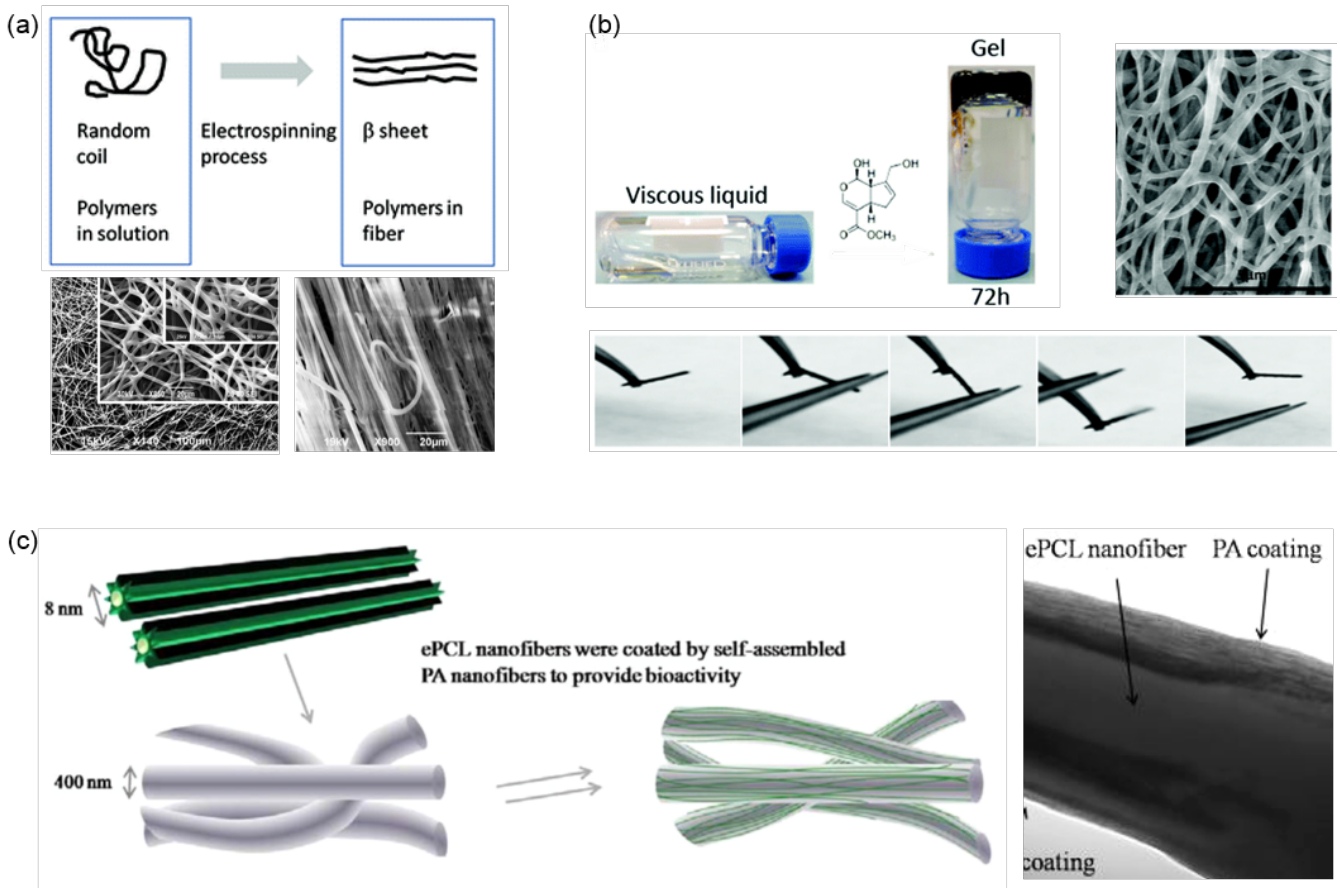

Figure 7 | Self-assembly in electrospinning. (**a**) Schematics and corresponding SEM images of polymers in solution and after electrospinning (Reproduced with permission from [120] ©2011 ACS Publications), (**b**) example of electrospinning self-assembling peptides under aqueous conditions leading to the formation of durable microchannels (Reproduced with permission from [121] ©2019 RSC), and (**c**) an example of PA fibres being used to coat a PCL-based scaffold to enhance the bioactivity (Reproduced with permission from [123] ©2009 IOP Publishing).

