ACS Biomaterials SCIENCE & ENGINEERING

Review

pubs.acs.org/journal/abseba

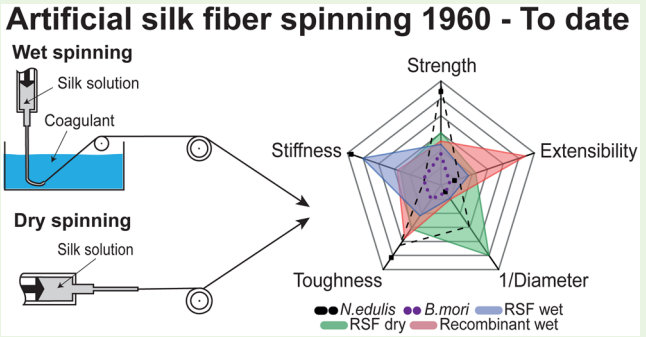
Progress and Trends in Artificial Silk Spinning: A Systematic Review

Andreas Koeppel and Chris Holland*

Department of Materials Science and Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, United Kingdom

Supporting Information

ABSTRACT: More than 400 million years of natural selection acting throughout the arthropoda has resulted in highly specialized and energetically efficient processes to produce protein-based fibers with properties that are a source of inspiration for all. As a result, for over 80 years researchers have been inspired by natural silk production in their attempts to spin artificial silks. While significant progress has been made, with fibers now regularly outperforming silkworm silks, surpassing the properties of superior silks, such as spider dragline, is still an area of considerable effort. This review provides an overview of the different approaches for artificial silk fiber spinning and compares all published fiber properties to date which has identified future trends and challenges on the road towards replicating high performance silks.



KEYWORDS: *silk, fibroin, fiber, bioinspired, spinning, recombinant, regenerated, spider, silkworm*

1. INTRODUCTION

Silks are structural proteins that are spun, on demand, into fibers for use outside the body by thousands of arthropod species.[1,2] However, the term "silk" is most commonly associated with textiles, specifically the fibers unravelled from cocoons spun by the silkworm *Bombyx mori*.[3] This "queen of textiles" has been used by humans for thousands of years in the production of luxury apparel due to its appearance, soft touch, and durability,[4] and is produced on a commercial scale in quantities of hundreds of thousands of tonnes per annum.[5] Yet, while plentiful in supply, commercial silkworm silks possess a relatively low strength (360 MPa)[6] and toughness (50.5 MJ/m^3),[6] especially when compared to spider silks, i.e., dragline (1150 MPa, 214.5 MJ/m^3),[7] which can even outperform most industrial fibers.[8−10]

Unlike silkworm silks, the first human uses of spider silk were in nonwoven formats; the ancient Greeks used bundled spider silk to heal bleeding wounds, Australian aborigines developed silk fishing lines, and New Guinea natives used spider silk to construct fishing nets and bags.[11] It was not until the beginning of the 18th century, René-Antoine Ferchault de Réaumur, a French naturalist, attempted to develop spider silk textiles to make stockings and gloves.[11] Unfortunately, he failed, due to the sheer number of spiders required to produce sufficient silk to weave into a textile, and herein lies the problem with spider silk applications. In fact, only very recently have full scale spider silk textiles been produced as an artistic endeavor, albeit at a cost of 1 million reeled spiders and ∼280 person years of work per garment.[12,13]

Therefore, for many years industry has been faced with the dilemma that silkworm silks are available in high quantity but lower quality, whereas spider silks yield low quantity yet very high quality. Solutions to this problem may be found both through the development of new technologies improving the output and quality of recombinant and regenerated silk proteins, and the design of artificial silk spinning processes which aim to produce high performance silk-based materials in a controlled and consistent manner. Such bespoke fibers can then be used for a range of new applications ranging from sutures, wound dressings, and scaffolds for tissue engineering[10,14−16] to reinforcing polymer composites.[17] However, while the field of artificial silk spinning focuses mainly on the use of regenerated and recombinant proteins from spiders and *B. mori*, canonical silks and nonmulberry silk varieties still remain an interesting area to be exploited in the future.[18,19]

Our systematic review presents the various approaches for artificial silk fiber spinning, discusses trends in fiber properties over time, and gives possible explanations as to why a truly biomimetic spider dragline silk has not been consistently reproduced to date.

2. THE NATURAL SILK SPINNING PROCESS

Before discussing artificial silk fiber production, it is important to appreciate how silk is naturally spun by spiders and silkworms. However, in order to maintain focus, should the reader wish to explore this area in more detail, the following papers and reviews are an excellent start: refs 20−23.

In general, silks are spun by a process of controlled protein denaturation as a result of shear. This is akin to polymeric flow-

Received: October 27, 2016
Accepted: January 17, 2017
Published: January 17, 2017

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

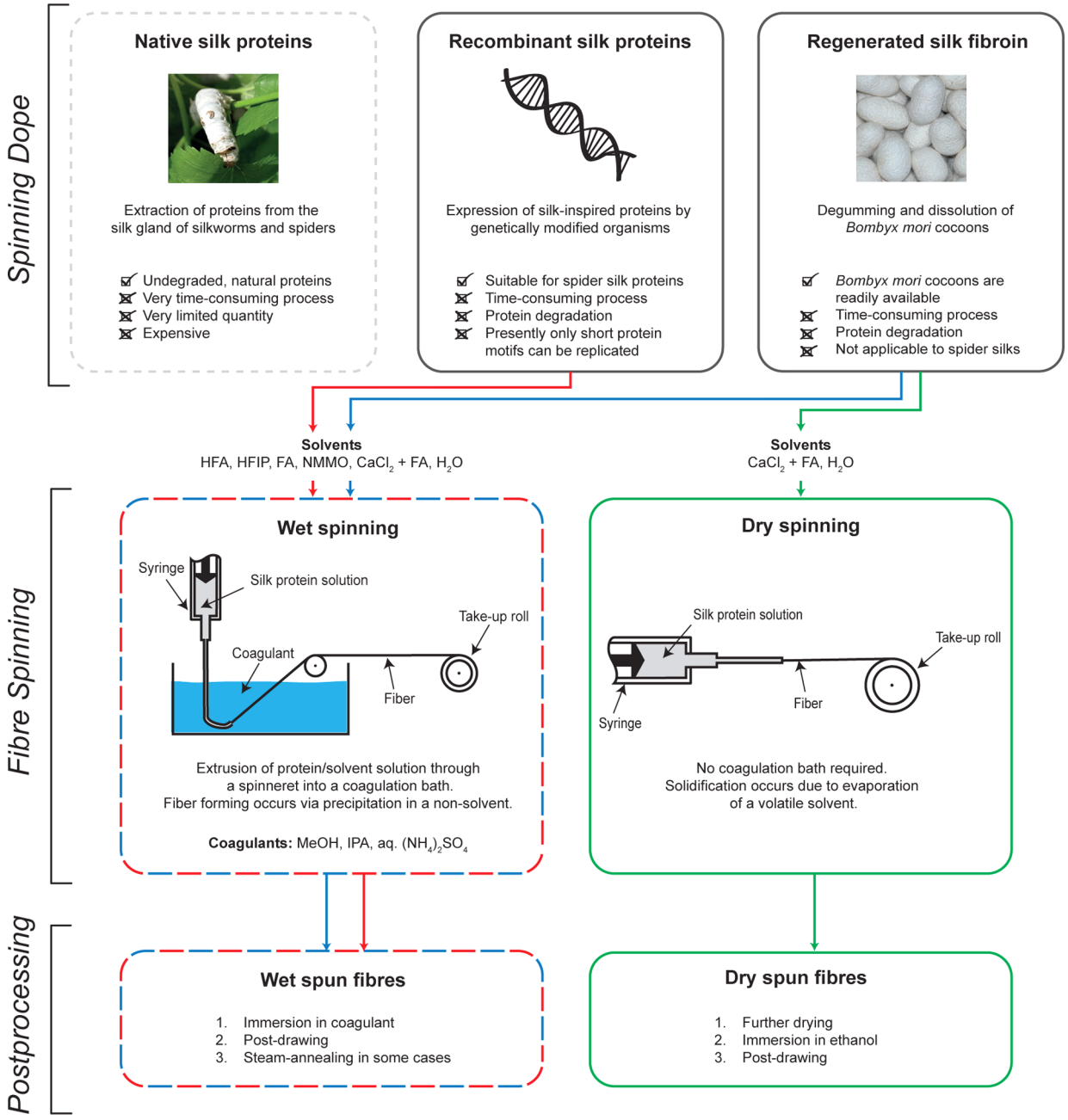


Figure 1. Scheme showing the different approaches for artificial silk fiber production. The different colors represent RSF wet spun fibers (blue), RSF dry spun fibers (green), and recombinant wet spun fibers (red). For consistency, this coloring is maintained throughout the whole review. Abbreviations: hexafluoroacetone hydrate, HFA; hexafluoroisopropanol, HFIP; formic acid, FA; *n*-methylmorpholine-*n*-oxide, NMMO; methanol, MeOH; isopropanol, IPA.

induced crystallization but uses a currently unknown mechanism that has been shown to be 1000 times more efficient.[24] Specifically, prior to spinning, silk proteins are synthesized and stored in specialized silk glands as a concentrated aqueous solution (spinning dope). Upon spinning, this protein solution flows down a specially shaped spinning duct and is subjected to shear and elongational flow fields alongside a pH and metal ion gradient.[23,25] Once sufficiently deformed, the silk proteins undergo a stress induced phase transition, spontaneously dehydrating, refolding, phase separating, and ultimately aggregating to form a solid, insoluble fiber.

3. ARTIFICIAL SILK FIBER PRODUCTION

3.1. Spinning Dope. The different approaches for spinning artificial silk fibers are illustrated in Figure 1. As in nature, artificial fiber spinning begins with the creation of a spinning dope, which we separate into native, recombination, and regeneration groups. Native dope is obtained by dissecting silkworms or spiders and extracting silk proteins directly from the silk gland.[26−28] While this feedstock is considered the gold standard, its preparation is both time-consuming and expensive and thus not feasible for large-scale production. The second approach is the recombinant synthesis of silk-inspired proteins. Various silk protein motifs have been expressed by genetically modified organisms such as bacteria,[29] yeasts,[30] and insect

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

Table 1. Overview of the Best Fiber Properties and the Respective Processing Parameters of All References Used in Our Analysis

