Minireview

Alginate microfibers as therapeutic delivery scaffolds and tissue mimics

Yubing Xie, Sujith Chander Reddy Kollampally, Matthew Jorgensen and Xulang Zhang

College of Nanoscale Science and Engineering, SUNY Polytechnic Institute, Albany, NY 12203, USA
Corresponding author: Yubing Xie. Email: xiey@sunypoly.edu

Impact Statement

Alginate hydrogel plays an important role in three-dimensional (3D) cell culture, cell encapsulation and transplantation, tissue engineering, and regenerative medicine. This work is a timely review of the fast-growing field of alginate hydrogel microfiber technology. It provides a concise but complete overview of the current state of the art of microfluidic synthesis of alginate hydrogel microfibers and their applications in 3D cell culture, cell delivery, and tissue engineering, in the context of historical review of alginate and its resultant hydrogel. The knowledge of what has been done, what can be done, and what needs to be done will further advance microfiber technology in biology and medicine, and promote multidisciplinary, collaborative, and convergent research in the field.

Abstract

Alginate, a naturally occurring polysaccharide, has been widely used in cell encapsulation, 3D culture, cell therapy, tissue engineering, and regenerative medicine. Alginate's frequent use comes from its biocompatibility and ability to easily form hydrogel in a variety of forms (e.g. microcapsules, microfibers, and porous scaffolds), which can provide immunoprotection for cell therapy and mimic the extracellular matrix for tissue engineering. During the past 15 years, alginate hydrogel microfibers have attracted more and more attention due to its continuous thin tubular structures (diameter or shell thickness $\leqslant$ 200 μm), high-density cell growth, high handleability and retrievability, and scalability. This review article provides a concise overview of alginate and its resultant hydrogel microfibers for the purpose of promoting multidisciplinary, collaborative, and convergent research in the field. It starts with a historical review of alginate as biomaterials and provides basics about alginate structure, properties, and mechanisms of hydrogel formation, followed by current challenges in effective cell delivery and functional tissue engineering. In particular, this work discusses how alginate microfiber technology could provide solutions to unmet needs with a focus on the current state of the art of alginate microfiber technology and its applications in 3D cell culture, cell delivery, and tissue engineering. At last, we discuss future directions in the perspective of alginate-based advanced technology development in biology and medicine.

Keywords: Alginate, hydrogel, microfiber, 3D culture, stem cell, cell delivery, tissue engineering

Experimental Biology and Medicine **2022; 247: 2103–2118. DOI: 10.1177/15353702221112905**

Introduction

Alginate is usually referred to the salt of alginic acid, mostly sodium alginate, or alginic acid itself. The majority of commercially available alginates are typically extracted from brown algae although alginate can also be produced through biosynthesis. Alginate is a biocompatible, enzyme-degradable, and versatile biopolymer. Sodium alginate has a unique property to form hydrogel once it encounters polyvalent cations, such as Ca^{2+}, forming calcium alginate hydrogel (usually simplified as alginate hydrogel), which is typically optically clear, simple to make, and easy to use.[1,2] Both sodium alginate and calcium alginate are on the list of Food and Drug Administration (FDA) Generally Recognized As Safe (GRAS) food substances, which have no significant toxicological effects from oral administration and also used as the excipient (pharmacologically inactive substance) in drugs.[3,4] Due to their biocompatibility, mechanical stability, high permeability, and processability, versatile alginate hydrogels (e.g. microbeads/microcapsules, microfibers/microtubes, fibrous matrices, and porous scaffolds) have been widely utilized in 3D cell culture, cell delivery, and tissue engineering, which shows great potential for cell therapy and regenerative medicine. For the past 20 years, alginate-related papers have increased exponentially as shown in PubMed (Figure 1(A)).

We start this article with a historical review of alginate as biomaterials and discuss the properties of alginate and alginate hydrogel in general. After summarizing current challenges in cell transplantation and tissue engineering, we focus on alginate hydrogel microfiber technology and discuss how the tubular-structured alginate hydrogel addresses needs in effective cell delivery and functional tissue engineering, followed by future perspectives.

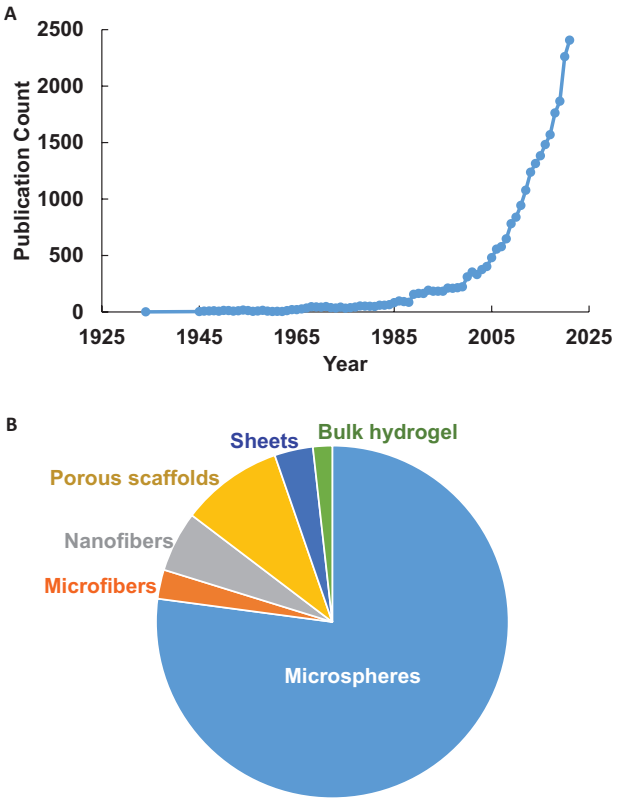

Figure 1. Survey of alginate-related papers in PubMed. (A) Increasing number of alginate-related papers published each year. (B) Spherical alginate hydrogels (e.g. microcapsules, microbeads, microspheres, microparticles, and microcarriers) dominating their biological and biomedical applications in comparison to alginate microfibers (e.g. microfibers, microtubes, microstrands, and microribbons), nanofibers, porous matrices or scaffolds, hydrogel sheets, and bulk hydrogels. (A color version of this figure is available in the online journal.)

Historical review of alginate as biomaterials

As shown in Figure 2, alginic acid was first discovered and extracted from brown algae by Stanford in 1881[5] and proposed as therapeutics by MacLennan in 1898.[6] Early work studied chemistry and crystalline structures of alginic acid,[7,8] showed decomposition of alginic acid by bacteria,[9] and demonstrated bacterial biosynthesis of alginic acid.[10] Early biomedical applications of alginic acid mainly focused on using alginate for dental impressions,[11] absorbable surgery products,[12] immunization/antigen depot,[13] or inhibition of intestinal absorption of radioactive strontium.[14,15] Starting in 1970s, alginate was used for immobilization of enzymes and microorganisms,[16] plant cells,[17] algae "living" electrodes as a photoconverter,[18] and animal cells for the production of antibodies,[19] in addition to being served as food additives.[20]

Almost one century after the discovery of alginic acid, Lim and Sun invented alginate microencapsulation in 1980, by which cells (e.g. insulin-secreting islets) mixed with low-viscosity alginate solution were introduced to Ca^{2+} solution (e.g. $CaCl_2$) dropwise to form alginate hydrogel microbeads, followed by coating with poly-L-lysine through polyelectrolyte complexation and liquefied by sodium citrate, leaving core–shell-structured alginate/polylysine microcapsules containing islets.[21] It demonstrated that microencapsulated

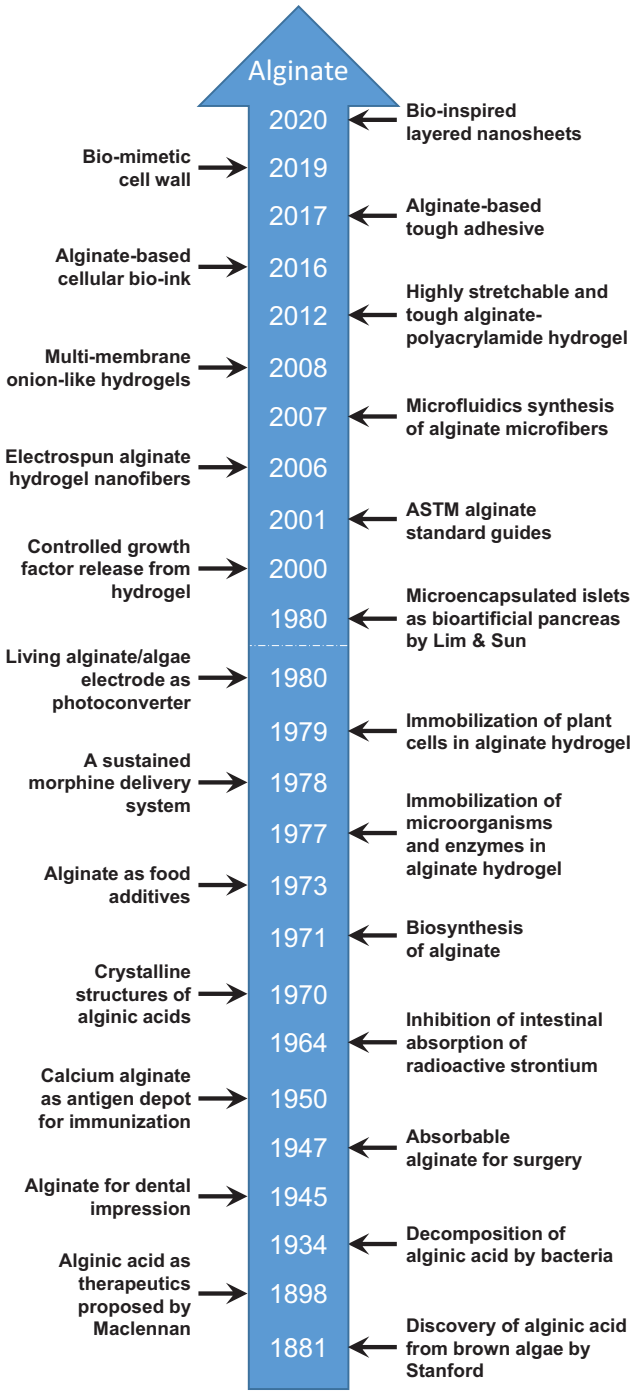

Figure 2. Timelines of the key alginate-based biotechnology developments. The white dashed line indicates the start of a new era of alginate as biomaterials for 3D cell culture, cell therapy, and tissue regeneration. (A color version of this figure is available in the online journal.)

islets maintained morphology and function during a 15-week *in vitro* culture and corrected diabetic state for 2–3 weeks after *in vivo* implantation in rat.[21] Although this milestone work stemmed from the concept of "Artificial Cell" in the form of semipermeable microcapsules, which were proposed by Chang in 1964,[22] it opened up a new era for using alginate hydrogels for cell therapy and tissue regeneration. The clinical benefits of alginate encapsulation systems were evidenced by maintaining tight glycemic control in a diabetic