3.4 Laser-assisted bioprinting

Laser-assisted bioprinting covers many techniques including for example laser direct writing (LDW) and laser-induced forward transfer (LIFT). Common to all laser-assisted techniques is their nozzle-free bioprinting method. For example, in LDW, a laser is applied onto a structure consisting of a glass slide, an absorbent layer and a gel with embedded cells. Focusing of the laser creates a local pressure point in the absorbent layer, which releases a droplet from the underlying material/cell layer. The technique has been used to arrange multiple cell-types in 3D structures made from collagen, generating skin mimetic scaffolds [125]. Conversely, the technique has also been used to create cell arrays to observe cell-cell behaviour, such as work by Gruene *et al.* using adipose-derived stem cells and endothelial colony-forming cells [126]. In this way, it is possible to control the layer height and composition as well as the cell-cell ratio and type. In another study, Corr and colleagues used LDW to place cells in predefined arrays, which then aggregate via cellular-driven self-assembly into embryoid bodies [127]. In this example, the bioprinting aspect complements the self-assembly by permitting control over colony size and cell density. While the area of laser-assisted bioprinting has offered many advantages in bioprinting [128], to our knowledge the work involving self-assembling materials has primarily focused on non-living materials such as self-assembling co-polymers to create nano-scale architectures [129]. In an effort to concentrate this review only on the fabrication of bioscaffolds, this topic was not explored further in this review.

4. Self-assembly-driven biofabrication techniques

The previous section provides an overview of different approaches that integrate self-assembling materials with conventional biofabrication techniques. However, given the distinct nature and inherent versatility of both self-assembly and biofabrication, new approaches are emerging and inspiring new ways of thinking about biofabrication. In this section, we describe how new biofabrication methods are using self-assembly as a central role in the process beyond its application as a SAB to offer higher levels of complexity and structural hierarchy.

4.1 Using external stimuli to direct self-assembly

Hydrodynamic forces developed through fluid flow offer an opportunity to guide molecular self-assembly and fabricate scaffolds with a higher degree of structural hierarchy [83,130,131]. For example, using confined unidirectional flow to direct the assembly of chitosan and gellan gum, Sant *et al*. reported on the fabrication of tubular hydrogel scaffolds (~ 1 mm diameter) comprising microscopic bundles of aligned fibrils of 1 - 5 μm in diameter that recapitulate the structure of native collagen bundles [132] (**Figure 8 (a)**). By modulating the hydrodynamic forces of the process (unidirectional flow or random mixing), it was possible to fabricate similar tubular structures with either aligned or randomly oriented fibrils. Using a similar mechanism, Patel *et al.* exploited the capacity to incorporate multiple components and demonstrated the possibility to fabricate polysaccharide fibres assembled with graphene flakes, which organised as horizontal sheets in response to the hydrodynamic forces [133]. This approach can also be used with self-assembling peptides. For example, Chin *et al.* used a cylindrical container attached to a rotating rod to direct PA nanofibers into circumferential alignment driven by shear forces from the rotating rod [134] (**Figure 8 (b)**). By simultaneously retracting the metal rod to allow an influx of calcium ions, the assembly was restricted to the walls of the cylinder, creating a hollow tubular gel made from aligned PA nanofibres. Moreover, the molecular versatility of the process permitted the incorporation of polymer-conjugated PAs, which enabled the fabrication of similar structures with the polymers selectively displayed on the surface.