		Processing parameters					Fibre properties				
		M_w	Protein conc.	Solvent	Coagulant	Draw ratio	Strength	Extensibility	Stiffness	Toughness	Diameter
	Reference	kDa	wt% or (w/v)%	-	-	-	MPa	%	GPa	MJ/m^3	μm
RSF wet spinning	Yazawa *et al.* 1960	-	n.s.[d]	concentrated magnesium nitrate	saturated ammonium solution	n.s.	2.5 g/den	20-25	n.s.	n.s.	n.s.
	Ishizaka *et al.* 1989	-	12	85 % phosphoric acid + 5.7 wt% dimethylformamide	25% aqueous sodium sulfate	9.3	2.1 g/den	10.1	n.s.	n.s.	n.s.
	Matsumoto *et al.* 1996	-	20	40 wt% LiBr·H_2O in ethanol; ethanol with different water contents	methanol, ethanol, isopropanol with 10% aq. LiBr	3.2	130[a]	11	6.7[a]	12.9[b]	118.5
	Yao *et al.* 2002	-	10	hexafluoroacetone hydrate (HFA)	methanol	3	321.2[a,b]	16.1[b]	5.3[a,b]	37.6[a,b]	40-50[a]
	Zhao *et al.* 2003	-	10	hexafluoro-iso-propanol (HFIP)	methanol	3	193[b]	19[b]	5.2[b]	28.2[b]	40-50[a]
	Um *et al.* 2004	-	15.6	98% formic acid	methanol	2	103.8[a,b]	40[a,b]	4.1[a,b]	38[a,b]	189[b]
		-	15.6	98% formic acid	methanol	5	257.5[a,b]	16.4[a,b]	5.5[a,b]	30.6[a,b]	119[b]
	Marsano *et al.* 2005	-	13	aqueous NMMO monohydrate + 0.7% n-propyl gallate	ethanol	2.7	120	35	7.2	38.9[b]	18.5 ± 0.8
	Ha *et al.* 2005	-	13	formic acid	methanol	3	1077.3 ± 173[a]	29.3 ± 11.9[a]	39.9 ± 6.1[a]	257.8[b]	35[a]
		-	13	trifluoroacetic acid (TFA)	methanol	3	959.0 ± 149.1[a]	18.1 ± 6.8[a]	43.2[a]	156.7[b]	21[a]
	Lee *et al.* 2007	-	15.6	98% formic acid	methanol	4.5	269.4[a,b]	19.5[a,b]	4.9[a,b]	38.7[a,b]	220-270
	Corsini *et al.* 2007	-	17	aqueous NMMO monohydrate + 0.7% n-propyl gallate	ethanol	2	127 ± 8	12.7 ± 1.9	5.3 ± 0.2	20.3[b]	73 ± 8
	Zuo *et al.* 2007	-	10	hexafluoro-iso-propanol (HFIP)	ethanol / methanol	n.s.	109.7[a]	25	n.s.	n.s.	68[a]
	Ki *et al.* 2007	-	12.3	98% formic acid	methanol	5	285.1 ± 10.7[a]	14.0 ± 1.7	7.2[a,b]	30.4[a,b]	100[b]
	Zhu *et al.* 2008	-	12 (w/v)	hexafluoro-iso-propanol (HFIP)	methanol	3	400.5[b]	20.7[b]	4.3[b]	51.3[b]	40[b]
	Sohn *et al.* 2009	-	29	PEG / LiBr	methanol/water	1.1	128.8[a,b]	7.6[a]	6[a]	6.8[a,b]	20-50
	Plaza *et al.* 2009	-	17	aqueous NMMO monohydrate + 0.7% n-propyl gallate	methanol	n.s.	313.6[c]	8.5[c]	13.4[c]	20.5[c]	41
		-	17	aqueous NMMO monohydrate + 0.7% n-propyl gallate	methanol	7.2	172.4[c]	48.4[c]	5.1[c]	55.5[c]	47
	Zhou *et al.* 2009	-	15	water	aqueous ammonium sulfate	6	450 ± 20	27.7 ± 4.2	12.5[b]	100.6 ± 6.3[a]	10.8 ± 2.4
	Zhu *et al.* 2010	-	15	hexafluoro-iso-propanol (HFIP)	methanol	3	408 ± 80	21 ± 3	7.3 ± 0.2	51.5[b]	n.s.
	Yan *et al.* 2010	-	16	water	aqueous ammonium sulfate	6	390 ± 50	32.1 ± 5.8	15.2 ± 3.3	109.1 ± 18.8[a]	n.s.
	Plaza *et al.* 2012	-	17	aqueous NMMO monohydrate + 0.7% n-propyl gallate	methanol	n.s.	336.4[c]	7.38[c]	18.5[c]	20.3[c]	n.s.
		-	17	aqueous NMMO monohydrate + 0.7% n-propyl gallate	methanol	5.3	257.6[c]	35.3[c]	7.4[c]	51.9[c]	18.4
	Ling *et al.* 2012	-	20	water	aqueous ammonium sulfate	4	221 ± 64	30 ± 4	11.2[b]	46.4[b]	100[b]
	Zhou *et al.* 2014	-	15	water	aqueous ammonium sulfate	9	314 ± 19	37 ± 4	10.4[b]	105.3 ± 10[a]	n.s.
	Zhang *et al.* 2015	-	12	$CaCl_2$-FA	water	4	470.4 ± 53.5	38.6 ± 6.3	6.9 ± 2.1	105.3 ± 15.5[a]	12.8 ± 4.6
	Fang *et al.* 2016	-	15	water	aqueous ammonium sulfate	9	450 ± 30	27.3 ± 4.6	18.9 ± 1.1	91.0 ± 7.4	15 ± 4.7[a]
	Chen *et al.* 2016	-	13	water	aqueous ammonium sulfate	4	98[b]	58.9[b]	37.8[b]	53.5[b]	~25
RSF dry spinning	Wei *et al.* 2011	-	20	water + (MES)-(Tris) buffer (pH adjustment) + $CaCl_2$ (Ca^{2+} adjustment)	-	3	301.5 ± 70.6	35.8 ± 21.9	6.2 ± 1.7	104.8 ± 37.8[a]	5.7
		-	20	water + (MES)-(Tris) buffer (pH adjustment) + $CaCl_2$ (Ca^{2+} adjustment)	-	n.s.	295.2 ± 92.2	74.8 ± 47.4	5.8 ± 4	155.9 ± 94.5[a]	6.4 ± 1.5
	Sun *et al.* 2012	-	50	water + (MES)-(Tris) buffer (pH adjustment) + $CaCl_2$ (Ca^{2+} adjustment)	-	4	337.7[b]	24.6[b]	11.1[b]	55.8[b]	10[b]
	Jin *et al.* 2013	-	40-60	water + $CaCl_2$ (Ca^{2+} adjustment)	-	4	357.3 ± 84.3	34.1 ± 8.1	8.8[b]	86.5[b]	6.3 ± 2.3
	Luo *et al.* 2014	-	50	water	-	2	614	27	19	136.4[a]	2
	Yue *et al.* 2014	-	20 and 25	formic acid + $CaCl_2$ (Ca^{2+} adjustment)	-	2	333[b]	35.1[b]	8.8[b]	90.9[b]	20-30
	Peng *et al.* 2015	-	44	water + $CaCl_2$ (Ca^{2+} adjustment)	-	4	541.3 ± 26.1	19.3 ± 4.8	9.4 ± 1.2	76.4 ± 22.8[a]	9.0 ± 1.3
Recombinant wet spinning	Lazaris *et al.* 2002	60	>23%		methanol and water	5	269.6[a]	43.4[a]	13.2[a]	101.4[a]	20[a]
	Teulé *et al.* 2007	62	25-30 (w/v)	hexafluoro-iso-propanol (HFIP)	90% isopropanol	n.s.	49.6 ± 19.4	15.8 ± 6.1	1.1 ± 1.0	10.6 ± 10.2	15.8 ± 6.1
	Brooks *et al.* 2008	71	10 to 12%	hexafluoro-iso-propanol (HFIP)	isopropanol	0	49.5 ± 7.8	3.6 ± 2.6	0.4 ± 0.3	4.7[b]	74.1 ± 33.9
	Xia *et al.* 2010	284.9	20 (w/v)	hexafluoro-iso-propanol (HFIP)	90 vol% methanol in water	5	508 ± 108	15 ± 5	21 ± 4	81.5[b]	n.s.
	Ellices *et al.* 2011	appr. 50	n.s.	hexafluoro-iso-propanol (HFIP)	isopropanol	5	246.7[c]	50.6[c]	4.5[c]	91.7[c]	46 ± 2
	An *et al.* 2011	70	30 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	n.s.	132.5 ± 49.2	22.8 ± 19.1	5.7 ± 2.4	23.7 ± 18.5	17.4 ± 5
	Teulé *et al.* 2012	58	26-27 (w/v)	hexafluoro-iso-propanol (HFIP)	90 % isopropanol / 10 % water	2-2.5	127.5 ± 23.0	52.3 ± 23.6	4.4 ± 1.0	54.6 ± 23.6	28.3 ± 6
		62	26-27 (w/v)	hexafluoro-iso-propanol (HFIP)	90 % isopropanol / 10 % water	2-2.5	96.2 ± 28.8	29.6 ± 20.5	3.8 ± 2.1	22.6 ± 15.7	14.0 ± 8.7
	An *et al.* 2012	66/48	30 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	3	37.6 ± 20.4	53.9 ± 68.0	3.4 ± 1.1	17.4 ± 20.1	29.1 ± 5.4
		66/48	30 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	3	59.6 ± 19.2	4.8 ± 8.6	4.3 ± 0.9	2.5 ± 5.4	29.1 ± 5.4
	Gnesa *et al.* 2012	45	20 (w/v)	hexafluoro-iso-propanol (HFIP)	95 % isopropanol	6	121.9 ± 5	18 ± 1	3.9[b]	17.4 ± 1.2	24.5 ± 0.3
		45	20 (w/v)	hexafluoro-iso-propanol (HFIP)	95 % isopropanol	3.5	95.1 ± 3.3	25 ± 4	2.6[b]	20.7 ± 3.8	30.5 ± 0.5
	Adrianos *et al.* 2013	66	15 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	3	150.6 ± 31.3	84.5 ± 37.8	4[b]	89.1 ± 23.9	15.1 ± 1.3
	Lin *et al.* 2013	378 dimer	8 to 10 %	hexafluoro-iso-propanol (HFIP)	$ZnCl_2$ and $FeCl_3$ in water	5	308 ± 57	9.6 ± 3	9.3 ± 3	24.4[b]	10
	Albertson *et al.* 2014	86.5	45-60 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	4	53.5 ± 18.0	18.0 ± 21.6	2.90 ± 1.1	9.3 ± 10.9	31.5 ± 4.5
		8.6	45-60 (w/v)	hexafluoro-iso-propanol (HFIP)	isopropanol	4	39.0 ± 7.4	181.3 ± 103.5	1.6 ± 0.4	59.3 ± 37.2	36.0 ± 5.9
	Copeland *et al.* 2015	65	25 (w/v)	hexafluoro-iso-propanol (HFIP) + >88% formic acid in 4:1 ratio	isopropanol	1.5/2	221.7 ± 11	56 ± 6.6	n.s.	102.5 ± 13.6	29.0 ± 1.1
	Jones *et al.* 2015	50-75	12 (w/v)	water	isopropanol	2-2.5	192.2 ± 51.5	28.1 ± 26	8.3[b]	33.8 ± 33.6	n.s.
	Heidebrecht *et al.* 2015	286	10-17 (w/v)	water + Tris/HCl or Na-phosphate buffer	water + isopropanol	6	370 ± 59	110 ± 25	4 ± 1	189 ± 33	27 ± 10
	Peng *et al.* 2016	47	12 (w/v)	NaCl/water	ethanol	n.s.	62.3 ± 17.2	3.5 ± 1.2	4 ± 2.8	1.6 ± 0.9	34[b]
		47	10-17 (w/v)	NaCl/water	ethanol	n.s.	286.2 ± 137.7	18.3 ± 12.8	8.4 ± 4.3	37.7 ± 28.8	14[b]
	Andersson *et al.* 2017	33	50 (w/v)	aqueous buffer at pH 8	aqueous solution (sodium acetate, pH 2.5 - 7.5)	0	162 ± 8	37 ± 5	6 ± 0.8	45 ± 7	12 ± 2

[a]Units converted. The density of silk was assumed to be 1.35 g/cm^3. A circular cross-section was assumed for the conversion of fineness values into diameter. [b]Values extracted from graphs/images. [c]Values converted from true stress/strain into engineering stress/strain. [d]n.s.: not specified.