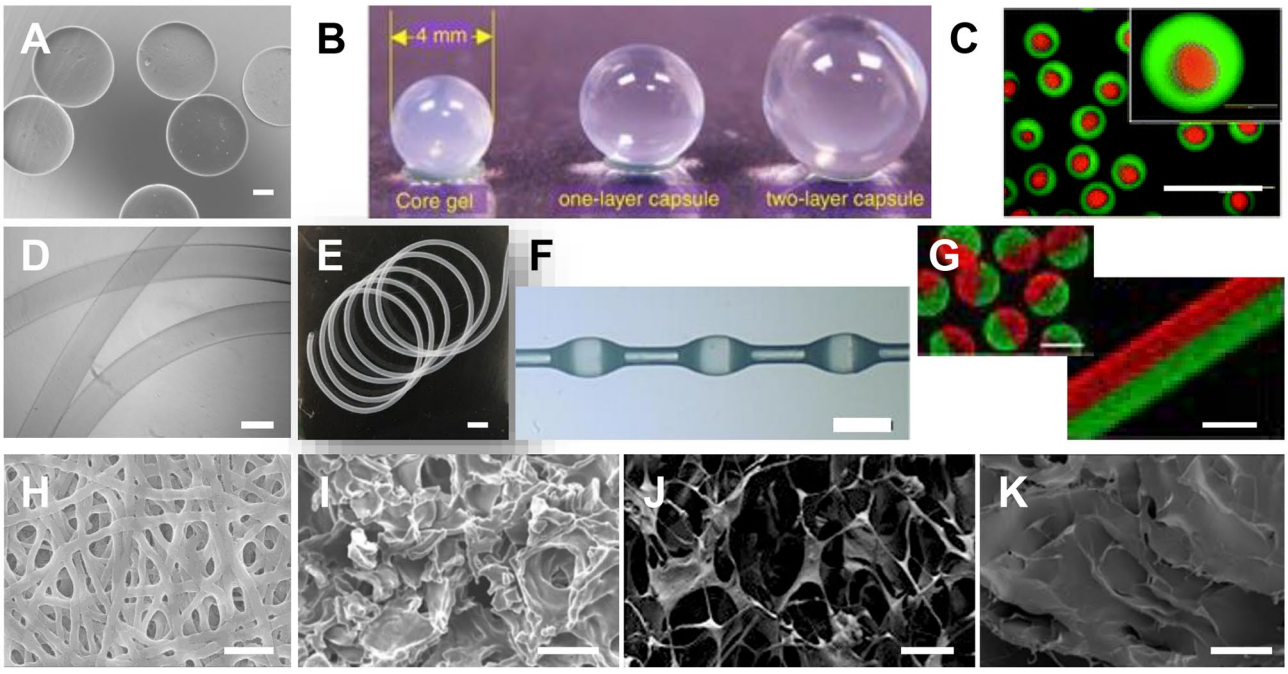

Figure 3. Representative versatile alginate hydrogel systems. (A) Optical image of alginate microcapsules. Scale bar = 100 µm. (B) Photo of alginate core (left), one-layer (middle), and two-layer onion-like hydrogel capsules (right).[28] (Adapted from Zarket and Raghavan, *https://creativecommons.org/licenses/by/4.0/*.) (C) Confocal image of multicompartmental alginate microparticles.[30] Scale bar = 1000 µm. (Reproduced from Lu *et al.* with permission from the Royal Society of Chemistry.) (D) Optical image of alginate hydrogel microfibers. Scale bar = 300 µm. (E) Photo of the preformed alginate microtube.[34] Scale bar = 4000 µm. (Adapted from Jorgensen *et al.*) (F) Optical image of the cavity microfiber.[35] Scale bar = 400 µm. (Adapted from Tian *et al.*, *https://creativecommons.org/licenses/by/4.0/*.) (G) Confocal image of multicompartmental microfibers.[36] Insert: Cross-sectional view. Scale bar = 200 µm. (Adapted from Cheng *et al.*, *Copyright © 2016 American Chemical Society*.) (H) Scanning electron microscopy (SEM) image electrospun alginate nanofibers. Scale bar = 1 µm. (I) SEM image of the IPN hydrogels composed of 15% PF127/0.25% alginate.[38] (Adapted from Chou *et al.*) (J) SEM image of alginate cryogel.[39] (Adapted from Bencherif *et al.*) (K) SEM image of bioprinted alginate hydrogel with minimal porosity.[41] (Adapted from Chaji *et al.*, *https://creativecommons.org/licenses/by/4.0/*.) (I–K) Scale bar = 100 µm.

patient for 9 months,[23] providing immunoprotection of human islet cells in four diabetic patients for three years,[24] and improving glycemic controls in eight diabetic patients for over 20 months even with encapsulated clinical grade porcine islets that could alleviate the shortage of donated human organs.[25] It leads to wide applications of alginate hydrogels for biomedical research.

To accommodate quick growth of the field, American Society for Testing and Materials (ASTM) provided ASTM alginate standard guides in 2001.[26] From PubMed, we have found that the majority of alginate hydrogels are spherical in shape (Figure 1(B)), including microcarriers, microspheres, microbeads, microcapsules (Figure 3(A)), onion-like multimembrane hydrogels[27] (Figure 3(B)),[28] and multicompartment microparticles[29] (Figure 3(C)).[30] The ability to fabricate multicompartment alginate hydrogels has enabled the development of alginate microbots that can be driven by rotating magnetic fields.[31] In addition, alginate hydrogel microfibers[32] or microstrands (Figure 3(D)), co-shell microfibers[33] or hollow microtubes (Figure 3(E)),[34] cavity microfibers (Figure 3(F)),[35] and multicompartment microfibers (Figure 3(G))[36] have been synthesized through microfluidic spinning and attracted more and more attention for the last 15 years. To mimic extracellular matrix (ECM), electrospun alginate nanofibers[37] (Figure 3(H)), interpenetrating polymeric network (IPN) hydrogels (Figure 3(I)),[38] cryogel (Figure 3(J)),[39] cryoelectrospun nanofiber sponge scaffolds,[40] and bioprinted hydrogel[41] (Figure 3(K)) were fabricated as well.

To simulate biological cues in ECM microenvironment, controlled release of growth factors from alginate hydrogel has been developed,[42] followed by on-demand delivery of macromolecules or cells controlled by magnetic,[43] mechanical, light, temperature, chemical, or biological triggers. With advancement in bioinspired nanotechnology and hydrogel engineering,[44] alginate-based biomaterials have been explored to design and develop biomimetic and advanced biomaterials, including onion-like, inside-out shape-morphing hydrogels,[27,28] spindle-knot microfibers for water collection,[35] adhesives that work in wet environments,[45] biomimetic cell wall,[46] layered nanosheets,[47] and bioinks,[48] highly stretchable, tough alginate composite hydrogels,[49] soft stretchable electronics,[50] and photonics.[51]

Chemical structure and properties of alginate

Chemical structure of alginate

Alginic acid is a naturally occurring, anionic polysaccharide. It is a linear copolymer, consisting of blocks of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues.[52] Sodium alginate aqueous solution has the unique feature to form alginate hydrogel on encountering divalent solution cations, such as Ca^{2+}. It is believed that G-blocks of alginate crosslink with divalent cations, such as Ca^{2+}, to form the alginate hydrogel, described by a prevailing simple "egg-box" model (Figure 4), which is true for initial association

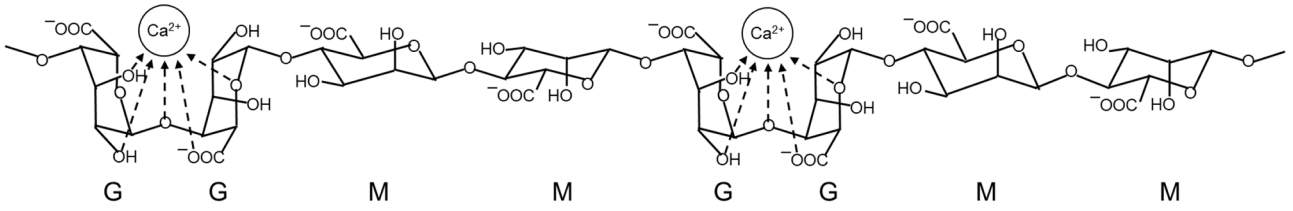

Figure 4. A simple egg-box model of calcium alginate hydrogel. G: α-L-guluronate residue; M: β-D-mannuronate residue. (A color version of this figure is available in the online journal.)

Table 1. Overview of alginate sterilization methods.

Sterilization methods	Alginate solution or powder	Alginate hydrogel
Sterile filtration	Filtration with ⩽ 0.22 μm filter: Time-consuming; reduced viscosity	—
Sterile filtration + lyophilization	Filtered 1% alginate through a 0.2 μm filter/frozen at –80°C and lyophilized for 48 h at 11 μbar: Maintained Mw and printability	—
Ethanol disinfection	—	Treated with 70% ethanol for 20 min: Maintained structural and mechanical properties of hydrogels; sufficient to eliminate bacteria persistence
Lyophilization	—	Freeze-dried for 36 h: Impacted hydrogel structure and mechanical properties
Autoclave	15 min at 121°C Reduced alginate Mw and viscosity	15 min at 250°C and 15 psi: Visual and structural defect; increased hydrogel stiffness
γ-irradiation	Degradation of alginate; reduced Mw; decreased viscosity	Degradation of hydrogel
Ethylene oxide gas	Residual ethylene oxide gas that is potentially carcinogenic and toxic for *in vivo* applications	
Ultraviolet (UV) irradiation	Alginate powder under a UV lamp at 254 nm, 2 cm distance for 1 h: Maintained printability of bioink; ineffective for sterilization	20 min per side at 250 nm in a biosafety cabinet: Maintained mechanical properties; insufficient to eliminate bacteria persistence

and certain conditions (such as low fractional Ca^{2+} saturation and low G content).[53] Other models include description of further lateral association of chain segments for other conditions (such as high Ca^{2+} saturation and high G content)[54] and two-component, broken rod-like model for competitive ligand exchange of chelated Ca^{2+} gelation.[55]

Solubility of alginic acids and its salts

Alginic acid is water-insoluble, but can be dissolved in sodium chloride solution (e.g. physiological saline – 0.9% NaCl), forming sodium alginate. Monovalent salts of alginic acid (sodium alginate, potassium alginate, and ammonium alginate) and propylene glycol alginate are soluble in water while polyvalent salts are insoluble or form hydrogels. All salts of alginic acids are insoluble to oils or organic solvents, except the tetrabutylammonium (TBA) salt of alginic acid that is soluble in water and ethylene glycol, and polar aprotic solvents, such as N,N-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), dimethylacetamide (DMAc), and 1,3-Dimethyl-2-imidazolidinone (DMI) in the presence of TBA fluoride trihydrate (TBAF).[56] The solubility of alginates in water depends on the structure of the biopolymer, viscosity, temperature, pH, and ionic strength of the dissolving medium. For example, it will be a little quicker to dissolve low-molecular weight and low-viscosity alginate in hot water, at neutral pH in the presence of 0.9% NaCl.

Molecular weight and viscosity of alginate

The molecular weight of commercially available sodium alginates ranges between 10,000 and 600,000 g/mol. The concentration of alginate used to form hydrogels is usually in the range of 0.5–5% (wt/vol). The pH of alginate solution for encapsulation of biomacromolecules or cells is usually maintained at 7.0–7.4, using physiological saline (0.9% NaCl) to make alginate solution or mixing alginate solution with cell culture media.

Viscosity is one of the important properties of alginate. The viscosity of alginate solution increases with increasing molecular weight and concentration of alginate, or decreasing pH, reaching a maximum viscosity at pH 3–3.5. Low-viscosity alginate is desirable for cell encapsulation or microfluidic processing, and therefore, alginate with low viscosity (e.g. 20–200 cp or 150–250 cp, 2%, 25°C) is usually used for making alginate hydrogel microbeads, microcapsules, microfibers, and microtubes. Medium-viscosity alginate (e.g. ⩾ 2000 cp, 2%, 25°C) is typically used for making electrospun nanofiber scaffolds or highly stretchable hydrogels in combination with other polymers, for example, polyethylene glycol (PEG) or polyacrylamide (PAAm) to increase electrospinnability or stretchability. Sterilization techniques, such as γ-irradiation,[57] autoclave, and filtration, through 0.22 μm membrane would decrease viscosity of alginate (Table 1).

Formation and properties of alginate hydrogels

Sterilization of alginate solution or alginate hydrogel

Methods for sterilizing the alginate solution or disinfecting alginate hydrogels are summarized and compared in Table 1.

Filtration through a filter (0.22 μm or less) in a biosafety cabinet was traditionally used to sterilize alginate solution for cell encapsulation (e.g. alginate concentration ≤ 1.5%). For sterilization of alginate bioink, filtration of alginate solution at even lower concentration (e.g. 1% alginate) followed by lyophilization was recommended.[58] Treatment with 70% ethanol was recommended for disinfection of alginate hydrogels.[59] Autoclave and γ-irradiation could sterilize alginate or its hydrogel, which, however, would reduce the molecular weight (Mw) and viscosity of alginate and cause structural defect of alginate hydrogels.[58–60] Ethylene oxide gas could sterilize alginate solution, powder, or hydrogel, but residual ethylene oxide gas is potentially carcinogenic and toxic, raising the concern for *in vivo* implantation and clinical applications. UV irradiation would not be an effective way to eliminate bacterial persistence at all.[58,59]

Gelation mechanisms and methods to form alginate hydrogels

Alginate hydrogels can be formed by ionic crosslinking, covalent crosslinking, photo crosslinking, free-radical polymerization, and cryogelation, and cell crosslinking.

Ionic crosslinking is the most common method since alginate has the ability to form hydrogel instantly in contact with polyvalent cations, for example, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Ce^{2+}, Co^{2+}, Cu^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+}, and Zn^{2+}. The crosslinking strength depends on the type of cations, G–M content, and sequence of alginate. For example, G-blocks of alginate displayed binding strengths as follows: $Ba^{2+} > Sr^{2+} > Ca^{2+} >> Mg^{2+}$, whereas for M blocks, $Ba^{2+} > Sr^{2+} \sim Ca^{2+} \sim Mg^{2+}$, and for alternating GM sequences, no significant differences between these ions.[30] More general, the affinity of alginate toward divalent cations was from high to low as follows: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ba^{2+} > Sr^{2+} > Ca^{2+} > Co^{2+}, Ni^{2+}, Zn^{2+} > Mn^{2+}$.[61] Even though Ca^{2+} does not exhibit the strongest binding to alginate, Ca^{2+}, in particular $CaCl_2$, is still the most widely used crosslinking cation for alginate gelation due to its relatively strong bridging interaction with alginate and non-toxicity. The gelation rate increased with increasing Ca^{2+} concentration, increasing temperature, and decreasing alginate concentration.[62] However, slower gelation could generate more uniform and mechanically stronger alginate hydrogels than faster gelation, which could be achieved by reducing Ca^{2+} concentration, lowering temperature, and increasing alginate concentration.

