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Silk-based biomaterials

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Abstract

Silk from the silkworm, *Bombyx mori*, has been used as biomedical suture material for centuries. The unique mechanical properties of these fibers provided important clinical repair options for many applications. During the past 20 years, some biocompatibility problems have been reported for silkworm silk; however, contamination from residual sericin (glue-like proteins) was the likely cause. More recent studies with well-defined silkworm silk fibers and films suggest that the core silk fibroin fibers exhibit comparable biocompatibility in vitro and in vivo with other commonly used biomaterials such as polylactic acid and collagen. Furthermore, the unique mechanical properties of the silk fibers, the diversity of side chain chemistries for 'decoration' with growth and adhesion factors, and the ability to genetically tailor the protein provide additional rationale for the exploration of this family of fibrous proteins for biomaterial applications. For example, in designing scaffolds for tissue engineering these properties are particularly relevant and recent results with bone and ligament formation in vitro support the potential role for this biomaterial in future applications. To date, studies with silks to address biomaterial and matrix scaffold needs have focused on silkworm silk. With the diversity of silk-like fibrous proteins from spiders and insects, a range of native or bioengineered variants can be expected for application to a diverse set of clinical needs.

Keywords: Silk; Sericin; Fibroin; Foreign body response; Suture; Tissue engineering; Biomaterial; Scaffold

1. Background

Silks are generally defined as protein polymers that are spun into fibers by some lepidoptera larvae such as silkworms, spiders, scorpions, mites and flies [1–3]. Silk proteins are usually produced within specialized glands after biosynthesis in epithelial cells, followed by secretion into the lumen of these glands where the proteins are stored prior to spinning into fibers. Silks differ widely in composition, structure and properties depending on the specific source. The most extensively characterized silks are from the domesticated silkworm, *Bombyx mori,* and from spiders (*Nephila clavipes* and *Araneus diadematus*). Many of the more evolutionarily advanced spiders synthesize different types of silks. Each of these different silks has a different amino acid composition and exhibits mechanical properties tailored to their specific functions: reproduction as cocoon capsular structures, lines for prey capture, lifeline support (dragline), web construction and adhesion.

Fibrous proteins, such as silks and collagens, are characterized by a highly repetitive primary sequence that leads to significant homogeneity in secondary structure, i.e., triple helices in the case of collagens and β-sheets in the case of many of the silks. These types of proteins usually exhibit important mechanical properties, in contrast to the catalytic and molecular recognition functions of globular proteins. Because of these impressive mechanical properties, this family of proteins provides an important set of material options in the fields of controlled release, biomaterials and scaffolds for tissue engineering. The relative environmental stability of these families of proteins, in comparison to globular proteins, in combination with their biocompatibility, unique mechanical properties, and options for genetic control to tailor sequence provides an important basis to exploit these natural proteins for biomedical applications.

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1.1. Silkworm silk

Silkworm silk has been used commercially as biomedical sutures for decades, and in textile production for centuries. The silk from the cocoon of *B. mori* contains at least two major fibroin proteins, light and heavy chains, 25 and 325 kDa, respectively. These core fibers are encased in a sericin coat, a family of glue-like proteins that holds two fibroin fibers together to form the composite fibers of the cocoon case to protect the growing worm. This structural arrangement contrasts with spider silks where these glue-like proteins are generally absent. Silkworm cocoon silk production, known as sericulture, produces high yields since the larvae can be maintained in high densities. The core sequence repeats in the fibroin heavy chain from *B. mori* include alanine–glycine repeats with serine or tyrosine.

1.2. Spider silks

Spider silks have not been commercialized for biomedical applications primarily due to the predatory nature of spiders and the relatively low levels of production of these silks when compared to silkworm cocoon silk. The molecular weights of spider silk proteins vary depending on source but can range from 70 to 700 kDa depending on the method of analysis. The dragline silk from *N. clavipes* is characterized by polyalanine and glycine–glycine-X regions, where X is often tyrosine, glutamine or leucine. Genetic engineering is being actively explored to construct, clone and express native and synthetic genes encoding recombinant spider silk proteins to overcome limits to use of the native organisms. This strategy has provided new opportunities in fundamental studies of spider silk genetics, silk protein structure and function, and materials processing [2,4–6]. In general, interest in spider silk has increased in recent years due to the differences in mechanical properties when compared to silkworm silk and the presence of the multi-gene family encoding this group of silks as a basis for the study of protein structure-function relationships [4,6–11].

