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Fabrication and Applications of Biological Fibers

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Fibers derived from processing of biological materials have long been implemented for commercial use as they are widely available from a diverse range of renewable materials. Moreover, their natural mechanical and functional properties continue to provide newfound sources of technological advancement in areas including medicine and filtration among other more traditional fields. While not only providing inspiration, the study of biological fiber formation in organisms such as spiders and silk worms has facilitated fundamental knowledge; however, this review concentrates instead on more recent artificial fabrication techniques that have been adapted for the formation of 1D (one dimensional) fibers from biological materials. In addition, we provide an extensive look at the various biological materials from the standpoint of their applications and fabrication methods. Areas encompassing the latest advancements in fiber processing techniques with respect to compositional and geometric control are discussed and provide a promising outlook of future growth in this continually growing field.

INTRODUCTION

The high aspect ratio geometry of one dimensional (1D) fibers provides them with key properties that are useful for a variety of applications. Because of their high surface-to-volume ratio, fibers feature favorable mass transport making them particularly suited for absorption, release, sensing, and catalysis (Matatov-Meytal et al., 2002; Huang et al., 2013). While being compact but able to transverse long distances, fibers have often been utilized in communication, with biological materials being recently explored as optical fibers for such applications (Huby et al., 2013). In addition, the flexible nature of fibers also allows them to be integrated into flexible materials, such as textiles (Schmucker et al., 2014). While synthetic polymer fibers have been fabricated by a range of physically, thermally, and electricallydriven techniques, the processing schemes used in producing biological fibers are more limited in scope but perhaps not in terms of application, as a diversity of functional biological materials may be implemented. Aside from native bio-spinning, some of the most common mechanical techniques employed for fabrication of 1D biological fibers include wet-spinning, electro-spinning, drawing, extrusion, solvent casting, and the use of microfluidics (Figure 1). In some ways, these synthetic fabrication techniques can produce fibers with improved characteristics over naturally generated fibers (Shao et al., 2002).

The potential applications of 1D biological fibers are broad owing to intrinsic properties including biocompatibility, biological activity, and in some case a stimuli responsive nature. In addition, unlike most synthetic systems, many biological macromolecules are capable of forming into well-defined structures across multiple levels of order owing to built-in sequences of chemical moieties. The results of utilizing natural biological materials to generate 1D fibers has hence yielded impressive systems capable of implementing selective catalysis, exquisite molecular recognition, and even structural order over long range (Sawicka et al., 2005; Caves et al., 2010). Ongoing efforts in generating 1D biological fibers have looked to recreating the structural and functional features present in native systems as well as increasing the mechanical properties and process ability of biological material by altering the fabrication process,treatment, or composition. Further extensions of the use of 1D biological fibers within diverse structures including membranes, weaves, meshes, gels, and even free-form architectures (Ang-atikarnkul et al., 2014) have afforded biologically active surfaces that continue to provide significant value as materials in biomedical technologies. When forming these structure, having the fibers in either random or aligned configurations can provide distinct benefits for different applications (Su et al., 2012). One such high impact area in which biological fibers are finding ever increasing interest is toward tissue engineering in which the fibers may provide a mimetic matrix for the growth and differentiation of cells (Chung et al., 2012; Roloff et al., 2014). Aside from tissue scaffolds, biological fibers have more classically been used in the textile industry; however, in this review we focus primarily on nano and microscale 1D fibers that have been prepared from natural building blocks with an emphasis on design and fabrication approaches that are pushing the boundaries of the field of biomaterials development.

In this review, we will highlight some of the fundamental

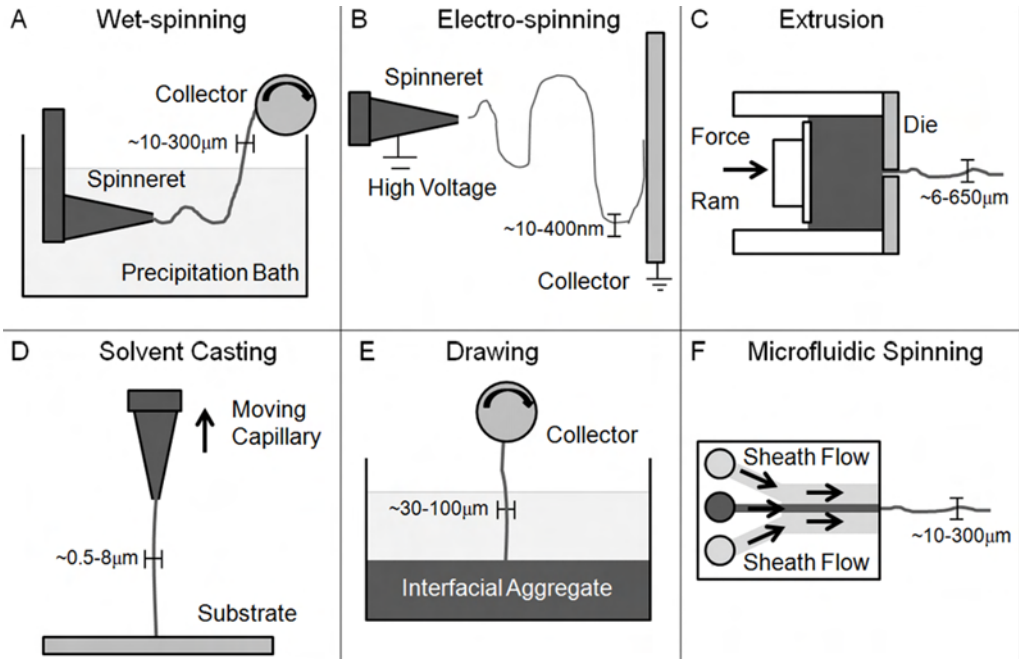

FIGURE 1 | Schematic representation of some of the more common 1D biological fiber processing approaches and their approximate diameter ranges.

aspects in several of the current approaches to biological fiber fabrication as well as provide a discussion of the progress made in controlling fiber design parameters including diameter and stability with respect to specific application areas. In addition, the following section will provide an overview of some of the common as well as emerging biological materials that have been explored for 1D fiber fabrication. Because the range of possible biological building blocks for fiber design is so diverse, an understanding of the structural biology of several categories of these materials is important in developing an intuition for which biological fibers may be most fitting to particular applications. Hence, we expect the following section reflecting on existing and new biological fiber materials will provide a good starting point for realizing how certain characteristics of specific biomolecules (or properties of particular classes of biomolecules) may be implemented for practical biological fiber design.