To control the gelation rate and form large-scale homogeneous hydrogels, less-soluble $CaSO_4$ or $CaCO_3$, or chelate complexes (e.g. Ca-ethylenediaminetetraacetic acid [CaEDTA] and Ca-ethyleneglycol tetra-acetic acid [CaEGTA]) were used as internal Ca^{2+} sources, triggered by hydrolysis of glucono δ-lactone (GDL) to provide a source of H^+ that dissolves solid $CaSO_4$ or $CaCO_3$, or substitute Ca^{2+} in chelate complexes.[55] "Caged calcium" held in photo-labile organic molecules was also used to release Ca^{2+} internally triggered by light.[63] In addition, the thermosensitive network of pluronic F127 (PF127) was used as a template to form Ca^{2+} crosslinked alginate IPN hydrogels, which were soft, elastic, and thermoresponsive.[38]

Ionically crosslinked alginate hydrogels tend to lose its mechanical integrity in a physiological environment due to exchange of polyvalent cations (e.g. Ca^{2+}) with monovalent cations (e.g. Na^+ and K^+) and association with phosphate groups in buffers, media, or body fluids. Therefore, covalent crosslinking was tried to improve mechanical properties of alginate hydrogels, using polymers.[64] In particular, alginate hydrogel that was both ionically crosslinked by Ca^{2+} and covalently crosslinked with long-chain PAAm exhibited high stretchability (> 20 times of elongation) and toughness (fracture energies of 9 kJ m^{-2}).[49] Further increasing alginate concentration while maintaining low viscosity (using both short- and long-chain alginate) and increasing crosslink density (using both $CaSO_4$ and $CaCl_2$ as crosslinkers) enhanced toughness (fracture energies of ~16 kJ m^{-2}) without jeopardizing the stiffness (maintaining constant elastic modulus of 1 MPa).[65]

Alginate can be chemically modified with methacrylate and covalently crosslinked under an argon laser or UV irradiation. Both covalent crosslinking and photo crosslinking could slow down gelation rate and provide stable covalent binding for tighter control over stability and mechanical properties of alginate hydrogel compared with ionic crosslinking; however, covalent-crosslinking reagents or photo-crosslinking by-products might be toxic, and unreacted chemicals may need to be removed thoroughly from alginate hydrogels.[66]

Free-radical polymerization has been used to prepare stimuli-responsive poly(N-isopropylacrylamide) (PNIPAM)/alginate semi-IPN hydrogel, which is sensitive to the temperature, pH, and ionic strength of swelling medium.[67] The semi-IPN structure was also generated using *in situ* copolymerization of N-isopropylacrylamide (NIPAAm) with poly(ethylene glycol)-*co*-poly(ε-caprolactone) (PEG-co-PCL) in the presence of alginate under UV irradiation, whose swelling ratio increases with alginate concentration at a constant temperature and decreases with the increase in temperature.[68]

Cryogelation, which is the process to form cryogels or porous hydrogel scaffolds under freeze-drying or freeze-thawing,[69] has also been used for gelation of alginate. For freeze-drying gelation, freshly formed alginate hydrogel is exposed to freezing condition to induce the growth of ice crystals, followed by vacuum drying to eliminate these ice crystals. For freeze-thawing gelation, freezing of alginate solution results in solvent crystallization that drives alginate polymer network crosslinked around ice crystals, and then subsequent thawing will leave behind alginate hydrogels with interconnective macroporous structures. For example, porous alginate hydrogel scaffolds can be fabricated by freeze-drying (freezing alginate solution that was immersed in aqueous ethanol solution of $CaCl_2$ at −20°C/drying at room temperature),[70] induced by freeze-thawing at low pH (freezing at −25°C/thawing at 4°C, pH 2–4).[71] Alginate blends with gelatin, hyaluronic acid, and polyvinyl alcohol (PVA) can also form cryogel through freeze-thawing.[72]

Mammalian cells can also crosslink with alginate that is modified with cell adhesion ligands (e.g. arginine–glycine–aspartic acid [RGD] peptides) to form hydrogel network structure even in the absence of any other crosslinker.[73] This cell-crosslinked gelation is governed by weak ligand–receptor interaction and reversible by applying shear force, making it ideal for cell delivery. For example, during injection,

Table 2. Gelation mechanisms to form alginate hydrogels.

Mechanisms	Pros	Cons
Crosslinking		
Ionic	Instant gelation; diverse crosslinkers	Loss of mechanical integrity
Covalent	Controlled gelation; stable binding	Potential toxicity of unreacted chemicals
Photo	Slow gelation; tight control over stability	Potential toxicity of photo-induced by-products
Cell	No need of other crosslinker; reversible	Need of modifying alginate with ligands
Free-radical polymerization	Temporal and spatial control of gelation; ease to incorporate a variety of chemistries	Complicated chemical reactions and use of organic solvent(s); toxicity of photoinitiator
Cryogelation	Highly interconnected porous structure	Low structural stability and other weak mechanical properties

the cell–alginate mixture will become fluid under flow shear while solidifying into hydrogel after injection into the body.[66] Cell-crosslinking gelation is also useful for encapsulation of cells that are sensitive to Ca^{2+}.

Advantages and disadvantages of these gelation methods are summarized in Table 2. The gelation mechanism can be chosen alone or combined based on desired hydrogel properties (e.g. stability, porosity, and mechanical properties) and applications (e.g. *in vitro* cell culture, *in vivo* cell delivery, and tissue engineering).

Chemical modification and alginate derivatives

Alginate is negatively charged at neutral pH due to the presence of a significant amount of carboxyl groups, and therefore, can interact with positively charged macromolecules (e.g. poly-L-lysine, chitosan) through polyelectrolyte complexation or crosslinking, for example, forming alginate-poly-L-lysine microcapsules,[21] alginate-poly-L-lysine microtubes,[74] or an alginate-chitosan membrane,[75] with altered permeability, mechanical properties, and biocompatibility compared to their counterparts of microbeads or microfibers.

Alginate can be chemically modified through its two secondary hydroxyl (-OH) positions (C2 and C3) and carboxyl (-COOH) position (C6) on the polysaccharide backbone, with improved properties. The reaction to synthesize alginate derivatives includes acetylation, phosphorylation, sulfation, oxidation, hydrophobic modification by covalent attachment of hydrophobic moieties (e.g. long alkyl chains, aromatic groups, and hydrophobic polymers), functionalization with cell-signaling moieties (e.g. 1-amino-1-deoxygalactose, RGD), covalent crosslinking, and graft copolymerization.[61] The chemical derivatization could improve or tune alginate properties, such as improved alginate solubility and hydrophobicity, tuned mechanical properties, stability, and degradability of alginate hydrogels, enhanced capacity for cell adhesion and growth, and increased biocompatibility.

Stability and mechanical strength of ionic-crosslinked alginate hydrogel

The stability and physical properties (e.g. pore size, porosity, swelling ratio, and mechanical properties) are important factors for applying alginate hydrogel in biomedical fields. The M/G ratio, sequence, G-block length, molecular weight and concentration of alginate, concentration of crosslinking cation, and gelation temperature affect physical properties of alginate hydrogels. It demonstrated that the mechanical strength of alginate hydrogel increased with alginate concentration, total Ca^{2+} content, molecular weight, and G content of the alginate.[62] Gelation at low temperature could slow down crosslinking, and therefore, increase crosslinking degree and increase stability and mechanical properties of alginate hydrogel.[62] Ba^{2+} was used to increase the stability of calcium alginate hydrogel, but potential toxicity at high concentration should be avoided.[76]

Although increasing the molecular weight of alginate can improve the mechanical properties of alginate hydrogels, high-molecular-weight alginate solution is associated with high viscosity. As mentioned early, for cell encapsulation and microfluidic synthesis of microfibers, low-viscosity alginate is desirable. The viscosity of alginate solution depends on both the concentration and chain length of alginate. One strategy is to manipulate the molecular weight and its distribution by combining high- and low-molecular-weight alginate,[65] which could produce alginate hydrogel with significantly increased elastic modulus while causing minimum increase in the viscosity of alginate solution.[77]

Biocompatibility of alginate hydrogel

Alginate–poly-L-lysine microcapsules have been initially designed to provide immunoprotection to encapsulated cells and prevent immune and autoimmune responses.[21] Biocompatibility of alginate hydrogel depends on alginate composition (G content versus M content), purity, crosslinking ions (Ca^{2+} versus Ba^{2+}), and polyelectrolyte membrane coating (poly-L-lysine versus poly-L-ornithine).[78] Although ultrapure alginate with defined G/M ratio could be chosen for cell encapsulation and implantation, the cellular overgrowth and fibrosis would often occur, which were caused by foreign body reaction against cell-containing alginate hydrogels, in particular, when using xenogeneic cells.[79] Chemically modified alginate derivatives containing antifouling triazole[79] or zwitterionic groups[80] were employed to improve alginate biocompatibility, mitigate cellular overgrowth and fibrosis, and prevent from foreign body response against cell-containing microcapsules.

Challenges in therapeutic cell delivery and tissue engineering

Needs of long-term survival, integration, and function *in vivo*

Cell transplantation is a procedure to transfer cells or stem cells, such as hematopoietic stem cells, mesenchymal stem

cells (MSCs), and induced pluripotent stem cells (iPSCs), to a damaged or diseased tissue. It holds promise for regenerative medicine to correct diseases that are traditionally incurable and regenerate tissues that typically do not regenerate. However, major challenges for clinical translation of cell transplantation lie in poor cell survival, impaired migration, and extremely poor engraftment into the host tissue.[81–83] After injection of cells in the body, loss of control over transplanted cells occurred, only around 1–20% of transplanted cells survived,[84] migration of cells from injection site was very limited, and in particular, the engraftment rate could be as low as 0.001%, typically $< 3\%$, and no more than 10% of cells engrafted.[85,86] Therefore, there is a great need for improving cell survival and engraftment of transplanted cells to achieve the full potential and therapeutic benefit of cell transplantation.

Alginate hydrogels for improved cell delivery and tissue function

Alginate hydrogels have been used for cell delivery in cell therapy and tissue engineering, demonstrating the potential to improve cell survival and engraftment of transplanted or implanted cells.[87] Alginate is inert and does not have any inherent cell instructive properties. Non-adhesive alginate hydrogels have demonstrated that they supported human iPSC-derived intestinal organoid growth *in vitro* and also supported *in vivo* survival and engraftment at the level comparable to conventional Matrigel.[88] Although Matrigel is widely used for organoid culture, it is an ill-defined ECM substrate that is derived from mouse tumors, and therefore, impractical for *in vivo* cell therapy. Alginate encapsulation has offered a Matrigel-free system for organoid culture and stem cell delivery although the organoid yield from alginate was lower than that from Matrigel.[88]

The alginate hydrogel is amenable for modification to further improve their properties and performance, such as incorporation of adhesive molecules in alginate to improve cell adhesion and function, chemical modification to augment biocompatibility, and blending with other polymers to enhance mechanical properties. For example, RGD-modified alginate hydrogel disks were used as scaffolds for cell encapsulation and transplantation and demonstrated the ability to engineer growing-bone tissue from small numbers of implanted cells,[89] which provides an attractive alternative to encapsulate or grow large numbers of cells prior to transplantation.[90] In particular, co-transplantation of osteoblasts and chondrocytes resulted in increased bone mass, mineral content, and cellularity compared to osteoblast alone,[89] highlighting the importance to recapitulate the cell–cell interaction for functional tissue engineering.

Based on tissue engineering principles,[91] alginate hydrogels can be used to simulate cellular microenvironments, including ECM, soluble factors, and neighboring cells. Alginate hydrogels could mimic biomechanical, adhesive, and topographic properties of ECM, which regulate cell processes, such as spreading, growth, proliferation, migration, lineage specification, differentiation, and organoid formation,[92–94] and therefore, modulate functionality.[95] Alginate-based microfluidic scaffolds could replicate soluble gradients of the 3D biochemical microenvironment.[96] In addition, alginate hydrogels could easily recapitulate cell–cell interactions by mixing two or multiple types of cells in alginate solution followed by gelation.

Alginate hydrogels in the spherical form (e.g. microcapsules, microbeads, microspheres, microparticles, and microcarriers), tubular structures (microfibers, microstrands, microribbons, core–shell microfibers, and hollow microtubes), and porous scaffolds (e.g. electrospun nanofibers, cryogel, and porous scaffolds) have been used for cell delivery and tissue engineering. Although alginate microcapsules demonstrate great potential and therapeutic benefits, alginate hydrogels that can be fabricated into long, thin microfibers or microtubes with a thin wall provide a new avenue for cost-effective, handleable, and retrievable cell delivery and versatile tissue construction. Compared to spherical or bulk hydrogels, tubular-structured hydrogels allow for easy transport of oxygen and nutrients, and therefore, allow for high-density cell growth (e.g. $\geqslant 10^8$ cells/mL) and mass production of cell aggregates or organoids with uniform size. In particular, one long alginate hydrogel microfiber or microtube has higher handleability and retrievability than individual microcapsules or microbeads. In addition, microfibers or microtubes also protect cells from mechanical stress during static or dynamic cell culture.

