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Microfibers as Physiologically Relevant Platforms for Creation of 3D Cell Cultures

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Microfibers have received much attention due to their promise for creating flexible and highly relevant tissue models for use in biomedical applications such as 3D cell culture, tissue modeling, and clinical treatments. A generated tissue or implanted material should mimic the natural microenvironment in terms of structural and mechanical properties as well as cell adhesion, differentiation, and growth rate. Therefore, the mechanical and biological properties of the fibers are of importance. This paper briefly introduces common fiber fabrication approaches, provides examples of polymers used in biomedical applications, and then reviews the methods applied to modify the mechanical and biological properties of fibers fabricated using different approaches for creating a highly controlled microenvironment for cell culturing. It is shown that microfibers are a highly tunable and versatile tool with great promise for creating 3D cell cultures with specific properties.

1. Introduction

Fibrous systems have numerous biomedical applications, including biomedical engineering, clinical treatments, 3D cell culturing and cell encapsulation.[1–4] Since the surface area-to-volume and strength-to-weight ratios of the fibers are high, they offer a highly useful and strong method for creating large-scale 3D tissue cultures.[5] They have the potential to guide cell growth, alignment, and migration. Additionally, the microfibers can be applied in order to perform drug delivery and time-controlled release of a variety of chemicals and materials for culturing and therapeutic applications.[6,7]

The mechanical properties of the fiber are important, since in biomedical areas, it is desirable to mimic the mechanical characteristics of natural tissues. In skeletal muscle tissue engineering, for example, the goal is to re-engineer damaged muscle tissues.[8] In order to regenerate the skeletal muscle tissue, it is necessary that the myoblast align on a scaffold to mimic the natural microenvironment. This area can also be applied in biorobotics,[9] biosensing,[10,11] cell-based assays,[12] and energy harvesting.[13–15] Additionally, scaffolds play a pivotal role in nerve tissue regeneration and modeling by providing an appropriate environment for cell adhesion and proliferation.[16–20]

Fibers are well suited for use in biomedical applications, since they consist of a group of 3D polymeric materials which have a hydrophilic structure which can hold large amount of water.[21] During the past decades in biomedical engineering, fibers were used as in vitro tissue models instead of native tissues due to the biocompatibility, ability to encapsulate bioactive molecules and cells and the efficient diffusion mass transfer of the hydrogels and polymers which form the fibers.[22] Cell-laden fibers can be divided into two main types: encapsulation type and surface type. Encapsulation type, where the cells are encapsulated within the body of the fiber, can be advantageous because the cells form a 3D culture that mimics tissues in vivo. The mechanical tension of the fibers can be a technical problem with culturing cells within a fiber because of the cell-derived extracellular matrix (ECM) that is secreted from the cells.[23] Alternatively, surface type cell-laden fibers have cells seeded on the surface of the fibers, which requires that the surface be cell-adhesive. Because the cells are seeded on the surface of the fibers, handling of surface type cell-laden fibers must to be delicate because the cells are exposed to the outside environment.

Although fibers can provide a desirable microenvironment which to emulate cell-cell and cell-ECM interactions, they can exhibit low mechanical properties and the appropriate mechanical strength might be found wanting.[24] Therefore, to provide better mechanical properties for biomedical applications, synthetic polymers have been used to create fibers.[22,25] This

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review aims to discuss different materials used and methods applied to improve the mechanical and biological properties of the fibers. While other reviews adequately cover topics ranging from microfibers' use in biomedical applications,[26] hydrogels in biology and medicine,[22] and scaffolding in tissue engineering,[17,20] this paper stands alone in its comprehensive one-stop approach to fiber fabrication. This paper provides details for creating fibers, highlights some common polymers for fiber creation, and gives an in-depth discussion over prominent biomedical applications.

2. Methods of Microfiber Creation

There are different methods for microfiber fabrication such as microfluidic techniques, Interfacial Polyelectrolyte Complexation (IPC), electrospinning, microfluidic spinning, drawing, wetspinning, meltspinning, and biospining.[27] Each of these techniques are discussed more thoroughly below.

2.1. Microfluidic Technique

The most common method to fabricate microfibers for biomedical applications is called laminar flow or microfluidic fiber fabrication.[26,28] In this approach, the fiber is created within a microchannel using coaxial flow of core (pregel) and sheath (gelator) fluids. Microchannels can be formed using embedded syringe needles or glass capillaries, or polydimethylsiloxane devices, which can be created from molds made with standard microfabrication techniques such as milling or photolithography.

Fiber polymerization in the laminar flow method can be accomplished via either chemical crosslinking or photopolymerization.[29] In chemical crosslinking, the pregel and gelator solutions flow through the microfluidic channel in coaxial or parallel laminar flow.[26,30] Exchange of ions or molecules occur via diffusion at the interface of the two distinct fluid flows, which leads to the polymerization of the pregel solution, and therefore the creation of the microfiber.[26] Alternatively, photopolymerization may be used to create the fiber from a microfluidic device. In this procedure, a photoinitiator (PI) is introduced into the pregel material, and the flow of the monomer solution is irradiated with ultraviolet (UV) light. After being irradiated, the PI causes crosslinking to occur within the pregel, and a polymer is formed.

The laminar flow procedure is advantageous over other methods because it gives precise control of the diameter and cross-sectional shape of the resulting microfibers.[26] This can be accomplished by varying the microfluidic channel dimension and shape, as well as modifying the pregel and gelator solutions' flow rates.[26] Moreover, the control of the alignment of the internal molecular direction can be achieved by the acceleration and deceleration of the flow,[31,32] which affects physical characteristics of the microfiber such as the optical polarization, electrical conductance and this process induces cell alignment that are on and inside the microfibers.[33,34]

The fibers created by this method are versatile and the process is continuous. Additionally, this technique is straightforward, cost-efficient, and compatible with many biological materials due to the fact that high temperature, high pressure, high voltages, or toxic materials are not required.[35] Different shapes, sizes, and morphologies can be obtained by changing the flow rate ratio, varying the concentration of the pregel material in the core fluid, and changing the shape or dimensions of the microchannel.[1,23,28,36–42] Many studies showed that different kinds of microfibers can be achieved using this method such as: solid,[28,36,39,43,44] tubular,[45,46] hybrid,[47] and flat.[48,49] Each is advantageous for various biomedical applications.

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The advantages of microfluidic spinning specifically in the domain of cell encapsulation includes: production of fibers with diverse morphology;[50] cell immobilization in solid or hollow configurations of fibers;[16] and formation of microchannel due to the elimination of alginate fibers from the 3D matrix.[50–55] Cell encapsulation via microfluidic spinning has been used for the in vitro reconstruction of complex 3D tissues emulating organs like the pancreas and improving immunity.[29]

2.2. Wetspinning

Wetspinning is an approach in which a pregel solution is injected into a coagulation bath, which must be either a poor solvent or a nonsolvent with respect to the polymer. The method is capable of making fibers with a wide range of diameters by simply adjusting size of the needle tip. The major challenge of using this method is that the pregel solution must be exposed to chemicals for a relatively long time, increasing the likelihood that the fibers will be placed in a situation which is not friendly to cells, either through increased toxicity, change in temperature or CO_2 level.[56–58]

2.3. Interfacial Polyelectrolyte Complexation

In IPC fiber production, two oppositely charged polyelectrolyte solutions interact with one another, leading to the formation of polyelectrolyte complex at the interface.[59] It is crucial that the two solutions do not mix prematurely, and this is ensured by the interfacial complex that takes the place of a viscous barrier between drops of both solutions. Forceps or pipette tips are used to draw the interfacial complex upward, which causes the disruption of the interface and creates scattered domains of complexation that behave as fiber nucleation sites. The exhaustion of the surrounding polyelectrolyte solution leads to the growth of nuclear fibers. As the mixed solution is drawn away from the interfacial complex, nuclear fibers join together to form a final, thicker fiber. This thicker fiber constitutes submicron nuclear fibers and gel droplets along its axis.

Wan et al. found that IPC fibers had more favorable mechanical properties than their original constituent polymers.[59] For instance, the tensile strength of chitosan-gellan fibers is 38.6 kg mm^{-2} whereas chitosan fibers spun from dilute acid has a tensile strength of 23.7 kg mm^{-2}.[60] Multi-Interfacial Polyelectrolyte Complexation (MIPC), in which the fiber is formed from the interfaces of multiple droplets of oppositely charged solutions placed against each other, has also proven advantageous for creating 3D patterned co-culture of cells which featured a variety of cell types that were encapsulated in desired patterns.[61,62] These experiments also revealed the cell migration, assembly and spreading within the fibers and also the process by which the traits were comparable to cell patterning. Tissue constructs having greater complexity and function can be created using MIPC. IPC fibers have the ability to create a conducive matrix for cell growth and differentiation, although to imitate the structure and function of a native tissue multiple cell types are generally required. MIPC fibers have been predicted to have a great use in biomedical applications especially of model system for cell biology and there are also used as basic components for the fabrication of human organs and tissues. IPC fibers are also used as drug delivery devices, light emitting diode and antireflection coating.[63–68] Syringe pumps and microfluidic channels are not required by this method.[26] Pregel solutions possessing polymers having high molecular weight not easily mixed by diffusion, can be treated using MIPC.

2.4. Electrospinning

Another method commonly used for the creation of micro- and nanofibers is electrospinning. Electrospinning is used due to its ability to create fibers consistently with highly controllable morphologies. By varying the solution, process parameters, and environmental conditions, a variety of fiber types can be created. These fibers can be used for a huge variety of unique applications including: highly porous, defect free, nonwoven nanofiber membranes used for water filtration;[69] in situ encapsulation of fungi enzymes;[70] and flexible, releasable guides for enhanced bone regeneration.[71] This small sample of the wide variety of application areas demonstrates the potential for a wide variety of uses for electrospun microfibers.