MATERIAL CONSIDERATIONS FOR BIOLOGICAL FIBERS

Fibers can be made from an extremely wide variety of biological materials, including carbohydrates, proteins, DNA, and even viruses (Figure 2). Biological materials are appealing for the fabrication of fibers because of their innate functionality as well as having great flexibility for chemical modification. Additionally, by starting with a proper biological material the resulting fibers may be biocompatible and biodegradable, making them particularly suited for medical applications and naturally more environmentally friendly (Albanna et al., 2013). Here we will describe some of these unique properties of specific materials in the context of the broader classes of biomolecule-derived 1D fibers.

Carbohydrates are among the most widely used biological materials in 1D fiber production. Cellulose, being one of the most common biopolymers in abundance, is frequently used in biodegradable packaging due to its impressive mechanical strength and low oxygen permeability (Larsson et al., 2014). Chemical functionalization of cellulose in itself is an active area of research in which dozens of derivatives have been generated in many cases for increasing its processing abilities or for adapting the fibers for particular applications in filtration or medicine. Cellulose acetate, for instance, can be readily dissolved in volatile solvents allowing for successful fabrication by electro-spinning, while solvents for traditional cellulose may not be completely volatile require further processing steps in order to yield stable fibers. To overcome such difficulties, 1D fibers produced from derivatives of cellulose are first generated by wet-spinning, electro-spinning, or other techniques and subsequent to this initial fabrication the fibers are then converted chemically back to cellulose fibers (Frey et al., 2008). Depending on the particular application of the intended cellulose fibers, it is also important to consider the fact that plant derived cellulose as compared to bacterial cellulose are vastly different, as these have strikingly diverse characteristics in terms of crystallinity, degree of polymerization, and water content.

Other carbohydrates have also proven to be notoriously difficult to process, such as hyaluronic acid and chitosan. Chitin for

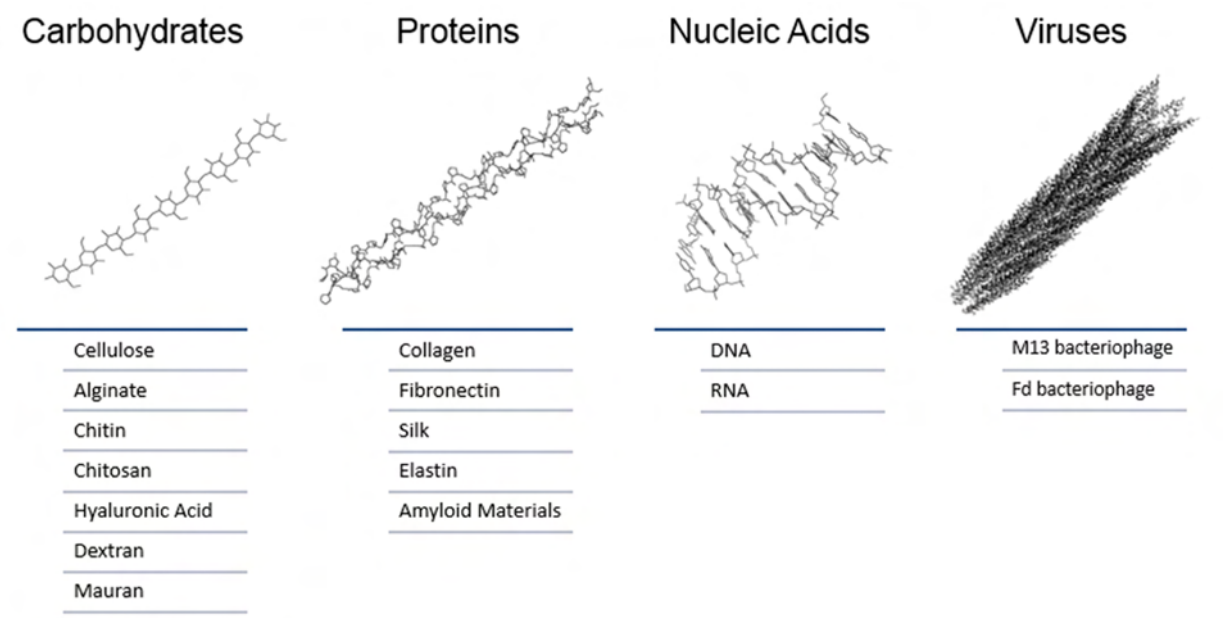

FIGURE 2 | Diagrams of several active applications areas which currently make use of 1D biological fiber structures.

instance, which is the second most abundant biopolymer, has impressive mechanical properties but is insoluble in many typical solvents thereby making processing highly problematic. Among the approaches to solubilize chitin, the formation of alkali chitin by dissolution in NaOH at low temperature has become well adapted for creating amorphous chitin that can in some casesbe dissolved in water (Rinaudo et al., 2006). The structurally similar derivative of chitin, known as chitosan, has a lower degree of acetylation and is in contrast soluble in acidic aqueous solutions. The chitosan is however insoluble in many organic solvents but has been found to be a biocompatible and biodegradable material that has been associated with having antimicrobial activity (Mi et al., 2014). A range of chemically modified and chemically treated derivatives of chitin and chitosan have been implemented as 1D fibers and more often as blends with other biological materials such as silk and cellulose via processes like wet-spinning (Hirano et al., 1999). The range of applications of fibers produced from these material are quite vast including the pharmaceutical, textile, biomedical, and cosmetics industries (Yusof et al., 2003). Difficulty in processing of certain biological materials does not necessarily stem from mere solubility issues, but rather due to physiochemical properties such as high viscosities and surface tension as in the case of hyaluronic acid. Due to such unfavorable characteristics, modified electro-spinning approaches, like electro-blowing, must be used in which heated air is delivered around the jet of electro-spun fiber to allow tuning of the solvent evaporation rate (Um et al., 2004).

