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Recent Advances in Microfluidically Spun Microfibers for Tissue Engineering and Drug Delivery Applications

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Abstract

In recent years, the unique and tunable properties of microfluidically spun microfibers have led to tremendous advancements for the field of biomedical engineering, which have been applied to areas such as tissue engineering, wound dressing, and drug delivery, as well as cell encapsulation and cell seeding. In this article, we analyze the most recent advances in microfluidics and microfluidically spun microfibers, with an emphasis on biomedical applications. We explore in detail these new and innovative experiments, how microfibers are made, the experimental purpose of making microfibers, and the future work that can be done as a result of these new types of microfibers. We also focus on the applications of various materials used to fabricate microfibers, as well as their many promises and limitations.

1. INTRODUCTION

1.1. Background

Because of the way that cells in the human body influence a myriad of applications, we know that incredibly small components can play a significant role in affecting and benefiting the body, and this is demonstrated extensively in microfibers. In this article, we discuss the various applications of microfibers in many fields. In fact, the connective tissues in our bodies have inspired new methods for producing microfibers. Naturally occurring fibers such as collagen and elastin, which can provide tensile strength and elasticity to blood vessels, have motivated scientists to fabricate microfibers (1) with highly porous scaffolds from biocompatible natural polymers on which to promote cell growth and adhesion for experiments (2) as well as to better improve tissue engineering applications such as wound dressing (3) and drug delivery (4). In addition, when 2D and 3D scaffolds are constructed from these fibers, cells can be adhered to them and can assemble and behave similarly when placed in an environment that mimics the extracellular matrix of the human body (5), making microfibers suitable for experiments on how cells react to drugs (6) and specific tissue growth (7). By modifying the flow rates and fluid concentrations (8) of the core and sheath flows in a microfluidic device, an operator can control the size (9), shape (10), and surface features (11) of a single microfiber and continuously generate uniform fibers with these same characteristics (12). Microfluidic spinning, as opposed to other methods, is an ideal choice for microfiber generation because it can produce large quantities of microstructures without a lot of equipment (13). These microfibers can be fabricated out of natural polymers that are biocompatible, biodegradable (14), nontoxic (15), environmentally safe (16), and cheap and easy to produce, making them ideal for use in biological experiments.

1.2. How Are Microfibers Made?

A tremendous advantage of microfibers is that they can be manufactured out of various types of biocompatible natural and synthetic materials, including polymers such as polyethylene glycol, polycaprolactone (17), and polyethylene glycol diacrylate (PEGDA) (18), which are naturally nontoxic and chemically inert and have a structurally rigid composition (19). Microfiber fabrication is accomplished by introducing an aqueous solution as the core fluid flows in a nozzle, which is continuously pumped into a sheath fluid solution. When the precursor is pumped into a gelation agent, the precursor solution becomes gelled and solidifies into fibers (20). With alginate fibers, aqueous sodium alginate (Na-Alg) is pumped into a calcium chloride ($CaCl_2$) solution (21). Because this gelation process does not involve temperature change and is mild, living cells and biomolecules are encapsulated without harm. The gelation speed of alginate is rapid and inexpensive, and the mechanical properties can be changed during fabrication, as is the case with all microfibers. A diagram showing how microfibers are manufactured is shown in **Figure 1**.

Microfluidic spinning is commonly used because numerous complications occur from electrospinning (23), wet spinning (24), biospinning (25), and melt spinning (26) methods. With the microfluidic method, by changing flow rate and the flow rate ratio (27), an operator can control the fiber size and aspect ratio (28). The fiber is created in a microchannel by using coaxial flow of prepolymer and sheath fluids with a dual-drive syringe pump to introduce simultaneously the core and sheath fluids into the microchannel. The microchannel contains three inlets: one in the middle for the core flow and one on each side of the middle inlet for the sheath flows. The shear force between the fluids focuses the core fluid in the center of the microchannel and fibers are formed from the core fluid. By altering the core and sheath flow rates, a microfluidic device can be used to continuously make microfibers, as fabrication ceases only when the core and sheath fluids stop flowing. When the flow rate ratio is decreased from 40:5 to 10:5, the tensile stress

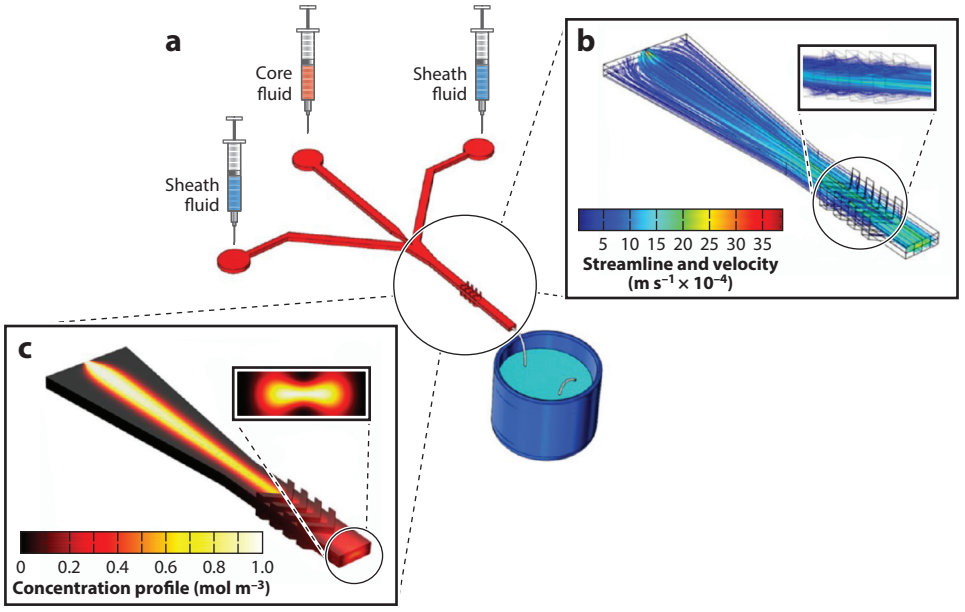

Figure 1

(*a*) Diagram of microfluidic fiber fabrication. (*b*) Fluid velocities in the channel. (*c*) Concentration profile of the microfluidic channel, showing how fluid flows inside, where the light and dark colors represent the core and sheath flows, respectively. Figure adapted with permission from Reference 22; copyright 2016 American Chemical Society.

at break, the tensile strain at break, and the Young's modulus all improve significantly, which is supported by studies showing that microfluidically spun microfibers possess excellent mechanical properties (29) while remaining flexible (30). These results show that the flow rate ratio of the core and sheath fluids plays a significant role in the mechanical properties of the fabricated microfibers (31).