The polymer solution in the syringe is slowly ejected into the electric field created between the needle tip and the grounded collector.[50] Different collector types allow for different fiber uses. Fibers collected on a rotating drum as shown are collinear; however collecting on a shaker bed can create meshes. The needle tip is connected to a high voltage electric field. That electric field begins elongating the droplet of polymer being ejected from the syringe forming a Taylor cone until the equilibrium with the surface tension the polymer solution is overcome. The static imbalance allows the polymer to jet toward the collector drum. Because the fibers inherently have a very high surface area to volume ratio, the solvent evaporates out of the fiber in the jet stream before it collects on the drum.

The morphology and material properties of the resultant fibers are dependent on the careful tuning of working parameters including polymer solution concentration, polymer molecular weight, polymer viscosity, polymer solution surface tension, polymer solution conductivity, voltage, flow rate, collector type, tip to collector distance, and ambient parameters.[72] With an abundance of controllable parameters significant advantages and limitations can be identified. The morphology of the fibers can be controlled through a wide range of sizes and shapes that allow for fibers that range from the nano- to microscale. The surfaces can also be controlled to include significant controllable porosity or exceptionally smooth surfaces depending on the application. The process notably also allows for a large range of polymer materials to be used. The wide variety of materials makes the process incredibly flexible and adaptable. Inherent drawbacks to this process come from the many parameters that also make the processes controllable. Carefully monitoring and manipulating each of the parameters that controls morphology can be difficult to accomplish. The higher precision applications typically need highly controlled environments that stabilize fluctuating ambient conditions and can create highly complex electric field patterns to manipulate the shape of the fibers.

2.5. Drawing

Mechanical drawing has been termed as the simplest and most effectual basis for microfiber creation, since the microfiber is usually drawn from solvated liquid polymer.[73] The solvent nature and polymer concentration are the important concerns. This process is not the preferred method due to difficulty in controlling the fiber morphology compared to other processes.

2.5.1. Drawing Using Glass Micropipettes

In this process, the polymer solution is continuously pumped through a micropipette made of glass.[74] The pipette can be controlled using a nanopositioner and is positioned perpendicular to the substrate. The substrate is raised continually till it touches the polymer droplet that is present at the glass micropipette tip end. To have reliable droplet formation and adhesion, a solvophilic substrate is taken. Before laterally drawing a suspended fiber, the pipette is transported vertically with a constant speed and brought to a constant height. The viscosity of the polymer solution is controlled during the waiting time after stopping. The solid polymer fiber is formed by evaporation of the solvent after the stage is transported along an established XYZ trajectory at a constant speed. The substrate is brought in contact with the glass micropipette after drawing the fiber, thereby creating a suspended fiber. Once completed, the process can be repeated, causing a string of fibers to be generated along the substrate's surface. For the final step, the needle is rapidly retracted causes it to lose contact with the droplet. Fibers having lengths of several millimeters and diameters as low as 37 nm with 1D and 2D network configurations could be fabricated using this method.

2.5.2. Direct Drawing

For direct drawing, the polymer solution heated to and maintained at a viscous state.[73] The tip of a heat-resistant rod, such as the end of a silica fiber, is immersed into the molten polymer before being slowly pulled away. As it moves away from the polymer it draws a thin fiber out of the surface of the molten polymer; this fiber can be quenched in air to induce rapid polymerization. The properties of fibers created with this method are affected by the pulling speed and the polymer viscosity.

Fibers produced with this method have a variety of applications; for instance, Ong et al. found that the fibers constructed from PMMA drawn with a 125 μm wide Silica Fiber could bend and curl very easily thereby having a potential use in optical sensors. It was also noted that the diameter was uniform and the surface was defect free making them useful for photonic applications.[73]

2.6. Meltspinning

Meltspinning is an approach in which continuous fibers are fabricated by heating a polymer to its melting point and extruding through a spinneret. While this method can be used to make a variety of synthetic fibers, it requires expensive equipment and a high temperature range (150–300 °C) in order to work.[75] High temperatures are known to damage cells and proteins, which means that meltspinning has limited applications for biomedical research or cell encapsulation applications.[76–78] The mechanical properties of the fibers made by this method are relatively low due to the rapid decrease of polymer viscosity during the process.[79] In addition, a high pressure gradient is required to move the melted polymer through the spinneret, which would cause potential harm for encapsulated cells.[80]

2.7. Biospinning

In the biospinning method, silk fibers are fabricated by insects. The tensile strength and biodegradability of silk is high. Additionally, it is not cytotoxic or inflammatory. Nevertheless, we face the limitation of resources for biospun fibers and the speed of fiber fabrication in this method is relatively slow, and so it is difficult to scale-up the process.[81,82] In the interfacial complexation method, two polyelectrolyte solutions oppositely charged are applied and the fibers are created at the interface of two polyelectrolyte solutions.[83] Different polyelectrolyte solutions are used in this technique such as chitosan, sodium alginate (SA), and hyaluronic acid.[83–85] This method can be applied for cell encapsulation purposes. However, in this method the variety of the materials that can be used for fiber fabrication is limited.

3. Biomedical and Mechanical Properties of Select Polymers

A variety of microfiber have broad potential applications in biomedical engineering, with the merit of their biocompatibility, biodegradability and mechanical property.[86] The most researched microfibers include poly(lactic-*co*-glycolic acid) (PLGA),[1,87–92] poly(ε-caprolactone) (PCL),[3,93–96] gelatin methacryloyl (GelMA),[97–101] alginate,[44,53–55,102–105] chitosan,[60,64,106–109] and more. To provide context for material properties of these biomedical microfibers, in the following section, the polymeric structure, biomedical property (biocompatibility, biodegradability, etc.) and mechanical property of select biocompatible polymers PLGA, PCL, GelMA, Alginate, and Chitosan will be reviewed.

PLGA or poly(lactide-*co*-glycolide is a copolymer formed from lactic and glycolic acid.[88] It is favorable for use in biomedical applications due to its approval for clinical use in humans by the U.S. Food and Drug Administration and its ability to be dissolved by a wide range of different common solvents.[90] Its uniquely controllable degradation rates can be modified by varying the concentration ratio of its two monomers within the polymeric chain, rendering various forms of PLGA that exhibit different physicochemical properties. Downsides to working with PLGA include its poor osteoconductivity and its low mechanical properties, which are not ideal for load bearing functions. To counter these negative characteristics, PLGA is often used alongside ceramics or

fiber-active glass in order to optimize clinical application in bone regeneration.

The fastest degradation rate is achieved by PLGA 50:50, a form of the copolymer that contains an equal concentration of the lactic acid monomer and the glycolic acid monomer within each chain.[90] Aside from the peak degradation rate of PLGA 50:50, forms of PLGA will generally possess faster degradation rates as the concentration of glycolic acid increases in the copolymer. Along with the concentration, degradation rates are also dependent on the molecular weight of PLGA. By increasing the molecular weight of PLGA, degradation rates have ranged from several weeks to several months.[88] The molecular weight of PLGA varies between the different forms of the copolymer, which are governed by the number of monomers and the ratio of lactic acid ($C_3H_6O_3$) to glycolic acid ($C_2H_4O_3$) within the chain.

PLGA is commonly used in 3D scaffolding for enhancing cell culturing, namely in bone substitute constructs.[88] Its highly tunable degradation rates allow for another level of control over factors that affect cell viability, growth, and interaction within the body.[91] Throughout degradation, there are noticeable changes to the mechanical properties of PLGA polymers, as they proceed through three stages of degradation: I) weight remains stable, dimension decreases; II) mechanical properties decrease, stable weight and dimensions; and III) weight and dimension decrease, acidic components of PLGA are released into the environment; if injected, interaction with bodily fluids will help to maintain a safe pH.

PCL is a synthetic polymer known and favored for its high degree of biocompatibility and biodegradability.[5] Its slow and tunable degradation rates ensure cell and tissue viability, as it does not change the chemical environment of the fibers by a rapid degradation process. These extremely slow degradation rates also have potential applications for long-term and targeted drug delivery systems, but in general PCL is mixed with other polymers to ensure a more rapid degradation rate.[110] Additionally, PCL fibers have highly tunable porosity which is suspected to be positively correlated to fiber diameter within electrospinning,[111] and is negatively correlated to PCL concentration for fibers created with the microfluidic approach.

Although pure PCL has a low tensile strength, its high elasticity still gains favor for use in biomedical engineering.[110] PCL fibers have been generated through electrospinning,[111] microfluidics,[5,94,112] and wetspinning.[113] They have been used for engineering model tissues of muscle cells,[113] neuronal stem cells,[94] and soft tissue cells.[111] Additional research indicates that PCL fibers created with different methods, such as different fluid flow rate ratios within the microfluidic fiber creation method, affect the behavior of cells along the fibers.

GelMA is a series of crosslinked hydrogels that holds properties of both gelatin solutions as well as methacryloyl groups. At the microscale level, GelMA is a very morphable material, making it popular for tissue engineering and the study of controlled cell behavior. Its spatial versatility allows for it to be formed into a wide range of shapes and structures to mimic properties of the native ECM. GelMA scaffolds also allow for cell proliferation, since they contain cell-attachment and matrix metalloproteinase responsive peptide motifs, as well as arginine-glycine-aspartic acid sequences.[97] GelMA is typically polymerized through photocrosslinking, which is favored due to its ability to work at mild temperatures and neutral pH values. Common PIs include 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959), lithium acylphosphinate salt, and 2,2-Azobis (2-methyl-*N*-(2-hydroxyethyl)propionamide).[97,114] However, photocrosslinking can be detrimental to cell encapsulation efforts if working with a cell line which is sensitive to UV light. GelMA has been used to mimic a variety of tissue types, including ocular,[101] cardiovascular,[99,100] and bone.[115]

GelMA's mechanical properties are highly tunable, and can be affected by the amount of methalcryloyl substitution, the concentration of GelMA and PI the prepolymer solution, and time of UV photopolymerization.[97] For instance, increasing the amount of methalcryloyl substitution is linked to a decrease in the average pore size of the hydrogel, as well as proportionally increasing the compressive modulus and decreasing the swelling ratio. Certain postprocessing techniques can also change the physical properties of the hydrogel; cryogenic treatments are linked to increasing the porosity of the surface of GelMA polymers.