Flow-directed assembly can be further modulated using processes such as liquid immiscibility, magnetic levitation, or immiscibility. For example, Shi *et al.* used a liquid-liquid moulding process which controls the assembly of nanoparticles such as cellulose nanocrystals at the interface between two immiscible liquids (oil/water) [135]. In another example, a liquid-in-liquid 3D printing technique was used to fabricate perfusable channels with high stability, which permitted printing of connecting bridge microchannel arcs formed by dragging the extrusion nozzle from one print to the next [136]. The authors used a dispersion of nanoclay printed within an oil-based surfactant to create a stable formation of nanoclay-polymer surfactant at the liquid-liquid interface forming the microchannel walls. In addition, the rapid self-healing properties of this material (~ milliseconds) permitted real-time disruption of bridge connections to redirect flow. In an elegant approach, Demirci and co-workers used magnetic levitation to develop a biofabrication approach whereby magnetic fields can be used to assemble microgels into defined complementary structures [137,138] (**Figure 8 (c)**). The process permits assembly of multiple building-blocks by adjusting parameters such as polymer composition, density, stiffness, elastic modulus, or porosity. Furthermore, cells can be encapsulated within each microgel. Interestingly, as a result in differences in cell density, different cell types exhibited variations in the level of levitation, which may be used to manipulate cells [137]. In another example, the immiscibility of oil and aqueous solutions can be used to drive and control self-assembly. For example, Villar *et al.* controllably ejected picolitre droplets of an aqueous solution within an oil bath, promoting the formation of lipid monolayers around each droplet and bilayers with neighbouring droplets. By precise printing, dynamic hierarchical structures were fabricated [139].

Another area is mesoscale assemblies driven by immiscibility. For example, Du *et al.* contained individual hydrogels within a prepolymer solution and subjected them to a secondary photocrosslinking step, which resulted in the self-assembly of the hydrogels by minimizing the surface tension [140]. In this way, the authors were able to create 3D assembled hydrogels of, for example, linear, branched, and lock-and-key shaped hydrogels. Another approach takes advantage of molecular recognition. For example, Harada *et al.* synthesised acrylamide-based gels which they functionalised with guest/host-moieties whereby the hydrogels subsequently assemble according to the specific recognition [141].

These examples demonstrate how fabrication processes can use exogenous forces to guide self-assembly while enabling hierarchical control. Furthermore, these techniques are advantageous as they are non-contact and non-invasive methods of assembly, which enable the incorporation of multiple types of building-blocks and can increase the overall cell viability and bioactivity of the system.