2. MICROFIBERS FOR HEALTH CARE APPLICATIONS

2.1. Biocompatibility and Use of Microfibers

Microfibers used in health care and biological experiments must possess certain properties to function properly. For example, microfibers designed for tissue engineering must have a large number of cells that can be encapsulated, a scaffold on which the seeded cells can develop, and be made of a biologically safe material. Various proteins and polysaccharides also need to be used as natural biopolymers. And for cell culturing and cell seeding experiments, extracellular matrix proteins such as collagen (32) and its denatured form, gelatin (33), are used in microfluidic applications because they are biocompatible and biodegradable. Gelatin in particular is a neutral, nontoxic, inexpensive, and water-soluble substance that is also biocompatible (34) with cell adhesiveness. It also possesses excellent physical and chemical properties that make it an ideal material for microfibers (35). Tissue engineering and cell encapsulation are discussed in detail in Section 3. Natural polysaccharides such as alginate, chitosan, collagen, and cellulose are also used in cell culturing, cell seeding, and tissue engineering experiments (36) and, with the exception of collagen, are inexpensive (37).

For example, gelatin microfibers were fabricated with a microfluidic device with four chevron grooves on the top and bottom of a microchannel, where the prepolymer stream was hydrodynamically focused to the center of the microchannel to allow it to adjust to specific shapes and sizes. When the concentration of the gelatin in the core solution was increased from 8% to 12%, the mechanical properties of the gelatin fibers were greatly improved; the Young's modulus and tensile stress at the break were 2.2 and 1.9 times greater than those measures at the 8% concentration break. The experiment also indicated that increasing the gelatin concentration by 1% increased the viscosity by 1.8 times on average, and the created fibers remained uniform in width. This finding indicates that the microfluidic process remains viable in a large viscosity range and is a feasible method for producing biocompatible fibers out of gelatin (38).

Alginate-based microfibers have recently received attention from the biomedical fields (39, 40) because they can encapsulate intact biomolecules or cells into the fiber matrix (41). Alginate (and Na-Alg), a powerful thickening, stabilizing, and gel-forming agent commonly used in foods to produce a variety of gel products, is safe for consumption and human use (42). Because alginate is a naturally occurring nontoxic polysaccharide, it is both biocompatible and abundantly cheap. Although these are ideal properties, alginate is difficult to construct into microstructures and tends to swell (43), making it unable to load or diffuse drugs at the desired rates. Microfluidics has overcome these issues by blending alginate with various materials to reduce swelling and control the shape to produce fibers with tubular structures (44). Na-Alg also has the unique property of cross-linking in the presence of cations, commonly calcium, which once cross-linked can reduce microfiber swelling in the presence of a solvent and the permeability of different substances mixed with the microfibers. In drug delivery applications, the release is delayed, a feature that can be used to control the rate of drug release (45).

Alginate was also used in a capillary-based microfluidics system to form gas-in-water microfibers, which consist of alginate-based composite (ABC) solution as the outer phase and nitrogen or air as the inner phase. Microfluidic spinning can precisely tune microfibers used for biorelated applications due to their control over small droplets (46) and microjets. But microfibers that have been fabricated by the traditional oil-in-water microfluidics method have uniform material composition and surface roughness (47), which are not ideal for experiments that require different fiber properties. Also, biorelated microfibers are generally used for water collection (48), so other experiments like cell culturing have not been sufficiently investigated, making this experiment cutting edge. Gases were used to generate bubbles to produce cavities because of the breakup of gas under the shear of the ABC solution. The ABC solution encapsulating gas bubbles was then extruded from the orifice into a $CaCl_2$ solution to fabricate microfibers with cavity knots, because the ABC solution can be cross-linked quickly upon reacting with the Ca^{2+} ions. Changing the flow of gas produced different types of cavity microfibers that could be assembled into a wide variety of 3D scaffolds, demonstrating a broad range of applications in different fields, because the cavity-knots of the cavity microfibers allow precise positioning and storage of cell growth medium for cell culturing. Because 3D scaffolds are widely used in cell culturing and tissue engineering, a wooden raft-like scaffold was used to culture human umbilical vein endothelial cells (HUVECs), which were uniformly seeded on the scaffold, and was able to attach the HUVECs after continuous cell culturing. All these properties show potential utility in drug delivery, cell culturing, and tissue engineering applications owing to the good cell viability of HUVECs and to the formation of a real 3D HUVEC framework on the scaffold (49).

2.2. Wound Dressing

New medical dressings that are moisture-absorbing and moisturizing are based on fibers (50). An ideal wound dressing can completely cover the wound without interfering with the body's natural

healing process (51), and would in fact enhance it by maintaining a moist environment that can both receive oxygen and prevent the growth of pathogens (52). Microfiber-type dressings meet these requirements and possess structural advantages such as high surface area, gas permeability, and controllable structure performance, making them well suited for wound dressing (53).

Microfibers produced via a mixed spinning solution of Na-Alg and PEGDA were prepared in a 1:1 ratio in distilled water to produce a fiber-based medical dressing. This mixed spinning solution was then loaded with an anhydrous $CaCl_2$ solution into a microfluidic pressure pump. The observed fibers were interlaced, overlapped, and disorganized, allowing the microfibers to be subjected to shear force during injection, yield to the hydrogel flow direction, and be effective at dressing deep wounds such as chronic ulcers and bedsores, which current fiber medical dressings are unable to do. The fibrous hydrogel also has a 3D network structure that possesses better air permeability and higher surface area than traditional fiber medical dressings, which will accelerate the wound healing cycle while maintaining moisture (54).

