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Electrospinning and Cell Fibers in Biomedical Applications

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Human body tissues such as muscle, blood vessels, tendon/ligaments, and nerves have fiber-like fascicle morphologies, where ordered organization of cells and extracellular matrix (ECM) within the bundles in specific 3D manners orchestrates cells and ECM to provide tissue functions. Through engineering cell fibers (which are fibers containing living cells) as living building blocks with the help of emerging "bottom-up" biomanufacturing technologies, it is now possible to reconstitute/recreate the fiber-like fascicle morphologies and their spatiotemporally specific cell-cell/cell-ECM interactions in vitro, thereby enabling the modeling, therapy, or repair of these fibrous tissues. In this article, a concise review is provided of the "bottom-up" biomanufacturing technologies and materials usable for fabricating cell fibers, with an emphasis on electrospinning that can effectively and efficiently produce thin cell fibers and with properly designed processes, 3D cell-laden structures that mimic those of native fibrous tissues. The importance and applications of cell fibers as models, therapeutic platforms, or analogs/replacements for tissues for areas such as drug testing, cell therapy, and tissue engineering are highlighted. Challenges, in terms of biomimicry of high-order hierarchical structures and complex dynamic cellular microenvironments of native tissues, as well as opportunities for cell fibers in a myriad of biomedical applications, are discussed.

1. Introduction

Human body tissues comprise cells, extracellular matrix (ECM), and bio-signals, which are organized in complex hierarchical manners.[1] Because trauma, disease, and dysfunction of tissues frequently occur in our daily life, there are huge and in many circumstances urgent demands for reconstituting/replicating the complicated hierarchical structures and functions of body tissues by harnessing and integrating these three elements, not only for building tissue/disease models for examining the effectiveness of therapies but also for offering suitable implants/substitutes as promising alternatives to organ transplantation. Over the past few decades, tissue engineering, which combines cells, bio-signals, and synthetic scaffolds that mimic the structures and properties of ECM, has attracted great attention and has also made significant advances in reconstituting/replicating the structures and functions of body tissues/organs.[2–4] Through incorporating cells by either cell seeding or cell recruitment within engineered cell microenvironments that are built via rational design of synthetic scaffolds and inclusion of specific bio-signals,[5–7] a variety of cell-laden structures resembling the anatomies of tissues/organs of interest have been formed by the "top-down" biomanufacturing approach, consequently facilitating the modeling, repair, and regeneration of diverse body tissues. However, such "top-down" biomanufacturing strategies face inherent challenges in recreating complicated hierarchical organizations as well as the interplays between cells and their native microenvironments as the 3D incorporation of cells within the engineered cell microenvironments would depend on the less controllable cell ingrowth. Accordingly, new strategies of "bottom-up" biomanufacturing that put modular assembly of cell-laden living building blocks into customized 3D tissue-mimicking architectures have emerged recently,[8–10] opening up new avenues in the endeavor of reconstituting/replicating the physiological complexities of body tissues.

Despite the high complexity, hierarchical architectures of human body tissues are assembled in specific spatial order by "base units" down to the micrometer scale with cells as a main component. The morphologies and geometries of the base units would directly determine the assembled structures and hence the hierarchical architectures of tissues. Using "bottom-up" biomanufacturing strategies, diverse living building blocks such as cell spheroids,[11] cell sheets,[12] cell-encapsulated microgels,[13] and functional cell organoids[14] have been employed for constructing physiologically relevant hierarchical structures. Nevertheless, it is far from satisfactory by employing these living building blocks that have combined cells, biomaterials, and bio-signals when seeking to reconstitute/recreate specific body tissues that have fiber-like fascicle morphologies. For fibrous tissues such as

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muscular fibers, blood vessels, nerves, and tendons/ligaments, a high volumetric density of elongated cells are aligned along a specific axis in the tissue. The specific fiber-like fascicle morphologies and ordered organization of cells into well-defined fibrous geometries also orchestrate their tissue functions, for example, high specific surface areas for facilitating metabolisms and oriented alignments of muscular cells for high-efficiency contraction/relaxation. Cell fibers, with a high-volume density of specific cells organized in defined fascicle morphologies, can therefore be highly promising and of great importance as appropriate living building blocks for reconstituting/recreating the hierarchical structures and even functions in the "bottom-up" biomanufacturing of fibrous tissues.

Free-floating cell fibers have been facilely produced through depositing cell aggregates in a microfabricated microchannel mold,[15] whereas these mold-assisted fabrication techniques are not as efficient as a few other methods for continuously producing cell fibers and for the ease of tuning fiber diameters, geometries, mesostructures, and multi-material compositions. It is notable that recent advances in fiber manufacturing technologies such as multi-interfacial polyelectrolyte complexation (MIPC), wet spinning, aerodynamic-/pressure-assisted spinning, microfluidic spinning, bioprinting, and electrospinning have offered extensive opportunities for continuous fabrication of centimeter-long or even meter-long cell fibers and assembling them into tissue-specific biomimetic hierarchical structures through stacking, bundling, or weaving, thereby enabling a broad range of applications of cell fibers in the diverse biomedical field.[16–18] In this article, we provide a concise review of the current research on cell fibers and their major applications, including fiber formation technologies, materials, and representative applications in modeling, therapy, and replacement or regeneration of body tissues. Electrospinning as a popular and heavily investigated "bottom-up" approach that can form ultrathin cell fibers with equivalent diameters to those of native body tissues (2–1000 μm) and produce ECM-resembling nanofibrous architectures is a focus of this review. We also provide a discussion on the bottlenecks of current cell fiber research in the "bottom-up" biomanufacturing of functional body tissues and point out possible future directions for both fundamental research and practical applications of cell fibers.

2. Technologies for fabricating cell fibers

2.1. Cell Assembly and Cell Aggregation

Through depositing cell aggregates within a microfabricated microchanneled mold/template (**Figure 1**a), cell fibers with well-defined geometries could be relatively easily formed. Using such straightforward techniques and specifically designed (and sometimes functionalized) molds/templates, it has been shown feasible to produce either solidified monolithic hydrogel fibers with the encapsulation of high-density (e.g., 10^6 cells mL^{-1}) mouse embryonic stem cells [19] or hollow structured fibers with the encapsulation of primary neonatal rat cardiomyocytes mimicking native cardiac bundles.[20] Using a similar principle, cell micropatterning methods that could confine cultured cells onto specific regions and geometries have also been investigated and developed for forming cell fibers on a chip-free substrate without the need of using microfabricated molds/templates. By tuning hydrophobicity/hydrophilicity of different surface areas of the mold/substrate, precise control may be achieved over the dispersion of highly concentrated cell suspensions into stripes on the hydrophilic areas. After gravity-driven deposition and then rapid remodeling of cells to form tight cell-cell junctions within a subsequent 24-h cell culture period, the cells confined in the stripes could self-organize into cell fibers without any supportive biomaterial.[21] Through functionalizing the micropatterned hydrophilic regions with thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAm), the surface wettability could be further tuned for the cell depositing regions for modulating cell attachment. Obtaining free-floating cell-only fibers with tubular organizations of co-cultured neurons and astrocytes has been demonstrated,[22] providing great promise for making living building blocks for "bottom-up" biomanufacturing of 3D cell-rich macroscopic structures mimicking the anatomies of unidirectional nerve bundles via modular assembly. Furthermore, molecular self-assembly has been investigated for making cell fibers. Through manually drawing a compound solution containing living cells and biocompatible amphiphilic peptides into a salty medium by a pipette, centimeter-long fibers with the encapsulation of aligned cells could be formed at physiological temperatures by the self-assembly of the amphiphilic peptides into macroscopic gel filaments via bundling of supramolecular nanofibers.[23] This "bottom-up" approach is particularly attractive for reconstituting/replicating native cell-ECM interplays by using customized peptide compositions, while challenges remain for the scale-up production of cell fibers.

2.2. Multi-Interfacial Polyelectrolyte Complexation (MIPC)

MIPC has been widely investigated as a facile method for producing gel fibers via an interfacial polyelectrolyte complexation process.[24] It has been found that gel fibers with beads spaced out at regular intervals along the fiber axis could be formed by drawing upward by means of a forceps or needle from the interface between two droplets that separately contain polyelectrolytes with opposite charges (Figure 1b). The formation of gel fibers via MIPC involves complex multi-step processes,[25] including initial formation of a polyelectrolyte complex barrier film at the interface of the two droplets, generation of "nuclear fibers" upon breaking the polyelectrolyte complex barrier film by drawing, and the growth and coalescence of "nuclear fibers" to the final gel fibers. By using water-soluble and biocompatible polyanions (usually alginate, heparin, or negatively charged DNA) and polycations (usually chitosan, methylated collagen, or positively charged histone proteins) as precursors, gel fibers with loaded biomacromolecules and living cells could be formed by MIPC, with pre-mixing the cargos within the precursor polyelectrolyte aqueous solutions.[26–27] With the assistance of a roll-up apparatus, meter-long cell fibers could be formed via MIPC in a continuous manner.[28] Moreover, MIPC could be used to generate multi-component fibers by drawing two or more interfaces of multiple droplets, which offers opportunities for co-encapsulation of different types of cells and for modulating their specific spatial organizations within the fibers.[29] Based on multi-component MIPC that enabled co-encapsulating of different types of cells,

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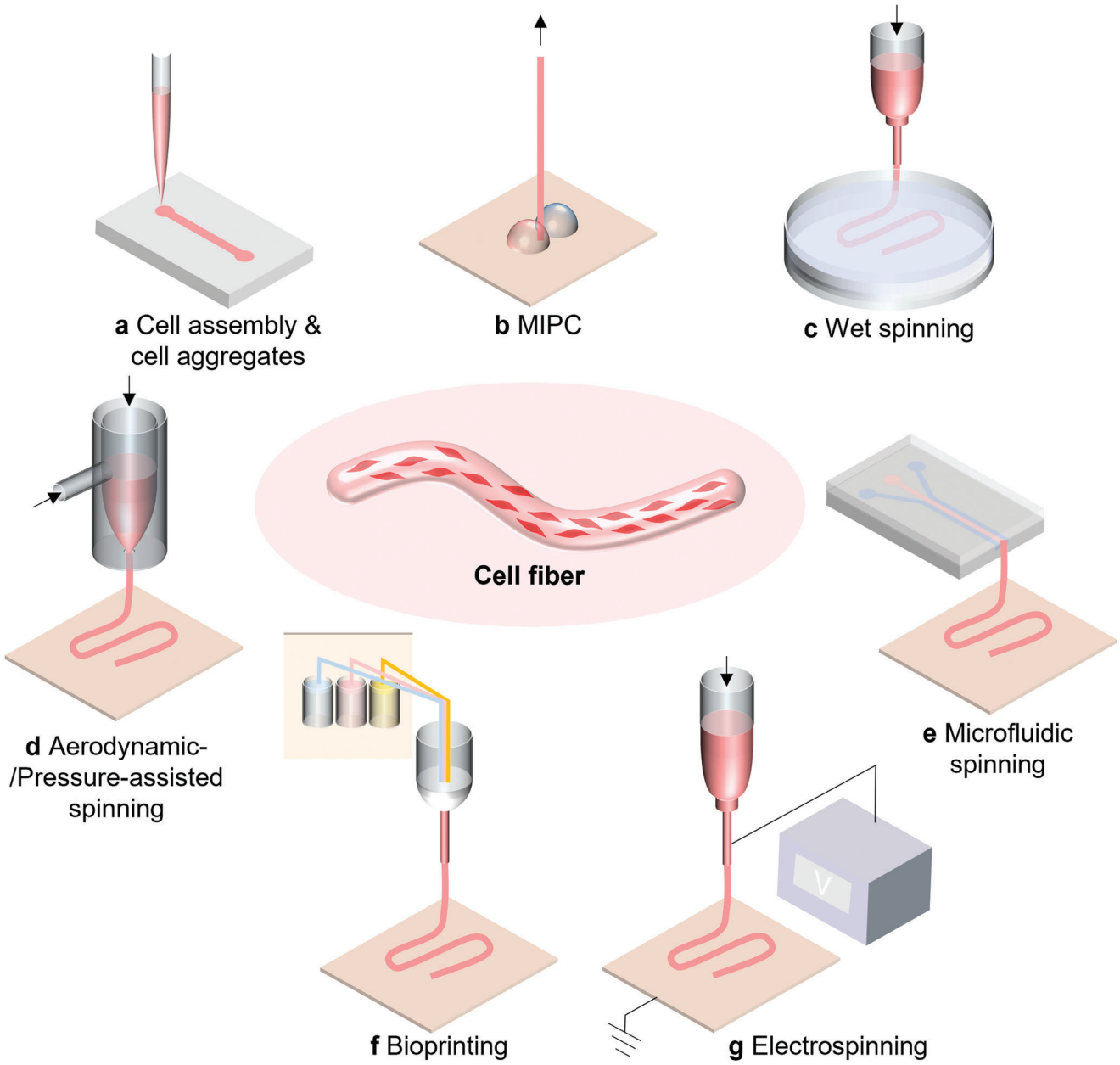