Not all carbohydrates have such issues with processing or dissolution. Dextran, for instance, represents a type of biomolecule that is highly soluble in aqueous solvents and DMSO, and it is capable of being fabricated into 1D fibers by processing methods such as electro-spinning (Jiang et al., 2004). As with many carbohydrates derived fibers, the biocompatible and biodegradable nature of dextran makes these polysaccharides amenable for tissues engineering, though requiring cross-linking to facilitate sufficient mechanical stability (Ritcharoen et al., 2008).

Aside from carbohydrates, the use of naturally occurring proteins, rather than synthetic polymers, is ever increasing in biomedical applications given the low immunogenicity and improved biocompatibility of many natural proteins. In addition, the sequence specific chemical motifs displayed as amino acid residues on the proteins can offer cell binding capabilities *via* different surface receptors displayed on certain differentiated cell lines. Fibronectin for instance, carries the arginine-glycine-aspartic acid motifs, allowing interaction with cell surface integrins in a variety of cell types (Harjanto et al., 2013), while laminin carries an isoleucine-lysine-valine-alanine-valine motif that is known to promote neurite extension and neural cell adhesion (Merzlyak et al., 2009). Fibrinogen, which is generally a soluble protein in blood plasma and naturally broken down during clot formation to form fibrin, has been widely used in tissue engineering applications to promote cell migration (Linnes et al., 2007). Such fibers made from protein based components are often formed by electro-spinning or wet extrusion processes to produce structurally stable mats that can be readily handled (Phillips et al., 2004); however, often once rehydrated such mats can lose stability over several days.

A protein-based material that can provide stable mechanical and physiochemical properties for extended periods of time is

collagen, which is a diverse material in itself given that it can be isolated from a number of different sources and can exist as a variety of types. In addition to its characteristic structural benefits as a component of natural extracellular matrices, collagen also shows minimal immunogenicity thus earning it a special place in tissue engineering applications (Caves et al, 2010). Processing of collagen fibers has been studied extensively and has successfully been developed by a range of fabrication techniques. The initial factors to consider in generating 1D collagen fibers include identifying an effective means of dissolving the collagen, which requires choosing a solvent capable of providing high enough concentrations of collagen in solution to facility fabrication. In the case of electrospun collagen for instance, this requires finding a solvent that is also capable of drying rapidly once fabricated. As discussed later, certain fabrication approaches provide improved strategies for forming collagen fibrils possessing hierarchical levels of structural control. In brief, this may require that the material be posttreated with a solvent which allows for molecular restructuring, and in some cases to increase the mechanical properties one of a number of cross-linking approaches may be pursued.

Among the protein-based materials found in nature, certainly silk has taken a lead role as a material of choice among 1D fiber researchers. Silk is itself comprised of a hydrophobic fibroin protein and a hydrophilic sericin protein. These proteins have been extracted from silkworms and spiders as part of natural biospinning processes to reveal unique properties in addition to their unprecedented mechanical strength that have found more than just niche applications. The fibroin protein has for instance shownexcellent biocompatibility and biodegradability (Meinel et al., 2005). For biomedical applications of silk fibers, such as ligament replacements, the sericin protein is usually removed from silk, as it is considered antigenic (Li et al., 2013; Panas-Perez et al., 2013). As always in electro-spinning techniques, the choice of solvent is important, and this is especially the case in generating 1D silk fibers in which the native structural features of the fibroin protein should not be lost. Often hexafluoroisopropanol is the solvent of choice to facilitate the proper high solubility necessary without compromising protein stability. Silk derived 1D fibers have also been generated by wet-spinning and solvent casting techniques with uniform cylindrical shapes. Aside from the traditional use of silk in the textile industry, there is newfound use of silk as a resist material for electron-beam lithography making it an interesting alternative to toxic materials used in nano and microfabrication (Kim et al., 2014). Silk has also been reported to be more biodegradable than other biomaterials (Kundu et al., 2013), making it particularly appealing for biomedical (Aytemiz et al., 2014) and tissue engineering applications (Kundu et al., 2013). Recent articles have looked into the molecular basis for the strength of silk (Tokareva et al., 2014; Xu et al., 2014) and its processing has been covered in depth (Asakura et al., 2014). Amyloid fibrils, being the second strongest protein material after silk, are also another promising biomaterial for fiber fabrication. The strength of amyloid fibers can be traced back to their hierarchical organization and molecular structure, therefore providing the potential for a wide range of physical properties (Knowles et al., 2011).

Natural biological materials having impressive intrinsic functionality are of course widely employed, but it is important to note that recombinant proteins, such as silk produced in genetically engineered organisms, can also be spun into fibers (Lazaris et al., 2002) and has been subsequently used in biomedical applications (Schacht et al., 2014). 1D biological fiber design is therefore not limited to natural proteins as recombinant hybrid proteins may also be implemented allowing benefits of combined protein domains or novel functional domains engineered by directed evolution or computational design. Recombinant elastin-like-polypeptide is one such protein that has been developed showing interesting structural features as well as a stimuli responsive nature to temperature changes (Huang et al., 2000; Wu et al., 2008). Elastin as a composite co-polymer with silk has also recently been implemented in 1D fiber processing techniques such as wet-spinning with pronounced success (Qiu et al., 2009). Certainly, there is plenty of room for new emerging 1D biological fibers and the exploration in developing this area further is expected to prove advantageous for new convergence technologies.