The high surface area–to–volume ratios and surface property control also make microfluidically spun microfibers well suited for ophthalmology sutures. A poly(lactide-co-ε-caprolactone) (PLCL) copolymer in chloroform solution was used as a core fluid and methanol as a sheath fluid for the microfluidic chip. Singular fibers were wound into PLCL rings, which were subsequently attached to a customized twisting machine and spun into fiber bundles. Properties were also controlled by adding or blending in biomolecules such as alginate. Alginate-coated PLCL fiber bundles showed greater initial Young's modulus values than uncoated fiber bundles did. The fiber bundles were then tested to exhibit the functions of ophthalmological sutures by inserting the bundle into a porcine eye. The microfibers showed superior mechanical properties; for example, a fiber bundle wound eight times was mechanically superior to traditional nylon sutures. These results show that microfiber fabrication methods open the door to create even more specialized biomaterials for use in biomedical research and health care that are superior to materials created by traditional methods (55).

2.3. Drug Delivery

Drug testing is a long and expensive process that requires multiple stages of development before the drugs ever get to human trials and experimental treatments. Microfibers offer a solution in that they can encapsulate cells and can be constructed into 3D structures that are similar to the organ they are built to represent, and they can be manufactured from a biocompatible material on which cells can be seeded and perform natural functions. These artificial 3D structures can be used to simulate how a new drug or treatment would affect human cells of a particular organ, and would greatly hasten breakthroughs in medicine.

Microfibers produced via microfluidic spinning for drug delivery possess structures that can be woven into patches that are used in in vivo and ex vivo applications to release the carried drug as the patch is biologically broken down (56). Transdermal patches are excellent for treatment because they can provide pain-free, self-administered drug delivery to patients (57). Therefore, patches fabricated from drug-laden microfibers would be an improvement because they would function better and be more durable than traditional patches. Microfiber patches are generally physically weaved, and it is difficult to weave microfibers into fabrics by nonphysical methods (58). Most microfibers used in drug delivery are composed of organic polymers such as alginate, chitosan, and collagen (59). Because biodegradable polymers provide sustained release of encapsulated drugs and degrade in the body to nontoxic small molecules that are easily excreted (60), the degradation rates can be either decreased or increased with higher percentages of natural polymers (61). Although alginate is commonly used in cell encapsulation and cell seeding experiments due to its low toxicity, it has been difficult to use as a drug carrier. At certain ratios of alginate the

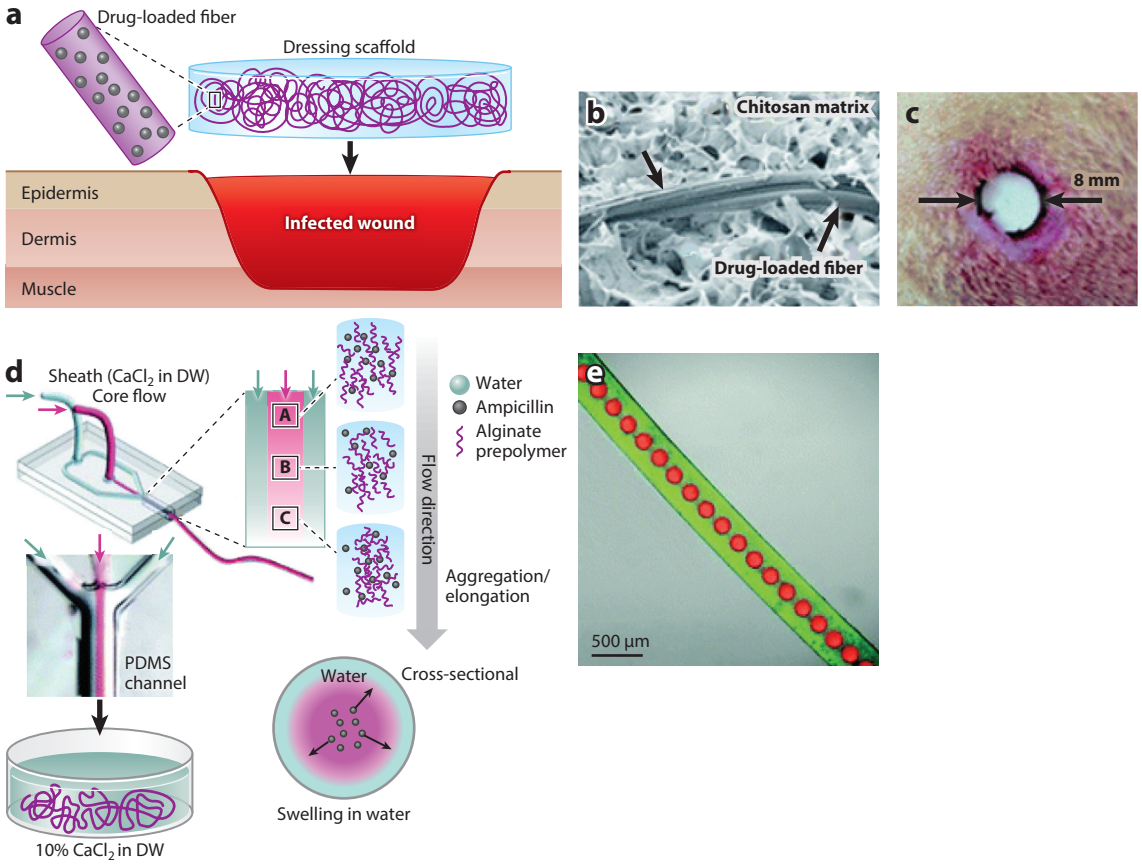

Figure 2

(*a*) How drug-loaded microfibers are commonly put into the body. Microfibers are (*b*) microfluidically spun and arranged into a dressing scaffold and (*c*) implanted into a wound to steadily release the encapsulated drug. (*d*) Diagram of how drugs are mixed in with the flow in the microfluidics approach to produce drug-loaded microfibers. Panels *a–d* adapted with permission from Reference 44; copyright 2015 Royal Society of Chemistry. (*e*) A microfluidically spun microfiber in which a tubular internal was produced and can easily encapsulate drugs. Panel *e* adapted with permission from Reference 64; copyright 2015 Royal Society of Chemistry.
Abbreviations: DW, deionized water; PDMS, polydimethoxysilane.

microfibers degrade rapidly (62), which would cause any loaded drug to be released spontaneously. Alginate in an aqueous condition tends to swell (63), which prevents microfibers from carrying a significant amount of a drug for treatment. A diagram demonstrating how microfibers are used to encapsulate and deliver drugs is shown in **Figure 2**.