Figure 1. A schematic diagram showing different technologies for producing cell fibers.

hierarchical multicellular constructs that mimic diverse body tissues such as vascularized adipose tissues,[30] hair follicles,[31] hepatic tissues,[32] and chondrogenic tissues,[33] could be built by weaving of different primary cell fibers into bundles.

2.3. Wet Spinning

Wet spinning is an old method for fiber production and can be employed for the continuous fabrication of cell fibers. Unlike bead-concomitant fibers formed by MIPC, cell fibers fabricated by wet spinning generally have uniform geometries. As shown in Liberski et al.'s work, in a typical wetting spinning process for making cell fibers (Figure 1c), a precursor cell-alginate suspension with an appropriate alginate concentration was extruded into a coagulation bath filled with calcium chloride ($CaCl_2$) aqueous solution by a pumped syringe at a suitable feeding rate. After ionic cross-linking between the alginate and Ca^{2+} ions in the bath, alginate hydrogel fibers with encapsulated living cells could be formed.[34] With the assistance of a roller collector that could roll at different speeds for continuous weaving, meter-long cell fibers with tunable diameters could be formed by wet spinning in an automated operation.[35] By using a suturing string as a core fiber ejector, the mesostructures of wet-spun cell fibers could be further modified into core-shell structures. Biomimetic 3D perfusable cell-laden constructs were shown to be formed via

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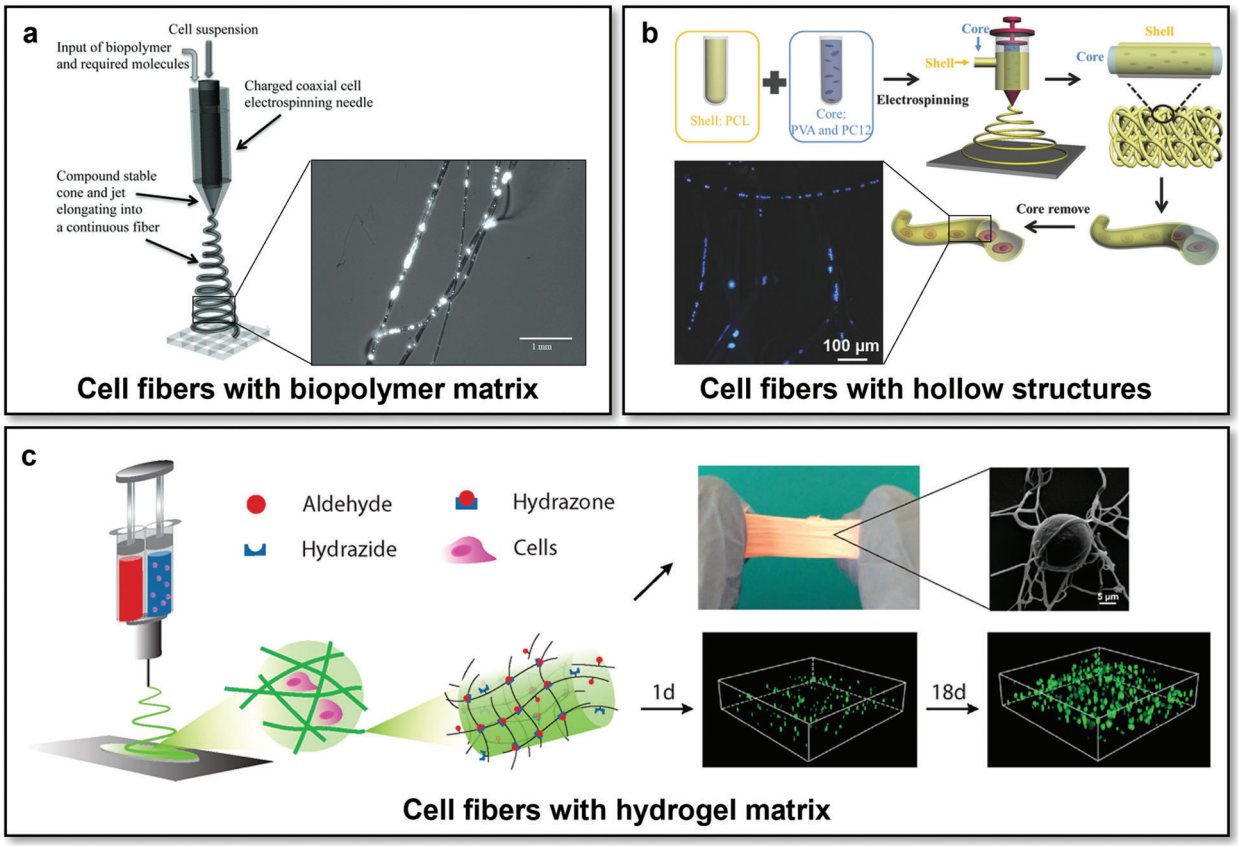

Figure 2. Cell fibers formed by electrospinning. a) Cell fibers with a biopolymer matrix fabricated by coaxial electrospinning where a cell suspension and a viscous biopolymer-based liquid were used as the inner and outer flows, respectively, through a coaxial spinneret. This figure has been reproduced with permission.[105] Copyright 2014, WILEY-VCH. b) Cell fibers with a hollow fiber structure fabricated via coaxial electrospinning where a cell-PVA suspension was used as the core flow. The PVA core would be removed in a culture medium, resulting in cell-encapsulated hollow microfibers. This figure has been reproduced with permission.[109] Copyright 2018, WILEY-VCH. c) Cell fibers with a hydrogel matrix formed by reactive electrospinning using two oligomer precursors that were readily cross-linked via hydrazone. This figure has been reproduced with permission.[115] Copyright 2018, American Chemical Society.

textile techniques by using wet-spun core-shell structured cell fibers as living building blocks.[36] Despite the ease of operation, extrusion-based wet spinning has an inherent shortage of spinning resolution (defined as fiber diameter) as the dimensions of wet-spun cell fibers are directly determined by the gauge size of spinnerets. Additionally, wet spinning is also less versatile in tuning different structures and/or components of cell fibers. Consequently, a variety of advanced spinning methods on the basis of wet spinning have been developed, aiming to form cell fibers with small diameters (sub-micron and nano) and/or customized fiber structures/components.

2.4. Aerodynamic-/Pressure-Assisted Spinning

Aerodynamic-/pressure-assisted spinning was developed upon the principle of using pressurized airflow to generate shear stress to overcome the surface tension of a jetting precursor solution to produce fine fibers and has been employed for obtaining cell fibers with enhanced resolutions beyond the limits of spinneret sizes.[37] The facility for aerodynamic-/pressure-assisted spinning is equipped with an air chamber, which contains an input tube for injecting air and a small orifice coaxially around the spinneret for generating pressurized airflows. Through aerodynamic-/pressure-assisted spinning, even polymeric fibers with diameters down to 50 nm could be formed from a high-viscosity poly(ethylene oxide) solution.[38] Cell fibers are generated by exerting shearing onto a precursor solution containing living cells by pressurized airflows (**Figure 2**d). Since the applied air pressure has a direct impact on the survivability of cells,[39] it should therefore be necessary to balance high resolution and cell viability for cell fibers fabricated using this technique by adjusting pressure of the air flows within an appropriate range. Using a viscous medical grade polydimethylsiloxane (PDMS) for the solution, stable jets for cell fibers were obtained and cell fibers with a resolution limit of ≈50 μm and good preservation of cell viability (>70%) were formed by aerodynamic-/pressure-assisted spinning when adjusting the applied pressure of the air chamber within the range of 0.01–5 bar.[40–42] Aerodynamic-/pressure-assisted spinning has provided an economical and also fast method for continuous and automated fabrication of cell fibers. But the

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fact that the manufacturing processes are highly sensitive to multiple processing parameters (e.g., viscosity, surface tension, feeding rate of precursor solution, and air pressure) may bring about difficulties in actual laboratory or commercial operations.

2.5. Microfluidic Spinning

Microfluidics has proved to provide excellent platforms for fabricating micro/nanomaterials with precisely tailored geometries, structures, and components.[43] Owing to the capabilities of integrating and precisely controlling multi-phase laminar flows within microchannels, easy-to-perform microfluidic spinning has also emerged for the design, preparation, and functionalization of micro/nanoscale fibers.[44–46] Using a microfluidic chip for generating coaxial laminar flows with an alginate-cell suspension and a $CaCl_2$ aqueous solution as the core and sheath flows, respectively (Figure 1e), Shin et al. showed that alginate hydrogel microfibers with the encapsulation of living mammalian cells could be continuously formed under the shear force and concurrent ionic cross-linking by the sheath flow.[47] Compared to the aerodynamic process for forming fibers, hydrodynamic microfluidic spinning that involves coaxial laminar flows is highly stable, showing obvious advantages in the ease of producing long and uniform cell fibers.[48] In addition, the volume of the precursor solution required for making cell fibers could be down to micro liters by miniaturizing the microfluidic device.[49] By adding microscale silk fibroin (SF) micelles within the alginate-cell suspension, the alignments of the encapsulated cells within the microfibers could further be controlled to be oriented by the elongated SF micelles that were co-sheared by the sheath flow.[50] The influence of different processing parameters in microfluidic spinning, including flow behavior and operation duration, on the viability and functions (e.g., proliferation) of cells encapsulated in cell fibers formed by typical coaxial laminar flow using alginate-cell suspensions as the precursor was systematically investigated, showing that excellent preservation of cell viability (>90%) and functions could be attained at a relatively low alginate concentration (1.5 w/v% better than 2.5 w/v%), a high cell density (1×10^7 cells mL^{-1} better than 5×10^5 cells mL^{-1}), and a short operation duration (30 s better than 120 s).[51] Even though these microfluidic spun cell fibers with alginate hydrogel as the matrix have various advantages, including easy-to-fabricate, high reliability, and desirable controllability, they still face challenges in mimicking the mechanical properties of fibrous tissues such as muscular bundles (Mechanical modulus: 50–200 kPa) and tendon/ligament (Mechanical modulus: 25–100 MPa) as they are limited by inadequate mechanical properties of alginate hydrogels (≈30 kPa).[52] To improve the strength of microfluidic spun cell fibers, polyacrylamide (PAAm) has been used to form double networks with alginate, resulting in the alginate/PAAm double-network hydrogel-based cell fibers with approximately six-fold higher strain (≈300%) and 10-fold higher tensile strength (≈150 kPa) than the alginate-only fibers (Fracture strain: ≈50%, ultimate tensile strength: ≈15 kPa).[52] Besides, cell fibers with enhanced mechanical properties could also be produced by microfluidic spinning by using precursors capable of forming chemically cross-linked networks, for example, poly(ethylene glycol dimethacrylate) (PEGDMA) that could be cross-linked via UV exposure [53] or 4-arm polyethylene glycol with maleimide end groups (PEG-4Mal) that could be chemically cross-linked with the assistance of a non-toxic and water-soluble dithiothreitol cross-linker.[54]