Alginate hydrogel microfibers for cell delivery and tissue mimics

Microfluidic fabrication of alginate microfibers and microtubes

The major methods to fabricate alginate hydrogel microfibers can be categorized into coaxial microfluidics using glass capillaries,[32,97] rectangular or cylindrical polydimethylsiloxane (PDMS) microfluidic channels,[98] wet spinning,[99] microfluidic spinning,[100] extrusion,[74] and bioprinting,[101] as shown in Figure 5(A) to (D). An array of microfibers has been produced through a microfabricated nozzle array[102] or porous filter-like microchannels.[103]

To make core–shell microfibers or hollow microtubes, double-coaxial or multicoaxial microfluidics (glass capillary[97,104] or microchannel-based),[105] microextruder,[106] and needle-based devices[2,107] were used (Figure 5(E) to (G)). In addition, gas bubble-, oil droplet-, and aqueous droplet-filled microfibers were fabricated using the gas-in-water (Figure 5(H)),[108] oil-in-water (Figure 5(I)),[109] and all-in-water microfluidic system (Figure 5(J)),[110] respectively. Combining multicoaxial microfluidics and a wet spinning process, bamboo-like hybrid microfibers were generated.[111]

Cells can be mixed with alginate or other solution and encapsulated in alginate hydrogel microfibers or core–shell microfibers during microfluidic fabrication. Alternatively, hollow microfibers (i.e. microtubes) were premade, followed by seeding cells inside the microtube through perfusion or injection.[2,112–114] In addition, hollow microfibers with a thin polyelectrolyte complexion membrane were produced by coating alginate microfibers with poly-L-lysine[74] or coating coiled alginate microfibers with chitosan,[115] followed by liquefying the alginate core using sodium citrate solution or using a multicoaxial microfluidic device to form hollow microfibers followed by dissolving away the outer calcium

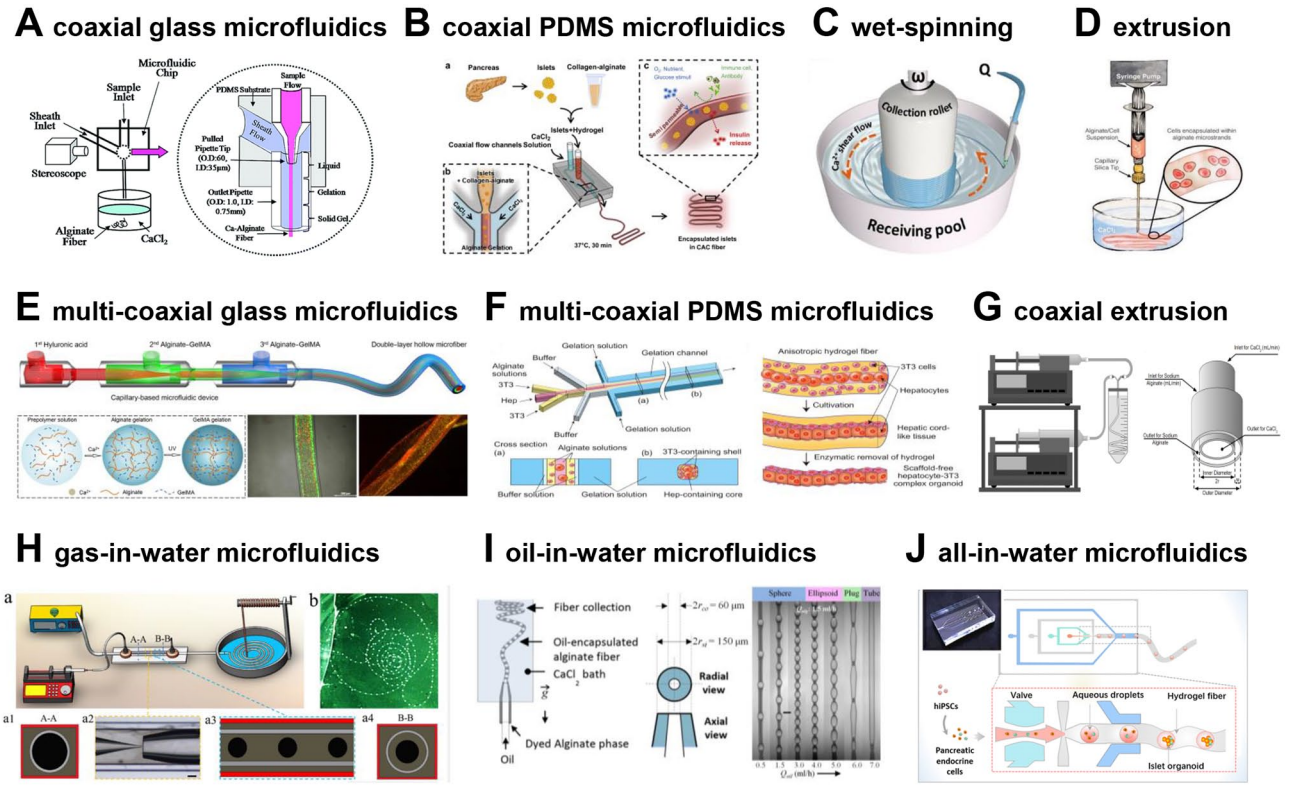

Figure 5. Versatile fabrication methods to make alginate hydrogel microfibers (A–D), core–shell microfibers or microtubes (E–G), or bead-in-microfiber structures (H–J). (A) Coaxial glass microfluidics embedded in a PDMS substrate.[32] (Reprinted with permission from Shin *et al.*, *Copyright © 2007 American Chemical Society*.) (B) Coaxial cylindrical PDMS microfluidic device.[98] (Reprinted from Jun *et al.*, Copyright (2013), with permission from Elsevier.) (C) Wet spinning.[99] (Adapted from Yang *et al.*, http://creativecommons.org/licenses/by/4.0/.) (D) Extrusion.[74] (Reprinted from Unser *et al.*, Copyright (2015), with permission from Elsevier.) (E) Multicoaxial glass capillary microfluidics for making core–shell microfibers.[104] (Reprinted from Zuo *et al.*, Copyright (2016), with permission from Elsevier.) (F) Multicoaxial PDMS microfluidics for making core–shell microfibers or co-cultured cell fibers.[105] (Reprinted from Yamada *et al.*, Copyright (2012), with permission from Elsevier.) (G) Extrusion through a needle-in-needle device to fabricate premade alginate microtubes.[2] (Reprinted with permission from Jorgensen *et al.*, *Copyright © 2021 IOP Publishing Ltd*.) (H) Coaxial glass capillary gas-in-water microfluidics to fabricate cavity microfibers.[108] (Adapted with permission from Tian *et al. Copyright © 2018 American Chemical Society*.) (I) Vertical, coaxial glass capillary microfluidics to fabricate oil droplet-filled microfibers that can be tuned by varying oil-phase flow rates.[109] (Reprinted from Chaurasia *et al.*, Copyright (2016), with permission from Elsevier.) (J) All-in-water microfluidics to fabricate aqueous-droplet-filled hydrogel microfibers.[110] (Adapted with permission from Wang *et al.*, *Copyright © 2021 American Chemical Society.*)

alginate hydrogel layer to obtain an ultra-thin alginate–chitosan membrane.[75]

Using multicoaxial microfluidic devices, multicomponent/compartment microfibers can be produced. One- to three-component microfibers were fabricated using multibarrel injection capillary microfluidics with multiple laminar flow[36,97] or a two-layer coaxial laminar-flow PDMS microchip,[116] and four-component microfibers with different arrangements were made by a membrane-sandwiched three-layer coaxial microchip.[116] By varying the design of the coaxial nozzle and multiple flows, complex microfibers (e.g. Janus, multilayered, wavy, double helix, lumen structures) could be fabricated. Using these cell-laden microfibers as building blocks, anisotropic or heterogeneous tissue mimics could be further constructed by manually weaving or stacking microfibers.[116]

In addition, alginate hydrogel microfibers can be produced manually through electrostatic interaction. For example, by bringing two individual drops of oppositely charged alginate and water-soluble chitin on a sterile parafilm into contact with each other using a pair of forceps and drawing upward, interfacial complexation occurred, forming a continuous fiber that was consecutively passed through $CaCl_2$ bath and collected on a rotating fiber holder.[117] Successive coating was another approach to forming the alginate hydrogel shell, which used a capillary tubing or needle to successively dip into $CaCl_2$ solution and then alginate solution, until the shell reaching 7 mm in diameter.[118] Both methods are simple to use and easy to access, without the need of any coaxial microfluidic devices, but lack of control and low throughput.

Automated and continuous microfiber production has been tried. For example, by adjusting the alginate flow rate through a needle-in-needle device, the "liquid rope-coil effect" occurred,[119] leading to the production of a continuous, coiled alginate hydrogel microtube that can be stacked and stored directly in a 50 mL centrifuge tube with hydrogel collection solution.[2] A wet spinning system was employed by a syringe pump, inlet pipe, and spinneret to continuously jet alginate prepolymer into a receiving pool where a coagulation wheel filled up with $CaCl_2$ solution to crosslink alginate jet into hydrogel microfibers. These were then automatically collected by a center roller in the middle of the receiving pool. Microfibers ranging from 20 to 600 μm were produced by controlling fabrication parameters, such as alginate flow rate, rotation rate of the central roller, and diameter of the spinneret.[120] These continuous microfibers were used as individual tubular structures for cell encapsulation/

Table 3. Representative cell expansion and differentiation in alginate microfibers and microtubes.

Alginate hydrogel	Cells	Fabrication	Outcomes	Ref.
AlgTubes	Human fibroblasts	Microextruder (cells/1.5% Alg/100 mM $CaCl_2$)	Reprogrammed/generated iPSCs with high purity (>95%) and yield (~5×10^8 cells/mL) in 30 days (400 μm)	Lin *et al.*[125]
Alginate/chitin microfibers	hESCs (HUES7, BG01V/hOG) and hiPSCs (PD-iPS5, hFib2-iPS4) ($2–5\times10^7$ cells/mL)	Interfacial complexation (1% Alg + cells/ 1% chitin/50 mM $CaCl_2$)	Maintained of self-renewal and differentiation potential of hPSCs for 10 passages; Cryopreservation efficiency 82.6% (200 μm)	Lu *et al.*[117]
AlgTubes	hESCs (H9) and hiPSCs ($1–10\times10^6$ cells/mL) Murine L Wnt3A cells	Microextruder (HA or MC + cells/1–2% Alg/100 mM $CaCl_2$)	Allowed long-term culture of hPSCs (>10 passages, >50 days) with high cell viability, expansion rate (500-fold), high purity (>95%) and yield (~5×10^8 cells/mL); High yield (6×10^8 cells/mL) (OD ⩽ 400 μm, θ ⩽ 70 μm)	Li *et al.*[106]
AlgTubes	hESCs (H9) and hiPSCs (1×10^6 cells/mL)	Same above	Generated hPSC-differentiated NSCs with high viability (~95%), purity (>90%), and yield (~5×10^8 cells/mL) in 12 days (OD = 250 μm, θ = 40 μm)	Lin *et al.*[126]
AlgTubes	hESCs (H9) and hiPSCs (1×10^6 cells/mL)	Microextruder (HA + cells/1.5% Alg/100 mM $CaCl_2$)	Generated hPSC-differentiated VSMCs with high viability, purity, and yield (~5.0×10^8 cells/mL) and contractile phenotype in 10 days (OD = 250 μm, θ = 40 μm)	Lin *et al.*[127]
Microfibers	hESCs (HUES7) ($1–2\times10^7$ cells/mL)	Extrusion (1% Alg + cells/1–100 mM $CaCl_2$)	Allowed long-term culture of hESCs (6–7 days × 5 passages) with pluripotency and differentiated to liver cells with efficiency comparable to Matrigel (OD ⩽ 350 μm)	Leong *et al.*[130]
AlgTubes	Human primary $CD3^+$ T cells (1×10^6 cells/mL)	Microextruder (HA + cells/1.5% Alg/10 mM $CaCl_2$)	Cultured T cells (three passages, 42 days) with high viability, expansion rate (320-fold), purity (98%), and yield (~3.2×10^8 cells/mL) in 14 days (OD ⩽ 400 μm, θ ⩽ 60 μm)	Lin *et al.*[128]
AlgTubes	Human primary glioblastoma TICs	Microextruder (HA + cells/1.5% Alg/100 mM $CaCl_2$)	Allowed long-term culturing (~50 days, 10 passages) of glioblastoma TICs with high cell viability, expansion rate (~700-fold), and yield (~3.0×10^8 cells/mL) in 14 days (OD: 100–400 μm)	Li *et al.*[129]

Alg: alginate; iPSCs: induced pluripotent stem cells; HA: hyaluronic acid; hESCs: human embryonic stem cells; hOG: hOct4-GFP reporter cells; MC: methylcellulose; hPSCs: human pluripotent stem cells; OD: outer diameter; θ: shell thickness; hiPSCs: human-induced pluripotent stem cells; NSCs: neural stem cells; PD: Parkinson's disease; VSMCs: vascular smooth muscle cells; TICs: tumor-initiating cells.