The capability of loading ampicillin into microfibers was tested by using isopropyl alcohol as a sheath flow to condense the alginate polymer chains to reduce swelling. A 3% (w/w) Na-Alg solution was prepared, and 100 mg of ampicillin sodium salt was mixed with 1 mL of alginate solution as the core flow. Sheath solution containing 3% (w/w) $CaCl_2$ in deionized water and low-polarity isopropyl alcohol was prepared to induce the instant gelation of alginate solutions. The resulting fiber structure was highly ordered and delayed degradation. The drug-laden fibers were immersed in a calcium ion bath containing 10% (w/w) $CaCl_2$ in deionized water and isopropyl alcohol to increase cross-linking within the alginate network. To test the effects of the fibers,

researchers infected wounded rats with *Staphylococcus aureus* and studied control and experimental groups: uninfected wound, bacteria-infected wound without treatment, and bacteria-infected wound treated with the fiber scaffold. The experimental group displayed a positive effect when compared with the control infected group, demonstrating that microfibers can encapsulate and gradually release antibiotics (44).

Electrospinning is commonly used to produce microfibers and nanofibers for drug delivery (65), but it is difficult for the operator to precisely control the internals of the fibers and fibers with the same internal and external properties are usually produced (66). Microfluidic spinning is better suited to produce microfibers with controllable internals, such as using a capillary microfluidic device by which glass capillary tubes are assembled on glass slides to produce microfibers with tubular and peapod-like internals. The tubular microfibers were tested as blood vessels by using an aqueous solution of methylene blue trihydrate and fresh rabbit blood plasma, and then embedded in an agar gel to mimic the extracellular matrix. The fibers moved both solutions smoothly, proving their use in transporting blood and nutrients in artificial tissue experiments. The peapod-like microfibers were used to demonstrate encapsulation of two drugs that normally are incompatible; the dyes Sudan Black, which is oil soluble, and Eosin Y, which is water soluble, were used as substitutes for the drugs. With both hydrophilic chitosan and hydrophobic oil in the microfibers, these drugs can be encapsulated together. This novel delivery would allow multiple drugs, including hydrophilic and hydrophobic drugs that are normally incompatible, to be encapsulated together. This type of delivery would allow for broader treatment and would be a huge step forward in research (64).

The inclusion of magnetic nanoparticles in microfibers can also enable controlled release of the encapsulated drug. Magnetic nanoparticles already have a role in biomedical applications such as drug and gene delivery and magnetic resonance imaging (67, 68). Here, diclofenac was used as the model drug, and magnetic iron oxide nanoparticles with an average particle size of 5 nm were loaded into the microfiber. The alginate microfiber solution consisted of 1 wt% alginate, 1 wt% diclofenac sodium, and 4 wt% magnetic iron oxide nanoparticles. Simultaneous injection of the dispersed and continuous phases into the microfluidic chip was carried out by a series of digitally controlled syringe pumps. After the dispersed phase streams were mixed, the microfiber was created. A control drug-encapsulated microfiber released up to $91.9 \pm 2.8\%$ of the encapsulated diclofenac and was emptied after 1.5 h without magnetic force, demonstrating steady release over time. Moreover, an in vitro study of cells encapsulated in microfibers supplemented with 10% (v/v) fetal bovine serum in a humidified incubator to simulate an in vitro environment showed that when a magnetic force is applied to microfibers at the beginning or at the twentieth minute for 10 min, $98.1 \pm 2.9\%$ and $96.5 \pm 3.0\%$ of diclofenac, respectively, was released from the microfibers by the fortieth minute. The faster release rate was a result of the nanoparticles making the microfibers more porous. When the same magnetic force was applied in 2-min bursts, total diclofenac was released at a controlled rate that would be favorable for treatment and biomedical experiments (69).

3. CELL ENCAPSULATION AND CELL SEEDING

3.1. 3D Cell Culturing

Microfluidics can be used for in vitro biological research and drug testing (70–78). Furthermore, microfluidically spun microfibers are ideal for testing cells because the fibers can be made of biomaterials and can mimic the extracellular matrix, allowing for cell adhesion, proliferation, differentiation, migration, and alignment and providing nutrients while maintaining sufficient mechanical properties. This means that cells cultured in microfibers will behave as they normally would in a human body, enabling researchers to observe how cells interact with new drug trials and artificial