The final class of biological material to be discusses are virus particles and DNA, which have the property that they can be produced as geometrically (*i.e.*, size and shape) and chemically homogeneous materials. DNA based fibers have been produced for decades by a myriad of techniques including wetspinning, drawing, and electro-spinning (Fang et al., 1997). The extended polymeric nature of DNA as well as an inherent negative charge has allowed for fiber processing of this material using means similar to that of traditional polyelectrolytes and will therefore not be reviewed in further detail here. Viruses on the other hand can have a range of surface properties owing to their ease of genomic modification and have recently found utility in self-assembly processes (He et al., 2010). The filamentous bacteriophage in particular has found extensive use as nanomaterials for biomedical applications which have been covered in details in recent reviews (Yang et al., 2013; Farr et al., 2014). The formation of 1D virus fibers had first been demonstrated by electro-spinning in the presence of a carrier polymer (Lee et al., 2004); however, recent techniques have shown that highly concentrated pure phage solutions could also be extruded into a cross-linking solution of glutaraldehyde to yield 1D fibers (Mao et al., 2010). In addition, solvent casting techniques have also proven to be an effective means for generating virus structures into films (Chung et al., 2011) or fibers (Oh et al., 2014). Filamentous bacteriophage possess a structure that is itself analogous to a nanoscale 1D fiber, possessing a structural diameter on the order of 6-7 nm with a length that is variable depending on the internal genetic cargo, generally 800-900nm for a 7-8 kilobase genome (Nasir et al., 2014). Because the coat of the viral particle is negatively charged and due to the high aspect ratio of the

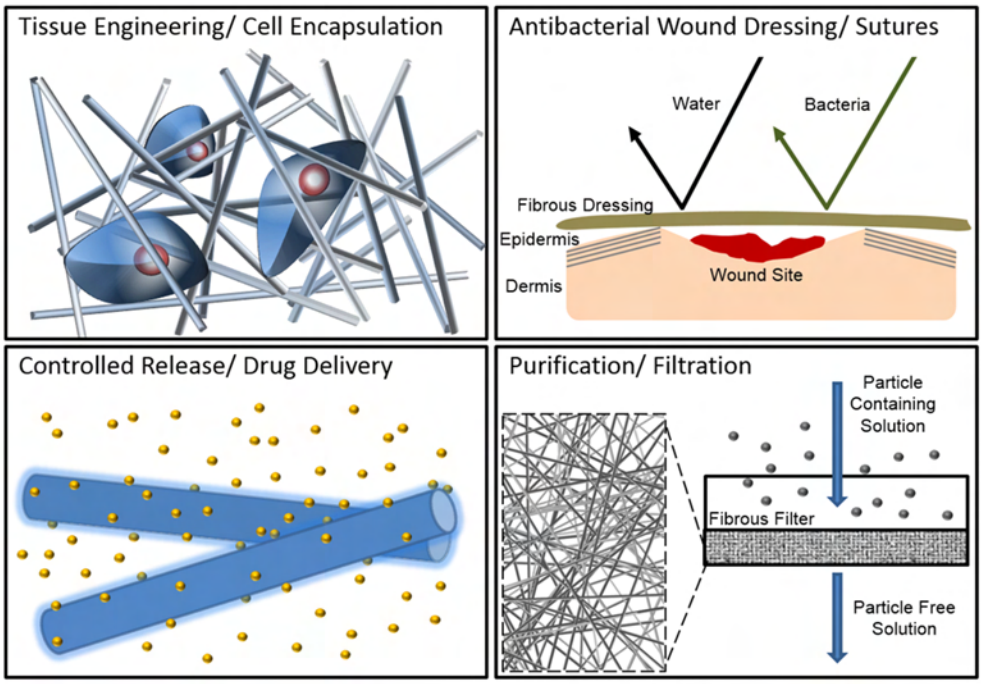

FIGURE 3 | Representative schematics of active applications areas which make use of 1D biological fiber structures.

structure, filamentous virus are known to readily display liquid crystalline properties wherein at high concentrations the virus take on mesophases including nematic and smectic phases (Dogic et al., 2006). The implications of this in processing of these biological materials as 1D fibers is the non-Newtonian fluid properties exhibited during formation, including the appearance of undulating and helical surface instabilities (Oh et al., 2014). These particular shapes that appear during fiber formation can be controlled by adjusting the ionic strength and concentration of virus particles. Nonetheless, a critical balance in the concentration and ionic strength should be maintained to provide the proper formation of a liquid crystalline phase, as the isotropic phase is refractory to fiber formation even at decreased fabrication speeds when examined by a solvent casted extrusion process (Oh et al., 2014). Because filamentous phage are readily modifiable by simple genetic engineering, the incorporation of custom recombinant proteins can be displayed directly on the virus coat or if necessary through exogenous chemical modification. While the solvent casting techniques have shown the feasibility of this virus fabrication approach, this has merely laid the groundwork for further adaptation of viruses to more intricate fiberderived technologies, since many scalable fabrication approaches have remain largely unexplored using this material.

OVERVIEW OF BIOLOGICAL FIBER FABRICATION APPROACHES

While processing of biological fibers has a history over thousands of years old, including fibers derived from flax for instance, the fabrication of micron and sub-micron 1D biological fibers is much more recent. For instance, spinneret devices had been invented and re-invented many times over to enhance control over the extruded structure of a range of biological material fibers. The viscous flow of many biological materials helps to align their structures during fiber fabrication. Depending on the particular processing technique, the alignment can be controlled to achieve particular characteristics of the resulting fibers. As such, fiber fabrication processes are often specific with respect to the intended application of the fiber.