culture or automatically assembled into highly aligned fibrous hydrogel mats or bundles with non-aligned hierarchical structure.[99] Alginate microfibers could also serve as bioink to be bioprinted into tissue patches (e.g. cartilage tissue patch).[113] In addition, alginate microfibers with grooved structures for guiding cell alignment were fabricated using microfluidic devices.[121,122]

Stimuli-responsive hydrogel microfibers, core–shell microfibers, or hollow microtubes could also be fabricated from alginate in combination with thermoresponsive polymers, such as poly(N-isopropylacrylamide-co-acrylic acid) (PNIPAM-AAc) using double-coaxial glass capillary microfluidics. By adjusting the outer to inner flow velocity ratio, tubular structures would be produced, showing isotropic or anisotropic shrinkage in response to temperature or pH.[123]

Alginate microfibers for long-term culture and cell expansion

Both alginate hydrogel microfibers and microtubes allow high-density cell encapsulation (such as 1×10^8 cells/mL),[33,103,124] and in particular, culturing cells in microtubes allows for high-fold expansion, leading to high cell numbers (volumetric yield of $3–6\times10^8$ cells/mL alginate microfibers) (Table 3). The usefulness of these cell microfibers or microtubes for long-term high-density cell culture is evidenced by allowing (1) effective generation of human-induced pluripotent stem cells (hiPSCs) from reprogrammed human fibroblasts,[125] high-efficiency cryopreservation, long-term self-renewal,[117] high-fold expansion,[106] and enhanced differentiation of human pluripotent stem cells (hPSCs) with high purity and yield[126,127]; (2) automated production of T cells for future adoptive immunotherapy[128]; and (3) scalable culturing of tumor-initiating cells for drug discovery.[129]

In general, alginate microfibers (without blending with any adhesive macromolecule) showed the ability to support the generation of hiPSCs,[125] maintain pluripotency of hESCs and hiPSCs,[130] and promote hepatic differentiation of hESCs with efficiency comparable to Matrigel.[130] Alginate microtubes, such as AlgTubes formed from alginate blended with hyaluronic acid, could consistently support long-term culture, producing a variety of cells in large quantity with high viability, high expansion rate, and high yield, including hESCs, hiPSCs, hPSC-differentiated neural stem cells (NSCs) and vascular smooth muscle cells (VSMCs), therapeutic T cells, and tumor-initiating cells.[106,126–129]

Alginate microfibers for cell delivery

Alginate hydrogel microfibers not only support high-density cell expansion and differentiation but also are injectable and transplantable. Alginate hydrogel microfibers, ribbons, and core–shell fibers that are incorporated with collagen or Matrigel were used as cell delivery vehicles for *in vivo* implantation through injection or conventional transplantation or implantation approaches (Table 4). The therapeutic potential was demonstrated by islet fibers normalizing blood glucose for more than four weeks with immunoprotection,[98] without fibrotic reaction in diabetic mice,[119] mouse mesenchymal stem cell (mMSC) fiber undergoing endothelization in response to angiogenic growth factors, showing biocompatibility in mice and having potential as small-diameter

Table 4. Representative applications of alginate tubular structures for *in vivo* cell implantation.

Alginate hydrogel	Cells	Fabrication	*In vivo* delivery/duration	Outcomes	Ref.
Core–shell microfibers	Rat pancreatic islet cells (1×10^8 cells/mL)	Glass double-coaxial microfluidics (cells + collagen/1.5% Alg–agarose/100 mM $CaCl_2$)	Injection into the subrenal capsular space of a diabetic mouse using a microcatheter (13 days)	Glucose-responsive insulin-secreting islet cell fibers (200 μm) normalizing blood glucose concentration without fibrotic reaction	Onoe *et al.*[33]
Microfibers	Rat pancreatic islets (5000 IEQ/mL)	PDMS cylindrical coaxial microfluidics (3% Alg + collagen + cells/3% $CaCl_2$)	Transplantation into the intraperitoneal cavity of BALB/c mice (4 weeks)	Islet fibers (250 μm) maintained normoglycemic blood glucose levels four weeks with immunoprotection	Jun *et al.*[98]
Microfibers	mMSCs (1×10^6 cells/mL)	Extrusion through glass microfluidics (2% Alg + cells + VEGF + FGF2/20 mM $CaCl_2$)	Implantation into the abdominal cavities of Kunming mice (14 days)	MSC fibers (500 μm) exhibited better *in vitro* cell proliferation and endothelial marker expression and *in vivo* biocompatibility	Liu *et al.*[131]
Microfibers	hMSCs (1×10^6 cells/mL)	Extrusion through a needle (2% Alg + fibrinogen + cells/100 mM $CaCl_2$ + thrombin)	Injection to athymic nude rats w/ critical-sized circular defects in both sides of the mandibular rami using a syringe (12 weeks)	MSC fibers (~250 μm) exhibited proliferation and osteogenic potential *in vitro* and achieved complete osseous bridging *in vivo*	Song *et al.*[132]
Microribbons	hiPSC-derived spinal cord neural stem cells (scNSCs) (1×10^8 cells/mL)	Extrusion through a needle (1.5% Alg + collagen + cells/100 mM $CaCl_2$)	Injection of the segment to Long–Evans rat cervical spinal cord hemi-contusion lesions (3–4 mm microfibers, ~5000 cells; 9 days)	Neural ribbons (60 μm) retaining cells in the spinal cord injury cavity and showing neuroprotection even at low delivery doses	Olmsted *et al.*[124]
Core–shell microfibers	hiPSC-derived hepatocytes (1×10^6 cells)	Glass double-coaxial microfluidics (cells + Matrigel/2% Alg/20 mM $BaCl_2$)	Spatula transplantation into abdominal cavity of immunodeficient NOD/SCID mice (3 days)	Hepatic fibers (~400 μm) secreted albumin at therapeutic level	Nagata *et al.*[133]

BALB/c: BALB/c: albino, laboratory-bred strain of the house mouse; hMSCs: human mesenchymal stem cells; mMSCs: mouse mesenchymal stem cells; MSC: mesenchymal stem cell; NOD: non-obese diabetic; PDMS: polydimethylsiloxane; SCID: severe combined immunodeficiency; VEGF: vascular endothelial growth factor.

vascular grafts,[131] osteogenic hMSC fibers achieving complete osseous bridging in rats with critical-sized circular defects,[132] and hiPSC-derived hepatic fibers secreting albumin at therapeutic level.[133] In particular, hiPSC-derived neural ribbons have shown the capacity of retaining cells in the rat spinal cord injury cavity and exhibiting neuroprotection even at the delivery dose as low as ~5000 cells in 3–4 mm microfibers.[124]

The major difference between alginate microfibers and microtubes for cell culture or cell delivery lies in microfibers that contain the solid alginate hydrogel throughout, so that cells are in direct contact with the hydrogel, and therefore, cells can only form small aggregates and are suitable for cell maintenance and storage. On the contrary, alginate microtubes contain a wall/membrane with a liquid core, in which cells can freely move and self-assemble into large aggregates or cell clusters, which can also reach a density as high as that of the tissue in the body, and are suitable for high-fold cell expansion, stem cell differentiation, cellular organization, and organoid formation.

Alginate microfibers and microtubes for 3D culture and complex tissue mimics

A variety of cells, including stem cells (e.g. ESCs, iPSCs, MSCs, and NSCs), endothelial cells, nerve cells, fibroblasts, epithelial cells, salivary gland cells, β-islets, hepatocytes, smooth muscle cells, myoblasts, myocytes, osteoblasts, T cells, and cancer cells, have been cultured in alginate hydrogel microfibers or microtubes to form cell fibers (Tables 3 to 5). Cells can be encapsulated in these microfibers, injected to premade microtubes or perfused in preformed core–shell cell fibers, or seeded onto the surface of microfibers. Co-culture can be arranged in diverse ways, for example, mixing endothelial cells with different cell types as part of the core flow, or subjecting endothelial cells to the core flow and other cell types to the shell flow to form cell fibers with an endothelial inner layer, or coating cell fibers with endothelial cells. It opens a new avenue to study epithelial–stromal interactions, vascularization, and innervation of tissue constructs to address challenges in tissue engineering,[134] recapitulate *in vivo*-like tissue physiology,[135] leading to functional tissue engineering.

Alginate microfibers and microtubes provide valuable co-culture platforms for studying cell–cell interactions, such as using ESC microstands to study pluripotent signaling that could restrict cancer metastatic potential,[136,137] using fibroblast fibers to supply secretome that promotes survival and proliferation of co-cultured myoblasts on a dish[138], exploiting the salivary gland epithelial–stromal cell interaction for salivary gland self-assembly,[34] recapitulating the hepatocyte–stromal cell interaction to enhance liver function,[105] and constructing meter-long hollow osteo fibers with the endothelial cell inner layer,[139] covering bundles of hepatic fibers with endothelial capillaries,[140] or injecting astrocytes into microtubes covered by endothelial cells to mimic the blood–brain barrier.[112]

Alginate microfibers have been shown to promote spontaneous stem cell differentiation (e.g. osteoblastic differentiation from mouse MSCs),[118] and in particular, directed

Table 5. Representative alginate-based tubular structures for tissue mimics *in vitro*.

Alginate hydrogel	Cells	Fabrication	Duration	Outcome	Ref.
Core–shell microfibers	Rat primary cardiomyocyte and cortical cells; HMVEC; mouse primary NSCs (~1×10^8 cells/mL)	Glass double-coaxial microfluidics (cells + ECM protein/1.5% Alg–agarose/100 mM $CaCl_2$)	3, 4, 35, 77 days	Functional cardiomyocyte, endothelial, and cortical cell fibers; differentiated NSC fibers (20–100 μm)	Onoe *et al.*[33]
Core–shell microfibers	Rat primary hepatocytes (3×10^7 cells/mL) and mouse Swiss 3T3 cells (1×10^7 cells/mL)	Multicoaxial microfluidic device (0.7% Alg + collagen + cells/20 mM $BaCl_2$)	90 days	*In vivo* hepatic cord-like organoid fibers (~80 μm) with long-term preservation of hepatic functions (albumin secretion, urea synthesis, and hepatocyte gene expression)	Yamada *et al.*[105]
Core–shell microfibers	HepG2 cells in microfiber (3×10^8 cells/mL) Co-cultured with bovine endothelial HH cells	Multicoaxial microfluidic device (0.7% GRGDSP-Alg + cells/1% Alg/20 mM $BaCl_2$)	2 + 5 days	Vascularized hepatic lobule-like fiber bundle (~90 μm fiber/1 mm bundle)	Yajima *et al.*[140]
Hollow microfibers	HepG2	Microfluidic chip with four channels (MC/2% Alg/1.1% $CaCl_2$/chitosan)	10 days	Proliferating and functional HepG2 fibers with ultra-thin membrane (~200 μm)	Liu *et al.*[75]
Core–shell microfibers	hiPSC-derived hepatocytes (1×10^8 cells/mL)	Double-coaxial laminar-flow microfluidics (cells + Matrigel/ 2% Alg/100 mM Ca^{2+})	7 days	High-density iPSC-hepatocyte fibers (~400 μm)	Nagata *et al.*[133]
Core–shell microfibers	HUVEC ($0.6–15 \times 10^8$ cells/mL)	Microfluidic coaxial (needle) printing (1.5% Alg + GelMA + SilkMA/160 mM $CaCl_2$)	3–4 days	Perfusable vascular constructs (inner 750 μm /outer 1250 μm)	Wu *et al.*[114]
Core–shell microfibers	Multipotent de-differentiated fat (DFAT) cells (1×10^7 cells/mL)	Multicoaxial glass microfluidics (cells + ECM proteins/1.5% Alg/100 mM $CaCl_2$)	30 days	Circumferentially oriented smooth muscle-like tissue constructs with marker expression (300–350 μm)	Hsiao *et al.*[142]
Core–shell microfibers	Human osteoblast MG63 and HUVECs (1×10^7 cells/mL)	Multicoaxial PDMS microfluidics (HA/1.5% RGD-Alg + HUVECs/1.5% RGD-Alg + MG63/300 mM $CaCl_2$)	3 weeks	Osteon-like microfibers with increased expression of osteogenic and vasculogenic genes (~450 μm)	Wei *et al.*[139]
Tissue strands as bioink	Cattle primary chondrocytes	Extrusion through a coaxial nozzle (4% Alg/4% $CaCl_2$) + cell injection	4 weeks	Implanted cartilage in a bovine explant exhibited GAG-rich ECM	Yu *et al.*[113]
Microfibers	Human primary chondrocytes (2×10^6 cells/mL)	Snake micromixing microfluidic device (2% Alg, 2% Alg + gelatin or 2% Alg + UBM/cells + 6.0 mM $BaCl_2$)	14 days	De-differentiated chondrocytes regained chondrocyte phenotype and recovered from cryopreservation with high viability (~700 μm)	Angelozzi *et al.*[144]
Microfibers	Mouse D1 MSC (5×10^7 cells/mL)	Successive alginate coating and cell-collagen injection (2% Alg/200 mM $CaCl_2$/cells + collagen)	21 days	MSC fibers showing spontaneous differentiation to osteoblastic lineage (200 μm)	Kalisky *et al.*[118]