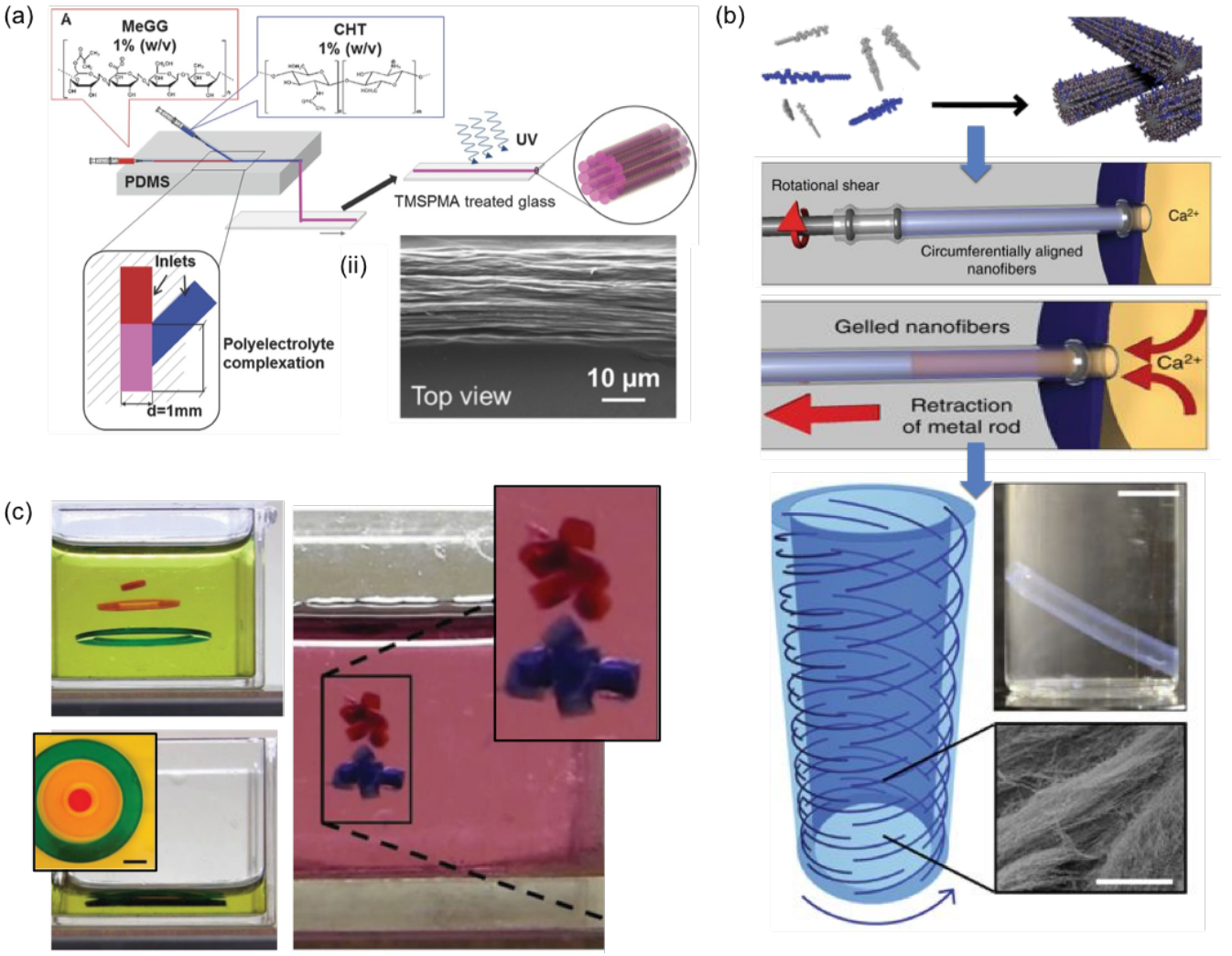

Figure 8 | External guidance of self-assembly. (**a**) A schematic illustrating the alignment of chitosan-based fibrils into a fibre bundle using confined flow within a PDMS mold (Reproduced with permission from [132] ©2017 John Wiley & Sons), (**b**) circumferentially aligned PA-fibres in a tubular structure through the application of directional shear stress (Reproduced with permission from [134] ©2018 Sprinter Nature Publishing AG), and (**c**) hierarchical organisation of microgels using magnetic levitation (Reproduced with permission from [138] ©2015 John Wiley & Sons).

4.2 Supramolecular biofabrication

By further approaching biofabrication from the bottom-up, it is possible to develop fabrication methods where the level of resolution and hierarchy is not limited by the printing technique but rather by the inherent nature of the self-assembly process and the capacity to modulate it. As such, self-assembly is not only able to define the molecular-to-nanoscale structure (*e.g.* nanofibers, fibrillar gels), but also guide the assembly at multiple sizescales. We name this approach “supramolecular biofabrication”. The underlying opportunity here is provided by the emergence of new assembling phenomena, structures, and material properties that can result from synergistic interactions between the building blocks and the top-down technique [77,142].

For example, taking advantage of a thermal pathway capable of turning isotropic solutions of PAs into liquid crystals and subsequently lamellae-to-fibre transitions, Stupp and colleagues developed a mechanism to generate higher-ordered bundles of PA nanofibers [74] (**Figure 9 (a)**), which can be further manipulated to incorporate topographical features [75]. This group also developed another hierarchical process based on the co-assembly of PAs with HA at liquid-liquid interfaces. In this case, the process leads to the formation of a diffusion barrier that prevents chaotic mixing and leads to a directional and organized molecular-nano-microscopic assembly. By modulating the mixing conditions, it is possible to fabricate membranes, sacs, or strings [76,143]. By applying an electrical current, the co-assembly mechanism can be modulated to create thinner or thicker membranes [144].