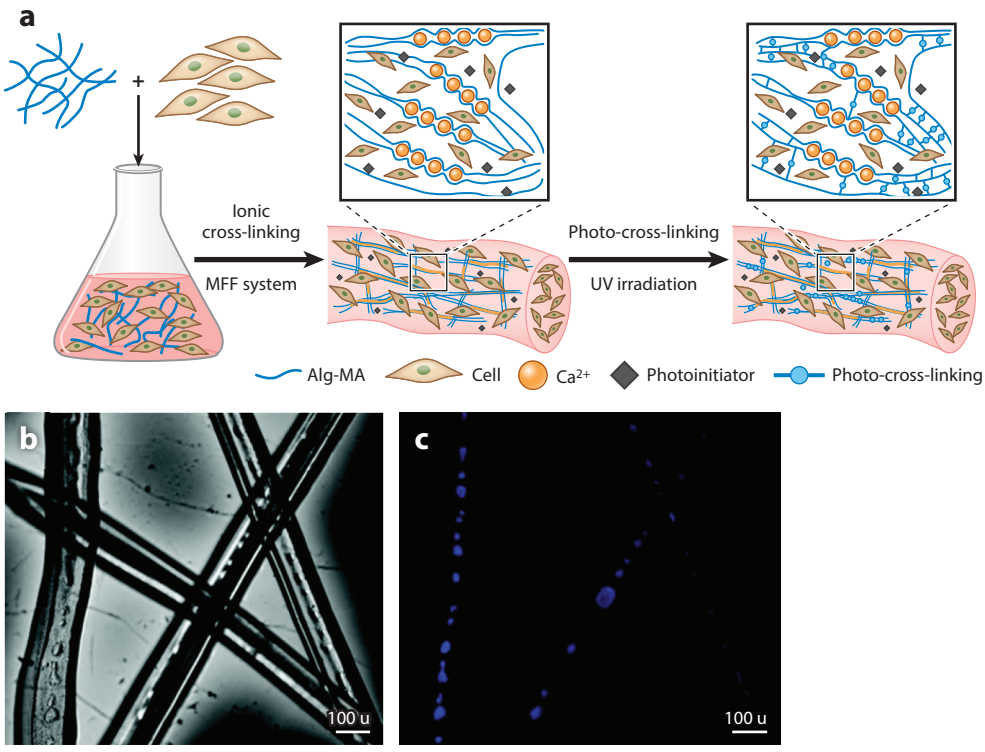

Figure 3

(*a*) How cells are commonly encapsulated by microfibers. Live cells are mixed with fabricated Alg-MA microfibers and cross-linked into and on the microfiber. (*b*) Corresponding optical and (*c*) fluorescent images of PC12 cells encapsulated inside hollow coaxial microfibers. Panel *a* adapted from Reference 80 under the terms of the Creative Commons Attribution (CC BY) License, **http://creativecommons.org/licenses/by/4.0**. Panels *b* and *c* adapted with permission from Reference 81; copyright 2020 Royal Society of Chemistry. Abbreviations: Alg-MA, methacrylated alginate; MFF, microfluidic fabrication.

organs without posing any danger to a person. In terms of culturing cells, microfluidically spun microfibers also have an advantage over electrospun microfibers, which are less favorable because the size of the fibers are limited to the nanoscale and the high voltages are harmful to biological material (79). Advancements in microfiber fabrication raise the possibility of creating synthetic tissue to substitute for, for example, heart muscle and spinal and nerve tissue. Natural polymers allow for cell growth along a defined axis but also for more complex, 3D matrices to form. (3D matrices are preferable to 2D matrices because natural tissues are 3D.) The tunability of the microfluidics processes used to create these fibers has the advantage of shaping the mechanical properties of the fibers without altering chemical structure or affecting biocompatibility. A diagram showing how microfibers are used for cell encapsulation and cell seeding, as well as what cells seeded in microfibers look like, can be found in **Figure 3**.

Nonmicrofluidics techniques for fabricating polylactic-coglycolic acid (PLGA) microfibers have undesirable trade-offs. For example, melt spinning requires heavy equipment, and the high temperatures involved exclude the addition of proteins for tissue development in the fiber. A microfluidics method using a small device to form and solidify PLGA microfibers can solve this problem with a core solution of PLGA, dimethyl sulfoxide, and proteins and a sheath solution of glycerin and water. This creates coaxial flows that precipitate the fiber as the dimethyl sulfoxide

flows out of the fiber and water flows in. L929 cells were cultured onto the PLGA fibers and were compared with a fibronectin-coated control; researchers found little significant difference in cell growth between the fibers, implying that the PLGA fibers are relatively biocompatible compared with fibronectin. Neuronal cells also attached to uncoated PLGA fibers, demonstrating that microfiber properties can be controlled without limiting their capability to culture cells (82), making PLGA fibers ideal for experiments involving cell growth.

Ultrathin microfibers are ideal for cell encapsulation because they easily transport nutrients and oxygen across the shell and into cells. Traditional microfluidic spinning methods, though able to control fiber structure and shell thickness, are unable to create ultrathin microfibers with a thickness of only a few micrometers. Moreover, the structure of the common calcium alginate (CaA) microfiber is more likely to disintegrate in a cell culture medium at low Ca^{2+} concentrations, and because CaA microfibers are several times thinner than traditional microfluidically spun microfibers, they are incredibly likely to fail at this stage and not be useful for long-term in vivo cell culturing experiments. The manufacture of ultrathin polyelectrolyte microfibers with hollow structures was done by fabricating CaA and chitosan (CS) composite microfibers via a microfluidic chip. Following fabrication, the microfibers were immersed in a phosphate-buffered saline solution, which removed the outer CaA shell of the microfiber and resulted in an ultrathin polyelectrolyte hollow microfiber. Human hepatocellular carcinoma (HepG2) cells were initially cultured in CaA/CS composite microfibers for 1 day to allow the liver cells to attach to the inner wall of the microfibers, and they were encapsulated in the hollow region of the ultrathin microfibers following immersion in phosphate-buffered saline. The cells maintained viability, demonstrated proliferation, and followed hepatocyte-specific functions while the microfibers remained intact. Ultrathin microfibers would be a breakthrough for cell culturing, as the thin shells can transport ample nutrients and oxygen, which would allow cells to remain healthy longer for long-term experiments (83).

Alginate is also used in experiments involving cell encapsulation and cell seeding. Alginate microfibers with a grooved structure were fabricated by a Y-shaped microfluidic spinning device consisting of a coaxial flow of two solutions. The grooved structure with wrinkles was obtained by adjusting the concentration of the flow rates and solutions and possessed the capability of mimicking the extracellular matrix for promoting cell and tissue growth, showing promise in future experiments on wound dressings and tissue scaffolds (84). Because cellular behavior is affected by the substrate (85), it is important that tests of regenerative medicine and tissue engineering scaffolds have cells grown on tissue following controlled behavior.

HepG2 liver cells are ideal for testing owing to their wide use in experiments to predict drug efficacy and toxicity, so creating an artificial environment similar to liver would greatly improve the accuracy of these experiments. To closely mimic the natural conditions, the culture system for liver cells must provide a uniform supply of oxygen and nutrients to the cells and a hierarchical assembly of multiple types of cells, and a large number of cells must be encapsulated. To meet these conditions, researchers established a perfusion system for hydrogel microfibers in which the obtained fibers were bundled together and packed into a perfusion chamber, followed by perfusion cultivation to structurally mimic the 3D structure of the liver. This culture system controlled liver-specific functions by tuning the perfusion conditions to change the formation of vascular network–like structures of densely packed HepG2 cells that behave similarly to in vivo tissues. This experiment would be a step forward in engineering liver tissue for experiments in drug development or for biochemical studies (86).