In addition to the capability of facilely forming straight long cell fibers, microfluidic spinning is also capable of tuning the geometries, mesostructures, multi-compartments, and multi-material compositions of micro-/nanofibers by tailoring microfluidic device configuration and processing parameters,[55–59] thereby offering opportunities for fabricating cell fibers with customized spatial organizations of multiple cells mimicking the complex structures of specific target tissues. For instance, cell-laden helical hydrogel microfibers, even the more complex helical structures such as multilayer helical microfibers and helical hollow microfibers with channels, could be formed by microfluidic spinning by simply adjusting the flow rates and/or the configuration of microfluidic device.[60] Apart from geometries, mesostructures of cell fibers could also be well controlled in microfluidic spinning. Using a configurable microfluidic spinning device with coaxial tri-phase flows (e.g., cell culture medium as the core flow, an alginate-cell suspension as the middle-layer sheath flow, and a $CaCl_2$ aqueous solution as the outer-layer sheath flow), cell fibers with hollow structures with the living cells encapsulated in the sheath could be formed,[61–63] providing a simple one-step method for constructing capillary-mimicking perfusable structures through the encapsulations of vascular endothelial cells (VECs).[64] By using a pair of biocompatible polymers containing opposite charges as precursors, for example, chondroitin sulfate C and chitosan [65] or alginate and chitosan,[66] hollow cell fibers could also be formed by simple flow-focus microfluidic spinning. The placement of the encapsulated cells within the fibers could be controlled at either the inner lining or the outer shell by suspending cells within either the core flow or the sheath flow.[65] By using a multi-barrel capillary microfluidic device for precisely controlling multiple laminar flows, cell fibers with tailored mesostructures (e.g., multi-core and multi-shell) and multi-compartments (e.g., bi-phase, tri-phase, and tetra-phase) could be formed,[67] realizing complex spatial organizations of multiple types of cells. In addition, microfluidic spinning could facilely generate hydrogel microfibers with encoded multi-material compositions at spatially programmed sites of the fibers with controllable intervals,[68] thus offering promising 3D cell culture platforms capable of placing different types of cells or organoids at designed distances which are suitable for fundamental cell biology study.[69] With the impressive capability for precisely controlling the geometries, mesostructures, multi-compartments, and multi-material compositions for cell fibers as well as the spatial organization of encapsulated cells, microfluidic spinning has inevitably been studied widely for forming cell fibers mimicking the anatomies of various fibrous tissues such as muscular bundles, blood vessels and nerves through the encapsulation and controllable organization of myocytes, VECs, and neural cells,[70] showing great promise for applications in drug testing and regenerative medicine.

2.6. Bioprinting

Bioprinting, as an emerging biomanufacturing platform over the past decade or so, has now been widely investigated for

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building 3D customized cell-laden constructs for modeling purposes or for repairing or substituting body tissues.[71–73] Using an extrusion-based bioprinter (Figure 1f), Zhang et al. have demonstrated that cell fibers could be formed on a 3D bio-plotting platform with the gelation of fibers following the principle similar to those for wet spinning and microfluidic spinning.[74] Typically, a coaxial spinneret is used, where a cell suspension and a liquid containing a coagulation agent are fed separately by the core and sheath flows. Through extrusion-based bioprinting, cell fibers could be programmed into designed patterns and/or stacked into customized 3D hierarchical architectures, for example, 3D cell-laden constructs with perfusable vascular networks which would be difficult to produce using any other spinning techniques.[75] The key to the success of bioprinting to produce cell fibers (or, cell struts in cell-scaffolds constructs for tissue engineering) is properly formulated bioinks, which should possess excellent cytocompatibility for preserving the viability and functions of encapsulated cells as well as appropriate viscosity for forming high-resolution cell fibers/cell structs via a mild gelation process. Alginate and gelatin methacryloyl (GelMA) are two of the most frequently used biopolymers for bioinks for fabricating cell fibers via bioprinting owing to their good biocompatibility, tunable mechanical properties, and mild gelation conditions, which could form individual networks via ionic and in situ UV cross-linking, respectively,[76–78] or form tough interpenetrating networks via blending them in the bioinks.[79] Both the tubular structure and mechanical properties of native tissues, such as small-diameter blood vessels, could be mimicked by bioprinted cell fibers with a tough interpenetrating networked matrix.[79] Despite the great promise of biomanufacturing, bioprinting to form cell fibers as living building blocks for "bottom-up" biomanufacturing of body tissues is facing many challenges, for example, optimizing the resolution of cell fiber/cell struct down to micron level approximating the "base units" of native tissues. Through electrohydrodynamic bioprinting in combination with microfluidic spinning and electrospinning,[80] it has been shown to be possible to form high-resolution bioprinted cell-laden struts (with strut diameters near to or <100 μm) with comparable sizes to those of the "base units" of native tissues (≈100 μm). Nevertheless, other challenges still remain in reconstructing/replicating the complex cell-ECM interplays in bioprinted 3D cell-laden structures.[81]

2.7. Electrospinning

Electrospinning, as one of the two typical electrohydrodynamic atomization techniques (the other is electrospraying), has been widely proven to be effective and efficient in producing nanofibers for a broad range of materials [82] for diverse applications, including those in the biomedical field. In electrospinning, a direct-current (DC) high-voltage power supply (in the range of several to tens of kilovolts) is used for generating an electrostatic field between an electrospinning spinneret and a fiber collector. A precursor solution, usually a polymer solution, with an appropriate viscosity is pumped by an automated syringe and flows through the spinneret to form a hemispherical droplet at the tip of the spinneret due to its surface tension, which will then be stretched into small jets by the electrostatic force overcoming the surface tension. After a series of physical processes including "bending instability" and "whipping motion",[83] the small jets are further stretched before being concentrated and solidified, resulting in fibers with diameters ranging from several micrometers to one hundred nanometers or less. Owing to the ease and versatility in producing submicron-/nanofibers and the capability of forming nanofibrous structures resembling the architectures of ECM and anatomies of various fibrous body tissues,[84–86] electrospinning has been extensively employed and developed in biomedicine.[87–92] It is can encapsulate diverse cargos, such as small-molecule drugs, biomolecules, genes, and nanoparticles, in submicron-/nanofibers through the use of appropriate electrospinning techniques, such as blend electrospinning, emulsion electrospinning, and coaxial electrospinning, and a suitable device.[93–101] Due to advantages in forming biomimetic nanofibrous structures and providing micro-/nanoencapsulation, electrospinning is regarded as an excellent method for fabricating cell fibers, particularly those with smaller diameters (<50 μm), as compared to other spinning methods.

Using living cells in electrohydrodynamic jetting to produce biomedical products was pioneered by Jayasinghe and his coworkers who initially investigated electrospraying, rather than electrospinning, to process directly a cell suspension into small-droplet (micron-sized) cell-containing jets with the assistance of an electric field.[102] By using a coaxial spinneret with a viscous biopolymer-based liquid, usually a medical grade PDMS liquid, as the sheath flow and a cell suspension as the core flow, the adopted coaxial electrospraying route could generate micron-sized cell-containing droplets from a highly concentrated cell suspension (>10^7 cells mL^{-1}).[103] By tuning the flow rate ratio between the biopolymer sheath flow and the cell suspension core flow into a suitable range, an electrospinning mode of electrohydrodynamic jetting would take place (Figure 1g), leading to the production of cell fibers.[104] The compound flow created by the coaxial electrospinning spinneret would be processed under the electrostatic field into a micron-sized single fiber with the microencapsulation of living mammalian cells with well-preserved viability (Figure 2a). To date, there have been extensive in vitro and in vivo investigations into the biosafety and reliability of such electrospinning techniques for processing a variety of living mammalian cells. It has been suggested that neither functions (e.g., adhesion, proliferation, and differentiation) of primary somatic cells nor pluripotency of stem cells would be affected by the electrospinning process,[105–107] indicating the great potential of the cell electrospinning technique for directly fabricating 3D cell-laden fibrous structures that resemble the physiological complexity of fibrous tissues.[108]

Despite the obvious advantages of producing high-resolution fibers over other fiber-spinning technologies and high efficiency in cell encapsulation, the above-described electrospinning techniques that use a viscous polymeric liquid for forming cell fibers face difficulties in realizing biomimetic cell-cell and cell-matrix interplays due to the physical confinements. A coaxial cell electrospinning method was developed accordingly to generate hollow microfibers with neuron-like cells (PC 12 cells) being encapsulated inside the fibers (Figure 2b).[109] Through coaxial electrospinning, uniform core-shell structured microfibers with a cell-suspended viscous poly(vinyl alcohol) (PVA) core and a biodegradable poly(ϵ-caprolactone) (PCL) sheath were initially

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formed. Upon the degradation of PVA by immersing cell-laden core-shell structured microfibers in a cell culture medium, PC 12 cells originally encapsulated in the PVA matrix could be released, resulting in cell-encapsulated hollow microfibers. Under suitable shearing conditions by optimizing the processing parameters in coaxial electrospinning, the encapsulated PC 12 cells could preserve well cell viability in the microfibers with a resolution of ≈50 μm. The hollow structure of microfibers could further direct neurogenic differentiation of encapsulated PC 12 cells and guide their directional axonal outgrowth along the microfibers to form connected neuronal networks. The coaxial electrospun cell fibers have the potential for use as in vitro models or analogs of nerves for drug testing or regenerative medicine application.