Inspired by these approaches, our group has focused on developing supramolecular biofabrication methods that take advantage of emerging phenomena arising from compartmentalization, concentration gradients, and controlled ionic transport. For example, Inostroza-Brito *et al.* developed a dynamic SAB based on the co-assembly of PAs with elastin-like proteins (ELPs) (**Figure 9 (b)**) [145]. A key molecular design element is the use of PAs as "molecular chaperones" that co-assemble with and modulate the conformation of the ELP molecules as a diffusion-reaction mechanism leads to a multi-layered membrane with the capacity to dis-assemble, seal to interfaces, and self-heal. Upon external manipulation, it is possible to grow the membrane in specific directions, resulting in a "touch-and-pull" interfacial fabrication process capable of generating macroscopic tubular structures exhibiting micro and nanoscale features [145,146]. Building on this, Hedegaard *et al.* used a co-assembling system based on PAs and structural proteins (*e.g.* keratin, fibronectin, collagen) with drop-on-demand printing to fabricate microgels with a spectrum of shapes (*i.e.* spherical, hollow, toroidal) and the capacity to be assembled into well-defined macroscopic structures [107] (**Figure 9 (c)**). This study also exploited hydrodynamic forces to guide the assembly to generate hydrogels with aligned or randomly aligned nanofibres, surface microtopographies, and distinct geometrical shapes. These approaches facilitate the engineering of new materials and material properties by systematically modulating the co-assembling components. Taking advantage of this opportunity, Wu *et al.* has recently reported on the use of ELPs to co-assemble with and modulate the organization of graphene oxide (GO) flakes into functional tubular structures [147] (**Figure 9 (d)**). As in the case of ELP/PA, these tubes form simply by injecting a droplet of ELP into a solution of GO, which initiates the assembly and eventually opening into a tube. However, in this case, the disordered nature of the ELP leads to a unique ELP-GO complex, which results in a material with radically improved properties and functionality. In this case, the material can be used as an extrusion SAB for fabricating functional macroscopic fluidic devices with resolutions down to ~ 10 μm in size, embedded cells, and a variety of material properties that resemble biological structures. This approach is being used to fabricate more biologically relevant organs-on-a-chip.

These studies demonstrate that self-assembly can be exploited to develop new fabrication processes based on the organization of molecular and nanoscale building-blocks at multiple scales. Furthermore, they inspire innovative biofabrication approaches that offer new opportunities for TE and RM by operating outside traditional conceptual boundaries.