Encapsulating cells in hydrogels protects them from hydrodynamic stresses that occur when they are plated on flat surfaces, and can reduce uncontrolled differentiation of stem cells and provide control for long-term trials. Further, alginate microcapsules can support the differentiation of

both mouse and human embryonic stem cells (87). Hydrogels can also be manipulated to release proteins and other chemicals to aid in the health of cell cultures. Small-size hydrogel materials are often used to ensure high cell viability and function (88). For these reasons, hydrogels were used to test mouse astrocyte cells, which were cultured and encapsulated within the produced alginate fibers, and up to 60% of the cells remained viable for testing for up to 264 h past the manufacturing process. The cells were encapsulated in a variety of fibers whose properties were controlled, showing promise in settings where alginate microfibers can be used to simulate an extracellular environment for testing the properties and effects of cells under different conditions and drug effects (89).

3.2. Organ Modeling and Tissue Engineering

Microfibers are ideal for biomedical (90) and tissue engineering (91) applications because their fibrous structure is similar to that of natural tissue (92). Many organic structures in the human body are fiber based (e.g., blood vessels, muscle fibers, and nerve bundles), so microfibers made from biomaterials hold potential for synthetic 3D tissue constructions, which better represent the spatial arrangement of cells in vivo than 2D environments do (93). 3D cell culturing can provide the structure of the extracellular matrix and offer the potential for scalability and reduced cost (94). These fibers are made with hydrogels. Hydrogels are useful for cell culturing because their diffusivity mimics an actual cellular membrane, including permeability to oxygen and nutrients (95), and they are biocompatible and have a tissue-like environment. Alginate hydrogel is ideal for use in cell encapsulation, cell seeding experiments, and in vivo applications because it is semipermeable; the membrane can inhibit the penetration of immune cells and antibodies while allowing oxygen and nutrients to pass through, keeping cells viable (96).

A specialized fabrication method that can finely tune the microfibers is necessary to replicate the 3D microstructures of natural tissue fibers in vivo. Multicompartmental microfibers were created with a modified device that introduced multiple core flows (Na-Alg and $CaCl_2$) into the channel. HepG2 cells and NIH 3T3 cells were seeded into different alginate solutions to test the biocompatibility of the fibers and the fabrication process. Approximately 80% of cells survived to encapsulation. This method can replicate the specialized morphologies found within organic fiber-based tissues and can improve research for tissue replacement applications (97). Microfluidic spinning also uses other techniques during fabrication, such as photopolymerization, in which microfibers made from PEGDA can be shaped like bowties yet their size can be modified by adjusting the shear force at the core fluid–sheath fluid interface. These microfibers can then be assembled into a 3D structure similar to the actual physiological environment for experiments in tissue engineering and regenerative medicine (98). A diagram demonstrating how microfibers are used in tissue engineering is shown in **Figure 4**.

Producing microfibers with specialized structures for particular functions often requires a wide variety of microfluidic devices due to the difficulty in preparing structures that simulate natural tissue (101). To avoid this, researchers use a facile two-flow microfluidics system that includes two replaceable fluids and a microfluidic device coaxially assembled from glass capillaries. The produced methacrylated gelatin hydrogel had cells both adhere and proliferate to it, indicating they are biocompatible. When the fluid flows were changed, three types of microfiber structures were produced: single layer, double layer, and hollow cell-laden. Double-layer microfibers encapsulated mouse embryonic osteoblast precursor cells (MC3T3-E1) and remained viable after 9 days of culturing. Confocal laser scanning microscopy showed that the cells were closely packed and formed a solid cell fiber. Natural tissues such as muscle and bone are composed of solid fibers, and the fibers in bone are specifically composed of the organic polymer collagen (102), so these double-layer microfibers would be suitable for engineering these tissues owing to their added stability and similarity to naturally occurring tissue (103). HUVECs were incorporated into

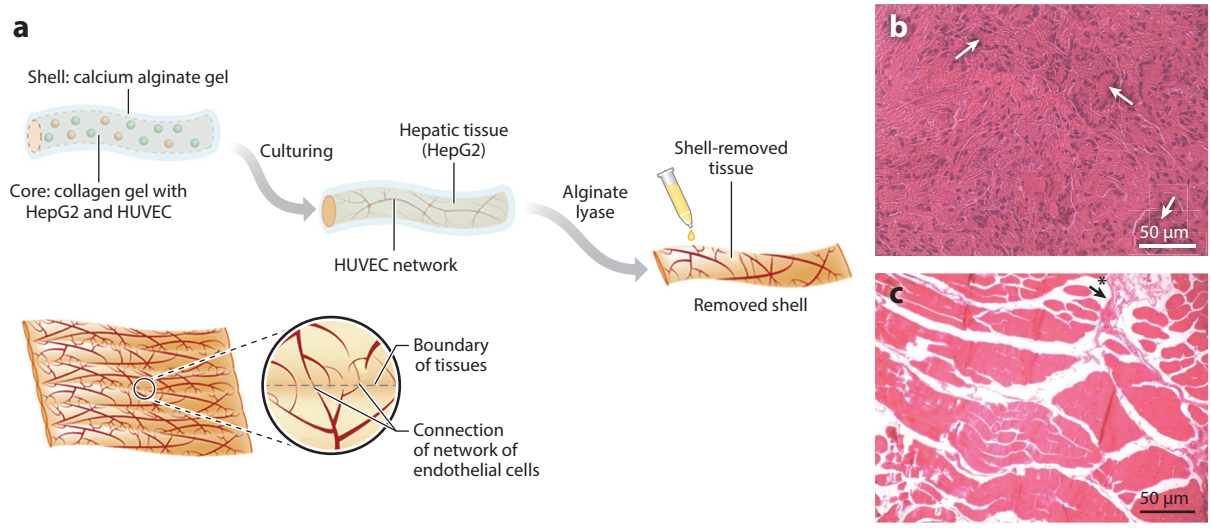

Figure 4

(*a*) HepG2 cells and HUVECs were encapsulated in a hydrogel microfiber and cultured for several days to form hepatic tissue with a HUVEC network. The hydrogel was then removed by an alginate lyase treatment and assembled with other hepatic tissue formed by microfibers to produce macroscopic tissue. Panel *a* adapted from Reference 99 under the terms of the Creative Commons Attribution (CC BY) License, **http://creativecommons.org/licenses/by/4.0**. (*b*) Polymer microfiber scaffolds in subcutaneous tissue 46 days after being implanted compared with (*c*) normal tissue. Panels *b* and *c* adapted from Reference 100 under the terms of the Creative Commons Attribution Non-Commercial (CC BY-NC) License, **http://creativecommons.org/licenses/by/4.0**. Abbreviations: HepG2, human hepatocellular carcinoma; HUVEC, human umbilical vein endothelial cell.

hollow cell-laden microfibers and were used to simulate blood vessels. The cells remained viable and formed a structure similar to blood vessels after 7 days. Each of these microfiber structures could be woven together and assembled into different 3D structures, demonstrating capability for tissue engineering applications and biocompatibility, which would be a great leap forward in promoting cell growth in bodies (104, 105).