Alginate, which is derived from seaweed, is made up of β-d-mannuronate (M) and α-l-guluronate (G) links that are often either block chains such as (AAA-BBB-AAA-BBB) or randomly chained polymers.[102] The physical form of Alginate is a gel-like substance and the specific characteristics of the natural polymer vary depending on the length of the polymeric chains and the concentrations of the links within the chain. Alginate is favored for use in healing of secreting lesions, as it creates a moist environment that promotes cellular regenerative processes. The gel-like textures and traits of Alginate are especially prevalent when applied to the wound due to the ion transfers between the alginate and the bodily fluids. Alginate also has a low toxicity and high tissue compatibility, which make it advantageous for application to open wounds, cell encapsulation and subcutaneous injection into living hosts.[116]

Gelation of alginate occurs via a chemical interaction wherein divalent cations bind to guluronate, which causes chaining to occur within the solution, thereby polymerizing the material.[116] Common crosslinkers include Na^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, and other cations to optimize tensile strength and hydration properties.[102] Choice of crosslinker play a crucial role in determining the physical properties of the resulting hydrogel, as seen in **Figure 1**.[105] For instance, using a crosslinker yields a film with a significantly higher tensile strength than a film created solely from SA which was polymerized via thermal crosslinking. Likewise, the presence of excess sodium during crosslinking affected the percentage of crosslinking cations present within the final sample, which causes significant differences in the mechanical properties of the resulting hydrogel. Some properties affected include the tensile strength, and the elastic modulus and percent elongation at break, which can be observed in Figure 1.

Chitosan is another polymer commonly used in tissue regeneration and localized drug delivery.[106,108] It is a naturally derived polymer with a high degree of biocompatibility and degradability within the human body, as well as exhibiting hydrophilicity, functional amino groups, and a cationic charge. These factors make it an ideal candidate for tissue engineering

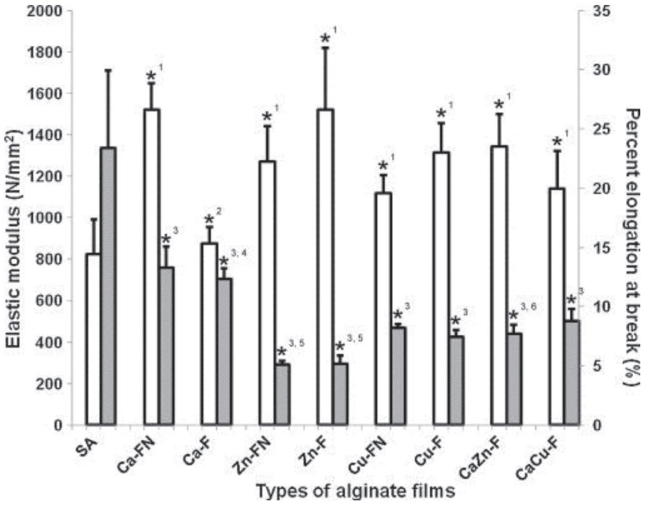

Figure 1. Changes in elastic modulus and percent elongation at break for films polymerized with different cations. Significance was denoted with the symbol ★, with each label referring to the following comparisons: 1: each of the film compared with SA film polymerized via thermal crosslinking; 2: Ca-F compared against Ca-FN, 3: each of the film compared with SA, 4: Ca-F compared against Cu-F or Zn-F; 5: Zn-FN or Zn-F compared with the rest of the films; and 6: CaZn-F compared with Zn-F. Reproduced with permission.[105] Copyright 2012, Elsevier.

and can be used for clinical applications such as therapeutics, subcutaneous injection, and oral delivery. It is also utilized for its ability to aid in cell transportation, since it can easily be processed into porous structures that can be used to create cell-permeable scaffolding.

Another incentive to use Chitosan includes the variety of ways in which it can be polymerized.[106] Gelation can be induced via chemical interaction, or thermal polymerization. Thermal polymerization of Chitosan is a reversible process; the solution remains liquid at room temperature but the onset of polymerization occurs at 25 °C. This allows for unique applications, such as subcutaneous injection of liquid Chitosan which will gel as it warms up to body temperature. Chitosan is often partially acetylated to produce a lower form of crystallinity.[108] When fully acetylated or fully deacetylated, chitosan exhibits a maximum crystallinity, whereas lesser crystallinity occurs at degrees of acetylation between zero and one hundred percent. Chitosan also carries a high charge density when in solution, which enables it to form insoluble ionic complexes with many different water-soluble anionic polymers.

4. Approaches for Improving Fiber Properties

As mentioned above, there are a variety of microfiber fabrication techniques, each of which creates unique fibers. Therefore, there are many ways to produce fibers with desired properties. This review aims to provide a comprehensive idea about methods for generating fibers with a wide range of mechanical properties, allowing for researchers to generate fibers with those properties that will be of most use in their respective fields.

4.1. Changing the Polymer Concentration

This approach can be applied in most of the fiber fabrication methods. In a study, Bai et al. fabricated gelatin fiber using the microfluidic fiber fabrication. In this paper, gelatin was dissolved in Dimethyl sulfoxide (DMSO) as the prepolymer solution (core fluid) with the concentration range of 8% to 12% that results in a wide range of core fluid viscosity from 446 to 5140 cP.[28]

Figure 2a–f exhibits the SEM images of the microfibers made by different concentrations of gelatin in DMSO with the core and sheath flow rates of 5 and 1500 mL min^{-1}, respectively.[28] This figure shows that the increase of the gelatin concentration results in an increase of the fiber roughness. The mechanical properties of the fibers made by different gelatin concentrations are provided in Figure 2f. This figure indicates that the mechanical properties of the microfibers are improved significantly by increasing the gelatin concentration. Additionally, Figure 2f and **Figure 3**e show the results of changing the gelatin concentration in DMSO. Based on this table, the increase of the gelatin concentration from 8% to 12% enhances the Young's modulus and tensile stress at break by 2.2 and 1.9 times, respectively.

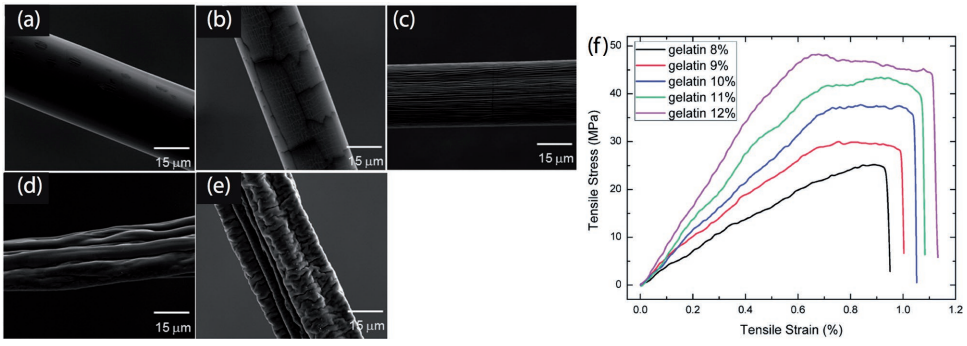

Figure 2. a) 8%, b) 9%, c) 10%, d) 11%, and e) 12% gelatin concentration in DMSO. The flow rate of the core and sheath fluids are 5 and 1500 mL min^{-1}, respectively. (g) Stress–strain curves of the gelatin microfibers fabricated with different gelatin concentrations in DMSO; the flow rate of the core and sheath fluids are 5 and 1500 mL min^{-1}, respectively. Adapted with permission.[28] Copyright 2014, RSC.

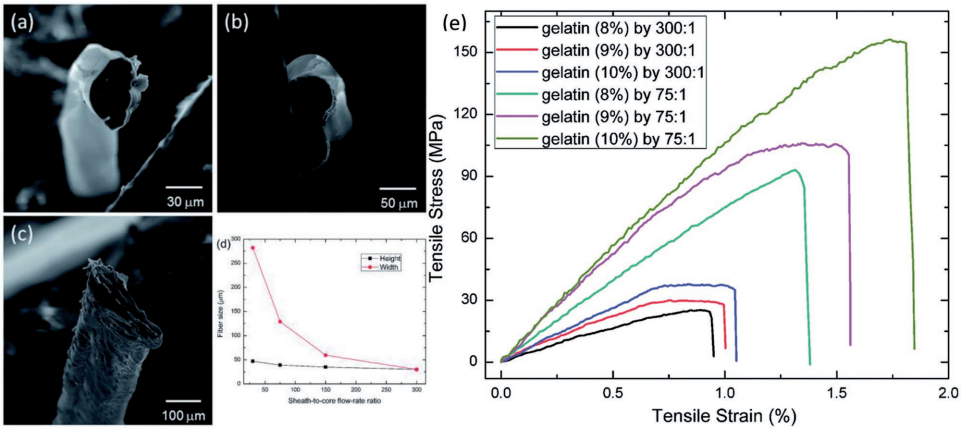

Figure 3. SEM images of the gelatin microfiber made by 9% gelatin in the core solution and the flow rate ratios of a) 150: 1, b) 75: 1, and c) 30: 1; d) the relation between flow rate ratio and the cross section of the fiber; e) the mechanical properties of the microfibers made by different flow rate ratios and gelatin concentrations. Adapted with permission.[28] Copyright 2014, RSC.