Current applications of biological fibers, while traditionally predominant in the textile industry, have expanded to innovative areas including tissue engineering, wound dressings, drug delivery, and filtration among others (Figure 3). As such, constraints in terms of morphological, compositional, and functional control have become much more demanding. To date, only a few techniques have shown the capability of producing 1D biological fibers that begin to meet such challenging criteria. As you will find in the following sections, many of these techniques have been devised with longstanding fiber fabrication principles in mind and in several cases utilizing the non-Newtonian fluid properties of the given biological material itself. By examining specific applications to the given fiber processing scheme, we may see that the level of morphological, compositional, or functional control that has been designed into a particular fabrication approach often stems from the ultimate purpose of the fiber. In addition, we provide discussion on the adaptability, complexity, and recent improvements in these established techniques. Here we cover aspects of fiber fabrication by wet spinning, electro-spinning, extrusion, solvent casting, drawing,

TABLE 1 | Common diameter ranges of recently fabricated 1D biological fibers and their respective applications as categorized by fabrication process.

Fabrication process	Biological material	Diameter range (μm)	Application area	Reference
Wet Spinning	*silk-elastin*	10-60	tissue engineering	(Qiu 2009)
	silk (fibroin)	20	-	(Sohn 2009)
	chitin	200-240	uranium extraction	(Barber 2014)
		30-90	wound dressing	(Huang 2014)
	alginate	102-124	antibacterial	(Khajavi 2014)
Electro-spinning	*chitosan*	0.08-0.12	water purification (virus adsorption)	(Mi 2014)
	mauran	0.12	cell culture, tissue regeneration, drug delivery	(Raveendran 2013)
	silk	0.36	incorporation of nanodisk codes	(Schmucker 2014)
	silk-gelatin	0.09-0.18	controlled release	(Somvipart 2013)
	silk-elastin	0.01-0.3	tissue engineering	(Qiu 2010)
Extrusion	*chitin (nanocrystalline)*	500	catalytic support	(Das 2012)
	chitosan	286-352	tissue engineering	(Albanna 2013)
	silk-collagen	6-70	tissue regeneration	(Panas-Perez 2013)
	filamentous bacteriophage	40-100	antibacterial	(Mao 2010)
		10-20	-	(Lee 2004)
	collagen	100-650	-	(Zeugolis 2008)
		200-300	tissue engineering	(Zeugolis 2010)
Solvent Casting	*filamentous bacteriophage*	0.5-8	direct writing of virus-based actuators	(Oh 2014)
Drawing	*collagen-alginate*	30-100	cell encapsulation	(Wan 2012)
	hen egg white lysozyme	50-80	-	(Meier 2011)
Microfluidic Spinning	*alginate*	19	cell culture, drug delivery, regenerative medicine	(Shin 2007)
		70-150	tissue engineering, drug delivery	(Kang 2010)
		70-90	tissue engineering	(Ghorbanian 2014)
		40-300	cell culture	(Cheng 2014)
		O.D.: 210, I.D.: 92	tissue engineering, regenerative medicine	(Onoe 2013)
		O.D.: 50-250, I.D.: 0.3	drug delivery, tissue engineering microvascularization	(Lee 2009)
	collagen-alginate	250	tissue engineering	(Jun 2013)
	silk	10-45	textiles, sutures, tissue engineering	(Kinahan 2011)
	chitosan	50-200	cell culture	(Yeh 2010)

and microfluidic spinning, which are summarized in Table 1 while the advantages and disadvantages of each technique are briefly reviewed in Table 2.

SCALABLE FIBER PROCESSING BY WET SPINNING

In the processing method of wet spinning, biological materials are often injected as solutions for the continuous formation of threads which can further coagulate into fiber-like structures. As will be discussed, a variety of fiber-based constructs can subsequently be produces for applications including tissue engineering. In the case of wet spinning of protein solutions, the process often involves the use of spinnerets or capillaries in the range of 10-50 μm in which the protein solution is driven by syringe pumps for extrusion of fibers into a coagulant bath. Generally the coagulation bath consists of a non-solvent or poor solvent for the biological materials. This step can be followed by washing/air-drying and fiber collection by implementation of a spinning carrier cylinder (Qiu et al., 2009). Many of the parameters involved throughout this form of processing can be tuned to alter the mechanical and physical properties of the resulting fibers. For instance, fiber diameters may be decreased by slowing the flow rate, lowering the protein concentration, or decreasing the spinneret diameter (Caves et al., 2010). The molecular weight of a protein itself may commonly affect the protein spinnability by intrinsically affecting the viscosity. For good wet spinnability (*i.e.,* continuous fiber formation), the particular process should be optimized with respect to the biological material in order to minimize the onset of bead formation or discontinuities, particularly at decreased viscosities associated

TABLE 2 | Advantages and disadvantages of common 1D biological fiber processing techniques.

Processing technique	Benefits	Drawbacks
Wet-spinning	- Well controlled fiber diameters	- Extensive setup
Electro-spinning	- Straightforward setup	- Fiber properties highly sensitive to concentration
	- Can be used to process most materials	
	- Relatively cheap	- Difficult to make multi-domain fibers
	- Nanoscale features sizes possible	
Extrusion	- Low cost	- May require wet-extrusion or cross-linking
	- Straightforward setup	- Limited to larger fiber diameter ranges
Solvent-Casting	- Can be used to form unique surface textures	- Low throughput
	- Fabrication of fibers into controlled shapes	- Limited solvent choices
		- Limited fiber dimensions
Drawing	- Simple setup	- Poor reproducibility of fiber diameters
	- Possible to generate multi-domain fibers (*i.e.,* laminar fibers)	- Low throughput
Microfluidic spinning	- Possible to generate multi-domain fibers (*i.e.,* laminar, coaxial, hollow, embedded)	- Limited number of commercial setups available - Potential clogging
	- Low sample consumption	- Extensive setup if built in-house

with low molecular weight species (Cho et al., 2012). Aside from traditional parameters, a recent case of wet spinning using silk fibroin molecules implemented tuning of the osmotic stress in an effort to restructure the random coiled silk fibroin molecules into a more oriented state (Sohn et al., 2009). They found that adjusting the wet spinning conditions to commence when the solution approached the phase boundary between silk I and silk II offered the best conditions for spin processing stability to afford 20 μm monofilament fibers with excellent physical properties.