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

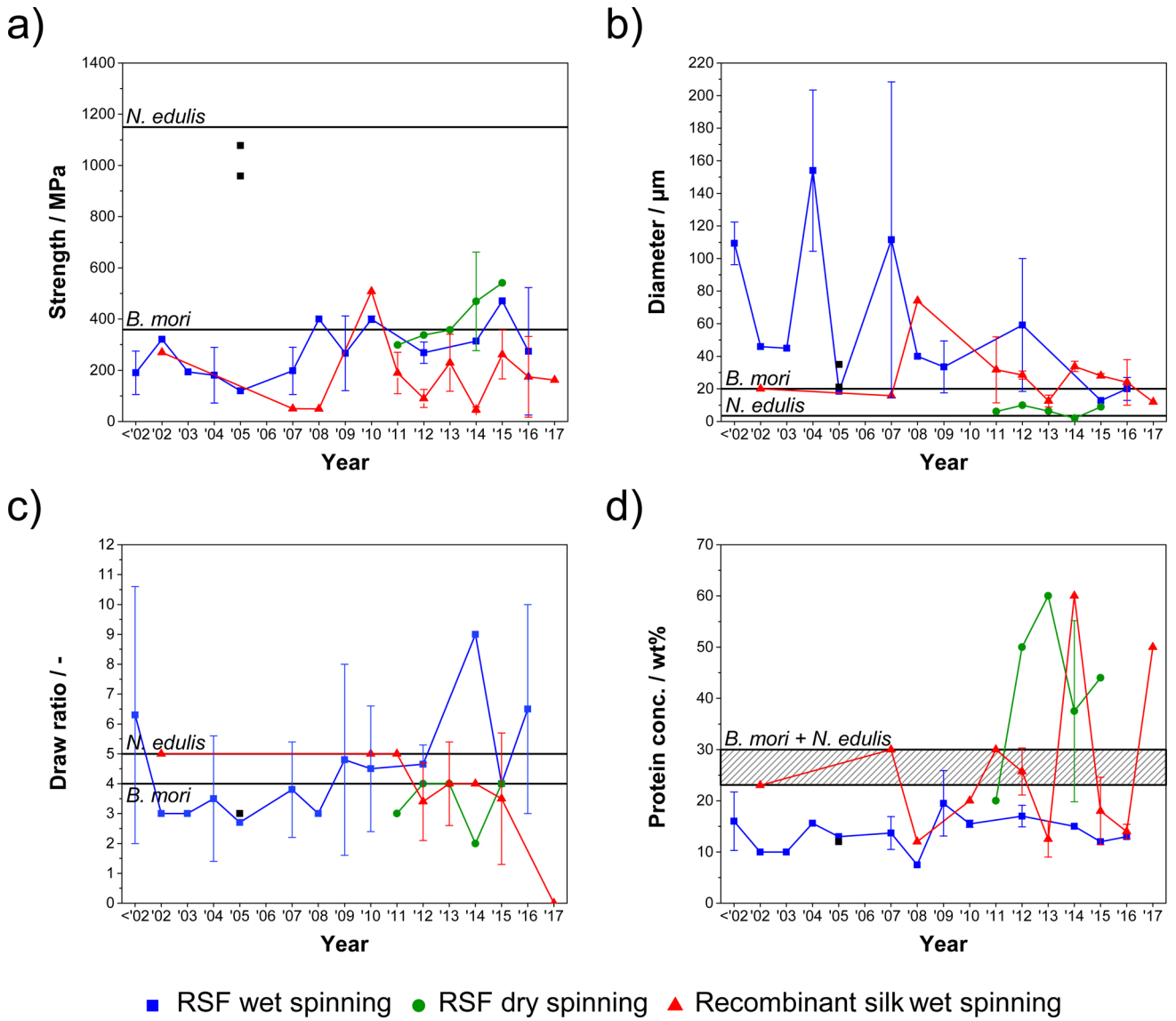


Figure 2. Fiber properties and processing parameters of the different artificial silk spinning approaches over time. Our analysis is based on the papers listed in Table 1. Further information on how the data was obtained can be found in the Supporting Information. The fiber properties of Ha et al.[72] are shown as black squares to demonstrate overall trends in fiber development as these findings have not yet been repeated. Fiber properties and processing parameters of *B. mori* and *N. edulis* are shown as references where strength and diameter values are extracted from Vollrath et al.[7] and Mortimer et al.;[6] the natural draw ratio was calculated by Zhou et al.[83], and the natural protein concentrations are given between 23 and 30 wt %. The error bars represent the standard deviation from the average values for each year. No standard deviation is shown for years with only one publication.

cells,[31] along with both mammalian[32] and plant cells.[33] While industrially scalable, it is currently limited by the fact that it is not possible to replicate the full length and sequence of a natural silk protein (i.e., 100s of kDa), and thus the resulting dopes contain silk-inspired proteins of a reduced molecular weight.[34−37]

Finally, it is possible to resolubilize previously spun silk fibers via a process called regeneration (aka reconstitution).[28,38−40] Spider silk regeneration is challenged by the fact that these animals regularly produce small amounts of silk throughout their lives; thus, acquiring sufficient raw material takes multiple spiders and several days of reeling. However, a few studies have achieved this technical feat and spun fibers from the resulting solution.[38,39]

This is in stark contrast to silkworms, which produce a large quantity of silk once in their life cycle for cocoon construction.[41] These cocoons are in plentiful supply and can readily be regenerated into large quantities of feedstock using well established techniques.[42] In general, *B. mori* silk regeneration is a three-step process: First is the removal of a glue-like coating of the fibers, sericin, by a process known as degumming.[43] In most cases, this is done by boiling the cocoons in water with either sodium carbonate,[44] marseilles soap,[45] or mixtures of both.[46] Second, fibers are dissolved in strong chaotropic agents (LiBr, $CaCl_2$, and $Ca(NO_3)_2$) which

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

disrupt their hydrogen bonded crystalline structure and enable rehydration of the proteins.[47,48] Finally, these chaotropic agents are dialyzed away, leaving a silk feedstock solution ready to use.

While regeneration is undoubtedly the most popular approach for silk feedstock preparation, over the past decade it has emerged that the silk proteins undergo partial degradation during this process.[49−51] This is likely due to the degumming step, and such degradation in turn affects the regenerated silks processing potential and ultimate mechanical properties.[28,52,53] As a result, there are currently concerted efforts to improve this process and enable higher quality regenerated silks with more native-like properties to be exploited.[50,54,55]

In summary, it is thus clear that there appear to be trade-offs for each approach in the production of an artificial silk dope with respect to achieving quality (native) or quantity (recombinant or regeneration).

3.2. Fiber Spinning. Due to their relative availability, regenerated and recombinant silk proteins have been used extensively by researchers to spin artificial fibers via both dry[56−64] and wet[29,32,35−37,44−46,65−107] extrusion based spinning, as well as electrospinning[52,87,108−114] processes and occasionally hand-drawn droplet spinning.[75,115] As this review focuses on published *individual* fiber properties from controlled spinning apparatus, as opposed to nonwoven mats, we will limit our discussion to dry and wet spun fibers (Figure 1) and direct readers to other studies that cover the electrospinning of silk.[116−119]

Dry spinning is the process by which solidification of the fiber occurs due to evaporation of a volatile solvent.[120] For wet spinning, the protein/solvent solution is extruded through a spinneret directly into a nonsolvent coagulation bath which initiates solidification into a fiber via precipitation.[120] A variation which bridges both wet and dry spinning processes also exists which involves a small air gap prior to the coagulation bath and is known as dry-jet wet spinning.[120]

3.3. Postprocessing. In general, as-spun silk fibers produced by both wet and dry spinning techniques are often brittle and have poor mechanical properties.[59,61,93,105] Therefore, different postprocessing methods have been applied to improve the mechanical performance via modulating protein order[6,57] and decreasing fiber diameter[93] (Figure 1). For wet spun fibers, the most common postprocessing methods are immersion in the coagulant for extended periods, manually or automatically applied postdrawing with different ratios, and in some cases steam-annealing.[45,69,83] It appears that dry spun fibers have to be further dehydrated and later immersed in ethanol for continuing crystallization.[56,57,61] Additionally, wet and dry spun fibers are postdrawn to increase both the order and alignment of the molecules.[37,62,64,91,93,105]

4. PROGRESS IN ARTIFICIAL SILK FIBER SPINNING OVER TIME

With so many variables in the process of artificial silk spinning, direct comparison of mechanical properties is often difficult. However, when analyzing the literature it is possible to observe some interesting trends over time that shed light onto both challenges that have been overcome and those still to be met (a complete list may be found in Table 1, with data summarized in Figure 2). For ease of discussion, we have split the field into regenerated silk fibroin (RSF) wet spinning, RSF dry spinning, and recombinant wet spinning.

4.1. Regenerated Silk Fibroin Wet Spinning. The first mention of wet spinning of silk fibers may be found in a patent by Esselen in 1933.[121] In the early days of silk fiber wet spinning, it was difficult to find an appropriate solvent/coagulant system, and therefore, Esselen began using those developed for cellulose fiber spinning. He found that silk fibroin is insoluble in typical cellulose solvents and therefore used a solution of blue copper hydroxide, ammonia, and sodium hydroxide to dissolve the silk fibroin before spinning it into sodium bisulfate. Yet while fibers were clearly produced by this process, to the best of our knowledge no mechanical property data exist. The first published mechanical property data of an artificially spun silk fiber came a quarter of a century later in the year 1960.[65] Yazawa, like Esselen, took inspiration from cellulose spinning and dissolved natural silkworm fibers in magnesium nitrate before extruding the dialyzed solution into saturated ammonium sulfate. The fibers produced had a tenacity of 2.5 g/den and an extensibility of 20−25%. From then until the turn of the century, artificial silk fibers showed little improvement,[44,66] which may be attributed to large fiber diameters (>100 μm), around five times that of a natural *B. mori* fiber (Figure 2a).[122,123]

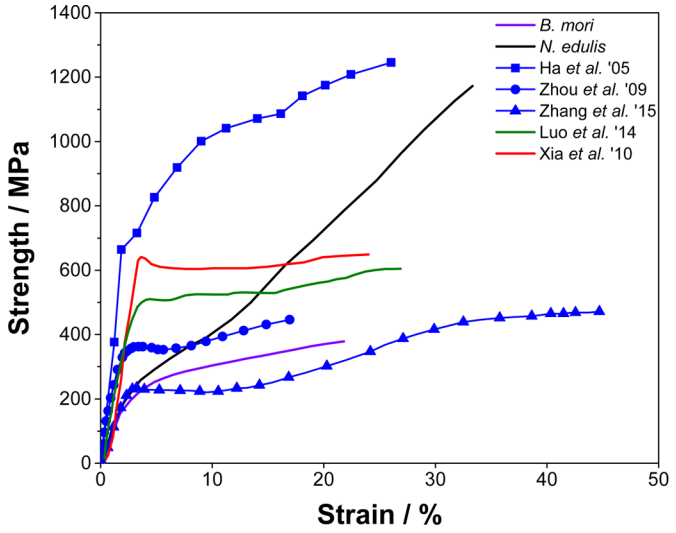
In 2002, Yao et al. reported promising results by spinning fibers with a performance close to that of silkworm silk.[45] This was achieved by using hexafluoroacetone hydrate (HFA) as a solvent for the spin dope which has been shown to possess very good solubility for silk proteins[45] and then spinning into a methanol bath to increase the degree of molecular order via further protein crystallization.[124] After drawing, their fibers were then steam-annealed at 125 °C for 30 min, resulting in a reduction of internal stresses and potentially a further increase in order via annealing of the disordered regions.[6,125,126] The resulting fibers exhibited a significantly reduced fiber diameter of 46 μm and a strength of 321.2 MPa (Figure 2a).