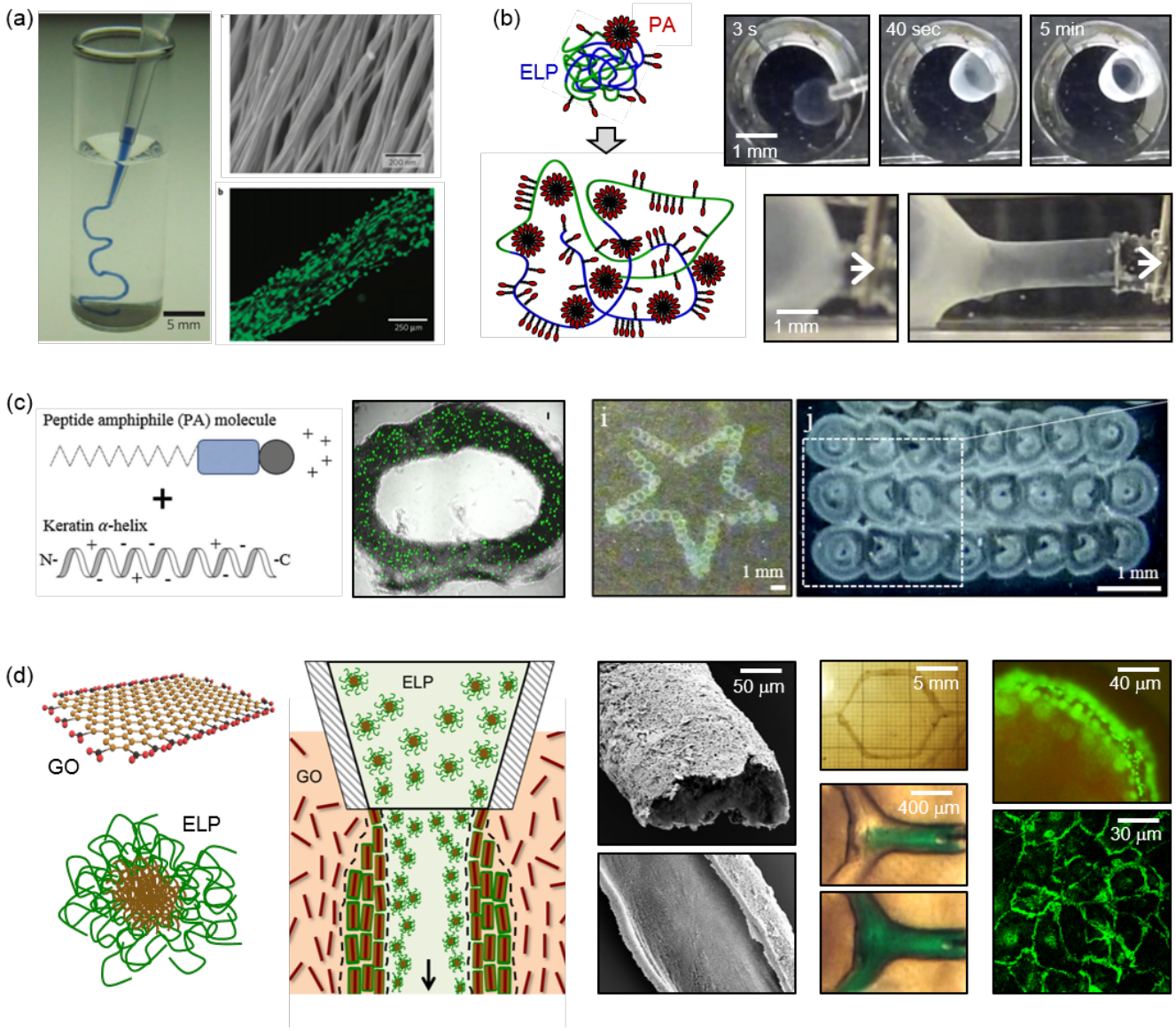

Figure 9 | Supramolecular biofabrication. (**a**) Concurrent assembly and alignment of PA nanofibres to form aligned 'noodle' hydrogels (Reproduced with permission from [74] ©Springer Nature Publishing AG), (**b**) supramolecular assembly of PA with ELP giving rise to tubular hydrogel structures (Reproduced with permission from [145] ©2015 Springer Nature Publishing AG), (**c**) co-assembly of PAs with proteins in a sequential inkjet set-up to form hierarchical 3D structures (Reproduced with permission from [107] ©2018 John Wiley & Sons), and (**d**) co-assembly of ELP with graphene oxide in an extrusion set-up leading to perfusable self-assembling fluidic devices ([147] @ 2019 Springer Nature Publishing AG).

5. Cellular self-assembly-driven biofabrication

Until now, we have concentrated our discussion on SABs based on either natural or synthetic molecules. However, cells alone can serve as self-assembling building-blocks of larger structures such as organoids or tissue spheroids. This section highlights biofabrication studies at the interface between bioassembly, bioprinting, and self-assembly. The general idea is to exploit the inherent need for cells to interact and communicate to prepare spheroids and assemble them using external stimuli such as fluid movement, physical confinement, or mechanical placement. For example, Bulanov *et al.* developed a method capable of positioning