Compared to solid synthetic polymer matrices, hydrogels are considered to be superior for constructing artificial cellular microenvironments owing to their permeability, biocompatibility, and tissue-like viscoelasticity.[110] Efforts have therefore been made in developing electrospinning techniques that are capable of producing hydrogel-based cell fibers. Using PVA grafted with UV-cross-linkable methacrylate groups or ion-cross-linkable alginate as precursors, cell-encapsulated micron-sized hydrogel microspheres (diameter: 50–1000 μm) were formed via electrospraying with the assistances of appropriate post-electrospraying cross-linking treatments (UV- or ion-cross-linking).[111,112] Using similar cross-linking strategies after electrospinning, hydrogel-based cell fibers were also formed.[113] Particularly, alginate hydrogel microfibers with high-density encapsulation of primary human VECs could be formed via electrospinning and collected on a substrate of agarose gels containing Ca^{2+}. Furthermore, using precursor aqueous solutions of two oligomers, e.g., hydrazide-functionalized poly(oligoethylene glycol methacrylate) (POH) and aldehyde-functionalized poly(oligoethylene glycol methacrylate) (POA) that could be cross-linked via hydrazone, hydrogel nanofibers could also be directly made via a reactive electrospinning process.[114] When adding cells within one oligomer precursor aqueous solution, thin hydrogel microfibers (with diameters of ≈2 μm when fully swollen, against the diameters above 50 μm for "normal" cell fibers formed by other techniques) with the encapsulation of living cells could be obtained (Figure 2c).[115] Reactive electrospinning is shown to be superior to other methods that require post-electrospinning cross-linking treatment in terms of operational convenience and fiber production efficiency, while the impact of the high shear stress during the formation of thin cell fibers on long-term cell viability and functions should be further investigated. Recently, we have also reported a direct electro-writing method for producing hydrogel-based cell fibers without the need for post-electrospinning cross-linking.[116] A coaxial spinneret was used during the direct electro-writing process, where the inner and outer needles were fed separately by a cell-alginate suspension and a calcium chloride ($CaCl_2$) solution. The compound jet coming out of the coaxial spinneret was stretched by electrostatic force into a single fiber, consequently yielding hydrogel-based cell fibers with diameters of several hundreds of micrometers after the concurrent cross-linking. The low shear stress exerted on cell fibers resulted in well-preserved viability of encapsulated cells. This facile method for fabricating hydrogel-based cell fibers could be easily integrated with other biomanufacturing technologies, for example, microfluidic spinning, bioprinting, and electrospinning to form polymeric cell fibers and cell-incorporated structures, offering the opportunities to construct hierarchical 3D cell-laden structures that mimic complex architectures of specific target body tissues.

2.8. Comparisons for Fiber Fabrication Technologies

As presented and discussed above, there are several technologies suitable for fabricating cell fibers. Brief comparisons of these technologies in terms of cell viability, resolution (defined by the fiber diameter), advantages, and disadvantages are summarized in **Table 1**. Overall, all of these technologies can provide desirable (or, acceptable) preservation of cell viability (at least 70%) within resultant cell fibers. Differences are mainly found in their manufacturing processes, facilities, and technical capability. The mold-/template-assisted cell assembly and cell aggregation approach provide straightforward methods for making cell fibers. However, the dimensions (length and diameter) of as-fabricated cell fibers are directly determined by the dimensions of the mold/template, which is regarded very often as a disadvantage. Additionally, these techniques also have limitations for scaling up the manufacture of cell fibers due to their low throughput. MIPC provides facile methods to produce centimeter-long cell fibers without any need for sophisticated equipment. But it can only be used for a limited number of materials, i.e., materials systems of two polyelectrolytes with opposite charges. Extrusion-based wet spinning has proven to be versatile for processing a broad range of materials into fibers and is also highly efficient in continuously producing cell fibers of lengths up to meters with the assistance of various textile strategies.[36] However, the resolution of wet spun cell fibers, which is determined by the diameter of spinneret and swelling behavior of the matrix material, is usually limited. To fabricate cell fibers with high resolutions below the nozzle size of spinneret, additional shear stress needs to be exerted during the jetting process. Aerodynamic-/pressure-assisted spinning utilizes air flows for shearing the jets to form fibers, which can yield much thinner cell fibers than those formed by wet spinning. But the involvement of high-pressure air flows may simultaneously bring about problems such as increasing jetting instability and hindering the formation of uniform fibers. On the other hand, both microfluidic spinning and electrospinning exhibit many respective advantages in versatility, compatibility, controllability, and reliability,[117] showing better promise in forming high-quality cell fibers. Between these two approaches, microfluidic spinning is particularly advantageous in precisely controlling the geometries, mesostructures, multi-compartments, multi-material compositions, and multi-cell organizations for cell fibers, while electrospinning is well and widely noted for its superiority in high resolution for fabricated cell fibers. In particular, both microfluidic spinning and electrospinning can be integrated with extrusion-based bioprinting by using specifically designed bioprinters for constructing customized 3D cell-laden structures. For instance, through integrating electrospinning and extrusion-based bioprinting, a cell electro-writing technique has been developed, enabling the fabrication of well-organized living fibrous structures with significantly improved resolution: from hundreds of micrometers by conventional extrusion-based bioprinting to several micrometers (down to 5 μm) by the

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Table 1. Comparisons for different technologies for fabricating cell fibers.

Technology	Cell viability[a)]	Resolution[b)]	Advantage	Disadvantage	Ref.
Cell assembly and cell aggregation	>90%	≈50 μm	Easy to perform	Discontinuous production, limited resolution, simple geometry	[19,20]
Multi-interfacial polyelectrolyte complexation (MIPC)	>80%	≈50 μm	Easy to perform	Limited materials	[28,29]
Wet spinning	>80%	≈200 μm	Continuous production, simple setup	Low resolution	[34–36]
Aerodynamic-/Pressure-assisted spinning	>70%	≈50 μm	High resolution	Requiring high-pressure, unstable jetting	[40–42]
Microfluidic spinning	>90%	≈50 μm	Highly controllable geometry, mesostructure, and multi-material composition	Potential high cost for microfabrication	[47–54,61–70]
Bioprinting	>80%	≈100 μm	Stacking of cell fibers into customized 3D structures	High cost for bioprinter	[75–79]
Electrospinning	>90%	≈2 μm	Formation of ultrathin fibers and 3D cell-laden structures	Sensitive to multiple processing parameters	[104–109,113–116]

[a)] here it means the survival rate of cells encapsulated in cell fiber; [b)] here it is meant as the minimum diameter of cell fiber.

Table 2. Popular materials for fabricating cell fibers.

Material Type	Material	Advantage	Disadvantage	Ref.
Synthetic polymer	PEO	Good mechanical properties; small batch-to-batch variation	Sometimes requiring organic solvent; lack of bioactivity	[119]
	PVA			[119]
	PDMS			[103]
	PEGDMA			[53]
	PEG-4Mal			[54]
	POH and POA			[114]
	PCL			[109]
Natural polymer	Alginate	High cytocompatibility and/or bioactivity	Insufficient mechanical strength and/or toughness	[34,47,51]
	HA			[121,122]
	Collagen			[20]
	GelMA			[78]
	Amphiphilic peptides			[23]
Blend of synthetic and natural polymers	GelMA and PEGDA	Balanced mechanical properties and bioactivity	Increasing complexities in handling, manufacturing, and processing	[126]

integrated technique.[118] Certainly, there are still large gaps between synthetic cell fibers formed by existing fabrication techniques and native fibrous tissues, which should bring about numerous opportunities for developing advanced technologies that either combine the advantages of existing techniques or revolutionize cell fiber fabrication.

3. Materials for Cell Fibers

3.1. Synthetic Polymers

For the different technologies for fabricating cell fibers, there are a variety of materials, ranging from synthetic polymers to natural polymers (**Table 2**), which can be processed into fiber matrices for the encapsulation/entrapment of living cells. While good cytocompatibility is on the top of the list for required properties for fiber matrix materials, in practice, good fiber formability/printability is the first property for any materials that will be used for making cell fibers, and cells should be properly encapsulated/entrapped in the fibers. Directly suspending living cells within a viscous network of a biopolymer is a straightforward way for cell entrapment (**Figure 3**a). It has been shown that fibers with cell-carrying microstructures could be formed via bio-electrospraying through the use of high-viscosity poly(ethylene oxide) (PEO) and PVA to entrap living cells within their networks.[119] Using high-viscosity medical-grade PDMS, cell fiber with cells entrapped in a viscous network could also be formed via coaxial electrospinning.[103] Although these viscous biopolymers did not present acute toxicity to the cells, the attachment of cells and exchanges of nutrients/oxygen within and through the viscous polymeric networks are constrained, thus bringing about difficulties in maintaining/promoting the

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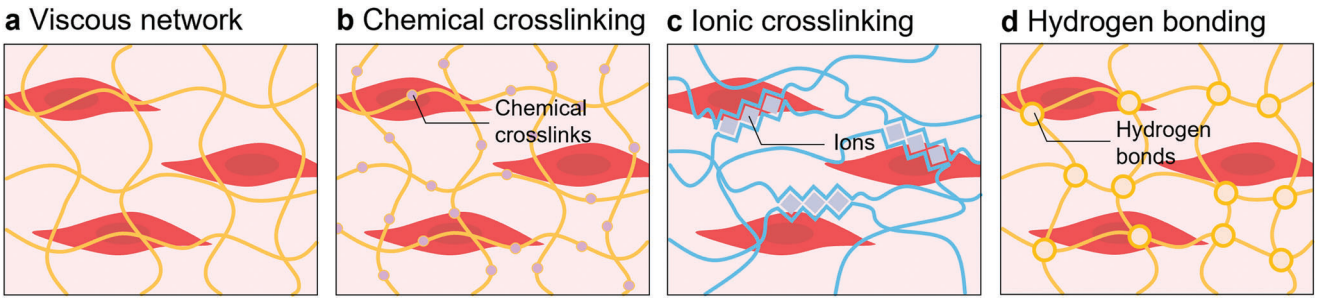

Figure 3. Schematical illustrations for representative network architectures for cell fibers.

viability and functions of entrapped cells in the subsequent in vitro and in vivo situations. To address this problem, synthetic polymers that can be cross-linked into stable water-abundant hydrogel networks that facilitate the growth and metabolism of encapsulated cells have been studied for forming the polymer hydrogel matrix for cell fibers (Figure 3b). For example, using PEGDMA [53] or PEG-4Mal,[54] hydrogel-based cell fibers were fabricated via flow-focus microfluidic spinning with the assistance of in situ chemical cross-linking. In addition, cell fibers with a hydrogel matrix could also be formed via reactive electrospinning by using two parallel syringes that fed aqueous solutions of POH and POA, which resulted in instant formation of chemically cross-linked networks through hydrazone-mediated reactions.[114] An alternative approach for producing cell fibers capable of maintaining or promoting the viability and functions of entrapped cells is to produce cell fibers with hollow structures. Such hollow cell fibers may be formed by coaxial electrospinning, where cells could be initially encapsulated in PVA-PCL core-shell coaxial electrospun microfibers and subsequently released after the degradation of PVA in a cell culture medium, resulting in cell-encapsulated hollow PCL microfibers.[109]

3.2. Natural Polymers

Compared to synthetic polymers, natural polymers such as polysaccharides, proteins, and peptides are much favored for producing cell fiber owing to their inherent excellent biocompatibility and bioactivity. Among these natural polymers, alginate, as one of the most abundant polysaccharides in nature,[120] has been frequently employed in cell fiber manufacture. It is compatible with almost all of the fiber formation technologies presented and discussed above (e.g., mold-/template-assisted cell aggregation, MIPC, wet spinning, microfluidic spinning, extrusion-based bioprinting, and cell electrospinning) and can be processed into solidified hydrogel-based cell fibers via mild ionic cross-linking (Figure 3c) or interfacial complexation. Hyaluronic acid (HA) is another commonly used polysaccharide for forming the matrix of cell fibers. It is readily remodeled by cells. Through microfluidic spinning or bioprinting, cell-instructive microfibers with an HA hydrogel matrix could be formed by using UV-cross-linkable methacrylated HA [121] or HAs functionalized with guest-host pairs (e.g., adamantane and β-cyclodextrin) [122] as precursors. Proteins such as gelatin, collagen, fibrinogen, and ECM proteins are also often used for fabricating cell fibers. They usually undergo spontaneous physical cross-linking via hydrogen bonding (Figure 3d) at the physiological/cell culture temperature of 37°C and are solidified into cell-encapsulated hydrogel fibers, of which the gel composition and features similar or identical to those of ECM could be achieved, promoting rapid remodeling of cells within fibers.[20] Through functionalizing these proteins with UV-cross-linkable groups, e.g., methacryloyl groups, hydrogel fibers with high resolution, well-defined geometry, and high-efficiency encapsulation of living cells could also be formed by microfluidic spinning or bioprinting with the assistance of in situ UV cross-linking.[78,123–124] As an important type of natural material extensively existing in body tissues, amphiphilic peptides have also been employed for making cell fibers via the molecular self-assembly approach. By selecting or synthesizing appropriate amphiphilic peptides capable of self-assembly into supramolecular nanofibers and then macroscopic gel filaments via bundling,[23] cell fibers with unidirectional alignment of encapsulated cells guided by self-assembled oriented supramolecular nanofibers could be formed, holding the promise to mimic body tissues with similar anisotropic architectures such as muscular bundles and nerve bundles.