In subsequent years, researchers continued to use methanol as a coagulant and examined alternative solvents for spinning.[46,69−79] Until 2007, the properties reported by Yao et al. were unsurpassed, most likely because the solvents used either heavily degraded the silk proteins, had low silk solubility,[69−71,76,79] or the spinning technique employed insufficient postprocessing[73] (as evidenced by the improved properties achieved by Lee et al.[46] and Ki et al.[77] by using higher draw ratios that year).

Post 2007, a clear upward trend in fiber strength can be observed (Figure 2a). Zhu et al.[80] was the first to report fiber properties exceeding those of natural silkworm silk, and from then onward, most studies reported fibers that were either better or close to the natural *B. mori* fiber.[83,85,86,89,92,93] From our analysis, concurrent with this improvement was both a decrease in fiber diameter (Figure 2b) and an increase in postprocessing draw ratio (Figure 2c). However, despite the artificial silk's material properties bearing a closer resemblance to the natural fiber, the concentration of the spinning dopes were generally lower than the natural dope protein concentration, (Figure 2d) ranging from 7.5 wt %[80] to 29 wt %,[82] with a mean of around 15 wt %.

To date, the most impressive properties have been reported by Ha et al.,[72] with fibers possessing a strength, extensibility, and toughness similar to that of a natural spider dragline silk but with four times higher stiffness (Figure 2, black squares). However, these fiber properties were based on a small number of hand-drawn fibers and as such have been difficult to replicate. Therefore, it was Zhang's efforts in 2015,[93] which has

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237



Fiber	*E* GPa	σ MPa	ε %	W_{break} MJ/m^3
B. mori [6]	8.5	360	18.5	50.5
N. edulis [7]	7.9	1150	39	214.5
Ha *et al.* '05 [72]	39.9	1077	29.3	257.8
Zhou *et al.* '09 [83]	12.5	450	27.7	100.6
Zhang *et al.* '15 [93]	6.9	470	38.6	105.3
Luo *et al.* '14 [61]	19	614	27	136.4
Xia *et al.* '10 [29]	21	508	15	81.5
Regenerated Wet	Regenerated Dry	Recombinant Wet		

Figure 3. Comparison of the best performing artificial silk fibers produced by regenerated wet (blue), recombinant wet (red), and regenerated dry spinning (green) with natural *B. mori* (violet) and *N. edulis* silk fibers (black).

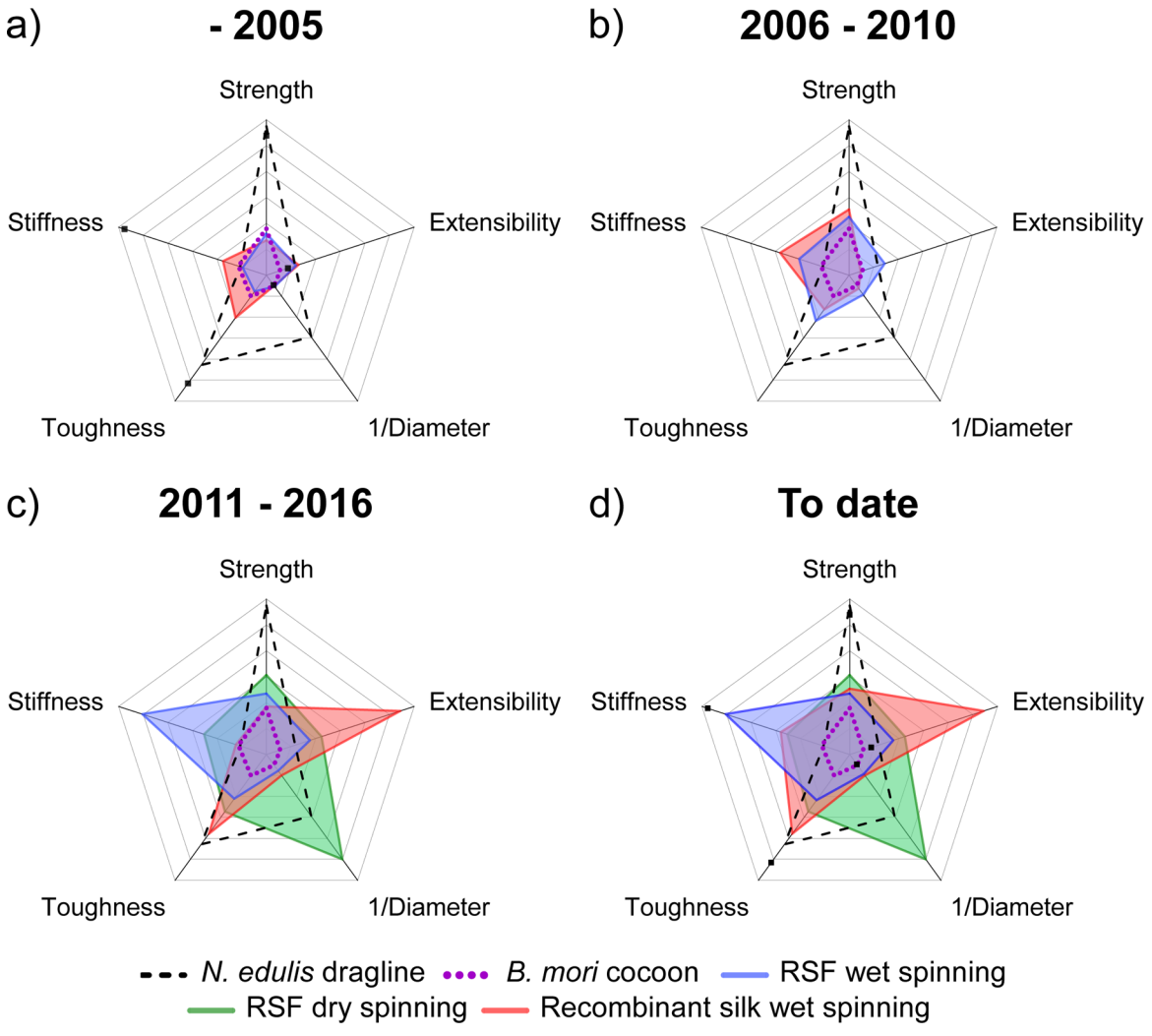


Figure 4. Performance space of the most important mechanical properties is shown for RSF wet and dry spun as well as recombinant silk wet spun fibers during different time periods. The area of each pentagon represents a performance space and is defined by the collective (not individual) best fiber properties that were achieved during each time period. In other words, all RSF wet spun fibers reported in the literature from 2011 to 2016 (see Table 1) lie within the blue pentagon area in image c. The single data points of *N. edulis*[7] dragline silk and *B. mori*[6] cocoon fibers represented by the dashed/dotted lines are included for reference. The fiber properties of Ha et al.[72] are shown as black squares (for explanation, see the main text).

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

to date reported the best fiber properties produced by wet spinning a reconstituted fibroin dope (Figure 3).

4.2. Regenerated Silk Fibroin Dry Spinning. Dry spinning of regenerated silk fibroin is a relatively recent innovation, with the first reports appearing in 2011, some 50 years after silk wet spinning began.[56,57,65] This area is dominated by the Zhang group with 6 of the 7 publications, and as they use the same degumming and dissolving conditions, it is much easier to directly compare fiber properties (Figure 2). From their first paper, fibers were produced that exhibited similar strengths to those of silkworm silk but had twice the toughness.[56,57] Since then, reports continued to show an improvement in fiber properties along with an increase in feedstock concentration from 20 to more than 50%,[56−59] and a decrease in diameter to ∼2 μm which is close to that of *Nephila edulis* dragline silk (Figure 2b), resulting in the best reported mechanical properties to date (Figure 3).[61]

Refinement of the process has been recently reported by Yue et al.[62] from another group. They reported similar properties to those of Jin et al.,[59] although using a much lower protein concentration for spinning. They spun fibers with a concentration of 20−25 wt % silk proteins into a calcium chloride/formic acid mixture. The natural silk proteins could be dissolved directly in this solvent and immediately processed, eliminating the dissolution and dialyzing steps which could significantly reduce the processing time. This time-saving way of using formic acid in silk regeneration seems to be a recent trend and was also used by Zhang and co-workers.[78,93]

4.3. Recombinant Silk Wet Spinning. In contrast to regenerated silk fibers, recombinant wet spun fibers do not show the same rate of improvement over time (Figure 2a). Starting in 2002, the widely publicized work by Lazaris et al.[32] reported fibers spun from spider silk-inspired proteins expressed from mammalian cell lines that had an elongation, stiffness, and toughness akin to *B. mori*. However, despite several attempts over the years,[29,35,97−103] a comparable strength to the fibers reported by Lazaris et al.[32] was not achieved until 2010.[29] Surprisingly, this improvement is not correlated with a reduction in diameter (like regenerated silk fibers), with fibers being generally larger than natural silks (Figure 2b). Thus, we propose that the primary improvement in properties from recombinant fibers is most likely due to an increase in molecular weight. While Lazaris et al. spun fibers from proteins of 60−140 kDa,[32] Xia and co-workers used multimerization of their gene construct to increase the molecular weight to 284.9 kDa[29] and reported improved properties. Furthermore, later reports from Lin et al.[104] and Heidebrecht et al.[105] reported a further increase in molecular weight to 286[105] and 378 kDa,[104] respectively, by using SUMO (small ubiquitin-like modifier) fusion technology and via disulfide bonding.

5. UNDERSTANDING THE DEVELOPMENT OF FIBER PROPERTIES

Above, we have seen single viewpoints on individual fibers, but we have not been able to see the *overall* development, i.e., "performance space" of the field. Here, we introduce a new means for comparing the most common fiber properties to enable us to understand the material property trade-offs in fiber development and determine possible areas for further improvement. As a result, a performance space is therefore derived from the best achieved fiber properties across all studies within a time period and not necessarily from an individual fiber (visualized as "web plots" in Figure 4).

Until 2005, the best properties from any wet spun fibers from regenerated feedstocks show a performance close to that of the natural *B. mori* fiber (Figure 4a). However, it is worth mentioning that it was not possible to spin an individual fiber that combines *all* of these properties. Of note is that the study from Ha et al. which reports fiber properties outperforming the performance of *N. edulis* dragline silk was set aside for discussion as they still remain to be reproduced.[72] During this time period, only one publication reported the spinning of fibers from recombinant silk proteins.[32] Those fibers possessed a higher stiffness and toughness compared to those of fibers from regenerated silk proteins, while the strength, diameter, and extensibility were comparable.

From 2006 to 2010, all regenerated silk fibers saw improvements (Figure 4b). For the first time, individual fibers with properties exceeding those of natural *B. mori* silk could be spun from regenerated feedstocks.[29,80] As discussed above, this is attributed to improved processing parameters that also account for the decrease in fiber diameter. This is in contrast to recombinant fibers, which do not show an overall improvement but rather a shift of properties: stiffness and strength were improved, albeit at the expense of extensibility and toughness.