Because of their strength, stability in water, and in some cases biocompatibility, the use of such micron sized protein fibers fabricated by wet spinning have a range of application. Even plant protein fibers, such as soy and wheat protein derived fibers produced from wet spinning, are finding uses toward tissue engineering, implants, and in some cases controlled delivery of drugs (Reddy et al., 2011). Traditional extracellular matrix components, such as collagen, are also utilized as monomeric solutions for the wet-spinning of extracellular matrices for biomedical applications. Recently, researchers have employed an interesting wet spinning approach to reveal the formation of 10-60 μm collagen fibers with triple helical structure and axially oriented periodic fibrils (Caves et al., 2010). In their system, a monomeric collagen solution was extruded through a spinneret in which the collagen stream emerged and aggregated into a gel-like fiber due to a surrounding buffer system present in the coagulation column. Next, the fiber entered a rinse bath of 70% ethanol where it subsequently underwent drying and collection onto a carrier cylinder. Interestingly, after a separate incubation step with phosphate buffer followed by rinsing with water and collection under tension the formation of periodic collagen bands appeared with fibril microstructures. The authors attributed the separate stages of processing systems as being necessary for allowing fibrillogenesis *via* tension. Such approaches allowed these materials with aligned structures to facilitate high tensile strengths. A different technique has also proven successful for blends comprising chitosan and alginate to yield fibers in the range of 50 μm in diameter. In this case, a colloidal suspension of chitosan whisker in alginate solution was mixed and passed through a spinneret into a coagulation bath comprised of $CaCl_2$/ethanol aqueous solution to promote the electrostatic and intermolecular interactions. Incorporation of chitosan into the yarns provided antibacterial properties, allowing for potential applications in wound dressing materials (Watthanaphanit et al., 2010).

Recombinant proteins have also been processed by wet-spinning techniques as shown recently for silk-elastin-like proteins. By implementing a copolymer having elastin and silk-derived sequences, the researchers found a means for enhancing control of the mechanical properties of the wetspun fibers, revealing not only adequate deformability but also high tensile strength (Qiu et al., 2009). As with natural silk, these recombinant protein-derived silk fibers also formed hierarchical self-assemblies, where the crystallization of molecular chains was more significant at smaller fiber diameters (20-30 μm) thereby offering improved mechanical properties.

SMALL DIAMETER BIOLOGICAL FIBERS DERIVED FROM ELECTRO-SPINNING

Electro-spinning can achieve the smallest fiber diameters

of the conventional mechanical fabrication techniques with diameters typically ranging from tens of nanometers to a few microns (Huang et al., 2013). Diameters as small 2 nm have been reported (Bhardwaj et al., 2010). For biological materials such as silk, smaller than natural diameters of 6 nm have been achieved (Zarkoob et al., 2004). Electro-spinning fabrication is straightforward, using three primary components including a high voltage power supply, a spinneret, and a collector counter electrode. At a high enough voltage, the electrostatic force on the polymer solution overcomes the surface tension and a liquid jet is generated. The solvent evaporates and continuous fibers are collected at the counter electrode (Huang et al., 2013). Parameters including the solution properties, flow rate, voltage, and distance of spinneret from counter electrode all affect fiber parameters (Huang et al., 2013). Electro-spinning fabrication has been covered in depth in various books (Huang et al., 2013) and a reviews (Bhardwaj et al., 2010). For fabrication of biological fibers, how the biological components in solution will affect solution properties such as viscosity, surface tension, conductivity, solvent volatility should be taken into consideration. Electrospun fibers can be made from a variety of biological materials including the extremophilic bacterial polysaccharide mauran (Raveendran et al., 2013), silk (Schmucker et al., 2014), chitosan (Mi et al., 2014), and bacteriophage (Lee et al., 2004).

The small and flexible nature of electrospun fibers makes them ideal for being embedded into other materials for counterfeiting and tracking applications, without disrupting the host material's properties. The flexible nature of fibers can even allow them to be incorporated into flexible materials and not be damaged during regular use. Silk fibroin fibers embedded with nanodisk codes were fabricated via electro-spinning (Schmucker et al., 2014). Due to the small diameter of electrospun fiber, resulting fibrous meshes can have very high surface areas useful for absorption applications. The chitosan derivative N-[(2-hydroxyl-3-trimethylammonium) propyl] chitosan (HTCC) has been shown to inactivate bacteria and viruses, yet has the downside of being water soluble. Researchers blended HTCC fibers with polyvinyl alcohol fibers and then crosslinked them to impart higher stability in water. The fibers were demonstrated to be effective at removing virus from water for purification applications (Mi et al., 2014). The high surface-to-volume ratio of fibers makes them effective at releasing reagents, but due to their small volumes, only small quantities of reagents can be released and for short time periods. To address this, researchers have developed electro-spinning techniques for the fabrication of homogeneous gelatin and silk fibroin beaded fibers, therefore enabling a more prolonged reagent release (Somvipart et al., 2013).

Electro-spinning is a well-established technique and can be used to make fibers from a variety of biomaterials. When using biomaterials, it should be considered that fiber properties are affected by the solution properties such as conductivity, viscosity, and surface tension. When the properties of biological materials prove unfavorable, modified approaches such as electro-blowing, might have to be used (Um et al., 2004). Despite the successes of electro-spinning, it is worth noting that the formation of electrospun fibers with controlled inhomogeneity and non-uniformity remains a challenge and is still an active area of research.