Despite various advantages, cell fibers made of natural polymers still have shortcomings of inherent poor mechanical properties (low strength and toughness) as compared to native tissues. To improve the mechanical properties of cell fibers made of natural polymers, e.g., GelMA, composite or blending strategies have been adopted. It was shown that the mechanical properties of GelMA-based cell fibers could be enhanced by forming interpenetrating networks with nano-clay via physical interactions [125] or with poly(ethylene glycol) diacrylate (PEGDA) via hydrogen bonding.[126] It can be speculated that in the future, the mechanical properties and bioactivity of cell fibers can be further balanced for better directing the behavior and functions of encapsulated cells by rationally designing the matrix of cell fibers by blending synthetic polymers and natural polymers.[127]

4. Biomedical Applications Of Cell Fibers

4.1. Organ-On-a-Chip

Organ-on-a-chip is a revolutionary concept and effort in medicine founded on the convergence of multidisciplinary subjects. It aims to provide promising alternatives to high-cost animal models for fundamental cell biology investigations and pre-clinical pharmaceutic testing through replicating key features of human pathophysiology.[128] It is also highly possible in the future to develop implantable organ-on-a-chip devices that can function well

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in human bodies, providing essential assistance to patients with internal organ failures. An organ-on-a-chip device may be conventionally built by patterning specific types of cells into different layers/sites of a microfluidic chip. But there will be inherent differences between the 2D cell patterns in a conventional organ-on-a-chip device and the 3D cell organizations in native pathophysiological conditions in tissues/organs. Since cell fibers can provide desirable 3D cell culture platforms capable of precisely controlling spatial organizations of different types of cells and mimicking natural cell microenvironments in terms of structures and mechanics,[16] they are therefore very attractive in organ-on-a-chip applications.

Cell fibers with designed and easily formed multi-compartment and multi-cell structures have made them desirable cost-effective in vitro cell co-culture models for fundamental cell biology research, helping to gain insights into areas such as cell-cell interactions. Through configurable microfluidic spinning, hollow cell fibers with co-encapsulation of VECs and osteoblast-like cells (MG63 cells) were formed.[129] The VECs and MG63 cells inside hollow cell fibers were encapsulated in the lumen and shell of the fiber, respectively (**Figure 4**a). Owing to their capillary-mimicking perfusable structures and location-specific organization of the two types of cells, these dual-encapsulation cell fibers could provide an excellent in vitro platform for investigating interactions between VECs and osteoblasts, as well as their impacts on osteogenesis and vascularization, during the neo-formation and regeneration of vascularized bone. Also, cell fibers can provide desirable in vitro 3D cell culture models for investigating the underlying mechanisms of stem cell fate determination. Through microfluidic spinning, a core-shell structured microfiber with a human mesenchymal stem cell (HMSC)-encapsulated collagen hydrogel core and an alginate hydrogel shell was formed.[130] The stiffness of the outer alginate hydrogel shell could be simply raised by adding cytocompatible strontium ions (Sr^{2+}) into the culture medium, thereby creating tunable mechanical stimulations on the encapsulated HMSCs without changes in the bulk shape and biochemistry of the cell microenvironment. Such cell fibers would expand the tools box for fundamental stem cell research, e.g., investigations of the effects as well as the underlying mechanisms of tunable mechanical stimulations on the osteogenic differentiation of HMSCs.

Leveraging cell fibers, 3D tissue-specific models for drug screening can also be built. For example, hollow microfibers with the encapsulation of VECs have been formed via microfluidic spinning for modeling vascular endothelium.[131] The hollow core throughout the microfiber made it perfusable for easy transportation of bio-fluids. In addition, due to excellent biocompatibility, the hollow microfibers could support complete coverage of VECs on the lumen to form a confluent endothelial cell monolayer, providing a biomimetic endothelial barrier model. By simply integrating the perfusable VEC-laden hollow microfiber with co-cultured astrocytes on a micro-chip, a blood-brain barrier-on-a-chip model was constructed by Nguyen et al. (Figure 4b). Since the blood-brain barrier plays a vital role in protecting brain's functions and mediating the brain-target delivery of chemotherapeutics, the blood-brain barrier-on-a-chip established in Nguyen et al.'s study would provide a simple in vitro model, which could potentially substitute inaccessible natural brain tissues for pre-clinical evaluations of drugs to be used for treating brain diseases. Cell fibers have also been investigated to model the convoluted proximal tubule or glomerular filtration barrier that has tubular structures and provides critical tissue functions in human kidneys through co-culturing kidney-specific epithelial cells and VECs.[132–134] Through microfluidic spinning using microfluidic devices with specifically designed configurations, perfusable hollow microfibers with either helical geometries (mimicking the structure of convoluted proximal tubule) or expanded shapes (mimicking the structure of glomerulus) were also formed.[134] In this study, a functional glomerular filtration barrier was successfully established by using the cell fibers after one-month incubation, indicating the potential of cell fibers for modeling another functional tissue.

In addition to 3D tissue-specific physiology-relevant models, different disease-specific pathology-relevant models may also be constructed using cell fibers. For instance, VEC- and vascular smooth muscle cell (VSMC)-laden hollow microfibers with tunable shapes mimicking different pathophysiological states of arteries, including regular, stenotic, or tortuous arteries, were successfully formed via microfluidic spinning by modulating the flow rates (Figure 4c).[135] By engineering cell fibers with stenotic or tortuous shapes, hallmark events in early atherosclerotic pathological conditions, e.g., stenotic and tortuous turbulent flows, could be recapitulated in vitro, thus offering useful atherosclerosis-on-a-chip models for pathophysiological investigations or screening potential drugs/therapies for atherosclerosis. An Alzheimer's disease in vitro model was also built based on the assembly of cell fibers formed by microfluidic-assisted bioprinting,[136] where the cell fibers were made with a core-shell structure containing an alginate hydrogel outer barrier shell and a Matrigel core encapsulated with genetically engineered human neural progenitor cells. The encapsulated cells in the Matrigel matrix could sufficiently self-organize, inter-connect, and express high amounts of amyloid-β proteins mimicking the pathology of Alzheimer's disease. These cell fiber-assembled constructs may be used for pre-clinical evaluation of drugs for treating currently incurable neurodegenerative disorders.

4.2. Cell Therapy

Cell therapies, by harnessing living cells as "bio-factories" to modulate or restore tissue/organ functions, hold great promise for treating diseases previously incurable by conventional treatments, for example, islet transplantation for type 1 diabetes (T1D) treatment, T cell delivery for cancer immunotherapies (delivery of genetically reprogrammed T cell has been approved by FDA in 2017 for the treatment of acute lymphoblastic leukemia and large B cell lymphoma), and stem cell/progenitor cell injection for regenerating tissues lacking innate renewal ability.[137–139] To achieve good or satisfactory therapeutic outcomes, cell therapies generally require a sufficiently high quantity of therapeutic cells as well as well-preserved cellular viability and functions in long-term transplantation.[140] It is, therefore, necessary and important to develop suitable biomaterials as carriers for high-efficiency microencapsulation of therapeutic cells and protect them from immune rejection.[141] Cell fibers with a hydrogel matrix, micron-sized diameter, and extremely high length-to-width

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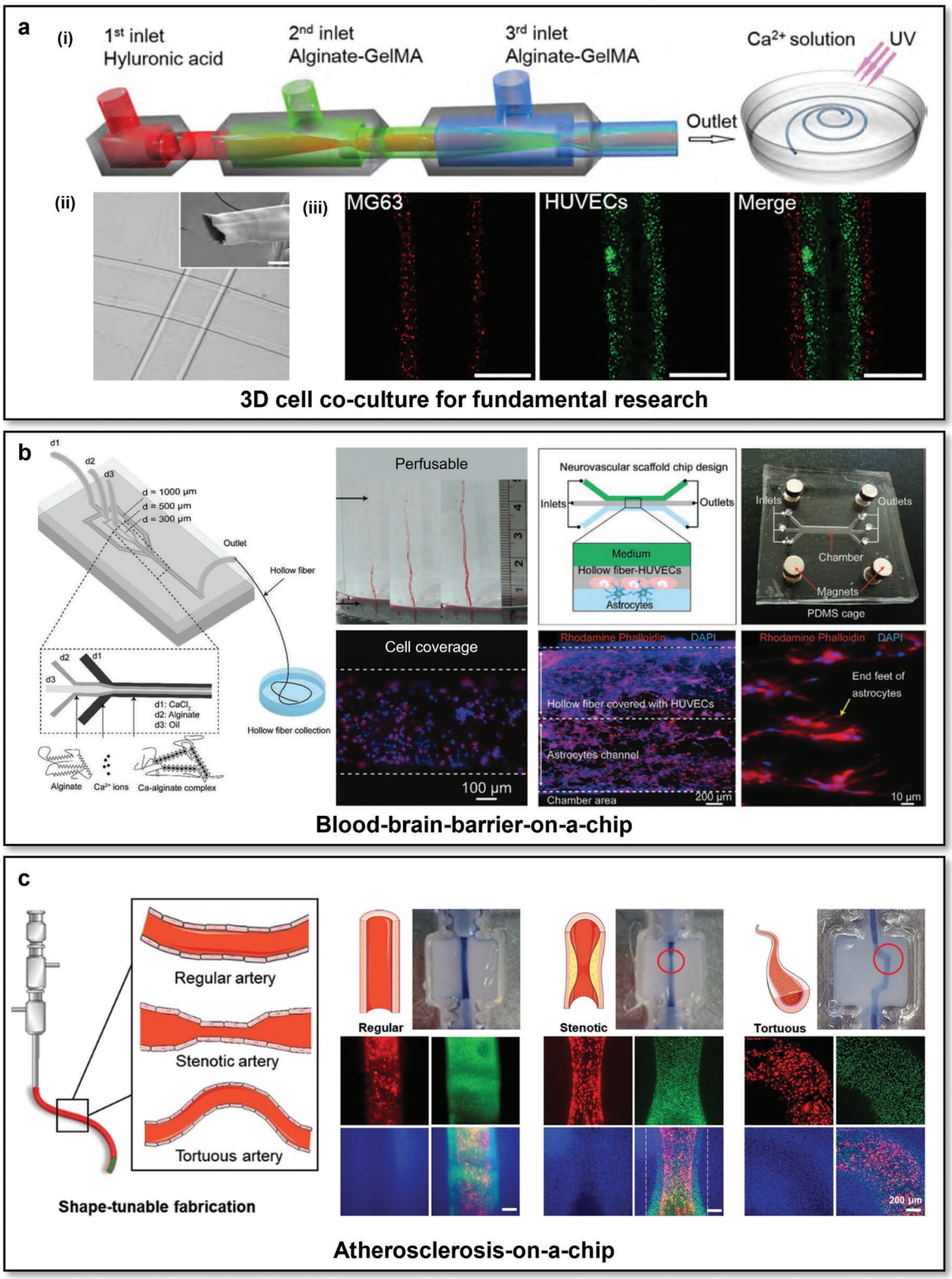

Figure 4. Organ-on-a-chip applications of cell fibers. a) Microfluidic spun microfibers used as a 3D cell co-culture model for investigating interactions between VECs and osteoblast-like cells (MG63 cells). Reproduced with permission.[129] Copyright 2016, Elsevier. b) Microfluidic spun hollow microfiber with encapsulation of VECs mimicking the structure and functions of endothelial barrier, which was further integrated with co-cultured astrocytes to form a simple blood-brain-barrier-on-a-chip model. Reproduced with permission.[131] Copyright 2018, Royal Society of Chemistry. c) VEC- and VSMC-laden hollow microfibers with tunable shapes formed by flow-focus microfluidic spinning as atherosclerosis-on-a-chip models. Reproduced with permission.[135] Copyright 2020, WILEY-VCH.