HMVEC: human microvascular endothelial cells; NSCs: neural stem cells; ECM: extracellular matrix; Alg: alginate; MC: methylcellulose; HH: bovine carotid artery normal endothelial cells; GRGDSP: Gly-Arg-Gly-Asp-Ser-Pro peptide; hiPSCs: human-induced pluripotent stem cells; iPSC: induced pluripotent stem cell; HA: hyaluronic acid; HUVEC: human umbilical vein endothelial cells; PDMS: polydimethylsiloxane; RGD: arginine–glycine–aspartic acid; GAG: glycosaminoglycans; UBM: urinary bladder matrix; MSC: mesenchymal stem cell.

differentiation (e.g. brown adipogenesis from mouse embryonic stem cells [mESCs],[74] neuronal differentiation from mouse NSCs,[33,141] and endothelial differentiation of mMSCs),[131] facilitate tissue *in vivo*-like construction (e.g. perfusable endothelial layer,[114] neural cell alignment,[99] smooth muscle-like spring),[142] and enhance cell function (e.g. hepatic function,[105,130,143] biominerization potential,[144] and cardiomyocyte contraction).[33]

These alginate hydrogel microfibers or microtubes can be used in versatile fashions to form cell fibers and further organize them into higher-order 3D cellular structures,[33,36,100,145] generate multicomponent heterogeneous microfibers for co-culture or multiculture of cells,[116] and create temperature-[67,146] or magnetic-responsive microfibers as microscale toroidal cellular building elements for constructing microvascular-like structures.[147]

Conclusions and future perspectives

Alginate hydrogel provides a versatile platform for 3D cell culture, cell therapy, tissue engineering, and regenerative medicine. In particular, alginate hydrogel microfiber technology opens a new avenue to therapeutic cell delivery and construction of tissue mimics. It has demonstrated that:

1. A variety of alginate microfibers and microtubes with high handleability and irretrievability could be fabricated through versatile microfluidics, spinning, and extrusion processes;
2. Alginate microfibers supported generation of iPSCs and long-term maintenance of pluripotency of PSCs at high density;
3. With incorporation of the ECM component (e.g. hyaluronic acid), alginate microtubes supported direct cryopreservation, high-fold expansion, and directed differentiation of PSCs with high purity and high yield;
4. With incorporation of ECM proteins (e.g. collagen, fibronectin, and Matrigel), alginate microfibers or microtubes supported functional cell fiber formation;
5. Alginate microfibers and microtubes provided co-culture and multiculture platforms and building blocks for engineering complex tissue mimics.

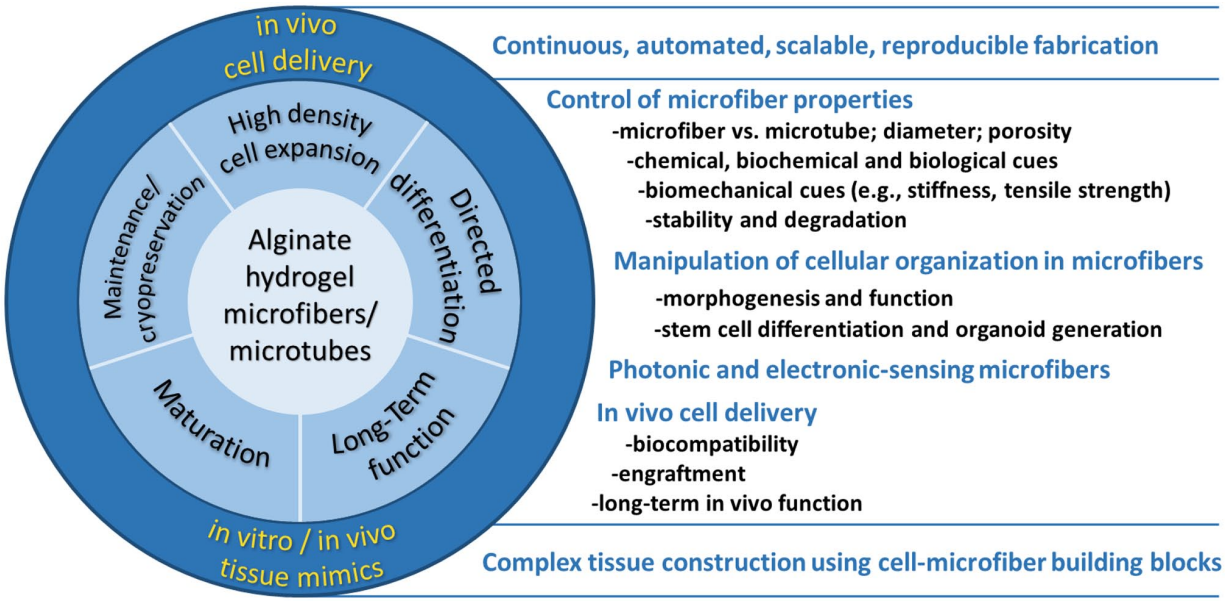

Figure 6. Perspectives of alginate hydrogel microfibers for cell delivery and tissue mimics. (A color version of this figure is available in the online journal.)

To realize the full potential and benefit of alginate hydrogel microfiber technology as shown in Figure 6, we suggest:

1. Establishing a standard of alginate and its resultant hydrogels and generating an "Alginate Materials Genome" because properties of alginate hydrogels are highly dependent on the source, molecular weight, viscosity, and concentration, and affected by long-term storage of alginate and sterilization methods, leading to fabrication of off-the-shelf alginate hydrogel tubular structures in a designable, continuous and automated, reproducible, scalable, and standardized manner;
2. Evaluating dynamic property changes of alginate hydrogel microfibers during long-term cell culture, such as morphology, diameter and shell thickness, porosity, permeability, mechanical properties, degradation, biocompatibility, and how these changes will affect cell organization and function;
3. Comparing genomics, proteomics, and metabolomics of cell–cell interactions and stem cell development in different alginate hydrogel microfibers (e.g. microfibers versus microtubes, alginate versus alginate-ECM microfibers/microtubes versus tissues), revealing design rules for directed stem cell differentiation, organoid formation, and tissue construction;
4. Developing photonic and electronic sensing microfibers for *in situ* monitoring of cell growth, organization, and function in microfibers and microtubes;
5. Studying vascularization and innervation of cell fibers *in vitro* and *in vivo*;
6. Evaluating *in vivo* biocompatibility, survival, and engraftment of cell fibers for long term and in large animal models.

AUTHORS' CONTRIBUTIONS

YX contributed to the conception and overall writing of this review article; SCRK and MJ contributed to drafting the section of alginate microfibers for cell delivery and tissue mimics; XZ contributed to discussion and figures of this review articles. All authors contributed to editing and revising the manuscript.

ACKNOWLEDGEMENTS

The authors thank Dr Andrea Unser for producing the scanning electron microscopy image of the electrospun alginate nanofiber matrix.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: M.J. is currently the founder of a startup company, Midux Laboratories LLC. Y.X. is an advisory board member and consultant for Midux. Y.X., M.J., and S.C.R.K. have a patent application (17/153,867), titled "Devices and Methods of Producing Tubular Systems for Cell Culture." The other authors declare no conflict of interest.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is partially supported by the National Institute of Health (NIH) National Institute of Dental and Craniofacial Research (NIDCR), Grant No. R01DE027953.

ORCID ID

Yubing Xie https://orcid.org/0000-0003-1395-7365

REFERENCES

1. Betz JF, Cheng Y, Tsao C-Y, Zargar A, Wu H-C, Luo X, Payne GF, Bentley WE, Rubloff GW. Optically clear alginate hydrogels for spatially controlled cell entrapment and culture at microfluidic electrode surfaces. *Lab Chip* 2013;**13**:1854–8
2. Jorgensen M, Gibbons A, Sui K, Carpenter R, Zhang X, Xie Y. Predictable fabrication of pre-made alginate hydrogel microtubes for stem cell aggregation using needle-in-needle devices. *Biofabrication* 2021;**13**:035043

3. Cattelan G, Guerrero Gerbolés A, Foresti R, Pramstaller PP, Rossini A, Miragoli M, Caffarra Malvezzi C. Alginate formulations: current developments in the race for hydrogel-based cardiac regeneration. *Front Bioeng Biotechnol* 2020;**8**:414
4. Khalid GM, Selmin F. Applications of alginates in the design and preparation of orodispersible dosage forms. In: Deniz I, Imamoglu E, Keskin-Gundogdu T (eds) *Properties and applications of alginates*. London: IntechOpen, 2021. DOI: 10.5772/intechopen.98610
5. Stanford E. British Patent **142**, 1881
6. Maclennan W. Notes on alginic acid and some of its compounds as therapeutic agents. *Glasgow Med J* 1898;**50**:24–6
7. Nelson WL, Cretcher LH. The alginic acid from macrocystis pyrifera1,2. *J Am Chem Soc* 1929;**51**:1914–22
8. Atkins EDT, Mackie W, Smolko EE. Crystalline structures of alginic acids. *Nature* 1970;**225**:626–8
9. Waksman SA, Carey CL, Allen MC. Bacteria decomposing alginic acid. *J Bacteriol* 1934;**28**:213–20
10. Larsen B, Haug A. Biosynthesis of alginate: part I. Composition and structure of alginate produced by *Azotobacter vinelandii* (Lipman). *Carbohydr Res* 1971;**17**:287–96
11. Axelband AA. Peripheral seal impression technique, using the alginates. *Dent Dig* 1945;**51**:694
12. Blaine G. Experimental observations on absorbable alginate products in surgery: gel, film, gauze and foam. *Ann Surg* 1947;**125**:102–14
13. Slavin D. Production of antisera in rabbits using calcium alginate as an antigen depot. *Nature* 1950;**165**:115–6
14. Skoryna SC, Paul TM, Edward DW. Studies on inhibition of intestinal absorption of radioactive strontium. I. Prevention of absorption from ligated intestinal segments. *Can Med Assoc J* 1964;**91**:285–8
15. Haug A, Smidsrod O. Strontium-calcium selectivity of alginates. *Nature* 1967;**215**:757
16. Kierstan M, Bucke C. The immobilization of microbial cells, subcellular organelles, and enzymes in calcium alginate gels. *Biotechnol Bioeng* 1977;**19**:387–97
17. Brodelius P, Deus B, Mosbach K, Zenk MH. Immobilized plant cells for the production and transformation of natural products. *FEBS Lett* 1979;**103**:93–7
18. Ochiai H, Shibata H, Sawa Y, Katoh T. "Living electrode" as a long-lived photoconverter for biophotolysis of water. *Proc Natl Acad Sci U S A* 1980;**77**:2442–4
19. Nilsson K, Scheirer W, Merten OW, Östberg L, Liehl E, Katinger HWD, Mosbach K. Entrapment of animal cells for production of monoclonal antibodies and other biomolecules. *Nature* 1983;**302**: 629–30
20. Wylie A. Alginates as food additives. *R Soc Health J* 1973;**93**:309–13
21. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980;**210**:908–10
22. Chang TM. Semipermeable microcapsules. *Science* 1964;**146**:524–5
23. Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet* 1994;**343**:950–1
24. Basta G, Montanucci P, Luca G, Boselli C, Noya G, Barbaro B, Qi M, Kinzer KP, Oberholzer J, Calafiore R. Long-term metabolic and immunological follow-up of non-immunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care* 2011;**34**:2406–9
25. Matsumoto S, Abalovich A, Wechsler C, Wynyard S, Elliott RB. Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. *EBioMedicine* 2016;**12**:255–62
26. Dornish M, Kaplan D, Skaugrud O. Standards and guidelines for biopolymers in tissue-engineered medical products: ASTM alginate and chitosan standard guides. *Ann N Y Acad Sci* 2001;**944**:388–97
27. Ladet S, David L, Domard A. Multi-membrane hydrogels. *Nature* 2008;**452**:76–9
28. Zarket BC, Raghavan SR. Onion-like multilayered polymer capsules synthesized by a bioinspired inside-out technique. *Nat Commun* 2017; **8**:193
29. Maeda K, Onoe H, Takinoue M, Takeuchi S. Controlled synthesis of 3D multi-compartmental particles with centrifuge-based microdroplet formation from a multi-barrelled capillary. *Adv Mater* 2012; **24**:1340–6
30. Lu Y-C, Song W, An D, Kim BJ, Schwartz R, Wu M, Ma M. Designing compartmentalized hydrogel microparticles for cell encapsulation and scalable 3D cell culture. *J Mater Chem B* 2015;**3**:353–60
31. Ali J, Cheang UK, Liu Y, Kim H, Rogowski L, Sheckman S, Patel P, Sun W, Kim MJ. Fabrication and magnetic control of alginate-based rolling microrobots. *AIP Adv* 2016;**6**:125205
32. Shin S-J, Park J-Y, Lee J-Y, Park H, Park Y-D, Lee K-B, Whang C-M, Lee S-H. "On the fly" continuous generation of alginate fibers using a microfluidic device. *Langmuir* 2007;**23**:9104–8
33. Onoe H, Okitsu T, Itou A, Kato-Negishi M, Gojo R, Kiriya D, Sato K, Miura S, Iwanaga S, Kuribayashi-Shigetomi K, Matsunaga YT, Shimoyama Y, Takeuchi S. Metre-long cell-laden microfibres exhibit tissue morphologies and functions. *Nat Mater* 2013;**12**:584–90
34. Jorgensen M, Ramesh P, Toro M, Evans E, Moskwa N, Zhang X, Sharfstein ST, Larsen M, Xie Y. Alginate hydrogel microtubes for salivary gland cell organization and cavitation. *Bioengineering* 2022; **9**:38
35. Tian Y, Zhu P, Tang X, Zhou C, Wang J, Kong T, Xu M, Wang L. Large-scale water collection of bioinspired cavity-microfibers. *Nat Commun* 2017;**8**:1080
36. Cheng Y, Yu Y, Fu F, Wang J, Shang L, Gu Z, Zhao Y. Controlled fabrication of bioactive microfibers for creating tissue constructs using microfluidic techniques. *ACS Appl Mater Interfaces* 2016;**8**:1080–6
37. Bhattarai N, Li Z, Edmondson D, Zhang M. Alginate-based nanofibrous scaffolds: structural, mechanical, and biological properties. *Adv Mater* 2006;**18**:1463–7
38. Chou H-Y, Weng C-C, Lai J-Y, Lin S-Y, Tsai H-C. Design of an interpenetrating polymeric network hydrogel made of calcium-alginate from a thermos-sensitive pluronic template as a thermal-ionic reversible wound dressing. *Polymers* 2020;**12**:2138
39. Bencherif SA, Sands RW, Bhatta D, Arany P, Verbeke CS, Edwards DA, Mooney DJ. Injectable preformed scaffolds with shape-memory properties. *Proc Natl Acad Sci U S A* 2012;**109**:19590–5
40. Ramesh P, Moskwa N, Hanchon Z, Koplas A, Nelson DA, Mills KL, Castracane J, Larsen M, Sharfstein ST, Xie Y. Engineering cryoelectrospun elastin-alginate scaffolds to serve as stromal extracellular matrices. *Biofabrication* 2022;**14**:035010
41. Chaji S, Al-Saleh J, Gomillion CT. Bioprinted three-dimensional cell-laden hydrogels to evaluate adipocyte-breast cancer cell interactions. *Gels* 2020;**6**:10
42. Lee KY, Peters MC, Anderson KW, Mooney DJ. Controlled growth factor release from synthetic extracellular matrices. *Nature* 2000;**408**: 998–1000
43. Zhao X, Kim J, Cezar CA, Huebsch N, Lee K, Bouhadir K, Mooney DJ. Active scaffolds for on-demand drug and cell delivery. *Proc Natl Acad Sci U S A* 2011;**108**:67–72
44. Zhang YS, Khademhosseini A. Advances in engineering hydrogels. *Science* 2017;**356**:eaaf3627
45. Li J, Celiz AD, Yang J, Yang Q, Wamala I, Whyte W, Seo BR, Vasilyev NV, Vlassak JJ, Suo Z, Mooney DJ. Tough adhesives for diverse wet surfaces. *Science* 2017;**357**:378–81
46. Shi P, Zhao N, Coyne J, Wang Y. DNA-templated synthesis of biomimetic cell wall for nanoencapsulation and protection of mammalian cells. *Nat Commun* 2019;**10**:2223
47. Zhao C, Zhang P, Zhou J, Qi S, Yamauchi Y, Shi R, Fang R, Ishida Y, Wang S, Tomsia AP, Liu M, Jiang L. Layered nanocomposites by shear-flow-induced alignment of nanosheets. *Nature* 2020;**580**: 210–5
48. Pataky K, Braschler T, Negro A, Renaud P, Lutolf MP, Brugger J. Microdrop printing of hydrogel bioinks into 3D tissue-like geometries. *Adv Mater* 2012;**24**:391–6
49. Sun J-Y, Zhao X, Illeperuma WRK, Chaudhuri O, Oh KH, Mooney DJ, Vlassak JJ, Suo Z. Highly stretchable and tough hydrogels. *Nature* 2012;**489**:133–6