From 2011 to 2016, only the stiffness of regenerated silk fibers increased, while other properties plateaued (Figure 4c). Recombinant fibers, however, saw a significant improvement with a toughness reported that was close to natural spider silk, a product of increased extensibility but at the expense of fiber strength and stiffness. This time period also saw the emergence of dry spun fibers, with properties reported that outperform regenerated wet spun silks and importantly show the highest strength of all fibers (alongside a very small diameter for dry spun fibers).

In summary, regenerated silk fibers have shown a gradual improvement in all properties over time, but upon closer inspection, it appears that the wet spinning approach has reached a limit in strength and toughness, yet a very high stiffness was recently reported by Chen and co-workers.[94] However, it has been the innovation in dry spinning that has led to improved properties in the field. On the other hand, the field of recombinant dope spinning appears to be currently faced with a trade-off; it is possible to spin fibers with either a high stiffness and strength[29] *or* high toughness and extensibility[105] but not both (Figure 4d). Very recently, however, Andersson and co-workers[107] have reported impressive mechanical properties of as-spun fibers from chimeric recombinant spider silk proteins without any postspinning modification.

6. CURRENT CHALLENGES

While the field has seen significant improvements in the production of artificial silks, it is arguable that the most significant challenges are still to come. In an effort to identify the general challenges faced, it is important to highlight that a fiber's mechanical properties are a product of both the feedstock *and* the means by which it is processed.

6.1. Feedstock. We propose one of the key problems leading to difficulties in replicating the properties of the higher performing silk fibers is the use of spinning dopes that may be considered unnatural, i.e., their protein constituents differ in both structure and function compared to those of the native proteins. For example, reconstituted and recombinant silk

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

dopes have been shown to have completely different mechanical/rheological properties, structure, and/or a lower molecular weight compared to those of natural silk proteins.[28,32,38,50−52,55,66,73,79,88,91,127] Furthermore, it is worth noting that reconstituted silk proteins typically originate from silkworms and hence are inherently different from spider silks and thus may not be able to be processed into a spider silk at all.[128]

6.2. Processing. As shown in this review, the current fiber forming processes for artificial silk fiber spinning are very different from the natural one. In nature, silk proteins are transformed into solid, insoluble fibers via a stress induced phase transition accompanied by an acidification and metal ion gradient along the spinning duct.[22,23,129] However, during wet spinning artificial fiber formation occurs via precipitation in a coagulant and without the presence of anisotropic stress (i.e., shear or post drawing under tension), which leads to a more isotropic molecular arrangement of the proteins. Even dry spun fibers formed by solvent evaporation require immersion and drawing in ethanol to get an acceptable mechanical performance. Yet there are currently several efforts to spin silk fibers in a more biomimetic fashion that move away from the more traditional means of spinning.[61,64,130−134]

6.3. Fiber Properties. From our analysis, the ability to produce thinner fibers appears to be linked to increased fiber strength for wet spun RSF fibers. This hypothesis is supported by fracture mechanics calculations performed by Porter et al.,[123] who proposed that natural silks are strong simply because they are thin. However, after plotting the data presented here for artificial silk fibers using the relationship for fracture strength provided by Porter et al.,[123] it can be observed that despite being thin, dry spun fibers and most wet spun fibers do not follow the trendline for the generic energy release rate for polymers (Figure 5). The majority of the fibers follow a fit that has a lower slope, meaning artificial silk fibers exhibit either a lower strength, a higher stiffness, or a smaller diameter compared to that of natural fibers. This suggests that apart from the external fiber structure (i.e., diameter and fiber surface), the internal structure (i.e., hierarchical structures, skin/core, and micro/nanofibrils, alongside control of the ordered and disordered regions) plays a vital part in defining the mechanical performance of silk fibers and is an area for future research.

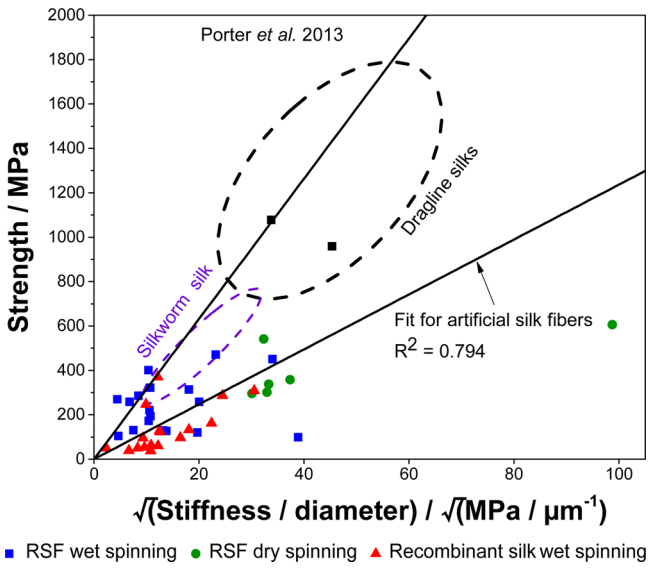


Figure 5. Fracture-strength relationship for polymer fibers according to Porter et al.[123] Synthetic polymers, natural silkworm, and spider dragline silk all follow the trendline for the generic energy release rate. Most artificially spun silk fibers, however, tend to follow a different fit, indicating more disordered regions compared to those of natural silks. The black squares represent the values from Ha et al.[72]

If further developments are to be made, we now have to go back to the beginning of our discussion and understand *all* properties of the best performing individual fibers (Figure 3) by looking at the complete regime of a stress−strain graph. When comparing the best performing fibers for every spinning approach to natural *B. mori* and *N. edulis* silk, some interesting differences can be observed (Figure 3). For example, all artificial fibers show very distinct yield points with clearly different pre- and postyield moduli that appear to be absent in natural silk fibers. Natural silks show a homogeneous, rubber like deformation to rupture, whereas the stress−strain behavior of the artificial silk fibers suggests that a previously less ordered structure is converted into more ordered areas by cold-drawing.[135] Studies that specifically probe this link and thus the continuum between natural and artificial silk fibers provide useful insight into this structure−function relationship. For example, Mortimer et al. using dynamic mechanical thermal analysis and thermal gravimetric analysis demonstrated that native silk fibers have less disordered regions compared to those of forced reeled and artificial silks.[6] Beyond the nanoscale, another factor that negatively influences the mechanical properties of artificially spun silks was previously discussed by Peng and co-workers.[106] They suggested that impurities enclosed within the fiber inhibit fibril assembly and lead to a porous structure affecting fiber performance. Such impurities are proposed to be very short protein fragments from highly degraded proteins,[53] remaining chaotropic salts[136] from insufficient dialysis, or entrapped air-bubbles during processing.

As a conclusion, it appears that the key to producing fibers with enhanced properties is to spin silk proteins as inherently thin fibers without impurities and at the same time develop molecular orientation during processing rather than by postdrawing.

7. SUGGESTIONS FOR COMPARISON AND CONSISTENCY

In this review, we conclude that in order to elucidate the underlying mechanisms behind successful biomimetic fiber production and present artificially spun fibers in a fair and comparable light, the community would do well to adopt a few consistent practices across studies. We suggest that in the future it would benefit all for the following parameters for single fiber tensile tests to be reported: manufacturer of the testing apparatus, maximum capacity of the load cell used and minimum resolution, test gauge length, strain rate, the number of samples per treatment tested, the testing temperature and humidity, and any fiber pretreatments (including storage conditions). With respect to the presentation of such data, tables with SI values and error alongside a comparison to other studies, or an average value for a natural fiber (taken from literature) would be beneficial.

One final topic not discussed so far but crucial to the successful application and scale-up of these processes is whether continuous spinning is possible. A process that can produce very short fibers with impressive properties is not

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

necessarily more attractive to industry when compared to one that allows continuous spinning of fibers with good properties. Therefore, to increase consistency and comparability in the field of silk fiber spinning, we suggest reporting the length of the longest continuously spun fiber in addition to the fibers' tensile properties. To date, only two publications,[57,107] to our knowledge, have reported either continuous spinning times or fiber lengths, and from other papers, it can only be inferred from the gauge length of the tensile samples and the number of repeats.

8. CONCLUSION

The successful production of artificial silk fibers has been a scientific and technological milestone for nearly a century. This review has made efforts to summarize the various artificial silk fiber spinning approaches and discuss the development and trajectory of each technology. In general, each approach has its own challenges to overcome surrounding aspects of the feedstock/dope, fiber spinning, and postprocessing: for the well-established wet spinning, it appears that postprocessing through the steady decrease in fiber diameter alongside an increase in postdraw ratio has seen significant improvements in mechanical properties. The more recent innovation of dry spinning has seen improvements through the use of highly concentrated dopes, microfluidics, and alternative protocols for regeneration. Finally, recombinant spinning, which has the added advantage yet complexity of truly bespoke protein configurations, has seen the greatest improvements through an increase in protein molecular weight.

From our review, it is clear that fibers outperforming natural *B. mori* silk can be produced by all spinning methods. Nevertheless, we are yet to reach the "gold standard" of an artificial fiber that matches the properties of a spider dragline silk. Extrapolation from our analysis (Figure 2) indicates that achieving this through simple iterative development of existing solutions will take a significant amount of time. Therefore, we must look toward first understanding the fundamental mechanisms behind natural silk production and performance in order to innovate. When such challenges are matched and systems developed, it will allow new exciting hypotheses to be tested that reach beyond engineering and hark back to biology, perhaps even helping unravel the fundamental evolutionary constraints placed on silk spinning that define this remarkable material.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.6b00669.

Details on the analysis for the information provided in Table 1 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +44 114 222 5477. E-mail: christopher.holland@sheffield.ac.uk.

ORCID

Andreas Koeppel: 0000-0002-4900-8797

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the EPSRC, project reference EP/K005693/1. We thank James Sparkes for proofreading the manuscript and Freya Harrison, Pete Laity, and Anastasia Brif for discussions.

■ REFERENCES

(1) Vollrath, F.; Porter, D. Silks as ancient models for modern polymers. *Polymer* **2009**, *50* (24), 5623−5632.

(2) Sutherland, T. D.; Young, J. H.; Weisman, S.; Hayashi, C. Y.; Merritt, D. J. Insect silk: One name, many materials. *Annu. Rev. Entomol.* **2010**, *55*, 171−188.

(3) Vollrath, F.; Porter, D.; Dicko, C. The Structure of Silk. In *Handbook of Textile Fibre Structure*; Woodhead Publishing: Cambridge, U.K., 2009; Vol. 2, pp 146−198. DOI: 10.1533/9781845697310.1.146.

(4) Babu, K. M. *Silk: Processing, Properties and Applications*; Woodhead Publishing: New Delhi, India, 2013.

(5) FAOSTAT - Food and Agriculture Organization of the United Nations Statistics Division. http://faostat3.fao.org/download/Q/QP/E (accessed Oct 2016).

(6) Mortimer, B.; Guan, J.; Holland, C.; Porter, D.; Vollrath, F. Linking naturally and unnaturally spun silks through the forced reeling of Bombyx mori. *Acta Biomater.* **2015**, *11*, 247−255.

(7) Vollrath, F.; Madsen, B.; Shao, Z. The effect of spinning conditions on the mechanics of a spider's dragline silk. *Proc. R. Soc. London, Ser. B* **2001**, *268* (1483), 2339−2346.