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ratios are particularly favorable to mass exchanges due to short diffusion distances. They offer excellent protective microenvironments for cell microencapsulation and hence hold great promise for cell therapies.

Microencapsulation of allogeneic pancreatic islets within alginate microgels has been proven highly effective in immune protection and long-term maintenance of islet viability and functions in the transplantation treatments of T1D.[142] Successful microencapsulation in microfluidic spun microfibers of pancreatic islets for effective immune protection was realized, with the hybrid hydrogel matrix consisting of alginate and collagen.[143] However, islet-encapsulated microgels with a sole hydrogel matrix usually possess poor mechanical stability, which will be difficult to be retrieved or replace after therapies. To address this issue, a composite cell fiber, termed thread-reinforced alginate fiber for islets encapsulation (TRAFFIC), was formed via template-assisted cell assembly,[144] where allogeneic pancreatic islets were coated on a twist-folded Nylon suture thread template and then encapsulated within an alginate hydrogel outer shell for immune protection (**Figure 5**a). Transplanted islets via TRAFFIC could attain long-term effectiveness in controlling blood glucose concentration in an immunocompetent T1D mouse model, suggesting good immune protection for the encapsulated islets by TRAFFIC carrier. Additionally, TRAFFIC possessed reinforced mechanical stability owing to the tough suture thread core, and it could also be conveniently retrieved or replaced through a minimally invasive laparoscopic procedure after therapies. Such composite cell fibers with excellent preservation of transplanted islet functions and good retrievability contribute to reliable and safe treatments for T1D patients.

Cell fibers also provide excellent 3D cell culture platforms for stem cells/progenitor cells, facilitating the development of stem cell- or progenitor cell-based therapies in regenerative medicine. When seeking the use of stem/progenitor cells for regenerative medicine, several essential requirements must be met, including 1) maintenance of stem/progenitor cell potency, 2) effective expansions of stem/progenitor cell amounts, and 3) inducing stem/progenitor cell differentiation into specific phenotypes.[138] Cell fibers have been shown to facilely encapsulate stem/progenitor cells within biomimetic hydrogel matrices in 3D cell culture manners, where the encapsulated different types of stem/progenitor cells, including human embryonic stem cells,[145] human adipose stem cells,[146] HMSCs,[147] neural stem cells (NSCs),[148] etc., could all achieve rapid expansion and well-preserved multi-potency via elaborately designed hydrogel matrix. Within cell fibers having an alginate hydrogel matrix, human lung progenitor cells (HLPCs) could be not only rapidly expanded but also differentiated into airway epithelial cells and alveolar type II cells in controllable manners, as revealed by upregulated expressions of phenotype-specific biomarkers, without the need of feeder fibroblasts (Figure 5b).[149] The cell-fiber-based strategy capable of supporting cell expansion and inducing bi-directional differentiation of HLPCs has the potential for cell therapies for lung diseases. Cell fibers have also been explored for cell therapies in regenerative medicine by modulating the host immuno-inflammatory response. Through encapsulating immune-related astrocytes, a neuro-supportive system was built based on astrocyte-laden microfiber having a hydrogel matrix made of the tripeptide Arg-Gly-Asp (RGD)-functionalized alginate which was formed via microfluidic spinning.[150] The microfluidic spun astrocyte-laden microfibers could effectively promote directional neurite outgrowth and synaptic formation of hippocampal neurons cultured on them even without any physical contact between the two types of co-cultured cells (Figure 5c), opening new avenues of cell therapies for treating neural injuries and neurodegenerative disorders through engineered immunomodulatory microenvironments. By means of simply adding low concentrations of cryoprotective agents in cell fibers, it was demonstrated to be feasible for long-term storage of cell fibers via cryopreservation.[151] It was shown that the encapsulated HMSCs in the core-shell alginate hydrogel microfibers could have high-percentage viability and maintain multi-potency after freeze-thawing cycles. Such cell fibers offer the possibility of scalable manufacturing of ready-to-use cell-based products, laying important foundations for future commercialization of reliable and standardized cell carriers for cell therapies.[152]

4.3. Tissue Engineering

4.3.1. Cell Fiber-Based 3D Structures

Cell fibers with tailored geometry, mesostructure, multi-material composition, and multi-cell organization are highly attractive as living building blocks in "bottom-up" biomanufacturing, whose ultimate goal is to reconstitute/replicate the physiological complexities of human native tissues. Realizing this goal requires not only placing specific cells into the desired 3D spatial forms but also mimicking complex cell-ECM organizations of native tissues. Through bioprinting, it is possible to make 3D cell-laden structures via programmed stacking of printed cell fibers. However, cells in the structures are usually embedded within the hydrogel matrix and hence difficult to be remodeled to attain sufficient cell-cell and cell-matrix interactions. Electrospinning is a versatile technology and can easily form ECM-mimicking nanofibrous architectures which can promote biomimetic interplays with cells. It is therefore highly promising to obtain hierarchical cell-laden structures to recreate the complex cell-ECM organizations of native tissues through integrating cell fibers with electrospun nanofibrous structures.

In our efforts to construct hierarchical cell-laden structures that would facilitate biomimetic cell-matrix interplays, we developed a new biomanufacturing method, i.e., concurrent emulsion electrospinning and coaxial cell electrospraying.[153] Biodegradable emulsion electrospun core-shell structured nanofibers with loadings of specific growth factors (GFs) and coaxial electrospray cell-encapsulated alginate hydrogel microspheres were co-deposited using this concurrent biomanufacturing process, resulting in the formation of bio-hybrid structures where cell-encapsulated microspheres were uniformly distributed within the nanofibrous polymeric matrix. Upon selective lysis of the alginate hydrogel via rapidly extracting Ca^{2+} by a low-concentration (0.055 M) sodium citrate aqueous solution, the encapsulated cells could be released through the triggered-release mechanism and hence placed within the GF-loaded nanofibrous matrix, thereby forming biomimetic 3D cell-laden structures. Compared to the 3D cell-laden structures formed by the established method of alternate electrospinning and bioprinting where cells were

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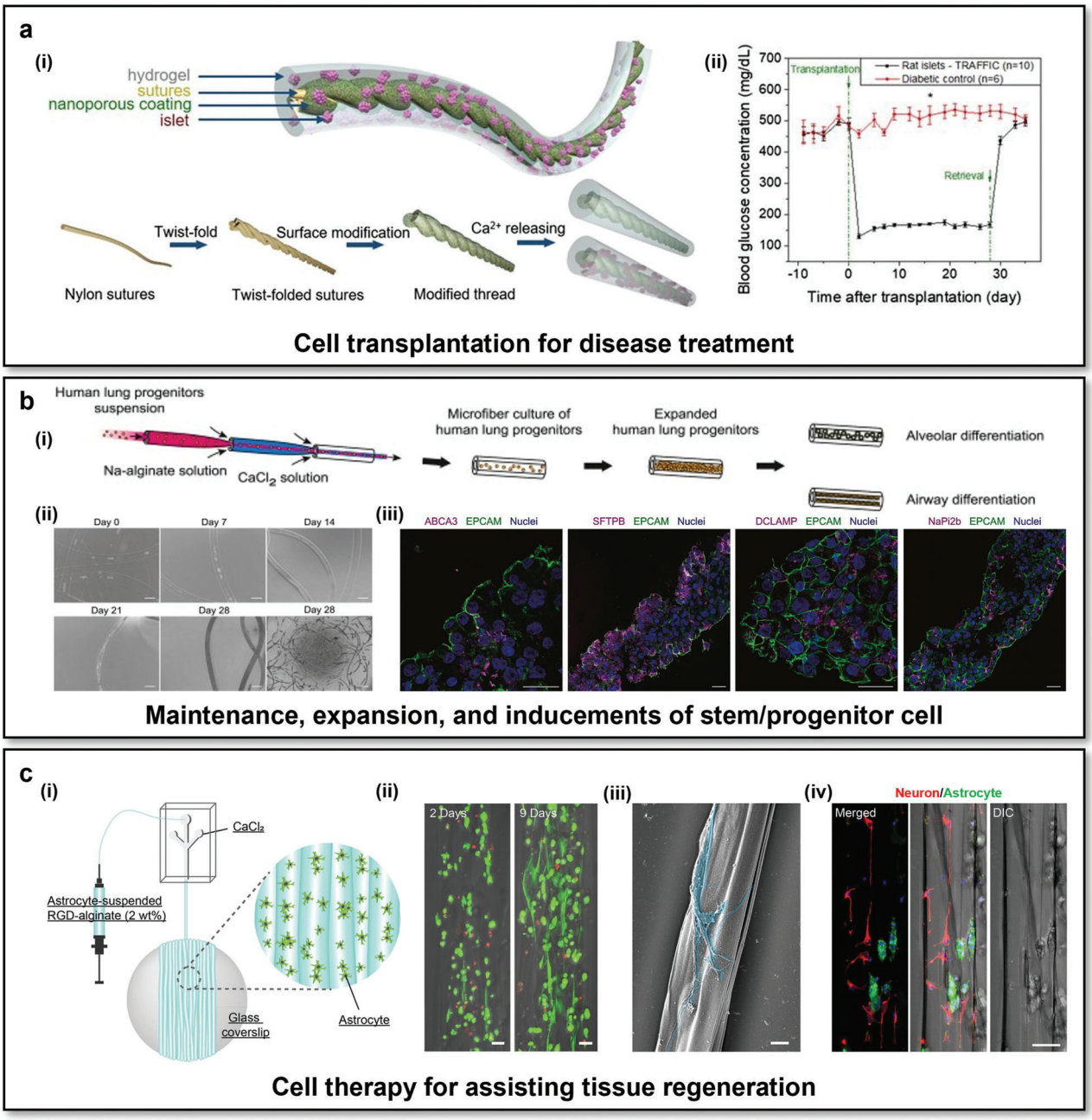

Figure 5. Cell fibers for cell therapy. a) Microfiber with encapsulated allogeneic islets for immune protection and transplantation treatment of T1D. Reproduced with permission.[144] Copyright 2018, National Academy of Science. b) Microfiber for 3D culture of human lung progenitor cells with excellent maintenance of viability and bi-potency to be differentiated into airway epithelial cells and alveolar type II cells. Reproduced with permission.[149] Copyright 2021, Elsevier. c) Astrocyte-laden microfibers formed by microfluidic spinning for enhancing directional neurite outgrowth and synaptic formation via immune modulation. Reproduced with permission.[150] Copyright 2020, WILEY-VCH .

placed within bioprinted parts sandwiched nanofibrous matrix layers,[154] the 3D cell-laden structures fabricated via concurrent emulsion electrospinning and coaxial cell electrospraying exhibited 3D uniform cell distribution within the bioactive nanofibrous matrix and also superior structural integrity.[153] Furthermore, the concurrent emulsion electrospinning and coaxial cell electrospraying technique could be slightly modified to form 3D nanofibrous multicellular constructs mimicking the cell-ECM organizations and also their interactions that are similar to those in body tissues with complex anatomies such as blood vessels.[155] In these 3D nanofibrous multicellular constructs, VECs and VSMCs were placed within separate construct layers consisting of biodegradable nanofibers with different orientations and capable of controlled release of different types of GFs. The behavior and functions of both 3D incorporated VECs and VSMCs within the multicellular constructs could be effectively

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guided by the cell-matrix interactions mediated by the controlled release of GFs and isotropic/anisotropic nanofibrous topographies, demonstrating a very promising "bottom-up" biomanufacturing strategy for building tissue analogs resembling body tissues in not only their structures but also their functions.