4.2. Changing the Microfiber Shape

In some of the microfiber fabrication methods, the shape of the fiber can be regulated by changing the variables involved. Microfluidic fiber fabrication is one the best techniques when producing different shapes of the microfiber is desirable. Some reports showed that the shape of the microfiber can be easily changed by using different flow rate ratios between the core and sheath fluid.[28,48] It was proven that decreasing the flow rate ratio between the core and sheath fluid results in the increase of the fiber aspect ratio and size (Figure 3a–d).[28] Figure 3d shows the direct relation between the flow rate ratio of two fluids and the size of the microchannel. The mechanical properties of the microfibers made by different flow rate ratios are shown in Figure 3e. This figure illustrates that the mechanical properties of the microfiber can be improved by decreasing the flow rate ratio of the sheath and core fluid as well as increasing the gelatin concentration in the core solution.

Additionally, in microfluidic fiber fabrication the cross section of the microfiber can be dictated by the design of the microchannel. Boyd et al. used thiol–ene and thiol–yne prepolymer solutions in order to fabricate fibers.[48] The SEM images of the microfibers made by two different channels are provided in **Figure 4**a–d. This clearly demonstrate the ability of

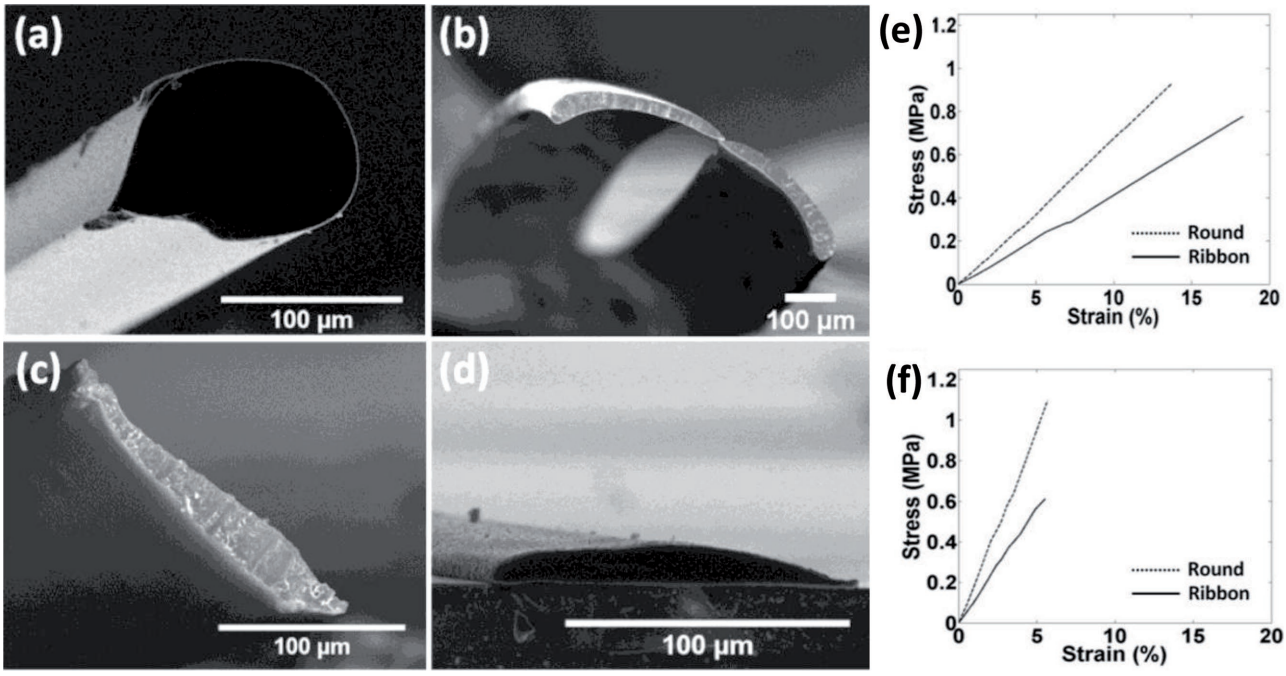

Figure 4. The SEM images of fiber cross section. a) Round thiol–ene fiber made by two inlet channel and b–d) ribbon-shaped fibers made by three inlet channels; e,f) stress–strain curves of the thiol–yne and thiol–ene fibers, respectively. Adapted with permission.[48] Copyright 2013, American Chemical Society.

the microfluidic approach for making fibers with a wide range of aspect ratio. Figure 4e,f shows the mechanical properties of the fibers made by thiol–ene and thiol–yne fibers, respectively. These figure exhibits that the stiffness of the round fibers is higher than that of the ribbon shaped fibers. Additionally, the thiol–yne fibers are stiffer than thiol–ene fibers.

4.3. Polymer Blending

In tissue engineering, one of the goals is to improve the cell adhesion, differentiation, and growth rate. Hydrogels created by synthetic polymers are usually nontoxic, homogenous, and tunable in terms of mechanical and chemical properties.[117] Despite many advantages, the cell affinity toward the synthetic polymers is weaker than that of the natural polymers due to their low hydrophilicity.[118] The cell affinity can be improved by using bioactive proteins onto the fibers.[93] Some studies proved that blending the synthetic and biological (BioSIN) polymers can modify the properties of the scaffold.[93,96,118,119] In some studies, the design of the scaffold was optimized in terms of the percentages of the synthetic and natural polymers.[109,120,121] However, there is always the possibility of incompatibility between the natural and synthetic components, such as phase separation and insolubility. Ghasemi-Mobarakeh et al. used electrospinning approach to fabricate biocomposite PCL:Gelatin nanofibrous scaffolds with weight ratios of 50:50 and 70:30.[119] The SEM images of the random and aligned PCL/gelatin nanofibers are shown in **Figure 5**a.

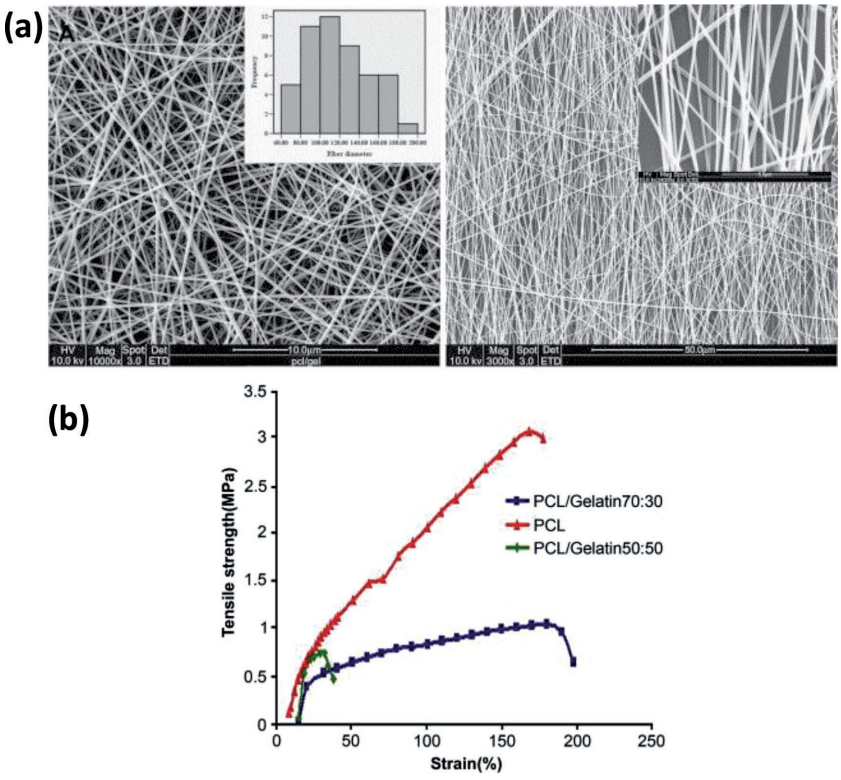

Figure 5. a) SEM images of the random and aligned PCL:Gelatin 50:50 nanofiber. b) Tensile properties of PCL, PCL/gelatin 50:50, and PCL/gelatin 70:30 nanofibers. Adapted with permission.[119] Copyright 2008, Elsevier.

In this study, the tensile properties of the electrospun PCL, PCL/gelatin 50:50, and PCL/gelatin 70:30 nanofibers were measured and the results are provided in Figure 5b.[119] The results demonstrate that the mechanical properties of the scaffold made by the nanofibers are affected significantly by the percentage of the gelatin in the blend. The flexibility of the PCL/gelatin 70:30 nanofibers were higher than that of the PCL fiber whereas PCL/gelatin 50:50 has weak mechanical properties. Therefore, the PCL/gelatin 70:30 nanofibers were applied for cell culture process since its cell adhesion properties is better than pure PCL and its mechanical properties does not change significantly.

Daniele et al. applied thiol-click and photopolymerization simultaneously in order to create BioSIN macromalecular interpenetrating networks (IPNs) with desirable mechanical properties and cytocompatibility.[98] They integrated GelMA and PEG by concurrent photoinitiated thiol-click reactions. In this study, three different IPNs were fabricated by the covalent ($BioSIN_x$) and physical ($BioSIN_p$) incorporation of proteins with a synthetic polymer network. The third one was PEG-*co*-GelMA, which was made in order to compare the effect of additional thiol-yen versus the thiol-ene network. The compressive elastic moduli of the three networks with different compositions are shown in **Figure 6**. Generally, the elastic modulus of BioSIN is more than others and it increases by increasing the percentages of PEG and GelMA.