(8) Gosline, J. M.; Guerette, P. A.; Ortlepp, C. S.; Savage, K. N. The mechanical design of spider silks: From fibroin sequence to mechanical function. *Journal of Experimental Biology* **1999**, *202* (23), 3295−3303.

(9) Kluge, J. A.; Rabotyagova, O.; Leisk, G. G.; Kaplan, D. L. Spider silks and their applications. *Trends Biotechnol.* **2008**, *26* (5), 244−251.

(10) Altman, G. H.; Diaz, F.; Jakuba, C.; Calabro, T.; Horan, R. L.; Chen, J.; Lu, H.; Richmond, J.; Kaplan, D. L. Silk-based biomaterials. *Biomaterials* **2003**, *24* (3), 401−416.

(11) Lewis, R. Unravelling the weave of spider silk. *BioScience* **1996**, *46* (9), 336−338.

(12) Morgan, E. *Gossamer Days: Spiders, Humans and Their Threads*; Strange Attractor: London, U.K., 2016.

(13) Peers, S. *Golden Spider Silk*; V&A Publishing: London, U.K., 2012.

(14) Vepari, C.; Kaplan, D. L. Silk as a biomaterial. *Prog. Polym. Sci.* **2007**, *32* (8−9), 991−1007.

(15) Mandal, B. B.; Kundu, S. C. Biospinning by silkworms: Silk fiber matrices for tissue engineering applications. *Acta Biomater.* **2010**, *6* (2), 360−371.

(16) Radtke, C. Natural Occurring Silks and Their Analogues as Materials for Nerve Conduits. *Int. J. Mol. Sci.* **2016**, *17* (10), 1754.

(17) Shah, D. U.; Porter, D.; Vollrath, F. Can silk become an effective reinforcing fibre? A property comparison with flax and glass reinforced composites. *Compos. Sci. Technol.* **2014**, *101*, 173−183.

(18) Kundu, S. C.; Kundu, B.; Talukdar, S.; Bano, S.; Nayak, S.; Kundu, J.; Mandal, B. B.; Bhardwaj, N.; Botlagunta, M.; Dash, B. C.; Acharya, C.; Ghosh, A. K. Invited review nonmulberry silk biopolymers. *Biopolymers* **2012**, *97* (6), 455−467.

(19) Walker, A. A.; Holland, C.; Sutherland, T. D. More than one way to spin a crystallite: Multiple trajectories through liquid crystallinity to solid silk. *Proc. R. Soc. London, Ser. B* **2015**, *282* (1809), 20150259.

(20) Heim, M.; Keerl, D.; Scheibel, T. Spider silk: From soluble protein to extraordinary fiber. *Angew. Chem., Int. Ed.* **2009**, *48*, 3584−3596.

(21) Eisoldt, L.; Thamm, C.; Scheibel, T. Review: the role of terminal domains during storage and assembly of spider silk proteins. *Biopolymers* **2012**, *97*, 355−361.

(22) Asakura, T.; Umemura, K.; Nakazawa, Y.; Hirose, H.; Higham, J.; Knight, D. Some observations on the structure and function of the spinning apparatus in the silkworm Bombyx mori. *Biomacromolecules* **2007**, *8* (1), 175−181.

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

(23) Vollrath, F.; Knight, D. P. Liquid crystalline spinning of spider silk. *Nature* **2001**, *410* (6828), 541−548.
(24) Holland, C.; Vollrath, F.; Ryan, A. J.; Mykhaylyk, O. O. Silk and Synthetic Polymers: Reconciling 100 Degrees of Separation. *Adv. Mater.* **2012**, *24* (1), 105−109.
(25) Foo, C. W. P.; Bini, E.; Hensman, J.; Knight, D. P.; Lewis, R. V.; Kaplan, D. L. Role of pH and charge on silk protein assembly in insects and spiders. *Appl. Phys. A: Mater. Sci. Process.* **2006**, *82* (2), 223−233.
(26) Laity, P. R.; Gilks, S. E.; Holland, C. Rheological behaviour of native silk feedstocks. *Polymer* **2015**, *67*, 28−39.
(27) Laity, P. R.; Holland, C. Native Silk Feedstock as a Model Biopolymer: A Rheological Perspective. *Biomacromolecules* **2016**, *17* (8), 2662−2671.
(28) Holland, C.; Terry, A. E.; Porter, D.; Vollrath, F. Natural and unnatural silks. *Polymer* **2007**, *48* (12), 3388−3392.
(29) Xia, X.-X.; Qian, Z.-G.; Ki, C. S.; Park, Y. H.; Kaplan, D. L.; Lee, S. Y. Native-sized recombinant spider silk protein produced in metabolically engineered Escherichia coli results in a strong fiber. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 14059−14063.
(30) Fahnestock, S.; Bedzyk, L. Production of synthetic spider dragline silk protein in Pichia pastoris. *Appl. Microbiol. Biotechnol.* **1997**, *47* (1), 33−39.
(31) Huemmerich, D.; Scheibel, T.; Vollrath, F.; Cohen, S.; Gat, U.; Ittah, S. Novel Assembly Properties of Recombinant Spider Dragline Silk Proteins. *Curr. Biol.* **2004**, *14* (22), 2070−2074.
(32) Lazaris, A.; Arcidiacono, S.; Huang, Y.; Zhou, J.-F.; Duguay, F.; Chretien, N.; Welsh, E. A.; Soares, J. W.; Karatzas, C. N. Spider Silk Fibers Spun from Soluble Recombinant Silk Produced in Mammalian Cells. *Science* **2002**, *295*, 472−476.
(33) Scheller, J.; Gührs, K.-H.; Grosse, F.; Conrad, U. Production of spider silk proteins in tobacco and potato. *Nat. Biotechnol.* **2001**, *19* (6), 573−577.
(34) Scheibel, T. Spider silks: recombinant synthesis, assembly, spinning, and engineering of synthetic proteins. *Microb. Cell Fact.* **2004**, *3*, 14.
(35) Albertson, A. E.; Teulé, F.; Weber, W.; Yarger, J. L.; Lewis, R. V. Effects of different post-spin stretching conditions on the mechanical properties of synthetic spider silk fibers. *Journal of the Mechanical Behavior of Biomedical Materials* **2014**, *29*, 225−234.
(36) Copeland, C. G.; Bell, B. E.; Christensen, C. D.; Lewis, R. V. Development of a Process for the Spinning of Synthetic Spider Silk. *ACS Biomater. Sci. Eng.* **2015**, *1*, 577−584.
(37) Jones, J. A.; Harris, T. I.; Tucker, C. L.; Berg, K. R.; Christy, S. Y.; Day, B. A.; Gaztambide, D. A.; Needham, N. J. C.; Ruben, A. L.; Oliveira, P. F.; Decker, R. E.; Lewis, R. V. More than just fibers: an aqueous method for the production of innovative recombinant spider silk protein materials. *Biomacromolecules* **2015**, *16*, 1418−1425.
(38) Seidel, A.; Liivak, O.; Calve, S.; Adaska, J.; Ji, G.; Yang, Z.; Grubb, D.; Zax, D. B.; Jelinski, L. W. *Macromolecules* **2000**, *33*, 775−780.
(39) Seidel, A.; Liivak, O.; Jelinski, L. W. Artificial spinning of spider silk. *Macromolecules* **1998**, *31* (19), 6733−6736.
(40) Cho, H. J.; Ki, C. S.; Oh, H.; Lee, K. H.; Um, I. C. Molecular weight distribution and solution properties of silk fibroins with different dissolution conditions. *Int. J. Biol. Macromol.* **2012**, *51*, 336−341.
(41) Zhao, H.-P.; Feng, X.-Q.; Yu, S.-W.; Cui, W.-Z.; Zou, F.-Z. Mechanical properties of silkworm cocoons. *Polymer* **2005**, *46* (21), 9192−9201.
(42) Rockwood, D. N.; Preda, R. C.; Yücel, T.; Wang, X.; Lovett, M. L.; Kaplan, D. L. Materials fabrication from Bombyx mori silk fibroin. *Nat. Protoc.* **2011**, *6* (10), 1612−1631.
(43) Wang, Y. J.; Zhag, Y. Q. Three-layered sericins around the silk fibroin fiber from Bombyx mori cocoon and their amino acid composition. *Adv. Mater. Res.* **2011**, *175*, 158−163.
(44) Ishizaka, H.; Watanabe, Y.; Ishida, K.; Fukumoto, O. Regenerated silk prepared from ortho phosphoric acid solution of fibroin. *Journal of Sericultural Science of Japan* **1989**, *58* (2), 87−95.
(45) Yao, J. M.; Masuda, H.; Zhao, C. H.; Asakura, T. Artificial spinning and characterization of silk fiber from Bombyx mori silk fibroin in hexafluoroacetone hydrate. *Macromolecules* **2002**, *35* (1), 6−9.
(46) Lee, K. H.; Baek, D. H.; Ki, C. S.; Park, Y. H. Preparation and characterization of wet spun silk fibroin/poly(vinyl alcohol) blend filaments. *Int. J. Biol. Macromol.* **2007**, *41* (2), 168−172.
(47) Sashina, E. S.; Bochek, A. M.; Novoselov, N. P.; Kirichenko, D. A. Structure and solubility of natural silk fibroin. *Russ. J. Appl. Chem.* **2006**, *79* (6), 869−876.
(48) Phillips, D. M.; Drummy, L. F.; Conrady, D. G.; Fox, D. M.; Naik, R. R.; Stone, M. O.; Trulove, P. C.; De Long, H. C.; Mantz, R. A. Dissolution and regeneration of Bombyx mori silk fibroin using ionic liquids. *J. Am. Chem. Soc.* **2004**, *126* (44), 14350−14351.
(49) Yamada, H.; Nakao, H.; Takasu, Y.; Tsubouchi, K. Preparation of undegraded native molecular fibroin solution from silkworm cocoons. *Mater. Sci. Eng., C* **2001**, *14* (1), 41−46.
(50) Wang, H.-Y.; Zhang, Y.-Q. Effect of regeneration of liquid silk fibroin on its structure and characterization. *Soft Matter* **2013**, *9* (1), 138−145.
(51) Perea, G. B.; Solanas, C.; Marí-Buyé, N.; Madurga, R.; Agulló-Rueda, F.; Muinelo, A.; Riekel, C.; Burghammer, M.; Jorge, I.; Vázquez, J.; Plaza, G. R.; Torres, A. L.; del Pozo, F.; Guinea, G. V.; Elices, M.; Cenis, J. L.; Pérez-Rigueiro, J. The apparent variability of silkworm (Bombyx mori) silk and its relationship with degumming. *Eur. Polym. J.* **2016**, *78*, 129−140.
(52) Ko, J. S.; Yoon, K.; Ki, C. S.; Kim, H. J.; Bae Do, G.; Lee, K. H.; Park, Y. H.; Um, I. C. Effect of degumming condition on the solution properties and electrospinnablity of regenerated silk solution. *Int. J. Biol. Macromol.* **2013**, *55*, 161−168.
(53) Rnjak-Kovacina, J.; Wray, L. S.; Burke, K. A.; Torregrosa, T.; Golinski, J. M.; Huang, W.; Kaplan, D. L. Lyophilized Silk Sponges: A Versatile Biomaterial Platform for Soft Tissue Engineering. *ACS Biomater. Sci. Eng.* **2015**, *1* (4), 260−270.
(54) Boulet-Audet, M.; Holland, C.; Gheysens, T.; Vollrath, F. Dry-spun silk produces native-like fibroin solutions. *Biomacromolecules* **2016**, *17*, 3198.
(55) Wang, Q.; Chen, Q.; Yang, Y.; Shao, Z. Effect of various dissolution systems on the molecular weight of regenerated silk fibroin. *Biomacromolecules* **2013**, *14* (1), 285−289.
(56) Wei, W.; Zhang, Y.; Shao, H.; Hu, X. Posttreatment of the dry-spun fibers obtained from regenerated silk fibroin aqueous solution in ethanol aqueous solution. *J. Mater. Res.* **2011**, *26* (09), 1100−1106.
(57) Wei, W.; Zhang, Y. P.; Zhao, Y. M.; Luo, J.; Shao, H. L.; Hu, X. C. Bio-inspired capillary dry spinning of regenerated silk fibroin aqueous solution. *Mater. Sci. Eng., C* **2011**, *31* (7), 1602−1608.
(58) Sun, M.; Zhang, Y.; Zhao, Y.; Shao, H.; Hu, X. The structure−property relationships of artificial silk fabricated by dry-spinning process. *J. Mater. Chem.* **2012**, *22* (35), 18372.
(59) Jin, Y.; Zhang, Y.; Hang, Y.; Shao, H.; Hu, X. A simple process for dry spinning of regenerated silk fibroin aqueous solution. *J. Mater. Res.* **2013**, *28* (20), 2897−2902.
(60) Jin, Y.; Hang, Y. C.; Zhang, Y. P.; Shao, H. L.; Hu, X. C. Role of Ca^{2+} on structures and properties of regenerated silk fibroin aqueous solutions and fibres. *Mater. Res. Innovations* **2014**, *18*, 113−116.
(61) Luo, J.; Zhang, L.; Peng, Q.; Sun, M.; Zhang, Y.; Shao, H.; Hu, X. Tough silk fibers prepared in air using a biomimetic microfluidic chip. *Int. J. Biol. Macromol.* **2014**, *66*, 319−324.
(62) Yue, X.; Zhang, F.; Wu, H.; Ming, J.; Fan, Z.; Zuo, B. A novel route to prepare dry-spun silk fibers from $CaCl_2$-formic acid solution. *Mater. Lett.* **2014**, *128*, 175−178.
(63) Zhu, J. X.; Zhang, Y. P.; Shao, H. L.; Hu, X. C. Effects of Environment Parameters on Sol-Gel Transition and Dry-spinnability of Regenerated Silk Fibroin Aqueous Solution. *Fibers Polym.* **2014**, *15* (3), 540−546.
(64) Peng, Q.; Shao, H.; Hu, X.; Zhang, Y. Role of humidity on the structures and properties of regenerated silk fibers. *Prog. Nat. Sci.* **2015**, *25* (5), 430−436.