The success of the concurrent emulsion electrospinning and coaxial cell electrospraying approach in forming biomimetic 3D nanofibrous multicellular constructs with implications for tissue engineering applications also inspired the research on cell-fiber-based 3D structures for the repair and/or regeneration of body tissues with fiber-like fascicle morphologies.[156] An important prerequisite for achieving these is to develop enabling techniques for producing cell fibers whose matrix could be selectively broken down on demand for releasing the encapsulated cells and the techniques should be easily integrated with electrospinning for forming the desired ECM-mimicking nanofibrous architectures. Through microfluidic spinning using phenolic-substituted alginate and gelatin for the hydrogel matrix accompanied by visible-light photo-cross-linking, cell fibers that could be degraded by the encapsulated cells via cell remodeling could be formed,[146] significantly alleviating the confinement of hydrogel networks on the cytoskeleton developments and proliferation of the encapsulated cells. Furthermore, microfluidic spun alginate hydrogel-based cell fibers could be completely degraded when alginate lyase-loaded nanoparticles were added to the core flow.[157] Encapsulated cells could then be released after the lysis of the alginate hydrogel matrix. However, this approach faces technical difficulties, such as a large difference in throughput between the two spinning methods, when seeking to integrate such microfluidic spinning with electrospinning, even though efforts have been made to optimize the throughput of microfluidic spinning in the fabrications of cell fibers, for example, developing a new method of in-air microfluidic spinning that could directly generate free-floating cell fibers in a chip-free platform by manipulating microscale liquid streams in the air.[158] Recently, we reported a method of concurrent cell electrospinning and emulsion electrospinning with similar throughputs for constructing cell-fiber-based 3D structures.[116] Through concurrent cell electrospinning and emulsion electrospinning, cell fibers with the encapsulation of either human dermal fibroblasts (HDFs) or HMSCs were co-deposited with biodegradable nanofibers that were loaded with basic fibroblast growth factor (bFGF), producing bio-hybrid scaffolds, which subsequently formed 3D homogeneous cell-laden nanofibrous structures after the encapsulated cells were released from cell fibers via selective lysis of the alginate hydrogel matrix (**Figure 6**a). The proliferation and cytoskeleton development of HDFs and HMSCs within the 3D cell-laden structures could be effectively promoted by the anisotropic topographical cues and controlled release of bFGF from nanofibers, with both being offered by the nanofibrous matrix. These 3D cell-laden structures have a high potential for repairing or regenerating body tissues with fiber-like fascicle morphologies such as tendons/ligaments. Extensive research should be conducted in the future to further optimize the structural integrity of cell fiber-based 3D structures by employing cell fibers with higher resolutions as living building blocks, as well as promoting translational research to apply these cell fiber-based 3D structures to repair/regenerate targeted body tissues.

4.3.2. Vascular Tissue Engineering

Popular strategies in vascular tissue engineering are the development of customized tubular scaffolds with tailored porous architectures for supporting attachment and ingrowth of VECs and VSMCs seeded manually or recruited from host tissues.[159–161] However, there have been challenges for these strategies, including in situ rapid endothelialization on the lumen of synthetic vascular scaffolds for long-term patency and organizing VECs and VSMCs into well-defined tissue layers for achieving successful vascular remodeling.[162] Despite successful 3D endothelialization and well-defined organization of different types of cells in different tissue layers which were achieved recently with the assistance of novel reconfigurable scaffolds for bioengineered vascular tissue analogs whose diameters were above 1 mm,[163–166] it would be difficult, if possible, to apply such reconfigurable scaffold strategy for engineering artificial veins or arteries with smaller diameters owing to increasing complexities in handling. On the other hand, microfluidic spinning and coaxial bioprinting, which have been proven highly effective and efficient in producing tubular cell-laden microfibers, have provided some promising solutions. Through microfluidic-assisted coaxial bioprinting using hybrid bioinks formulated by mixing vascular-tissue-derived decellularized ECM and alginate, hollow microfibers with encapsulated living endothelial progenitor cells were produced,[64] mimicking the endothelial layer of blood vessels. Artificial small-diameter blood vessels with biomimetic biphasic cell layers (i.e., a VEC-laden inner layer and a VSMC-laden outer sheath) could be further built up using microfluidic-assisted coaxial bioprinting when suspending VECs and VSMCs in the middle-layer and outer-layer sheath flows, respectively.[126] By formulating bioinks using natural proteins (e.g., Matrigel), cells encapsulated in the matrix of hollow cell fibers could even self-organize and sufficiently remodel into well-defined vascular tissue layers that possess respective specific vascular functions (e.g., quiescence, perfusability, and contractility).[167] In addition to the spatial organization of specific tissue layers, mechanical properties (e.g., toughness, strength, anti-fatigue performance, and pressure-bearing capability) of native blood vessels could also be mimicked and attained by this type of hollow cell fibers formed by using bioinks capable of forming mechanically reinforced networks (Figure 6b), for example, using hybrid bioinks consisting of nano-clay, *N*-acryloyl glycinamide, and GelMA that could have robust physical interactions among them,[125] or, alternatively, using hybrid bioinks consisting of gelatin and alginate that could yield interpenetrating double networks.[79] It appears promising that the repair, regeneration, or even replacement of injured or diseased small-diameter blood vessels will be achieved by using bioengineered hollow cell fibers with biomimetic structures and adequate mechanical properties.

4.3.3. Muscular Tissue Engineering

Muscular tissues, ranging from skeletal muscle to myocardium, mostly consist of axially aligned myotubes with fiber-like fascicle morphologies through bundling of unidirectionally aligned

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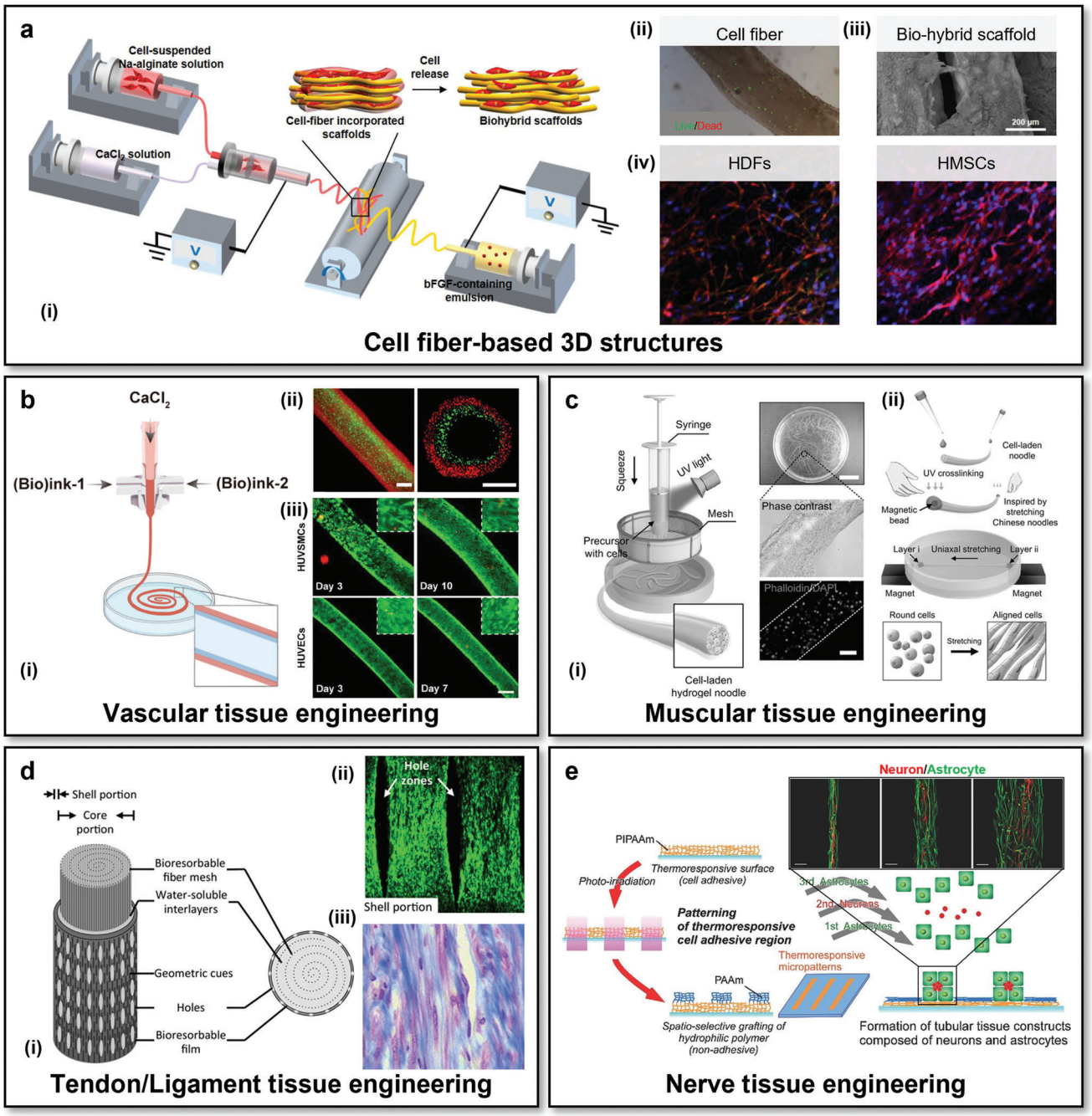

Figure 6. Tissue engineering applications of cell fibers. a) Concurrent cell electrospinning and emulsion electrospinning for forming bio-hybrid scaffolds consisting of bioactive bFGF-loaded nanofibrous matrix and 3D incorporated cells (HDFs and HMSCs), where HDF- and HMSC-encapsulated cell fibers formed by cell electrospinning were used as vehicles for placing cells in the bioactive nanofibrous matrix to achieve biomimetic 3D multicellular organization. Reproduced with permission.[116] Copyright 2021, World Scientific Publishing Co Pte Ltd. b) Microfluidic bioprinting of tough hydrogels into vessel-mimicking cell-laden hollow microfibers with VECs and VSMCs encapsulated in the inner lining and outer sheath, respectively. Reproduced with permission.[79] Copyright 2022, American Association for the Advancement of Science. c) Myocyte-laden microfibers formed by wet spinning and in situ UV cross-linking. The encapsulated myocytes were stretched by external magnetic fields to be unidirectionally aligned mimicking the anatomies of muscular bundles. Reproduced with permission.[169] Copyright 2015, WILEY-VCH. d) A microfiber with engineered surface topography as the template for directing alignment and remodeling of human tenocytes, aiming to promote functional recovery of tendon/ligament. Reproduced with permission.[175] Copyright 2018, American Association for the Advancement of Science. e) Free-floating cell fibers composed of neurons and astrocytes with specific spatial organization resembling the anatomies of anisotropic nerve bundles. Reproduced with permission.[22] Copyright 2016, WILEY-VCH.