The stress–strain curves of the BioSIN and neat hydrogels under uniaxial compression are provided in **Figure 7**a. This figure demonstrates that all of the formulations have a linear region.[98] Additionally, in $BioSIN_x$ and PEG-*co*-GelMA network, the modulus increases by increasing the strain whereas the modulus of $BioSIN_p$ does not change significantly. The viscoelastic properties of different formulations are shown in Figure 7b. This figure indicates that the loss and storage modulus are independent of frequency for all of the networks. $BioSIN_x$ shows an elastic behavior since its storage modulus is higher than its loss modulus whereas in $BioSIN_p$, the difference between these two modulus is lower compared to $BioSIN_x$.

4.4. Inclusion of Nanomaterial

Many synthetic or natural polymers have been used to fabricate fibers in the biomedical engineering area. As mentioned above, the natural polymers have a better functionality in terms of cell affinity compared to synthetic polymers, but their mechanical properties and electrical conductivity are very weak. These shortcomings of natural polymers limit the application of natural polymers in adapting the cellular activity such as

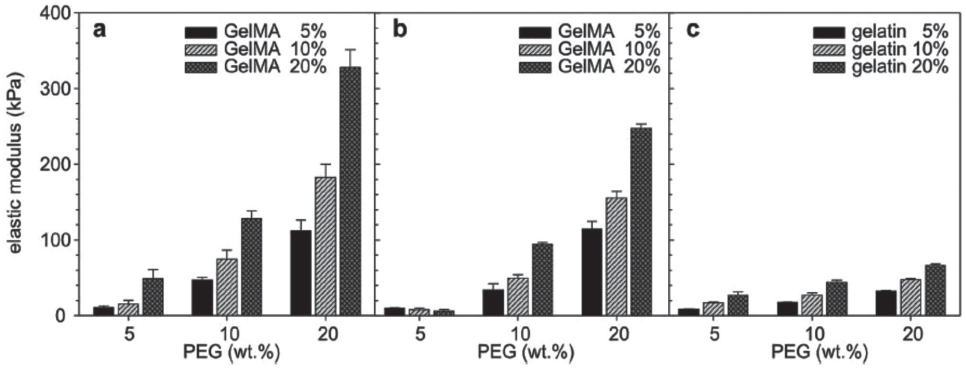

Figure 6. Compressive elastic modulus (kPa) of a) BioSIN$_x$, b) PEG-*co*-GelMA, and c) BioSIN$_p$. Reproduced with permission.[98] Copyright 2014, Elsevier.

skeletal muscle cells.[122] Gelatin, for instance, is a biocompatible and biodegradable natural polymer, which is obtained from native collagen.[123] Some studies show that the inclusion of nanomaterials is another way to improve the mechanical and biological properties of biomaterials. Another advantage of using this method is to increase the electrical properties of the polymers.[99]

In one study, it was shown that the mechanical properties and conductivity of GelMA hydrogels can be enhanced by adding carbon nanotubes (CNTs).[100] However, in this research, it was not possible to create the hydrogel with the shape of natural ECM. Ostrovidov et al. handled this problem and fabricated gelatin fibers using the electrospinning approach and crosslinked them with glutaraldehyde vapor followed by rinsing them in water for two days.[124] A schematic of electrospinning fiber fabrication method is illustrated in **Figure 8**a. Figure 8b,c shows TEM images of the gelatin fiber including the multiwalled carbon nanotubes (MWCNTs). The effect of MWCNT percentage on Young's modulus of the gelatin nanofibers is demonstrated in Figure 8d–f. Based on this figure, the Young's modulus for a gelatin nanofiber increases from 509 ± 37 kPa (without MWCNT) to 1077 ±266 kPa and 1170±168 kPa with 0.5 and 5 mg mL^{-1} MWCNT, respectively.

4.5. Using Textile Manufacturing Processes

It was recently found that the textile technologies have high potential to control the size, shape, and porosity of natural and synthetic fibers.[27,125,126] The textile technologies can essentially be divided into different types, such as weaving, knitting, and braiding.[23,87] These technologies can be applied in order to mimic mechanical properties of natural tissues, such as cardiac muscle, tendon, and vascular walls.[87,127,128] In a study, a 3D scaffold was made by weaving poly(glycolic acid) (PGA) microfibers.[129]

The variables involved are the drawing speed and the polymer concentration.[130] The increase of the gel concentration and drawing speed results in increasing the thickness of the layers (**Figure 9**a–c). After making composite living fibers (CLFs), the most common textile manufacturing processes were applied in order to obtain different structures of the fibers.

In this study, they used braiding technology to combine three different CLFs containing NIH 3T3 cells, HepG2 cells, and HUVECs, respectively, in order to model the liver.[130] The tensile test was applied to compare the mechanical properties of the CLFs and alginate (**Figure 10**). The results of Young's modulus and tensile tests reveal the possibility of obtaining a wide range of mechanical properties for the fiber by using braiding.

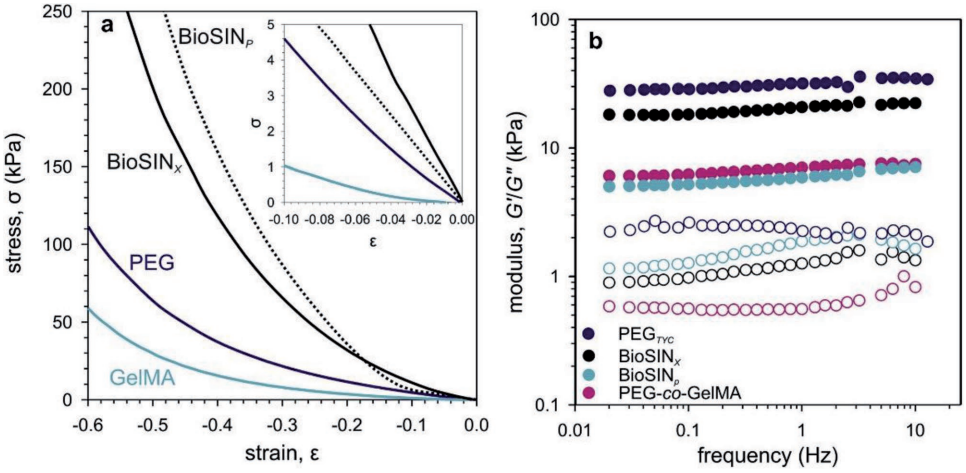

Figure 7. a) Stress–strain curves and b) the viscoelastic properties of different formulations. Adapted with permission.[98] Copyright 2014, Elsevier.

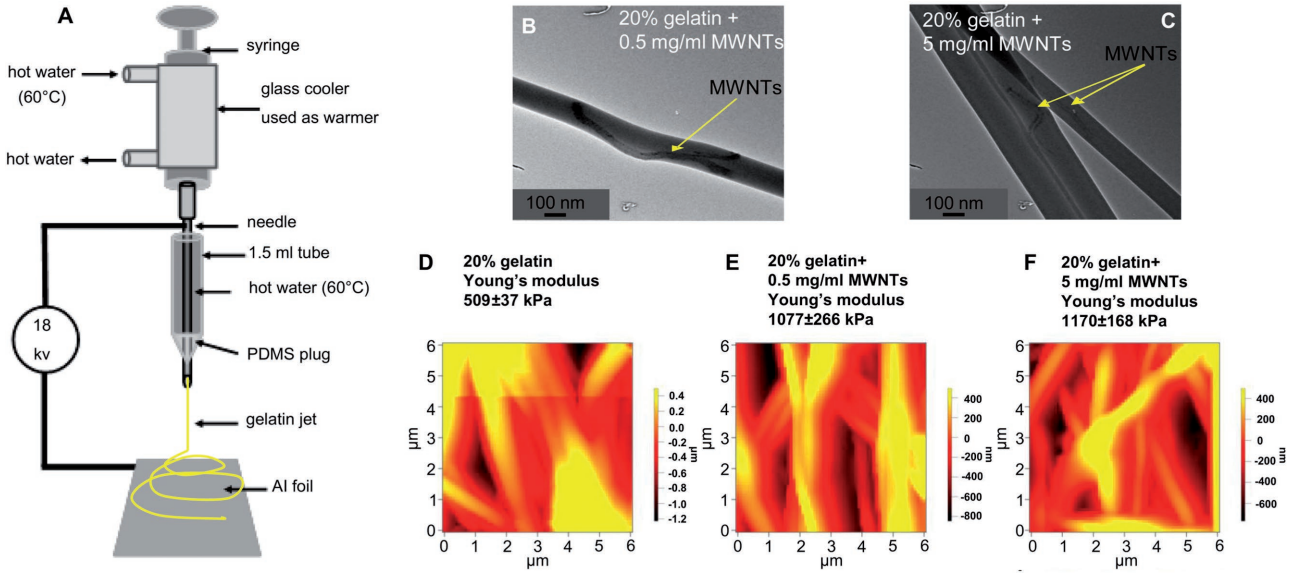

Figure 8. a) Schematic of electrospinning fiber fabrication method; b,c) TEM images of the gelatin nanofiber that includes MWNTs; d–f) Young's modulus of the nanofibers made by 0, 0.5, and 5 mg mL^{-1} of MWNTs. Adapted with permission.[124] Copyright 2011, Dove Medical Press Ltd.

5. Use in Biomedical Applications

Fibers have enjoyed attention within biomedical fields, including biomedical engineering, due to their versatility and highly tunable mechanical properties, which allows for precise control over the microenvironment when used for cell culturing. They are favored within these fields for their ability to deliver cells to a specific target region in a targeted and protected matter, as well as providing support for tissue engineering purposes. Cell-laden fibers can be classified into two main types of culture: encapsulation type and surface type. Modifications to the topography and chemistry of the surface of the fibers can help to aid in cell adhesion, and creating a 3D structure of fibers can ensure a more physiologically correct model for use in biomedical research.