GENERATION OF LARGE DIAMETER FIBERS BY EXTRUSION

Extrusion provides a straightforward route to fiber formation with minimal consumption of energy; hence, it has newfound appeal for generating materials from sustainable resources such as biomaterials. However, among the techniques for fiber formation from biological materials, extrusion tends to result in the largest diameter cross-sections often in the hundreds of micrometer range, although fibers down to 10 μm have been fabricated (Kew et al., 2011). While the diameter is inherently related to the size of the die used for extrusion, the final diameter of the formed fiber depends heavily on the postprocessing methods, including cross-linking, dehydration, or solvent immersion.

In the case of collagen extrusion, which has been thoroughly investigated since first reported (Kato et al., 1989), the fibers are typically formed from a gel-like substance produced by extensive hydration and homogenization of natural collagen (Enea et al., 2011). Processing of the acidified collagen by extrusion into a neutral buffer is followed by incubation and washing in a dehydrating solvent to remove water from the structure. In many cases, the osmotic pressure between the fiber and the dehydrating solution can be tuned by addition of components such as poly(ethylene glycol) to speed dehydration. In a specific example, the implementation of a syringe pump allows pushing of a gel-like collagen substance at less than 1 mL per minute through a sub-millimeter inner diameter tubing and into a phosphate buffer containing PEG for fiber formation and subsequent washing and drying under tension (Kew et al., 2012). Under such conditions, the resulting collagen fibers, having diameters of 60 μm, were able to reveal axial alignments reminiscent of those found in natural systems; however, such observation may indicate collagen assembly only at localized nanoscale regions within a much larger extended structure (Kew et al., 2011). While outside the scope of this review, it is worth mentioning that the products immediately resulting from this process, at least for the case of collagen extrusion, lack sufficient strength and thereby requires cross-linking treatment before use as a biomaterial for soft or hard tissue repair (Zeugolis et al., 2010). Recent works with biological materials exhibiting larger inelastic deformations have used extruded chitin. It is important to note however that we distinguish this from melt spinning extrusion in which a material would first be heated, melted, and extruded. Because chitin will decompose prior to melting, melt spinning extrusion cannot be performed; hence, solubilized or nanocrystalline forms of chitin are utilized instead (Pillai et al., 2009). Specific applications have made use of this structurally robust biomaterial for the generation of fiber structures capable

of supporting functionalized catalysts (Das et al., 2012). In their approach, fibers were prepared by extrusion of gelled chitin nanofibrils through a syringe needle into a coagulation bath containing ethanol or THF followed by drying. Because of the high structural integrity of the fibers, they could be handled directly and may have potential in being further applied to areas requiring robust biological material fibers.

CONTROL OF FIBER SURFACE TEXTURES BY SOLVENT CASTING

Examining the approaches presented thus far, reveals that most processes tend to utilize extrusion or spinning of the fibers into a liquid environment. Associated results also support that the mechanical properties are improved by processing into liquid rather than air by improved chain orientation of the biomolecular species (Guinea et al., 2005). Researchers have even tested drawing of silk fibers directly from the glands of spider at a consistent reeling speed to show that fibers produced underwater have increased strength albeit with decreased breaking strains. It is assumed that the aqueous environment gives more time for the molecules to extend and align thereby allowing changes of intramolecular hydrogen bonds into more intermolecular hydrogen bonds (Chen et al., 2006). However not all biological 1D fiber processing techniques are performed into liquids, and one processing approach, known as solvent casting, distinctively utilizes the evaporation of liquid solvent for generation of 1D fibers. Solvent cast fibers of chitosan have been produced by a technique involving submersion of an existing fiber or rod into a mixture containing the biomaterial of interest and then drying/heating the sample. This type of process was repeated several times to achieve the preferred diameter or coating thickness of biological material (Bhattarai et al., 2009). Inherently, solvent casing approaches are slow and time consuming; however, they may offer more dimensional and textural control of the final fibers. As an example, researchers have utilized glass capillaries filled with filamentous virus to produced 1D fibers of custom shape by forming a meniscus between the capillary tip and a surface and controlling the speed and direction of the capillary tip withdrawal (Oh et al., 2014). As the capillary is moved, the resulting liquid bridge meniscus solidifies leaving behind a trail of filamentous virus fiber. Interestingly, this approach revealed that different pulling speeds could yield unique surface textures on the fiber including helical and undulating fiber structures with diameters in the range of 500 nm up to 8 μm. These smart materials, capable of responding to external stimuli, are oftenimplemented for applications in sensing and actuation.

DRAWING METHODS FOR MULTI-DOMAIN FIBERS

The fabrication of fibers drawn from interfaces can yield continuous fibers that can be collected by means of a carrier cylinder. In this approach, a stable interface is created between two biological materials having opposite charges where in a complex interface (*i.e.*, film) forms that is then pulled and retracted to result in fiber formation. While the setup is relatively simple, the types of biomaterials that can be used and the working fiber diameter range is limited to larger ranges compared to that of other fiber fabrication techniques. In a specific example of drawing from an interface, researchers have shown that fibers can be spun from films generated by the interfacial self-assembly of negatively charged gellan molecules with positively charge amyloid fibers (Meier et al., 2011). In this work amyloid nanofibers were generated from lysozyme protein aggregates and the resulting electrostatic assembly with gellan gum provided a sufficiently strong interfacial film for withdrawal and spinning into 50-70 μm fibers having strength and stiffness values among the highest for existing biological fibers. These charged, multi-domain fibers have potential for pH-triggered release of reagents for drug delivery, and because of their high strength, they can also be potentially used for structural re-enforcement.

More recently, drawing from multiple interfaces has offered an impressive means for creating multicomponent fibers (Wan et al., 2012). Researchers have utilized biological polyelectrolyte solutions, including alginate as a polyanion and chitosan or chitin as a polycation, to generate fused composite fibers. A stable interface is formed after contact between droplets containing the respective polyelectrolyte solutions and the draw process is initiated by contacting the interfaces with a pipette tip and withdrawing at a speed of 0.4 mm/s. The resulting fibers spontaneously form parallel 30-40 μm domains comprised of the distinct biological materials, wherein fibers having up to 4 different laminar domains were demonstrated. The application of this technique toward cell patterning gave promising results as the sizes of the fiber were sufficiently large for encapsulation of cells. These different domains enable cell co-cultures, which are required to achieve the desired growth and differentiation needed for tissue regeneration applications. Even smaller diameter fibers, down to 10 μm, have been drawn from alginate/chitosan interfaces using faster drawing speed of 10 mm/s by attachment of the drawn fibers to a roller/carrier cylinder (Yim et al., 2006).