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smooth muscle cells. Cell fibers with encapsulated myocytes can mimic the structures consisting of axially arranged myotubes, providing the promise for muscular tissue engineering.[168] Through microfluidic spinning or coaxial bioprinting, it is shown to be convenient to form myocyte-laden cell fibers with well-defined geometry and well-preserved cell viability and functions. The shape of encapsulated myocytes could be further elongated and the cells could be unidirectionally aligned, mimicking the anatomies of myotubes, which was achieved by means of magnetic-field-assisted uniaxial stretching [169] (Figure 6c) or by manual stretching with the assistance of an elastic stretching device.[170] Also, cell fibers with anisotropic alignments of encapsulated myocytes could be formed via cell electrospinning by using a myocyte-alginate/PEO suspension as the precursor.[171] The alignment of myocytes could be directly guided by the sheer force during electrospinning as well as the anisotropic topographical cues endowed by concomitant aligned nanofibers that were produced during concurrent electrospinning. By simply shifting the alignment of cell fibers, inter-woven aligned cell-laden structures could be formed,[172] mimicking the anisotropic structures of myocardium. Myocyte-laden cell fibers alone can only be used as the living building blocks for "bottom-up" biomanufacturing of myotubes, whereas the bulk structures of muscular tissues may be assembled from bundles of myotubes and perfused with vascularized networks. Through concurrent cell electrospinning and bioprinting, micro-/nano-hierarchical structures containing both myocytes and VECs have been made,[154] offering the prospect of forming biomimetic vascularized 3D cell-laden constructs mimicking the physiological complexity of native skeletal muscles. A new method of acoustofluidics has been reported recently for patterning muscle progenitor cells (myoblasts) into fascicle morphologies with tunable diameters in a spinning-free, contactless, and high-throughput manner.[173] Leveraging on the acoustofluidic forces generated by an external piezoelectric transducer for patterning cells in a precursor cell-GelMA suspension into parallel lines with controllable distances, 3D cell-laden structures with parallelly aligned myoblast filaments mimicking the assembled structures of skeletal muscles were subsequently formed via acoustofluidics and in situ UV cross-linking. Acoustofluidics appears to be a simple yet effective method for constructing 3D cell-laden structures assembled by multiple cell fibers. It is promising not only for muscular tissue engineering but also for engineering many other tissues that consist of bundles of a fibrous cellular "base unit".

4.3.4. Tendon/Ligament Tissue Engineering

In addition to blood vessels, skeletal muscle, and myocardium, tendon/ligament with typical anisotropic anatomies can also be repaired or regenerated with the assistance of cell fibers. Due to high axial strength and toughness of native tendon/ligament tissues, it is highly challenging for conventional cell fibers with generally viscous or hydrogel matrix to meet the specific biomechanical requirements in tendon/ligament tissue engineering. Consequently, template-assisted cell assembly methods are normally used for constructing composite cell fibers as tissue analogs for tendons/ligaments. Specifically, using suture threads as templates, human tendon-derived cells (HTCs) have been put on the surfaces of suture threads, followed by encapsulation in an alginate hydrogel outer shell that would be cross-linked via in situ ionic cross-linking, resulting in composite cell fibers.[174] The encapsulated HTCs could be remodeled within the composite cell fibers, which was evidenced by up-regulated expressions of tendon-relevant genes. Using textile methods, the composite cell fibers could also be weaved into bundled 3D structures mimicking the structures and mechanics of native tendon tissues, showing their high attractiveness for the repair or regeneration of tendons. By using a microfiber template with engineered anisotropic surface microgrooves, composite cell fibers with aligned human tenocytes could also be formed (Figure 6d).[175] It was shown that tendon-specific phenotype, remodeling, and maturation of the incorporated human tenocytes in vitro and functional restoration of injured tendons in vivo could all be promoted by the topographical cues of the composite cell fibers with the engineered anisotropic surface microgrooves. The studies on cell fibers for tendon/ligament tissue engineering applications also imply an attractive future direction of combining cell fibers and conventional scaffold-based strategies for assisting the repair or regeneration of specific hard tissues.

4.3.5. Nerve Tissue Engineering

Human nerves, including central nervous system (CNS) and peripheral nervous system (PNS), play vital roles in integrating and controlling functions of various body tissues/organs yet lack sufficient innate regeneration ability. It is, therefore, necessary and also urgent to develop appropriate tissue engineering strategies for assisting the reconstruction or restoration of structures and functions of neural tissues upon their injury or disease. Scaffold-based strategies, with the aim of developing appropriate scaffolds of synthetic or natural materials for guiding neurons, Schwann cells (SCs), and/or glial cells (e.g., microglial cells and astrocytes) to be anisotropic nerve bundles via engineered topographical and biochemical cues, have been widely investigated in nerve tissue engineering,[176] with the majority of studies focusing on regenerating PNS nerves while CNS regeneration faces huge challenges. PNS scaffolds normally require elaborate design of surface micro-/nano-structured topography, biophysical properties, and biochemistry for effectively guiding the reconstruction/regeneration of anisotropic nerve bundles. For example, engineering anisotropic surface micro-/nano-structured topography for enhancing axonal ingrowth and/or introducing electroactive agents/neurogenic factors for directing neural differentiation of stem cells were reported.[177,178] In comparison, cell fibers enable straightforward approaches by directly organizing different types of neural cells into biomimetic anisotropic forms within cell-conductive microenvironments. For example, microfibers with well-defined spatial organizations of NSCs and SCs mimicking structures of peripheral nerves could be simply formed by wet spinning using a coaxial spinneret.[179] Furthermore, conductive agents such as graphene could be easily incorporated into neuron-laden cell fibers formed by microfluidic spinning by dispersing it within the core flow.[180] It was shown that the incorporated graphene not only significantly lift the conductivity of cell fibers to a level similar to that of native neural tissues but also promoted the maturation

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of the encapsulated neural cells. Using a thermoresponsive culture substrate, neurons, and astrocytes could be initially patterned into stripes and then released from the substrate to become free-floating cell fibers upon decreasing the temperature to trigger the wettability change of the thermoresponsive culture substrate. These biomaterial-free cell fibers could attain physiologically relevant communications between the neurons and astrocytes and subsequently self-organize the two types of cells into unidirectional alignments mimicking the structures of anisotropic nerve bundles (Figure 6e),[22] giving the promise of alternative "bottom-up" approaches for nerve tissue engineering.

4.3.6. Other Tissue Engineering Applications

Apart from the tissue engineering applications presented and discussed above, cell fibers have also been investigated for other tissues. For example, through MIPC involving two or more interfaces, it is possible to generate cell fibers with co-encapsulation of different types of cells that will be organized in specific spatial manners. It has been shown that by incorporating different types of cells within cell fibers via multi-phased MIPC, multicellular structures assembled by the cell fibers mimicking the anatomies of different tissues could be formed (e.g., assembly of dermal papilla and keratinocytes for mimicking the structure of hair follicle,[31] assembly of induced pluripotent stem cell-derived hepatocytes and VECs for mimicking the structure of liver, and assembly of HMSCs for mimicking the structures of cartilage.[33]), offering various promising platforms for the repair or regeneration of these tissues. Through microfluidic-assisted bioprinting using a composite bioink containing alginate, NIPAAm monomer, PEGDA, black phosphorus (BP) nanosheets, nano-hydroxyapatite, and HUVECs, hierarchical constructs were formed by 3D stacking of HUVEC-laden hollow microfibers after in situ ionic and UV cross-linking.[181] Upon exposure to near-infrared (NIR) irradiation, the hollow microfiber structs within the hierarchical constructs could be expanded into perfusable architectures arising from the photothermal effect of the BP nanosheets and the thermoresponsive property of PNIPAAm. The hierarchical constructs containing osteogenic components and possessing NIR-controlled perfusable architectures would therefore promote the regeneration of vascularized bone tissues. Furthermore, since fiber-like fascicle morphologies are very often seen in various tissues/organs of the human body, it can be envisaged that cell fibers, which can be made using different technologies and can have different structures, will have a wider range of applications in the tissue engineering field.

5. Summary and Outlook

With advances in different disciplines and fields such as materials sciences and engineering, biology, and manufacturing technologies, cell fibers have emerged as promising living building blocks for "bottom-up" biomanufacturing of human body tissues, especially those with fiber-like fascicle morphologies. A variety of enabling technologies have been developed for forming cell fibers with tailored geometry, structure, composition, mechanical properties, and 3D cell spatial organization, while electrospinning has shown its particular superiority among them in high resolution and in ease of forming tissue-mimicking 3D hierarchical cell-laden constructs. Cell fibers and 3D structures based on them have shown numerous opportunities for applications in organ-on-a-chip, cell therapy, tissue engineering, and many other biomedical areas. Despite their high promise in biomedicine, great challenges still remain for producing cell fibers to recapitulate the complicated physiology or pathophysiology of native tissues in terms of structure, mechanics, and biochemistry. Not only rational design and clever and delicate formation of higher-order hierarchical structures but also proper engineering of dynamic cell microenvironments for directing the behavior and functions of encapsulated cells are required so as to meet the in-service demands. The fulfillment of these requirements will depend on new inventions/innovations in materials and engineering and manufacturing technologies and also advances in biological and clinical sciences, for example, integrating intelligent responsive materials whose shape, stiffness, and other properties can be dynamically tuned by external stimuli,[182–185] developing 4D bioprinting for the synthesis of structures with changeable geometries and mechanics,[186–189] incorporating and delivering bioactive molecules for further directing cell behavior,[190–192] introducing new concept of synthetic biology for coupling programmable cell behavior and synthetic cell-instructive microenvironment,[193] and/or gaining new insights into both cell-cell and cell-material interplays.[194,195] It can be envisaged that cell fibers with improved and expanded functions, including sensing, responding, and even self-adaption in a closed-loop manner for better simulating/accommodating the native cell microenvironments, can and will be developed. Such future cell fiber developments will offer new diagnostic and/or therapeutic platforms and open up a myriad of biomedical applications for novel and advanced cell fibers.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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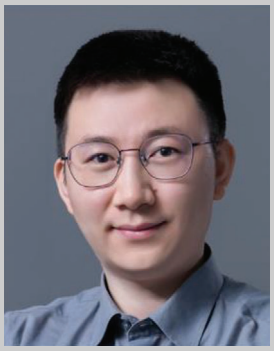

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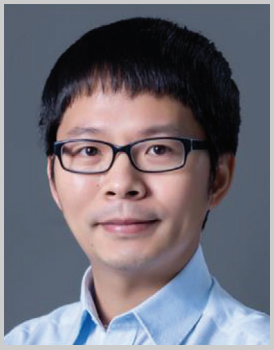

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