5.1. Encapsulation Type

For encapsulation type cell-laden fibers, cells are originally dispersed in prepolymer solutions and then after gelling the solutions the cells become encapsulated forming cell-laden fibers. As shown in **Figure 11**, there are multiple ways to introduce cells to fibrous scaffolds. Figure 11a shows surface

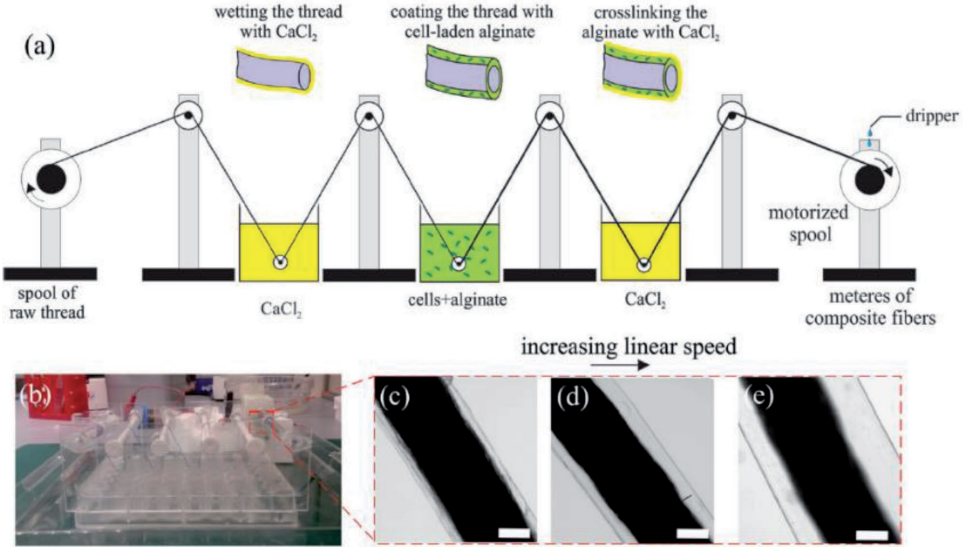

Figure 9. a) Schematic of cell-laden composite living fibers (CLFs); b) the experimental setup; c–e) fibers coated with hydrogel at different drawing speed.[130]

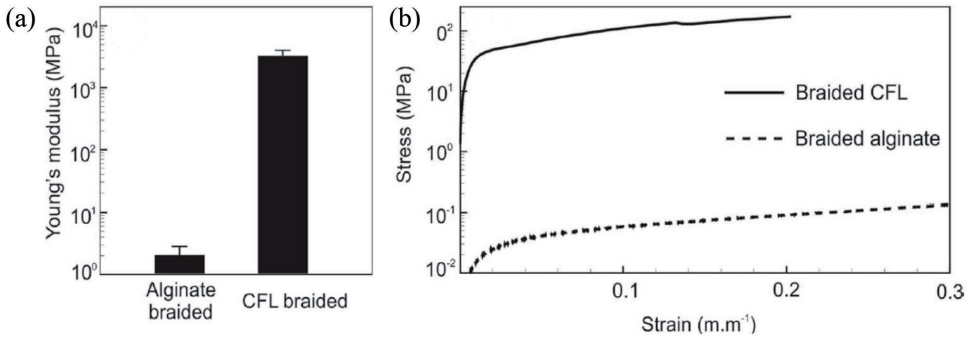

Figure 10. a) Young's modulus and b) stress–strain curve of braided CLFs and alginate fiber.[130]

type cell-laden fibers, while Figure 11b shows that, encapsulation type cell-laden fibers can have multiple fiber geometries including: standard, tubular, core-shell, and compartmentalized. An advantageous feature of encapsulation type fibers is that cells form a 3D culture that mimics tissues in vivo. This provides a critical cell culture platform that contributes to a number of different fields of study.[26] The fiber material used in the fabrication process for encapsulation type cultures must, obviously, be biodegradable and nontoxic to the cells. Because of this, the mechanical tension of the fibers can be a technical problem with culturing cells within a fiber. As the cells spread and proliferate over time, the fiber often changes as the encapsulated cells secrete extracellular matrix (ECM).[23] Conversely, if the polymer degradation happens too slowly, a buildup of ECM will occur and may influence cell function.[132] To maintain the shape of the cell construct long term, the main issues that are to be considered are: type of cells, and degradation speed and mechanical stiffness of the fiber material.[23]

Successful encapsulation has occurred using a wide variety of materials and microfiber fabrication techniques. Perhaps the most common technique used for creating a variety of types of encapsulated fibers is the microfluidic technique, which is favored for this application due to the fact that it is capable of producing fibers in mild conditions with biocompatible materials,[24,25,27] However, MIPC and Extrusion are also common methods for creating fibers with encapsulated cells. Electrospinning has also produced encapsulated fibers, but in this case caution must be used, since the diameter of electrospun fibers is typically smaller than that of the encapsulated cells.[27] Alginate is particularly suited for encapsulating cells due to its ability to polymerize under conditions which are appropriate to cell culturing; it is possible to ensure a neutral pH, and lower

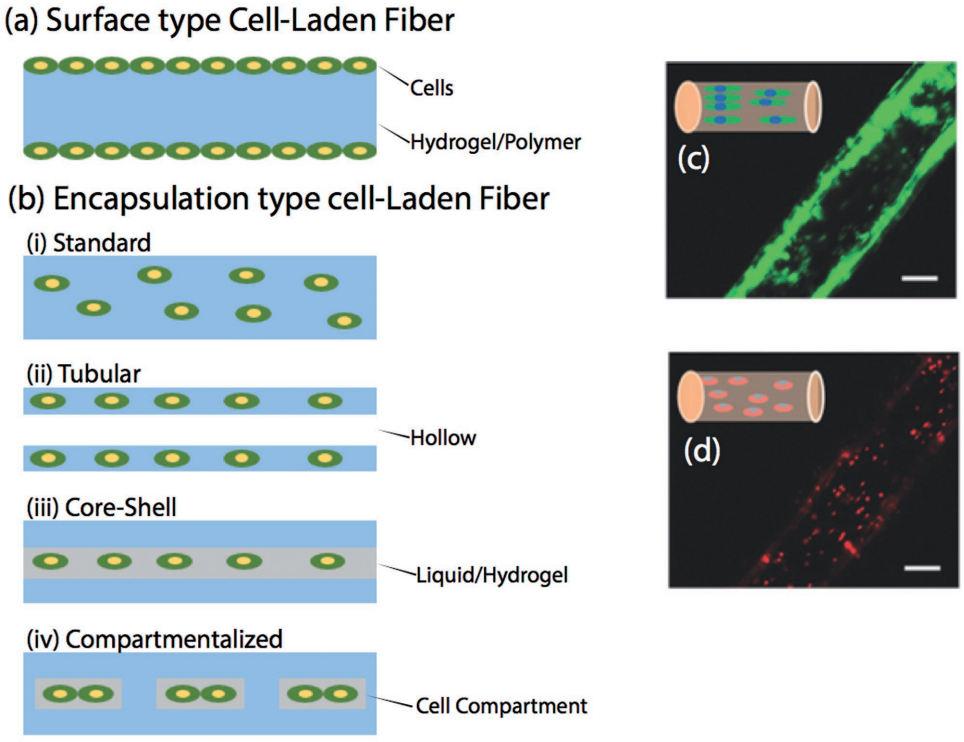

Figure 11. a) Cells seeded on the surface of the fiber. b) Encapsulated fibers: (i) standard, (ii) tubular, (iii) core–shell, and (iv) compartmentalized. c) Myoblasts seeded on GelMA fibers. d) HUVEC cells encapsulated in GelMA microfibers. Scale bars in (c) and (d) are 150 µm.[131]

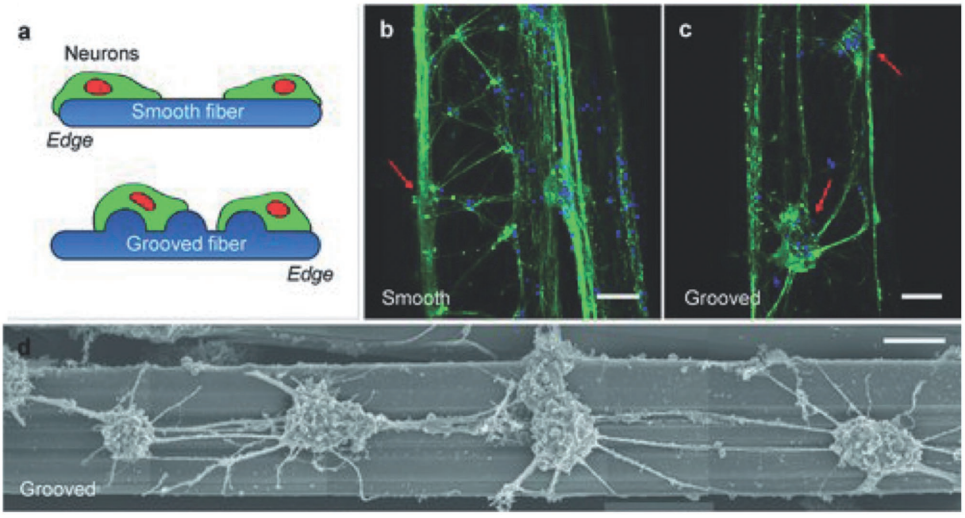

Figure 12. Cell behavior on grooved fibers. Scale bars indicate 50 µm.[104]

concentrations of Alginate do not need to be heated to ensure viscosity.[26] However, a wide variety of polymers have successfully encapsulated cells, including Poly(ethylene glycol) diacrylate,[121,133,134] PCL,[135,136] Alginate,[53,55,116] and more.[26,110]

5.2. Surface Type

Surface type cell-laden fibers have cells seeded on the surface of the fibers, which can be seen in Figure 11a. This requires that the surface is cell-adhesive and biocompatible. For surface type cell-laden fibers, cells are not present during the production of the fibers; therefore, it is possible to use toxic solutions or severe conditions that might be harmful to cells. This expands the possibilities of different materials that can be used in surface type fibers. Because the cells are seeded on the surface of the fibers, handling of the cell-laden fibers has to be delicate because the cells are exposed to the outside environment.