Creating physiologically accurate cultures of cells on fibers is a challenge, because encapsulated cells on hydrogel microfibers tend to form relatively isotropic layers, whereas those found in vivo are often anisotropic (106) with specialized growth patterns. This challenge necessitates the creation of bioactive microfibers that are physiologically relevant. Microfibers were fabricated with a device that contains capillaries and injects flows of the pregel solution into a cylindrical channel, in which HepG2 and NIH 3T3 cells were seeded into different alginate flows asymmetrically. Bioactive extracellular matrix was also added to the cell encapsulations to better mimic the structure of living tissues. Albumin secretion and urea synthesis were used as measures of HepG2 cell activity. Over 10 days, all fibers showed increases in albumin secretions and urea synthesis. When methacrylated gelatin was used as a bioactive additive to the alginate solution, primary normal HUVECs were seeded into fibers, and vessel-like structures were found in the created microfiber, possibly creating synthetic human blood vessels. Different woven structures were also made from the microfibers to demonstrate their suitability for creating synthetic tissue networks (107).

Microfibers are also useful for producing artificial adipose tissue, which is difficult to fabricate and is in high demand for reconstructive and plastic surgeries. In this experiment, microfibers were produced from solubilized collagen type I and seeded mouse adipocytes on 3D tissue, showing a viability of 96 ± 2% after 14 days. 3D culturing was determined to be needed because 2D cell

culturing caused many of the seeded adipocytes to dedifferentiate after a few days. In addition, to replicate in vivo adipose tissue, the seeded adipocytes needed to be structured in multilayers. The microfibers had higher porosity than did solubilized collagen type I by itself and as such allowed for a higher concentration of collagen. The higher porosity allows for better nutrient diffusion, making it more similar to natural tissue, and will be a step forward in producing adipose tissue for medical purposes (108).

Embryonic stem cells are known for their ability to differentiate into any type of cell in the body (109), including neurons with nerve fibers (110). As such, experiments are using stem cells to repair nerve injuries (111). But embryonic stem cell–derived neurons cultured in laboratories often display randomly oriented rather than properly aligned neurite growth (112), which would be needed for successful nerve replacement. To address this, researchers tested a microfiber system to develop a way to culture neurons so that the neurites develop into an aligned system (113). PLGA was selected owing to its biocompatibility, biodegradability, and plasticity, which remained intact in microfibers into which PLGA was manufactured. A modified microfluid chip fabrication system was established to create a novel aligned contiguous microfiber platform (ACMFP) for the neuronal differentiation of embryonic stem cells and the guidance of nerve fibers into highly parallel systems with no or very limited gaps. All-*trans* retinoic acid–treated cells were seeded on 60-, 90-, and 120-μm ACMFP microfibers as well as on flat PLGA to establish a control group. After 6 days of incubation, neurite outgrowths grew in random directions in a culture dish, where the alignment value of the control group was on average 41.07 ± 22.72°. In the ACMFP groups, neurite outgrowths were much more parallel, with the average alignment values of 60-, 90-, and 120-μm ACMFP microfibers being 9.38 ± 5.18°, 14.27 ± 8.82°, and 18.87 ± 9.02°, respectively. This experiment shows that ACMFP microfibers can culture embryonic stem cells with much more parallel alignments compared with conventional means. This platform will hopefully be used to develop treatments for nerve injuries (113).

These aligned nerve tissue growths are also seen where adult hippocampal stem/progenitor cells (AHPCs) were grown on different polycaprolactone microfiber scaffolds. These scaffolds controlled the direction of tissue growth of grown cells such that it was similar to natural nerve tissue, showing potential for guiding nerve regeneration within the central and peripheral nervous systems and for facilitating the repair of spinal cord injuries (22).

Microfibers are also used to create 3D scaffolds for culturing self-renewing stem cells for transplantable tissue (114) and for encapsulating endothelial cells and bioprinting to simulate heart functions (115). 3D structures, as opposed to 2D structures, that can culture cells can accurately represent real tissue structures, which allows for more accurate growth and function of both cells and artificial tissues (116). A 3D structure would also be useful in soft tissue regeneration, as a 3D structure composed of cellulose microfibers was found to have high protein absorption and cell seeding capabilities when tested in vitro with fibroblasts (117).

3.3. Hollow Microfibers

Hollow microfibers are commonly used in biomedical experiments, for example, as blood vessels for hemodialysis, and in cell culturing applications for both cells and proteins (118). They are best suited for microvascular applications due to their lumen channel, which allows flexible fluid conveyance, and they are most commonly made of alginate. Hollow microfibers are commonly produced by a microfluidic-based coaxial flow system in which the core fluid flow is altered, which requires an inert property to prevent turbulence from occurring between the adjacent streams so that the hollow fiber walls do not collapse following gelation.

Hollow microfibers can be used to mimic the structure of blood vessels and can imitate blood vessel behavior and facilitate routine cell behavior. Here, a triple-flow microfluidic device produced hollow microfibers for three inlets into which a sheath fluid ($CaCl_2$), a sample fluid (Na-Alg), and a core fluid (mineral oil) were introduced. The microfibers were tested with ink and behaved similarly to actual blood vessels in terms of flow and permeability. HUVECs were then seeded to mimic a blood vessel, and hollow fibers were integrated into a neurovascular scaffold to mimic the blood-brain barrier. The results showed that a polydimethoxysilane-based cylindrical microchannel can produce biomimetic microfibers that act as blood vessels, making them a promising model for studying drug interactions at the blood-brain barrier and how drugs move throughout the brain (119).