individual spherical tissue spheroids within a collagen matrix to allow tissue fusion and maturation [148,149]. Manning *et al.* used agarose-based moulds to create defined shapes of microtissues, such as toroidal or honeycomb, which were then stacked using a free-fall chamber to allow tissue fusion [150]. In another example, aggregated cell strands were used as an extrusion ink, thereby creating layer-by-layer structures of aggregated cell tubes, which over time mature to form solid tissue blocks [151]. Alternatively, spherical microtissues can be injected into a pre-fabricated porous support structure to permit tissue fusion across the inert scaffold [152]. The same method can also be used with a suspension of individual cells, removing the need to pre-fabricate microtissues [153,154]. In contrast, non-contact methods such as using magnetic fields are being explored as less invasive methods of assembly. For example, by dispersing magnetic particles in an alginate/cell solution, toroidal bundles can be fabricated from magnetic fibres which fuse through cellular driven assembly [155,156]. More recently, it has been shown that magnetic levitation can be used to controllably assemble single cells into constructs without the presence of additional materials [137,157,158]. This method permits the organization of multiple cell types within a microscale structure without direct contact. A recent study by Kingsley *et al*. used laser-based bioprinting to controllably fabricate microcapsules of cells within an alginate-chitosan shell [159]. In this way, the authors are able to create arrays of cellular microbeads with cells aggregating to fill each sphere.

In this topical review, we have focused our discussion on systems that are based on the use and manipulation of organic molecules or cells. However, it is important to keep in mind that self-assembly can also be exploited to grow and fabricate hierarchical structures based on inorganic components. We refer the interested reader to other review articles where these approaches have been thoroughly discussed [5,160–162].

6. Conclusion and future trends

The success of tissue engineering and regenerative medicine relies on the ability to recreate the structures and functions of biological systems. In this regard, biofabrication is playing an increasingly important role. The possibility to biofabricate with the capacity to manipulate and control the assembly of biomolecules and nanostructures into functional hierarchical structures is an exciting one. In this review, we have demonstrated that by combining biofabrication and self-assembly, a variety of opportunities are emerging where the advantages of one approach are helping to overcome the limitations of the other. We propose that through this strategy it is possible to enhance conventional bioprinting methods, expand the traditional biofabrication tool-box, and develop new ways of thinking about building, fabricating, and growing more biologically relevant and functional structures (**Figure 10**).

We have featured methods that integrate self-assembly with biofabrication to create structures with unprecedented hierarchy that expand from the precise presentation of molecular signals to the creation of anatomical geometries. **Table 2** provides an overview of the main highlighted examples summarising key advantages arising from either the self-assembly side or the biofabrication side. These approaches also enable enhanced biomimicry, molecular versatility, communication with cells, and overall bioactivity. However, there are also important challenges to overcome, such as the capacity to self-assemble immediately robust structures, high costs, and scalability constraints. Nonetheless, given the need to better recreate the distinctive molecular, structural, and cellular complexity of biology, we envision that self-assembly will continue to be integrated with biofabrication through both emerging self-assembling platforms

as well as enhanced printing methods. For example, the ability to inkjet print within complex environments enabling simultaneous extrusion and growth of self-assembling structures [147] could significantly enhance resolution, bioactivity, and level of biomimicry. Another important step will likely come from improved self-assembling systems that enhance structural integrity for example through the addition of host-guest interactions [163], modulation of mechanical properties via interactions between different components through non-covalent [164] or covalent co-assembling processes [165].

Overall, advances in recombinant technologies, nanotechnologies, and supramolecular chemistry, as well as, a growing understanding of fundamental processes emerging from fields such as structural and systems biology are likely to continue enhancing the integration of these two approaches and accelerating incorporation within industrial manufacturing processes.