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

(65) Yazawa, S. Spinning of concentrated aqueous silk fibroin solution. *J. Chem. Soc. Jpn.* **1960**, *63*, 1428−1430.
(66) Matsumoto, K.; Uejima, H.; Iwasaki, T.; Sano, Y.; Sumino, H. Studies on regenerated protein fibers. III. Production of regenerated silk fibroin fiber by the self-dialyzing wet spinning method. *J. Appl. Polym. Sci.* **1996**, *60* (4), 503−511.
(67) Liivak, O.; Blye, A.; Shah, N.; Jelinski, L. W. A microfabricated wet-spinning apparatus to spin fibers of silk proteins. Structure-property correlations. *Macromolecules* **1998**, *31* (9), 2947−2951.
(68) Trabbic, K. A.; Yager, P. Comparative structural characterization of naturally- and synthetically-spun fibers of Bombyx mori fibroin. *Macromolecules* **1998**, *31* (2), 462−471.
(69) Zhao, C.; Yao, J.; Masuda, H.; Kishore, R.; Asakura, T. Structural characterization and artificial fiber formation of Bombyx mori silk fibroin in hexafluoro-iso-propanol solvent system. *Biopolymers* **2003**, *69* (2), 253−259.
(70) Um, I. C.; Ki, C. S.; Kweon, H.; Lee, K. G.; Ihm, D. W.; Park, Y. H. Wet spinning of silk polymer. II. Effect of drawing on the structural characteristics and properties of filament. *Int. J. Biol. Macromol.* **2004**, *34* (1−2), 107−119.
(71) Um, I. C.; Kweon, H.; Lee, K. G.; Ihm, D. W.; Lee, J. H.; Park, Y. H. Wet spinning of silk polymer. I. Effect of coagulation conditions on the morphological feature of filament. *Int. J. Biol. Macromol.* **2004**, *34* (1−2), 89−105.
(72) Ha, S. W.; Tonelli, A. E.; Hudson, S. M. Structural studies of Bombyx mori silk fibroin during regeneration from solutions and wet fiber spinning. *Biomacromolecules* **2005**, *6* (3), 1722−1731.
(73) Marsano, E.; Corsini, P.; Arosio, C.; Boschi, A.; Mormino, M.; Freddi, G. Wet spinning of Bombyx mori silk fibroin dissolved in N-methyl morpholine N-oxide and properties of regenerated fibres. *Int. J. Biol. Macromol.* **2005**, *37* (4), 179−188.
(74) Phillips, D. M.; Drummy, L. F.; Naik, R. R.; De Long, H. C.; Fox, D. M.; Trulove, P. C.; Mantz, R. A. Regenerated silk fiber wet spinning from an ionic liquid solution. *J. Mater. Chem.* **2005**, *15* (39), 4206−4208.
(75) Xie, F.; Zhang, H.; Shao, H.; Hu, X. Effect of shearing on formation of silk fibers from regenerated Bombyx mori silk fibroin aqueous solution. *Int. J. Biol. Macromol.* **2006**, *38* (3−5), 284−288.
(76) Corsini, P.; Perez-Rigueiro, J.; Guinea, G. V.; Plaza, G. R.; Elices, M.; Marsano, E.; Carnasciali, M. M.; Freddi, G. Influence of the draw ratio on the tensile and fracture behavior of NMMO regenerated silk fibers. *J. Polym. Sci., Part B: Polym. Phys.* **2007**, *45* (18), 2568−2579.
(77) Ki, C. S.; Kim, J. W.; Oh, H. J.; Lee, K. H.; Park, Y. H. The effect of residual silk sericin on the structure and mechanical property of regenerated silk filament. *Int. J. Biol. Macromol.* **2007**, *41* (3), 346−353.
(78) Ki, C. S.; Lee, K. H.; Baek, D. H.; Hattori, M.; Um, I. C.; Ihm, D. W.; Park, Y. H. Dissolution and wet spinning of silk fibroin using phosphoric acid/formic acid mixture solvent system. *J. Appl. Polym. Sci.* **2007**, *105* (3), 1605−1610.
(79) Zuo, B. Q.; Liu, L.; Wu, Z. Effect on properties of regenerated silk fibroin fiber coagulated with aqueous Methanol/Ethanol. *J. Appl. Polym. Sci.* **2007**, *106* (1), 53−59.
(80) Zhu, Z. H.; Ohgo, K.; Asakura, T. Preparation and characterization of regenerated Bombyx mori silk fibroin fiber with high strength. *eXPRESS Polym. Lett.* **2008**, *2* (12), 885−889.
(81) Plaza, G. R.; Corsini, P.; Marsano, E.; Perez-Rigueiro, J.; Biancotto, L.; Elices, M.; Riekel, C.; Agullo-Rueda, F.; Gallardo, E.; Calleja, J. M.; Guinea, G. V. Old Silks Endowed with New Properties. *Macromolecules* **2009**, *42* (22), 8977−8982.
(82) Sohn, S.; Gido, S. P. Wet-spinning of osmotically stressed silk fibroin. *Biomacromolecules* **2009**, *10* (8), 2086−2091.
(83) Zhou, G.; Shao, Z.; Knight, D. P.; Yan, J.; Chen, X. Silk Fibers Extruded Artificially from Aqueous Solutions of RegeneratedBombyx moriSilk Fibroin are Tougher than their Natural Counterparts. *Adv. Mater.* **2009**, *21* (3), 366−370.
(84) Ko, J. S.; Lee, K. H.; Bae, D. G.; Um, I. C. Miscibility, Structural Characteristics, and Thermal Behavior of Wet Spun Regenerated Silk Fibroin/Nylon 6 Blend Filaments. *Fibers Polym.* **2010**, *11* (1), 14−20.
(85) Yan, J.; Zhou, G.; Knight, D. P.; Shao, Z.; Chen, X. Wet-spinning of regenerated silk fiber from aqueous silk fibroin solution: discussion of spinning parameters. *Biomacromolecules* **2010**, *11* (1), 1−5.
(86) Zhu, Z.; Kikuchi, Y.; Kojima, K.; Tamura, T.; Kuwabara, N.; Nakamura, T.; Asakura, T. Mechanical properties of regenerated Bombyx mori silk fibers and recombinant silk fibers produced by transgenic silkworms. *J. Biomater. Sci., Polym. Ed.* **2010**, *21*, 395−411.
(87) Cho, H. J.; Yoo, Y. J.; Kim, J. W.; Park, Y. H.; Bae, D. G.; Um, I. C. Effect of molecular weight and storage time on the wet- and electro-spinning of regenerated silk fibroin. *Polym. Degrad. Stab.* **2012**, *97* (6), 1060−1066.
(88) Ling, S.; Zhou, L.; Zhou, W.; Shao, Z.; Chen, X. Conformation transition kinetics and spinnability of regenerated silk fibroin with glycol, glycerol and polyethylene glycol. *Mater. Lett.* **2012**, *81*, 13−15.
(89) Plaza, G. R.; Corsini, P.; Marsano, E.; Perez-Rigueiro, J.; Elices, M.; Riekel, C.; Vendrely, C.; Guinea, G. V. Correlation between processing conditions, microstructure and mechanical behavior in regenerated silkworm silk fibers. *J. Polym. Sci., Part B: Polym. Phys.* **2012**, *50* (7), 455−465.
(90) Chung, D. E.; Um, I. C. Effect of Molecular Weight and Concentration on Crystallinity and Post Drawing of Wet Spun Silk Fibroin Fiber. *Fibers Polym.* **2014**, *15* (1), 153−160.
(91) Kim, H. J.; Um, I. C. Effect of degumming ratio on wet spinning and post drawing performance of regenerated silk. *Int. J. Biol. Macromol.* **2014**, *67*, 387−393.
(92) Zhou, H.; Shao, Z. Z.; Chen, X. Wet-spinning of regenerated silk fiber from aqueous silk fibroin solutions: Influence of calcium ion addition in spinning dope on the performance of regenerated silk fiber. *Chin. J. Polym. Sci.* **2014**, *32* (1), 29−34.
(93) Zhang, F.; Lu, Q.; Yue, X.; Zuo, B.; Qin, M.; Li, F.; Kaplan, D. L.; Zhang, X. Regeneration of high-quality silk fibroin fiber by wet spinning from $CaCl_2$-formic acid solvent. *Acta Biomater.* **2015**, *12*, 139−145.
(94) Chen, Z.; Zhang, H.; Lin, Z.; Lin, Y.; van Esch, J. H.; Liu, X. Y. Programing Performance of Silk Fibroin Materials by Controlled Nucleation. *Adv. Funct. Mater.* **2016**, *26*, 8978.
(95) Fang, G.; Huang, Y.; Tang, Y.; Qi, Z.; Yao, J.; Shao, Z.; Chen, X. Insights into Silk Formation Process: Correlation of Mechanical Properties and Structural Evolution during Artificial Spinning of Silk Fibers. *ACS Biomater. Sci. Eng.* **2016**, *2*, 1992.
(96) Teulé, F.; Furin, W. A.; Cooper, A. R.; Duncan, J. R.; Lewis, R. V. Modifications of spider silk sequences in an attempt to control the mechanical properties of the synthetic fibers. *J. Mater. Sci.* **2007**, *42*, 8974−8985.
(97) Brooks, A. E.; Stricker, S. M.; Joshi, S. B.; Kamerzell, T. J.; Middaugh, C. R.; Lewis, R. V. Properties of synthetic spider silk fibers based on Argiope aurantia MaSp2. *Biomacromolecules* **2008**, *9*, 1506−1510.
(98) An, B.; Hinman, M. B.; Holland, G. P.; Yarger, J. L.; Lewis, R. V. Inducing beta-sheets formation in synthetic spider silk fibers by aqueous post-spin stretching. *Biomacromolecules* **2011**, *12*, 2375−2381.
(99) Elices, M.; Guinea, G. V.; Plaza, G. R.; Karatzas, C.; Riekel, C.; Agulló-Rueda, F.; Daza, R.; Pérez-Rigueiro, J. Bioinspired Fibers Follow the Track of Natural Spider Silk. *Macromolecules* **2011**, *44*, 1166−1176.
(100) An, B.; Jenkins, J. E.; Sampath, S.; Holland, G. P.; Hinman, M.; Yarger, J. L.; Lewis, R. Reproducing natural spider silks' copolymer behavior in synthetic silk mimics. *Biomacromolecules* **2012**, *13*, 3938−3948.
(101) Gnesa, E.; Hsia, Y.; Yarger, J. L.; Weber, W.; Lin-Cereghino, J.; Lin-Cereghino, G.; Tang, S.; Agari, K.; Vierra, C. Conserved C-terminal domain of spider tubuliform spidroin 1 contributes to extensibility in synthetic fibers. *Biomacromolecules* **2012**, *13*, 304−312.
(102) Teulé, F.; Addison, B.; Cooper, A. R.; Ayon, J.; Henning, R. W.; Benmore, C. J.; Holland, G. P.; Yarger, J. L.; Lewis, R. V. Combining flagelliform and dragline spider silk motifs to produce tunable synthetic biopolymer fibers. *Biopolymers* **2012**, *97*, 418−431.