Hollow microfibers can also be used to create multicompartmental structures for culturing multiple different cells simultaneously (120). The tubular internals of hollow microfibers make them favorable for loading drugs and supporting cells (121). In fact, the wall of a hollow microfiber can be fabricated out of stimuli-responsive material, which stimulates the release of encapsulated drugs in the presence a wound. Wounds have an increased concentration of K^+ around the affected area due to inactive Na^+-K^+ pumps on dead cells. This feature was tested, and the hollow microfibers released drugs in an increased K^+ environment, which would allow drug release to be customized to the patient and would be extremely beneficial in wound healing (122).

4. CONCLUSIONS

The most recent advances in microfluidics and microfluidically spun microfibers have shown a tremendous amount of progress in the biomedical and tissue engineering fields. Frequent discoveries have led to varied fabrication methods and growing applications of microfibers. These innovations are important, because a large number of microfibers with controllable structures and properties can be produced cheaply and easily. Microfibers are ideal for a wide variety of applications, and their benefits could hasten medical testing and help improve lives.

In this review, we analyze the biomedical applications of microfluidics and microfluidically spun microfibers, focusing on the materials from which they can be manufactured and their applications, as shown below in **Table 1**. We also discuss the most recent advances in microfluidics and microfibers and what this means for related work in the future. With the advantages that a microfluidics system offers, innovations in biomedical applications will continue to be found, and it is easy to imagine that medical testing will eventually be shaped by studying how human cells react to new drugs in a realistic but artificial in vitro system developed by microfluidics.

With the ability to control the size, shape, and properties of microfibers produced by microfluidic spinning, it is no wonder that revolutionary experiments in the biomedical field can be accomplished. Wound dressings created from microfibers have been used to dress deep wounds and accelerate the natural healing cycle, which improves the function of bandages. Microfibers that encapsulate drugs can be woven into patches or directly implanted into the body to steadily release the encapsulated drug. Moreover, microfluidics methods help create specialty fibers that can encapsulate hydrophilic and hydrophobic drugs simultaneously, which can provide a novel way of delivering multiple drugs at once. Microfibers can also encapsulate and coculture cells in a controlled medium that closely resembles the extracellular matrix, which will allow cells to behave more naturally for experiments. Cell-encapsulated microfibers can also be constructed into 3D structures that can be used to create artificial tissue and to determine how in vitro cells of a particular organ would react to a new drug, which will lead to breakthroughs in the development of medicine and demonstrate how multiple types of cells can interact with each other.

Table 1 Specific applications for microfibers made of various kinds of materials that share the same characteristics as microfibers (controllable properties, high surface area–to–volume ratios, and surface property control)

Material	Applications	Advantages	Disadvantages
Alginate	Drug delivery (59), cell culturing, cell seeding due to high porosity (123), tissue engineering (36); can be woven into similar structure as biological tissue (124)	Gelation is rapid, mild, and inexpensive (21); encapsulates intact biomolecules or cells (41); can be used to simulate extracellular matrix (89)	Difficult to construct into microstructures; tends to swell (43); at certain ratios and cross-links (80) can degrade rapidly (62)
Cellulose	Cell culturing, cell seeding due to high porosity (125), and tissue engineering experiments (36)	Common and inexpensive, environmentally friendly, hydrophilic (126), low solubility (127), low density, good stiffness (128)	Very high absorption of water (126), which can cause problems; medium mechanical strength (129)
Chitosan	Cell scaffold cell culturing; cell seeding due to high porosity (123); tissue engineering experiments (36), especially liver tissue (130); and drug delivery (59)	Structurally similar to glycosaminoglycans (liver extracellular matrix) (130), biocompatible, biodegradable; provides sustained release of encapsulated drugs (60)	Mechanically weak in pure form and must be cross-linked to be effective (130); dressings made of fibers have poor stability (131)
Collagen	Drug delivery (59), tissue regeneration, cell culturing (132), specialized growth factors for constructing bone (133)	Biocompatible, biodegradable, inexpensive (33); develops 3D structures; is a major part of the extracellular matrix (132)	Expensive (37), cannot be used in injectable scaffold due to insufficient injectable property (132), weak mechanical properties and thus must be mixed to be effective (133)
Gelatin	Cell culturing (134), cell seeding due to high porosity (33), extrusion bioprinting (135, 136); supports physiologic cell functions (124)	Biocompatible, biodegradable, inexpensive (33), neutral, nontoxic (34); possesses cell adhesiveness and good physical and chemical properties (35)	Chemical cross-linking may be performed at temperatures harmful to gelatin morphology (130); can dissolve into colloidal sol at 37°C (137)
Poly(lactide-co-ε-caprolactone)	Wound dressing and sutures (55), tissue engineering (138)	Biocompatible (138) and biodegradable (139); has a slower degradation rate than natural polymers (140)	Properties can only be controlled by adding or blending in biomolecules (55)
Polycaprolactone	Tissue engineering (17); provides scaffolds for cell culturing (22) and bone tissue regeneration (133)	Biocompatible, nontoxic, chemically inert, rigid composition (19), rubbery property (139)	Slow degradation rate bad for drug delivery; poor mechanical properties, including Young's modulus, tensile and compressive strength (141)
Polyethylene glycol	Tissue engineering (17); used to mimic crowded cellular conditions (142)	Biocompatible, nontoxic, chemically inert, rigid composition (19)	Inhibits cellular uptake (143), must be mixed with natural polymer for experiments (64)
Polyethylene glycol diacrylate	Tissue engineering (17); produces interlaced and overlapping microfibers for wound dressing (54)	Biocompatible, nontoxic, chemically inert, rigid composition (19)	Possesses weak mechanical rigidity in pure form (83)
Polylactic-coglycolic acid	Cell culturing cell seeding; incorporates natural plasticity into microfibers (113)	Biocompatible, biodegradable nature, plasticity (113)	Scaffolds with low porosity lack interconnectivity between cells (144)

Future work in microfiber development will include the creation of new spinning techniques to fashion new shapes that will be used in cell encapsulation and drug delivery experiments. Further, the discovery of new blend combinations and new materials that can be spun into microfibers will lead to stronger microfibers with improved mechanical properties. Eventually, operating microfluidic devices will become universal and widespread such that scientists will obtain fibers with desired properties simply by following a recipe. Microfibers are extremely useful in the biomedical field; their properties can be controlled and they are cheap to manufacture, biocompatible, biodegradable, and environmentally safe—features that assure microfibers will have a boundless future ahead of them.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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