50. Lin S, Yuk H, Zhang T, Parada GA, Koo H, Yu C, Zhao X. Stretchable hydrogel electronics and devices. *Adv Mater* 2016;**28**:4497–505
51. Choi M, Humar M, Kim S, Yun S-H. Step-index optical fiber made of biocompatible hydrogels. *Adv Mater* 2015;**27**:4081–6
52. Draget KI, Smidsrød O, Skjak-Braek G. Alginate from algae. In: Steinbüchel A, Rhee SK (eds) *Polysaccharides and polyamides in the food industries: properties, production and patents*. Weinheim: WILEY-VCH Verlag Gmbh & Co. KGaA, 2005, pp.1–30
53. Grant GT, Morris ER, Rees DA, Smith PJC, Thom D. Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett* 1973;**32**:195–8
54. Stokke BT, Draget KI, Smidsrød O, Yuguchi Y, Urakawa H, Kajiwara K. Small-angle X-ray scattering and rheological characterization of alginate gels. 1. Ca−alginate gels. *Macromolecules* 2000;**33**:1853–63
55. Yamamoto K, Yuguchi Y, Stokke BT, Sikorski P, Bassett DC. Local structure of Ca2+ alginate hydrogels gelled via competitive ligand exchange and measured by small angle X-ray scattering. *Gels* 2019;**5**:3
56. Pawar SN, Edgar KJ. Chemical modification of alginates in organic solvent systems. *Biomacromolecules* 2011;**12**:4095–103
57. Lee DW, Choi WS, Byun MW, Park HJ, Yu Y-M, Lee CM. Effect of γ-irradiation on degradation of alginate. *J Agric Food Chem* 2003;**51**:4819–23
58. Lorson T, Ruopp M, Nadernezhad A, Eiber J, Vogel U, Jungst T, Lühmann T. Sterilization methods and their influence on physicochemical properties and bioprinting of alginate as a bioink component. *ACS Omega* 2020;**5**:6481–6
59. Stoppel WL, White JC, Horava SD, Henry AC, Roberts SC, Bhatia SR. Terminal sterilization of alginate hydrogels: efficacy and impact on mechanical properties. *J Biomed Mater Res B Appl Biomater* 2014;**102**:877–84
60. Lee DW, Choi WS, Byun MW, Park HJ, Yu YM, Lee CM. Effect of gamma-irradiation on degradation of alginate. *J Agric Food Chem* 2003;**51**:4819–23
61. Pawar SN, Edgar KJ. Alginate derivatization: a review of chemistry, properties and applications. *Biomaterials* 2012;**33**:3279–305
62. Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. *Biomaterials* 2001;**22**:511–21
63. Cui J, Wang M, Zheng Y, Rodríguez Muñiz GM, del Campo A. Light-triggered cross-linking of alginates with caged Ca2+. *Biomacromolecules* 2013;**14**:1251–6
64. Yang J, Steck J, Suo Z. Gelation kinetics of alginate chains through covalent bonds. *Extreme Mech Lett* 2020;**40**:100898
65. Li J, Illeperuma WRK, Suo Z, Vlassak JJ. Hybrid hydrogels with extremely high stiffness and toughness. *ACS Macro Lett* 2014;**3**:520–3
66. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. *Prog Polym Sci* 2012;**37**:106–26
67. Ju HK, Kim SY, Kim SJ, Lee YM. pH/temperature-responsive semi-IPN hydrogels composed of alginate and poly(N-isopropylacrylamide). *J Appl Polym Sci* 2002;**83**:1128–39
68. Zhao S, Cao M, Li H, Li L, Xu W. Synthesis and characterization of thermo-sensitive semi-IPN hydrogels based on poly(ethylene glycol)-co-poly(epsilon-caprolactone) macromer, N-isopropylacrylamide, and sodium alginate. *Carbohydr Res* 2010;**345**:425–31
69. Memic A, Colombani T, Eggermont LJ, Rezaeeyazdi M, Steingold J, Rogers ZJ, Navare KJ, Mohammed HS, Bencherif SA. Latest advances in cryogel technology for biomedical applications. *Adv Ther* 2019;**2**:1800114
70. Ho MH, Kuo PY, Hsieh HJ, Hsien TY, Hou LT, Lai JY, Wang DM. Preparation of porous scaffolds by using freeze-extraction and freeze-gelation methods. *Biomaterials* 2004;**25**:129–38
71. Zhao Y, Shen W, Chen Z, Wu T. Freeze-thaw induced gelation of alginates. *Carbohydr Polym* 2016;**148**:45–51
72. Gurikov P, Smirnova I. Non-conventional methods for gelation of alginate. *Gels (Basel, Switzerland)* 2018;**4**:14
73. Lee KY, Kong HJ, Larson RG, Mooney DJ. Hydrogel formation via cell crosslinking. *Adv Mater* 2003;**15**:1828–32
74. Unser AM, Mooney B, Corr DT, Tseng YH, Xie Y. 3D brown adipogenesis to create "Brown-Fat-in-Microstrands." *Biomaterials* 2016;**75**:123–34
75. Liu H, Wang Y, Chen W, Yu Y, Jiang L, Qin J. A microfluidic strategy to fabricate ultra-thin polyelectrolyte hollow microfibers as 3D cellular carriers. *Mater Sci Eng C Mater Biol Appl* 2019;**104**:109705
76. Mørch YA, Qi M, Gundersen POM, Formo K, Lacik I, Skjåk-Bræk G, Oberholzer J, Strand BL. Binding and leakage of barium in alginate microbeads. *J Biomed Mater Res A* 2012;**100**:2939–47
77. Kong H-J, Lee KY, Mooney DJ. Decoupling the dependence of rheological/mechanical properties of hydrogels from solids concentration. *Polymer* 2002;**43**:6239–46
78. de Vos P, Faas MM, Strand B, Calafiore R. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 2006;**27**:5603–17
79. Vegas AJ, Veiseh O, Gürtler M, Millman JR, Pagliuca FW, Bader AR, Doloff JC, Li J, Chen M, Olejnik K, Tam HH, Jhunjhunwala S, Langan E, Aresta-Dasilva S, Gandham S, McGarrigle JJ, Bochenek MA, Hollister-Lock J, Oberholzer J, Greiner DL, Weir GC, Melton DA, Langer R, Anderson DG. Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat Med* 2016;**22**:306–11
80. Liu Q, Chiu A, Wang L-H, An D, Zhong M, Smink AM, de Haan BJ, de Vos P, Keane K, Vegge A, Chen EY, Song W, Liu WF, Flanders J, Rescan C, Grunnet LG, Wang X, Ma M. Zwitterionically modified alginates mitigate cellular overgrowth for cell encapsulation. *Nat Commun* 2019;**10**:5262
81. Mitrousis N, Fokina A, Shoichet MS. Biomaterials for cell transplantation. *Nat Rev Mater* 2018;**3**:441–56
82. Templin C, Lüscher TF, Landmesser U. Cell-based cardiovascular repair and regeneration in acute myocardial infarction and chronic ischemic cardiomyopathy-current status and future developments. *Int J Dev Biol* 2011;**55**:407–17
83. Caplan H, Olson SD, Kumar A, George M, Prabhakara KS, Wenzel P, Bedi S, Toledano-Furman NE, Triolo F, Kamhieh-Milz J, Moll G, Cox CS Jr. Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. *Front Immunol* 2019;**10**:1645
84. Sortwell CE, Pitzer MR, Collier TJ. Time course of apoptotic cell death within mesencephalic cell suspension grafts: implications for improving grafted dopamine neuron survival. *Exp Neurol* 2000;**165**:268–77
85. Mooney DJ, Vandenburgh H. Cell delivery mechanisms for tissue repair. *Cell Stem Cell* 2008;**2**:205–13
86. Cooke ME, Jones SW, Ter Horst B, Moiemen N, Snow M, Chouhan G, Hill LJ, Esmaeli M, Moakes RJA, Holton J, Nandra R, Williams RL, Smith AM, Grover LM. Structuring of hydrogels across multiple length scales for biomedical applications. *Adv Mater* 2018;**30**:e1705013
87. Bidarra SJ, Barrias CC, Granja PL. Injectable alginate hydrogels for cell delivery in tissue engineering. *Acta Biomater* 2014;**10**:1646–62
88. Capeling MM, Czerwinski M, Huang S, Tsai YH, Wu A, Nagy MS, Juliar B, Sundaram N, Song Y, Han WM, Takayama S, Alsberg E, Garcia AJ, Helmrath M, Putnam AJ, Spence JR. Nonadhesive alginate hydrogels support growth of pluripotent stem cell-derived intestinal organoids. *Stem Cell Rep* 2019;**12**:381–94
89. Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. *Proc Natl Acad Sci U S A* 2002;**99**:12025–30
90. Langer R, Tirrell DA. Designing materials for biology and medicine. *Nature* 2004;**428**:487–92
91. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;**260**:920–6
92. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005;**310**:1139–43
93. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;**126**:677–89
94. Chaudhuri O, Cooper-White J, Janmey PA, Mooney DJ, Shenoy VB. Effects of extracellular matrix viscoelasticity on cellular behaviour. *Nature* 2020;**584**:535–46
95. Green JJ, Elisseeff JH. Mimicking biological functionality with polymers for biomedical applications. *Nature* 2016;**540**:386–94
96. Choi NW, Cabodi M, Held B, Gleghorn JP, Bonassar LJ, Stroock AD. Microfluidic scaffolds for tissue engineering. *Nat Mater* 2007;**6**:908–15