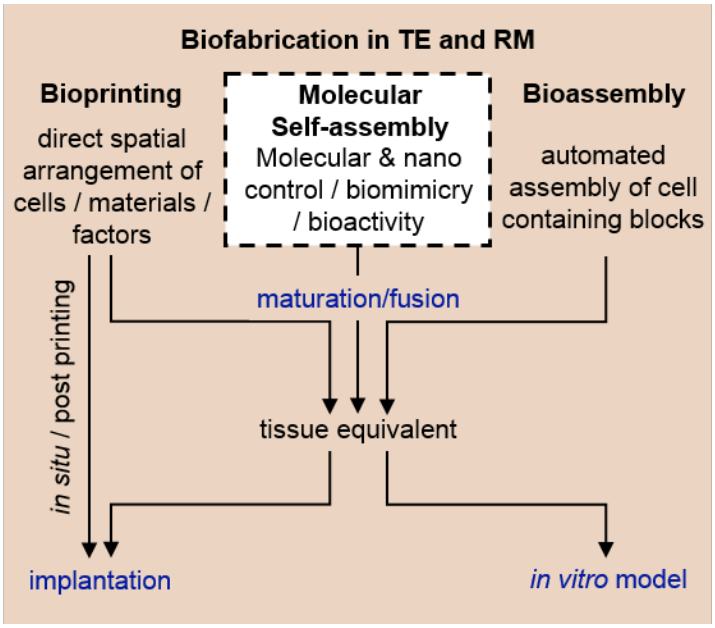

Figure 10 | Self-assembly: An emerging field within biofabrication. Schematic representation of the two established fields within biofabrication (Reproduced with permission from [8] ©2016 IOP Publishing), with the addition of a bridging third field integrating self-assembly and biofabrication.

Table 2: Examples benefiting from advantages provided by both biofabrication and self-assembly

Biofabrication	Self-assembly	Integration	Advantages biofabrication	Advantages self-assembly	Reference
Extrusion	Guest / host moieties	Extrusion within a hydrogel	Complex patterns within a hydrogel/ Free standing structures	Permits the printing of a hydrogel within a hydrogel through reversible non-covalent bonds	[93,94]
	PA / thiolated gelatin	Immersion in bath of PA	Reproducible grid-matrix for experimental consistency	Bioactive hydrogel with functionalized sequences	[91]
	ELP / GO	Extrusion within a solution	Defined internal 3D prism structures	Formation of reproducible tubular structures	[150]
Inkjet	DNA / polypeptide	Sequential printing	Fabrication of 3D structures with up to 20 defined layers	Integration of DNA in the printed material	[105]
	PA / PBS	Sequential printing	Formation of multidomain hydrogels with spatially defined cell positioning	Reversible formation of secondary molecular structures	[101]
	Polymer / silica	Sequential printing	Precise placement of microgels down to 15 µm diameter	Embed particles/selective presentation and density of binding sites	[109]
	PA / protein	Inkjet into a liquid	Precise positioning of microgels creating 2D/3D structures	Macromolecular versatility and ability to control the nano-micro structure	[107]
Electrospinning	PA / PCL	Coating post electrospinning	Reproducible and structurally stable fibres	Fibrous network and surface display of cell adhesive sequence	[123]
	PA	Spinning with genipin and organic solvent	Bundling of polymer microfibres	Nanofibers shifting from random to aligned	[121]
	Polypeptide	Spinning in aqueous solution	Spinning into durable tubes	Selective presentation of binding sites and biomimetic sequences	[120]
Laser-assisted	Cellular	LDW with hydrogel incl. cells	Controllable matrix array, cell density and positioning	Ability to go from independent units to functional bodies with natural maturation	[127]

Self-assembly-driven fabrication	Material	Advantages assembly method	Advantages material	Reference
Shear	PA / PBS	Ability to bundle and align microfibres and tube formation by constraint	Assembly into microfibres	[132, 134]
Magnetic	Polymer-based hydrogels	Cell friendly and touch-free organisation of microgels	Compatibility with a range of materials + uses magnetism of cells directly	[137, 138]
Liquid - liquid attraction / Immiscibility	Polymeric solution	Cell friendly and touch-free organisation of microgels	Complex shapes through delicate interactions	[136, 140]
Supramolecular	PA/ELP	Self-driven assembly into a tubular shape	Selective presentation and density of epitopes	[145]

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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