CO-AXIAL AND PARALLEL LAMINAR FIBERS BY MICROFLUIDIC SPINNING

Microfluidic devices can be used for the formation of fibers with feature sizes in the micron scale. Microfluidics allow for tunable fiber properties (such as fiber diameter) by controlling parameters such as flow rate, solution viscosity, and channel geometries (Chung et al., 2012). The ability to tune such characteristics is achieved by the laminar flow arising from low Reynolds numbers typical in microfluidics, which enables controlled kinetics, predictable concentration gradients, and reproducible flow patterns (Daniele et al., 2014). The flow patterns in microfluidics are relatively simple to model mathematically, and thus fibers with tunable and predictable features can be produced, as demonstrated for silk fiber fabrication (Kinahan et al., 2011). The development of laminar flows permits multiphase flows,

coaxial flows, and parallel flows which can be used to fabricate complex fiber structures (Chung et al., 2012). Recent reviews have covered in depth the different configurations useful for fiber fabrication (Chung et al., 2012; Daniele et al., 2014; Jun et al., 2014). An additional advantage of microfluidic fiber fabrication are the small volumes (50 uL) required for fiber fabrication (Kinahan et al., 2011). These small volumes allow microfluidic fabrication to be used for fabrication of fibers from small batches of precious samples, such as for screening libraries of recombinant spider silk (Kinahan et al., 2011).

Given the small feature size of microfluidic channel geometries, clogging can be a problem for reliable continuous fabrication of long fibers. Researchers have worked to overcome these issues by introducing automated declogging mechanisms that deliver gel dissolving solvent into their devices (Ghorbanian et al., 2014). Other approaches to minimize clogging include novel methods to produce microfluidic devices with cylindrical, instead of square, microchannels (Kang et al., 2010). Of particular interest to biomedical applications is the ability to use microfluidic to fabricate fibers embedded with viable cells (Shin et al., 2007). Researchers have exploited the reproducible and predictable fabrication of microfluidics to embed viable cells into alginate fibers at specific densities using a microfluidic direct writer. This ability to embed cells at specific positions is critical for 3D culture systems and is an advantage microfluidic fabrication has over competing conventional techniques (Ghorbanian et al., 2014). The ability of microfluidics for the fabrication of complex fiber structures that mimic *in vivo* tissues has been exploited for tissue regeneration applications. Using a double-coaxial laminar flow, core-shell fibers were generated with embed ECM proteins and pancreatic islet cells. These fibers were implanted into diabetic mice to restore normal glucose levels (Onoe et al., 2013). From a different group, collagenalginate fibers were also fabricated in microfluidics for immunoprotection for the same application (Jun et al., 2013). Recent advances have enabled fabrication of hollow alginate fibers to mimic microvasculature (Lee et al., 2009) and multi-compartment alginate fibers for cell co-culture models (Cheng et al., 2014). Complex chitosan-based geometries are required for liver tissue engineering, yet fabrication of complex pure chitosan fibers is challenging given the mechanical weakness of pure chitosan. Using microfluidics, complex, pure chitosan fibers suitable for cell culture were fabricated without chemical additives (Lee et al., 2010). Different approaches have also explored the use of microfluidic fabrication of chitosan for cell culture (Yeh et al., 2010).

As shown by the highlighted demonstrations, microfluidic fiber spinning has unique advantages in its ability to generate non-homogenous fibers with tunable properties and complex geometries, making these fibers well suited for biomedical applications. An additional advantage is the small sample volumes consumed, enabling fabrication with limited samples such as recombinant libraries of biomaterials. Given these differentiating advantages, mass adoption by other laboratories will depend on the future availability of commercial devices that can be operated without microfabrication expertise.

CONCLUSIONS AND FUTURE PERSPECTIVES

Without a doubt there is an extensive history of 1D fibers from biological materials, and certainly we have seen that significant advances have been made in increasing control over the mechanical and physiochemical properties of biological materials. It is important to note that the techniques discussed in this review are not exhaustive as the field is extensive and new approaches continuously evolve. Selfassembly, for instance, is one area of 1D fiber development that is rich with potential but remains limited with respect to the fiber length scales capable by this approach (Zhang et al., 2003). Even the traditional approach of natural biospinning has seen recent advances (Roloff et al., 2014); an interesting example revealing that hybrid biomaterial fibers could be produced by feeding magnetic nanoparticles (Wang et al., 2014a) and carbon nanotubes (Wang et al., 2014b) to silk producing organisms. As discussed, wetspinning, and electro-spinning can accommodate the diversity of biological materials to generate fibers over a range of diameters. Nonetheless, one key area that remains to be improved exists in processing, specifically controlling the fabrication of 1D fibers into more customizable geometries. Such breakthroughs will substantially extend the potential application areas outside of amorphous mats and meshes as have traditionally been prepared. As this area continues to develop, we will undeniably see researchers continue to make great advancements in generating multi-domain functional fibers by processes such as drawing and microfluidics. Combining such techniques with the custom geometries achievable through solvent casting may someday afford a means for generating spatially complex fiber architectures in which distinct biologically active domains are embedded. While solvent casting and microfluidic techniques have generally remained as laboratory-based processing approach, we expect adoption of commercial and scalable variations of these systems may become available in the future for industrial fabrication of such biological fiber structures. Based on historic success and owing to the growing diversity of structures and functions discovered in biological materials, one question that remains of interest is in which industry can we next expect a major adoption of biological fiber technologies?

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