5.3. Surface Properties of Fibers

Using surface type fibers in biomedical applications can be difficult, as it requires cells to attach to the fibers' surface. This process can be aided by modifying the chemical or topographical properties to produce a situation which encourages cellular attachment. Additionally, the topography and surface chemistry of the fiber plays a large role in the behavior and health of cells growing along its surface, and in controlling the differentiation of neural progenitor cells.[27] Modification of fibers' surface environment can be accomplished by introducing chemicals or polymer bases to the surface of the fiber, or by changing the surface texture.

One can change the chemical properties of the fibers through several procedures. An example of a cell adhesion component can be the addition of the oligopeptide sequence Arg-Gly-Asp.[137] Additionally, coating the surface with a component of the ECM can help by shifting the fibers to be more biologically compatible. In addition to modifying the surface with biological agents typically found in the cells' native environments, it is possible to tether polymer chains onto the surface of fibers to enhance cell adhesion. For instance, researchers grafted poly(ethylene glycol) methacrylate chains onto the surface of electrospun fibers, which enhanced cell adhesion.[138]

Researchers have also shown that changing the surface texture of the fibers aided with cell adhesion and adjusted cell behavior. For instance, creating grooves on the surface of the fiber not only helps with cell adhesion, but also aids in cell orientation.[104] An example of this can be seen in **Figure 12**.

Another method for changing the surface of fibers includes increasing the porosity, which allows for homogeneous cell distribution and interconnection, as well as potentially aiding in nutritional diffusion to the cells.[25] There are multiple methods to adjust the porosity of fibers; for instance, it is possible to mix a dissolvable particle into the pregel solution which can then be washed out of the solidified fiber. Postprocessing methods can also create porosity on fiber surfaces after their creation; these include freeze-drying the hydrogels, which causes thermodynamic instability and phase separation within the structure. Additionally, plasma etching can be used to modify the surface, which not only helps by causing fibers to become more hydrophilic and thereby increasing their interactions with biomolecules, but also by etching the surface and increasing wettability and roughness without influencing the bulk properties.[139,140] More modifications can be borrowed from surface engineering, such as: ion beam implantation, which promotes cell adhesion; electron beam texturing, which gives precise control of nanofeatures; and laser texturing, which provides precise control over even complex features.[141]

5.4. Fibrous Scaffolds

Once fibers have the appropriate chemical and biocompatible properties, it is possible to use them to create 3D scaffolds to aid in the support and health of a 3D cell culture. This is preferred over 2D cell culturing techniques due to its increased physiological relevance. To create scaffolds, fibers of various

surface topologies can be wound, woven, or manipulated as described above so that it gains a 3D component with a highly controllable microenvironment. Methods include circumferential winding, where a fiber is repeatedly wound around a base and allowed to dry. This forms a mesh of fibers, the width of which is determined by the number of rotations performed in the winding process.[142] Additionally, by alternating the pregel solution, it is possible to generate circumferential scaffolds with spatially distinct chemical or physical properties, thereby allowing for a more complex 3D environment for cell culturing or regenerative medicine.[143]

Another method for the creation of scaffolding includes extruding the fiber onto a flat, mobile surface. As the fiber is created, the platform below it moves, causing it to fall in a uniformly flat mesh.[95] The height of these meshes can be adjusted by changing the amount of time the fiber is extruded, or by stacking multiple meshes on top of one another.

Additional techniques to assemble fibrous scaffolds can be borrowed from the textile industry.[23] Weaving, knitting and braiding can affect the mechanical properties of a linear fiber, as discussed above, but can also be very effective when applied to the issue of creating a 3D culture. For instance, Onoe et al. detail a method for creating woven 3D structures of fibers, which can be seen in **Figure 13**a.[23] In the same paper, the group mentioned a method for creating helical fibrous scaffolds, which can be seen in Figure 13b.

There is also a possibility that IPC fibers can be used as a building block or "biostructural unit" for engineering 3D constructs, For example, IPC fibers formed from water soluble chitin and alginate was used to encapsulate cells and proteins while leaving the quality of the biologicals uncompromised.[62] In order to assemble these biostructural units in a spatially governed arrangement so as to attain multicellular tissue constructs MIPC can be used. In the MIPC process, there is fusion of various interfaces within the polyelectrolyte droplets in order to create an IPC fiber with several sections.[59]

In another study, knitting textile technology was applied to combine type I collagen and PLGA for the cartilage regeneration applications.[87] Akbari et al. fabricated the CLFs by passing the fibers into several reservoirs of cell-laden prepolymer (Na-alginate) and crosslinking reagents ($CaCl_2$).[130] The schematic of this fiber fabrication method and the experimental setup are illustrated in Figure 9a,b, respectively. Some of the resulting scaffolding can be seen in **Figure 14.**

Fibrous scaffolds also allow for a tunable environment between the fibers, which affects the behavior and health.[94] It is known that even slight changes to the mechanical properties of a cells' environment can affect their health and behaviors by changing the way they interact with their surroundings and each other.[144] Sharifi et al. showed that scaffolds created with fibers of certain sizes allowed for cells to bridge across gaps within the scaffolding.[94] The size of the fibers and the tightness of the scaffolding affect the density and amount of empty space within the scaffold, thereby affecting the 3D microenvironment through which the cells exchange nutrients and secrete signaling chemicals and waste. This plays an important role in determining cell health, but also can affect studies which aim to understand cell-to-cell interactions by examining the chemical makeup of the interstitial fluid within the scaffold. For this reason, it is crucial to design scaffolding with

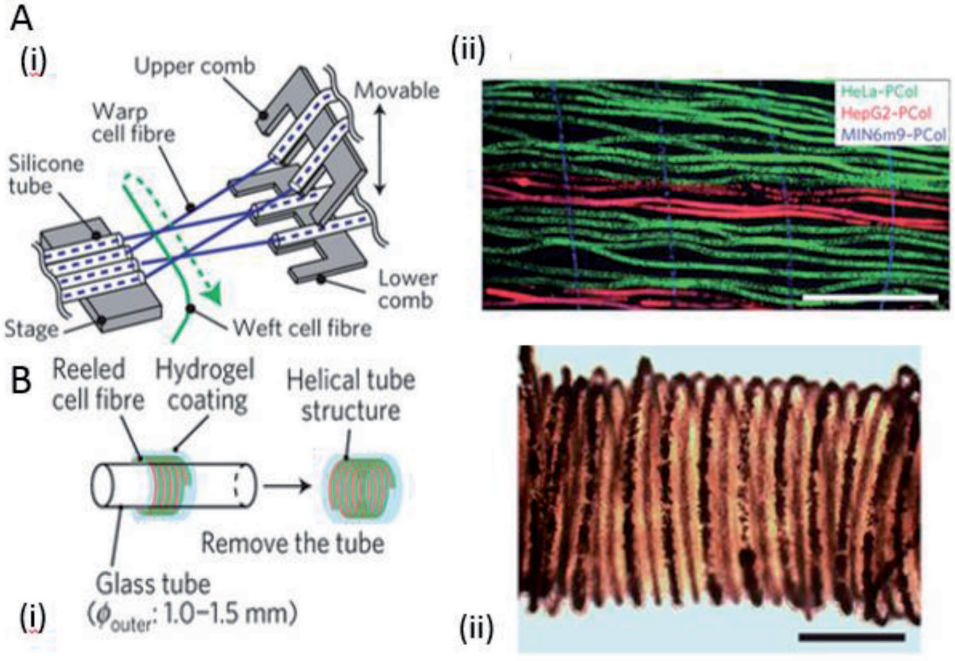

Figure 13. a) (i) Method for weaving a 3D structure from fibers with a loom submerged in cell media; (ii) example of woven 3D cell culture formed with fibers encapsulating three different cell lines. Scale bar represents 1 mm. b) (i) Schematic for creating a helical fibrous scaffold, (ii) resulting helical tube, created with two cellular fibers, one with NIH/3R3-ACol cells and the other with HepG2-PCol fibers. Scale bars represent 1 mm. Adapted with permission.[23] Copyright 2013, Nature Materials.

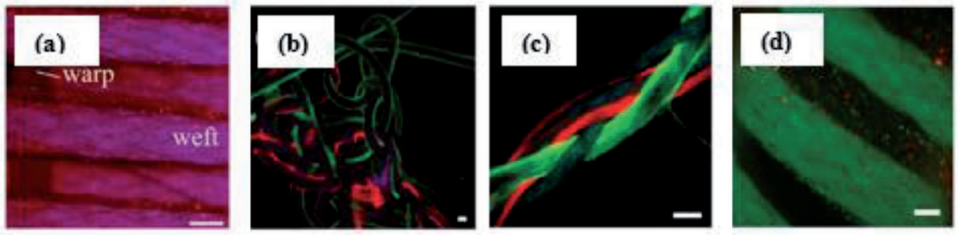

Figure 14. Using the most common textile manufacturing types: a) weaving; b) knitting; c) braiding; d) winding. Reproduced with permission.[130]

the original tissue environments and ECM matrices in mind, taking care to mimic the original architecture, bioactivities, and mechanical properties.[20]

5.5. Microfibers for Topical and Subcutaneous Drug Delivery and Regenerative Aides

Another strong motivation to progress knowledge of biocompatible microfibers is the possibility of subcutaneous injection for targeted, time-controlled drug delivery or aided regenerative medicine. An example of a subcutaneously injected electrospun fibrous scaffold can be seen in **Figure 15**.