97. Yu Y, Shang L, Guo J, Wang J, Zhao Y. Design of capillary microfluidics for spinning cell-laden microfibers. *Nat Protoc* 2018;**13**:2557–79
98. Jun Y, Kim MJ, Hwang YH, Jeon EA, Kang AR, Lee SH, Lee DY. Microfluidics-generated pancreatic islet microfibers for enhanced immunoprotection. *Biomaterials* 2013;**34**:8122–30
99. Yang Y, Sun J, Liu X, Guo Z, He Y, Wei D, Zhong M, Guo L, Fan H, Zhang X. Wet-spinning fabrication of shear-patterned alginate hydrogel microfibers and the guidance of cell alignment. *Regen Biomater* 2017; **4**:299–307
100. Xie R, Liang Z, Ai Y, Zheng W, Xiong J, Xu P, Liu Y, Ding M, Gao J, Wang J, Liang Q. Composable microfluidic spinning platforms for facile production of biomimetic perfusable hydrogel microtubes. *Nat Protoc* 2021;**16**:937–64
101. Armstrong JP, Burke M, Carter BM, Davis SA, Perriman AW. 3D bioprinting using a templated porous bioink. *Adv Healthc Mater* 2016;**5**:1724–30
102. Sugiura S, Oda T, Aoyagi Y, Satake M, Ohkohchi N, Nakajima M. Tubular gel fabrication and cell encapsulation in laminar flow stream formed by microfabricated nozzle array. *Lab Chip* 2008;**8**:1255–7
103. Raof NA, Padgen MR, Gracias AR, Bergkvist M, Xie Y. One-dimensional self-assembly of mouse embryonic stem cells using an array of hydrogel microstrands. *Biomaterials* 2011;**32**:4498–505
104. Zuo Y, He X, Yang Y, Wei D, Sun J, Zhong M, Xie R, Fan H, Zhang X. Microfluidic-based generation of functional microfibers for biomimetic complex tissue construction. *Acta Biomaterialia* 2016;**38**: 153–62
105. Yamada M, Utoh R, Ohashi K, Tatsumi K, Yamato M, Okano T, Seki M. Controlled formation of heterotypic hepatic micro-organoids in anisotropic hydrogel microfibers for long-term preservation of liver-specific functions. *Biomaterials* 2012;**33**:8304–15
106. Li Q, Lin H, Du Q, Liu K, Wang O, Evans C, Christian H, Zhang C, Lei Y. Scalable and physiologically relevant microenvironments for human pluripotent stem cell expansion and differentiation. *Biofabrication* 2018;**10**:025006
107. Takei T, Kitazono J, Tanaka S, Nishimata H, Yoshida M. Necrotic regions are absent in fiber-shaped cell aggregates, approximately 100 μm in diameter. *Artif Cells Nanomed Biotechnol* 2016;**44**:62–5
108. Tian Y, Wang J, Wang L. Microfluidic fabrication of bioinspired cavity-microfibers for 3D scaffolds. *ACS Appl Mater Interfaces* 2018; **10**:29219–26
109. Chaurasia AS, Jahanzad F, Sajjadi S. Flexible microfluidic fabrication of oil-encapsulated alginate microfibers. *Chem Eng J* 2017;**308**: 1090–7
110. Wang H, Liu H, Zhang X, Wang Y, Zhao M, Chen W, Qin J. One-Step generation of aqueous-droplet-filled hydrogel fibers as organoid carriers using an All-in-Water microfluidic system. *ACS Appl Mater Interfaces* 2021;**13**:3199–208
111. Yu Y, Wen H, Ma J, Lykkemark S, Xu H, Qin J. Flexible fabrication of biomimetic bamboo-like hybrid microfibers. *Adv Mater* 2014;**26**: 2494–9
112. Nguyen TPT, Tran BM, Lee NY. Microfluidic approach for the fabrication of cell-laden hollow fibers for endothelial barrier research. *J Mater Chem B* 2018;**6**:6057–66
113. Yu Y, Moncal KK, Li J, Peng W, Rivero I, Martin JA, Ozbolat IT. Three-dimensional bioprinting using self-assembling scalable scaffold-free "tissue strands" as a new bioink. *Sci Rep* 2016;**6**:28714
114. Wu Z, Cai H, Ao Z, Xu J, Heaps S, Guo F. Microfluidic printing of tunable hollow microfibers for vascular tissue engineering. *Adv Mater Technol* 2021;**6**:2000683
115. Liu Y, Yang Y, Shen Y. Tubular microcapsules with polysaccharide membranes based on a co-axial microfluidic chip. *ACS Biomater Sci Eng* 2019;**5**:6281–9
116. Yao K, Li W, Li K, Wu Q, Gu Y, Zhao L, Zhang Y, Gao X. Simple fabrication of multicomponent heterogeneous fibers for cell co-culture via microfluidic spinning. *Macromol Biosci* 2020;**20**:e1900395
117. Lu HF, Narayanan K, Lim SX, Gao S, Leong MF, Wan AC. A 3D microfibrous scaffold for long-term human pluripotent stem cell self-renewal under chemically defined conditions. *Biomaterials* 2012;**33**: 2419–30
118. Kalisky J, Raso J, Rigothier C, Rémy M, Siadous R, Bareille R, Fricain J-C, Amedée-Vilamitjana J, Oliveira H, Devillard R. An easy-to-use and versatile method for building cell-laden microfibres. *Sci Rep* 2016;**6**:33328
119. Liu R, Kong B, Chen Y, Liu X, Mi S. Formation of helical alginate microfibers using different G/M ratios of sodium alginate based on microfluidics. *Sens Actuators B Chem* 2020;**304**:127069
120. Yang Y, Liu X, Wei D, Zhong M, Sun J, Guo L, Fan H, Zhang X. Automated fabrication of hydrogel microfibers with tunable diameters for controlled cell alignment. *Biofabrication* 2017;**9**:045009
121. Kang E, Choi YY, Chae S-K, Moon J-H, Chang J-Y, Lee S-H. Microfluidic spinning of flat alginate fibers with grooves for cell-aligning scaffolds. *Adv Mater* 2012;**24**:4271–7
122. Zhao M, Liu H, Zhang X, Wang H, Tao T, Qin J. A flexible microfluidic strategy to generate grooved microfibers for guiding cell alignment. *Biomater Sci* 2021;**9**:4880–90
123. Nakajima S, Kawano R, Onoe H. Stimuli-responsive hydrogel microfibers with controlled anisotropic shrinkage and cross-sectional geometries. *Soft Matter* 2017;**13**:3710–9
124. Olmsted ZT, Stigliano C, Badri A, Zhang F, Williams A, Koffas MAG, Xie Y, Linhardt RJ, Cibelli J, Horner PJ, Paluh JL. Fabrication of homotypic neural ribbons as a multiplex platform optimized for spinal cord delivery. *Sci Rep* 2020;**10**:12939
125. Lin H, Li Q, Du Q, Wang O, Wang Z, Akert L, Carlson MA, Zhang C, Subramanian A, Zhang C, Lunning M, Li M, Lei Y. Integrated generation of induced pluripotent stem cells in a low-cost device. *Biomaterials* 2019;**189**:23–36
126. Lin H, Du Q, Li Q, Wang O, Wang Z, Liu K, Elowsky C, Zhang C, Lei Y. Hydrogel-Based bioprocess for scalable manufacturing of human pluripotent stem cell-derived neural stem cells. *ACS Appl Mater Interfaces* 2018;**10**:29238–50
127. Lin H, Qiu X, Du Q, Li Q, Wang O, Akert L, Wang Z, Anderson D, Liu K, Gu L, Zhang C, Lei Y. Engineered microenvironment for manufacturing human pluripotent stem cell-derived vascular smooth muscle cells. *Stem Cell Rep* 2019;**12**:84–97
128. Lin H, Li Q, Wang O, Rauch J, Harm B, Viljoen HJ, Zhang C, Van Wyk E, Zhang C, Lei Y. Automated expansion of primary human T cells in scalable and cell-friendly hydrogel microtubes for adoptive immunotherapy. *Adv Healthc Mater* 2018;**7**:e1701297
129. Li Q, Lin H, Rauch J, Deleyrolle LP, Reynolds BA, Viljoen HJ, Zhang C, Zhang C, Gu L, Van Wyk E, Lei Y. Scalable culturing of primary human glioblastoma tumor-initiating cells with a cell-friendly culture system. *Sci Rep* 2018;**8**:3531
130. Leong MF, Lu HF, Lim TC, Narayanan K, Gao S, Wang LY, Toh RP, Funke H, Abdul Samad MH, Wan AC, Ying JY. Alginate microfiber system for expansion and direct differentiation of human embryonic stem cells. *Tissue Eng Part C Methods* 2016;**22**:884–94
131. Liu M, Zhou Z, Chai Y, Zhang S, Wu X, Huang S, Su J, Jiang J. Synthesis of cell composite alginate microfibers by microfluidics with the application potential of small diameter vascular grafts. *Biofabrication* 2017;**9**:025030
132. Song Y, Zhang C, Wang P, Wang L, Bao C, Weir MD, Reynolds MA, Ren K, Zhao L, Xu HHK. Engineering bone regeneration with novel cell-laden hydrogel microfiber-injectable calcium phosphate scaffold. *Mater Sci Eng C Mater Biol Appl* 2017;**75**:895–905
133. Nagata S, Ozawa F, Nie M, Takeuchi S. 3D culture of functional human iPSC-derived hepatocytes using a core-shell microfiber. *PLoS ONE* 2020;**15**:e0234441
134. Griffith LG, Naughton G. Tissue engineering–current challenges and expanding opportunities. *Science* 2002;**295**:1009–14
135. Griffith LG, Swartz MA. Capturing complex 3D tissue physiology in vitro. *Nat Rev Mol Cell Biol* 2006;**7**:211–24
136. Mooney B, Abdul-Raof N, Tian YI, Xie Y. Restriction of cancer metastatic potential using embryonic stem cells encapsulated in alginate hydrogel microstrands. *ACS Biomater Sci Eng* 2017;**3**:1769–79
137. Mooney B, Tian YI, Rousseau E, Xie Y. Understanding convergent signaling regulation in metastatic breast cancer cells using a bioengineered stem cell microenvironment. *J Cancer Metastasis Treatment* 2019;**5**:19

138. Shima A, Itou A, Takeuchi S. Cell fibers promote proliferation of co-cultured cells on a dish. *Sci Rep* 2020;**10**:288
139. Wei D, Sun J, Bolderson J, Zhong M, Dalby MJ, Cusack M, Yin H, Fan H, Zhang X. Continuous fabrication and assembly of spatial cell-laden fibers for a tissue-like construct via a photolithographic-based microfluidic chip. *ACS Appl Mater Interfaces* 2017;**9**:14606–17
140. Yajima Y, Lee CN, Yamada M, Utoh R, Seki M. Development of a perfusable 3D liver cell cultivation system via bundling-up assembly of cell-laden microfibers. *J Biosci Bioeng* 2018;**126**:111–8
141. Onoe H, Kato-Negishi M, Itou A, Takeuchi S. Differentiation induction of mouse neural stem cells in hydrogel tubular microenvironments with controlled tube dimensions. *Adv Healthc Mater* 2016;**5**:1104–11
142. Hsiao AY, Okitsu T, Onoe H, Kiyosawa M, Teramae H, Iwanaga S, Kazama T, Matsumoto T, Takeuchi S. Smooth muscle-like tissue constructs with circumferentially oriented cells formed by the cell fiber technology. *PLoS ONE* 2015;**10**:e0119010
143. Park DY, Mun CH, Kang E, No DY, Ju J, Lee SH. One-stop microfiber spinning and fabrication of a fibrous cell-encapsulated scaffold on a single microfluidic platform. *Biofabrication* 2014;**6**:024108
144. Angelozzi M, Penolazzi L, Mazzitelli S, Lambertini E, Lolli A, Piva R, Nastruzzi C. Dedifferentiated chondrocytes in composite microfibers as tool for cartilage repair. *Front Bioeng Biotechnol* 2017; **5**:35
145. Luo Y, Lode A, Gelinsky M. Direct plotting of three-dimensional hollow fiber scaffolds based on concentrated alginate pastes for tissue engineering. *Adv Healthc Mater* 2013;**2**:777–83
146. Imani KBC, Kim D, Kim D, Yoon J. Temperature-controllable hydrogels in double-walled microtube structure prepared by using a triple channel microfluidic system. *Langmuir* 2018;**34**:11553–8
147. Sun T, Shi Q, Huang Q, Wang H, Xiong X, Hu C, Fukuda T. Magnetic alginate microfibers as scaffolding elements for the fabrication of microvascular-like structures. *Acta Biomater* 2018;**66**:272–81