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237

(103) Adrianos, S. L.; Teulé, F.; Hinman, M. B.; Jones, J. A.; Weber, W. S.; Yarger, J. L.; Lewis, R. V. Nephila clavipes Flagelliform silk-like GGX motifs contribute to extensibility and spacer motifs contribute to strength in synthetic spider silk fibers. *Biomacromolecules* **2013**, *14*, 1751−1760.
(104) Lin, Z.; Deng, Q.; Liu, X.-Y.; Yang, D. Engineered large spider eggcase silk protein for strong artificial fibers. *Adv. Mater.* **2013**, *25*, 1216−1220.
(105) Heidebrecht, A.; Eisoldt, L.; Diehl, J.; Schmidt, A.; Geffers, M.; Lang, G.; Scheibel, T. Biomimetic Fibers Made of Recombinant Spidroins with the Same Toughness as Natural Spider Silk. *Adv. Mater.* **2015**, *27*, 2189−2194.
(106) Peng, Q.; Zhang, Y.; Lu, L.; Shao, H.; Qin, K.; Hu, X.; Xia, X. Recombinant spider silk from aqueous solutions via a bio-inspired microfluidic chip. *Sci. Rep.* **2016**, *6*, 36473.
(107) Andersson, M.; Jia, Q.; Abella, A.; Lee, X.-Y.; Landreh, M.; Purhonen, P.; Hebert, H.; Tenje, M.; Robinson, C. V.; Meng, Q.; Plaza, G. R.; Johansson, J.; Rising, A. Biomimetic spinning of artificial spider silk from a chimeric minispidroin. *Nat. Chem. Biol.* **2017**, DOI: 10.1038/nchembio.2269.
(108) Jin, H. J.; Fridrikh, S. V.; Rutledge, G. C.; Kaplan, D. L. Electrospinning Bombyx mori silk with poly(ethylene oxide). *Biomacromolecules* **2002**, *3* (6), 1233−1239.
(109) Kim, S. H.; Nam, Y. S.; Lee, T. S.; Park, W. H. Silk fibroin nanofiber. Electrospinning, properties, and structure. *Polym. J.* **2003**, *35* (2), 185−190.
(110) Ohgo, K.; Zhao, C. H.; Kobayashi, M.; Asakura, T. Preparation of non-woven nanofibers of Bombyx mori silk, Samia cynthia ricini silk and recombinant hybrid silk with electrospinning method. *Polymer* **2003**, *44* (3), 841−846.
(111) Sukigara, S.; Gandhi, M.; Ayutsede, J.; Micklus, M.; Ko, F. Regeneration of Bombyx mori silk by electrospinning - part 1: processing parameters and geometric properties. *Polymer* **2003**, *44* (19), 5721−5727.
(112) Zarkoob, S.; Eby, R. K.; Reneker, D. H.; Hudson, S. D.; Ertley, D.; Adams, W. W. Structure and morphology of electrospun silk nanofibers. *Polymer* **2004**, *45* (11), 3973−3977.
(113) Chen, C.; Cao, C. B.; Ma, X. L.; Tang, Y.; Zhu, H. S. Preparation of non-woven mats from all-aqueous silk fibroin solution with electrospinning method. *Polymer* **2006**, *47* (18), 6322−6327.
(114) Yoon, K.; Lee, H. N.; Ki, C. S.; Fang, D.; Hsiao, B. S.; Chu, B.; Um, I. C. Effects of degumming conditions on electro-spinning rate of regenerated silk. *Int. J. Biol. Macromol.* **2013**, *61*, 50−57.
(115) Weisman, S.; Haritos, V. S.; Church, J. S.; Huson, M. G.; Mudie, S. T.; Rodgers, A. J. W.; Dumsday, G. J.; Sutherland, T. D. Honeybee silk: recombinant protein production, assembly and fiber spinning. *Biomaterials* **2010**, *31*, 2695−2700.
(116) Zhang, J.-G.; Mo, X.-M. Current research on electrospinning of silk fibroin and its blends with natural and synthetic biodegradable polymers. *Frontiers of Materials Science* **2013**, *7* (2), 129−142.
(117) Zhang, F.; Zuo, B.; Fan, Z.; Xie, Z.; Lu, Q.; Zhang, X.; Kaplan, D. L. Mechanisms and Control of Silk-Based Electrospinning. *Biomacromolecules* **2012**, *13* (3), 798−804.
(118) Zhang, X.; Reagan, M. R.; Kaplan, D. L. Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv. Drug Delivery Rev.* **2009**, *61* (12), 988−1006.
(119) Singh, B. N.; Panda, N. N.; Pramanik, K. A novel electrospinning approach to fabricate high strength aqueous silk fibroin nanofibers. *Int. J. Biol. Macromol.* **2016**, *87*, 201−207.
(120) Takajima, T. *Advanced Fiber Spinning Technology*; Woodhead Publishing: Cambridge, U.K., 1994.
(121) Esselen, G. J. Production of Silk Fibers. US Patent US1934413 A, 1933.
(122) Mortimer, B.; Drodge, D. R.; Dragnevski, K. I.; Siviour, C. R.; Holland, C. In situ tensile tests of single silk fibres in an environmental scanning electron microscope (ESEM). *J. Mater. Sci.* **2013**, *48* (14), 5055−5062.
(123) Porter, D.; Guan, J.; Vollrath, F. Spider silk: super material or thin fibre? *Adv. Mater.* **2013**, *25* (9), 1275−1279.
(124) Hu, X.; Kaplan, D.; Cebe, P. Determining Beta-Sheet Crystallinity in Fibrous Proteins by Thermal Analysis and Infrared Spectroscopy. *Macromolecules* **2006**, *39* (18), 6161−6170.
(125) Hu, X.; Shmelev, K.; Sun, L.; Gil, E.-S.; Park, S.-H.; Cebe, P.; Kaplan, D. L. Regulation of Silk Material Structure by Temperature-Controlled Water Vapor Annealing. *Biomacromolecules* **2011**, *12* (5), 1686−1696.
(126) Guan, J.; Porter, D.; Vollrath, F. Thermally Induced Changes in Dynamic Mechanical Properties of Native Silks. *Biomacromolecules* **2013**, *14* (3), 930−937.
(127) Koyanagi, R.; Zhu, Z.; Asakura, T. Regenerated Bombyx mori silk fiber with enhanced biodegradability. *J. Insect Biotechnol. Sericol.* **2010**, *79* (1), 27−30.
(128) Teulé, F.; Miao, Y.-G.; Sohn, B.-H.; Kim, Y.-S.; Hull, J. J.; Fraser, M. J.; Lewis, R. V.; Jarvis, D. L. Silkworms transformed with chimeric silkworm/spider silk genes spin composite silk fibers with improved mechanical properties. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (3), 923−928.
(129) Willcox, P. J.; Gido, S. P.; Muller, W.; Kaplan, D. L. Evidence of a cholesteric liquid crystalline phase in natural silk spinning processes. *Macromolecules* **1996**, *29* (15), 5106−5110.
(130) Kinahan, M. E.; Filippidi, E.; Köster, S.; Hu, X.; Evans, H. M.; Pfohl, T.; Kaplan, D. L.; Wong, J. Tunable silk: using microfluidics to fabricate silk fibers with controllable properties. *Biomacromolecules* **2011**, *12*, 1504−1511.
(131) Martel, A.; Burghammer, M.; Davies, R.; DiCola, E.; Panine, P.; Salmon, J.-B.; Riekel, C. A microfluidic cell for studying the formation of regenerated silk by synchrotron radiation small- and wide-angle X-ray scattering. *Biomicrofluidics* **2008**, *2* (2), 024104.
(132) Martel, A.; Burghammer, M.; Davies, R. J.; Di Cola, E.; Vendrely, C.; Riekel, C. Silk Fiber Assembly Studied by Synchrotron Radiation SAXS/WAXS and Raman Spectroscopy. *J. Am. Chem. Soc.* **2008**, *130* (50), 17070−17074.
(133) Rammensee, S.; Slotta, U.; Scheibel, T.; Bausch, A. R. Assembly mechanism of recombinant spider silk proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 6590−6595.
(134) Rising, A.; Johansson, J. Toward spinning artificial spider silk. *Nat. Chem. Biol.* **2015**, *11* (5), 309−315.
(135) Guan, J.; Porter, D.; Vollrath, F. Silks cope with stress by tuning their mechanical properties under load. *Polymer* **2012**, *53* (13), 2717−2726.
(136) Yang, Y.; Kwak, H. W.; Lee, K. H. Effect of Residual lithium ions on the structure and cytotoxicity of silk fibroin film. *International Journal of Industrial Entomology* **2013**, *27* (2), 265−270.

DOI: 10.1021/acsbiomaterials.6b00669
ACS Biomater. Sci. Eng. 2017, 3, 226−237