Creating fibers for targeted drug delivery systems is accomplished by encapsulating the drug within the fiber; this can be done by injecting them within the core of hollow microfibers, encapsulating them like the cell encapsulation which was discussed at length previously, and crosslinking or absorbing the drugs on the surface of created microfibers.[144] Due to the potential sensitivities of therapeutic chemicals, only certain microfiber creation techniques are suitable for drug encapsulation. These include wet extrusion/spinning,[143,144,146] microfluidic fabrication,[43] and electrospinning.[104,147,148] Fibers or scaffolds injected into a patient might include a variety of therapeutic agents, including antibiotics, proteins, growth factors, genes, vitamins, liposomes, and chemotherapy medications.[6,144,149–151]

5.5.1. Topical Drug Delivery and Wound Protection

Topical drug delivery systems remain on and are absorbed through the skin instead of entering the body through oral or subcutaneous injection. This system has some key advantages over internal drug delivery: it avoids the potentially harsh and changing environment within the body, it shows high levels of efficiency, treatment can be easily terminated at any time, and more.[152] However, difficulties arise due to the possibility of contact dermatitis, damage to the drug due to its interactions with the skin, and the inability to absorb large particles through the skin.

Topical fibrous treatments are typically used in healing wounds, where they can both protect damaged tissue and deliver medications to increase healing rate. They can take the form of sutures, which are used to hold the wound shut and therefore must have high tensile properties; wound dressings, which are temporarily attached to the surface of the wound; and grafts, which holds living tissue that will hopefully take the place of tissue which is missing due to injury or disease.[152,153] Each of these must come in direct contact with a wound, and therefore must be stable and biocompatible so as not to further damage already injured tissues.

Suturing is a widely used and accepted method of treating a wound, such as a cut. Sutures are microfibers which must be strong, since they are being used to draw and hold wounded skin together, but they also must be able to stretch and recoil to accommodate the shifting of the wound and of the patient.[152] They can be made from both synthetic and natural polymers, and common methods of creation include dry spinning, melt spinning and gel spinning. Modifications include embedding antibiotics, and including radioactive isotopes. Examples of sutures include an absorbable PGA suture and poly(lactic acid) suture anchors; however, these are known to have poor interactions with cells, and therefore newer materials with more favorable surface chemistry have been generated.[154]

Wound dressings come in a variety of types; passive dressings only cover the wound, while interactive dressings allow passage for water vapor and oxygen but protect the wound from

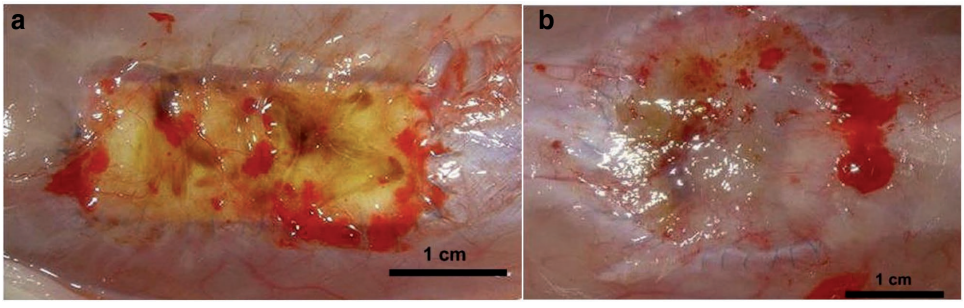

Figure 15. Electrospun poly(ester urethane) urea implanted into the abdominal wall of rats a) at the time of implant and b) after four weeks. Reproduced with permission.[145] Copyright 2011, Elsevier.

damaging material, and bioactive dressings contain and provide helpful components to aid in healing.[152] Micro- and nanofiber mats can be applied directly to the wound can allow for necessary evaporation and oxygen permeability, which provides a more ideal environment for wound healing. Fibrous mats which contain therapeutics can also create a bioactive dressing, with highly tunable characteristics for absorption and release of drugs, as well as of fluid from the wound.

Historically, grafts have constituted living tissue which has been relocated in order to aid in healing a wound. Typically grafts are taken from the patient in order to minimize threat of rejection or other postsurgery complications. However, microfibers may lead the way to a new generation of grafts which would nullify the need of harvesting healthy tissue, which by necessity would create another wound in the process. Inclusion or replacement of standard grafts with those created by fibers can create an ideal environment for healing while housing new cells which help to speed up the healing process. Skin grafts used on mice showed significant improvement in healing rates over untreated mice.[155] Additionally, microfiber composites show promise in increasing the effectiveness of vascular grafts.[153]

5.5.2. Subcutaneous Injection for Drug Delivery and Regenerative Aide

Injection of both microfibers and fibrous scaffolds can be an excellent way to aid in tissue regeneration and provide targeted drug delivery.[35] However, any material injected into a living body must be both biocompatible and stable. Depending on the application it might also be necessary for injected microfiber and fibrous scaffolds to either degenerate safely or be reabsorbed into the body; if not, the injection would either be a permanent addition to the patient, or would need to be removed at some later point.

Different applications may call for different volumes of injected material or amounts of therapeutic agent; therefore, it may be more advantageous to inject on the microfiber-scale or to go through the more invasive technique of injecting scaffolding. While microfibers are particularly well suited for targeted drug delivery, or for aiding in small-scale cellular regeneration and guiding, cases where larger volumes of tissue must be regenerated call for the insertion of a fibrous scaffold. Both microfibers and their 3D counterparts might be modified in any of the ways listed above, which can help to increase their hydrophilicity, change their mechanical properties, or adjust cell behavior as they interact with the surface of the fiber. Additionally, it should be noted that the addition of therapeutic chemicals may alter the mechanical properties of the fibers, including factors such as ultimate tensile strength and strain at failure.[144]

Microfiber injection has been proposed for a variety of applications, including using wetspun Poly(l-lactic acid) fibers for targeted release of inflammatory drugs, which occurs linearly for up to 8 weeks in vitro.[144] Additionally, Lin et al. used the microfluidic method to create alginate microfibers whose release of drugs was controllable through magnetic stimulation; if left alone, the fibers released the drug diclofenac steadily, but the fibers experienced the application of an external magnetic field, they rapidly released diclofenac.[43]

Injection of fibrous scaffolds can be a powerful technique to aid in regrowth of damaged tissue, and has shown promise for a wide variety of biomedical engineering applications. For instance, Park et al. showed the efficacy of titanium microfiber scaffolds in aiding bone regeneration; furthermore, they showed that modifying the surface of the scaffold with long-term exposure to UV light improved its hydrophilicity and dramatically improved bone regeneration and strength after implantation in vivo.[156] Other researchers, such as Chet et al., have begun incorporating smart biomaterials into microfiber drug delivery by creating a microfiber mesh with the ability to release drugs in an electrically controlled manner.[148] These studies show high degrees of biocompatibility and excellent capabilities in time and location-controlled drug release. Likewise, injecting fibrous scaffolds of a variety of materials and creation methods has been utilized in areas such as smooth muscle,[107] soft tissue,[145] teeth,[92] and bone.[156,157] Others have focused on using scaffolding for regenerative treatments for intervertebral disc tissues,[20] or creating viable tumeroid models for testing cancer treatments, including ones which might host cells gathered from biopsies for the generation of patient-specific treatment plans in clinical settings.[89] While scaffolding injections can provide critical tissue-specific clinical treatments, it should be noted that critical concerns have arisen over the gaps in mechanical properties needed for scaffolding to succeed as well as metallic stents.[158]

Scaffolding can gain another level of complexity, since they can also be modified to emit drugs and act as a therapeutic device. For instance, Ranganath and Wang mixed Paclitaxel, a drug used in chemotherapy, into the pregel solution before using electrospinning to create PLGA fibers.[147] They showed that Paclitaxel was released continuously over a span of at least 80 d, and the amount released varied based on the amount of available surface area over the different samples.

6. Conclusions

This review paper provided different methods that can be applied in order to regulate the mechanical and biological properties of microfibers for applications within biomedical engineering. The most common fiber fabrication methods have been discussed, as well as the parameters for tuning the properties of the fibers. There are some general modifying methods that are functional for all of the fiber fabrication approaches. Through modifying the method of creation, materials, or additional postprocessing, it is possible to obtain a wide range of mechanical and chemical properties. This can be accomplished through changing the polymer concentration, changing the fiber cross section, using a textile manufacturing process, polymer blending, coating the fibers in ECM, inclusion of nanomaterials, and more.

Because of their versatile and useful properties, microfibers have specifically received a great amount of attention in 3D cell culturing and other biomedical applications. For surface type cell seeding, modifications to the topography and chemistry of the fiber's surface can help to aid in cell adhesion and

proliferation, whereas the mechanical and chemical properties of the fibers themselves play a significant role in the viability and behavior of cells encapsulated within.

As microfibers gain prominence in 3D cell culturing and biomedical engineering applications, they continue to show promise for creating physiologically correct models and other clinical applications. Their continued contributions toward these fields shows their power as a tool capable of generating new technologies which can aid in biomedical research and provide new venues for designing highly complex but controlled experiments.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

3D cell culture, biological properties, mechanical properties, microfiber, nanofiber

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