During the past 10 years a great deal of progress has been made in understanding silk genetic and protein structures. Cloning and expression of native and synthetic silks has been achieved in a variety of host systems. The sequences of cDNAs and genomic clones encoding spider silks illustrate the highly repetitive structures [4,6,8–11], which can be readily exploited to construct genetically engineered spider silk-like proteins using synthetic oligonucleotide versions of the consensus repeats or variants of these repeats. This highly repetitive sequence was also recently fully described for the fibroin heavy chain from the silkworm, *B. mori* [12]. The unique organization of the family of silk genes in more advanced spiders provides a fertile area for the exploration of structure-function relationships in protein design. For example, the dragline spider silk from the golden orb weaver *N. clavipes* displays impressive toughness, and a balance of stiffness, strength and extensibility reflecting the native function of the silk orb web construction [7,13,14]. Transgenic expression of spider silks in plants (tobacco and potato) and mammalian epithelial cells has been reported [15,16] and may point the way toward more substantive production of these proteins in the future.

Furthermore, since native and genetically engineered versions of the silks tend to self-assemble into microfibrils, causing precipitation and leading to loss of protein during purification [3,17], alternative methods to maintain solubility of the recombinant versions of these proteins have been explored. For example, tweaking the native or consensus sequences of spider silks to include encoded 'triggers' to regulate molecular-level assembly of the proteins has been demonstrated [17–21]. Chemical redox triggers, or a kinase recognition site for biochemical phosphorylation, flanking the β-sheet forming regions of the proteins have been demonstrated. These modifications encoded in the synthetic gene designs enabled improved control over solubility of the genetically engineered silk protein.

1.3. Properties of silk

The enhanced environmental stability of silk fibers in comparison to globular proteins is due to the extensive hydrogen bonding, the hydrophobic nature of much of the protein, and the significant crystallinity. Silks are insoluble in most solvents, including water, dilute acid and alkali. Detailed structural analysis of spider dragline silk proteins has yielded information on the organization and orientation of the numerous but very small β-sheet crystals in the fibers, and a high level of organization of the protein even in the less crystalline domains [2]. Liquid crystalline phases and conformational polymorphism have been implicated in the biological processing of these proteins to contribute to the architectural features within the fibers [22–24]. These nanoscale features, factoring in the small, orientated and numerous β-sheet crystals, a fuzzy interphase between these crystals and the less crystalline domains, and the shear alignment of the chains, provides a basis for the origin of the novel mechanical properties exhibited by silk fibers. A comparison of mechanical properties (Table 1) suggests that they provide a remarkable combination of strength and toughness. The distinguishing features of the spider silks are the very high strength in combination with excellent elasticity in comparison with these other biomaterials. In addition, these fibers display resistance to failure in compression that distinguishes them from other high performance fibers, such as Kevlar [13].

Table 1
Comparison of mechanical properties of common silks (silkworm and spider dragline) to several types of biomaterial fibers and tissues commonly used today

Material	UTS (MPa)	Modulus (GPa)	% Strain at break	Authors
B. mori silk (w/ sericin)[a]	500	5–12	19	Perez-Rigueiro et al. [68]
B. mori silk (w/o sericin)[b]	610–690	15–17	4–16	Perez-Rigueiro et al. [68]
B. mori silk[c]	740	10	20	Cunniff et al. [13]
Spider silk[d]	875–972	11–13	17–18	Cunniff et al. [13]
Collagen[e]	0.9–7.4	0.0018–0.046	24–68	Pins et al. [69]
Collagen X-linked[f]	47–72	0.4–0.8	12–16	Pins et al. [69]
PLA[g]	28–50	1.2–3.0	2–6	Engelberg and Kohn [70]
Tendon (comprised of mainly collagen)	150	1.5	12	Gosline et al. [71]
Bone	160	20	3	Gosline et al. [71]
Kevlar (49 fiber)	3600	130	2.7	Gosline et al. [71]
Synthetic Rubber	50	0.001	850	Gosline et al. [71]

[a] *Bombyx mori* silkworm silk—determined from bave (multithread fibers naturally produced from the silk worm coated in sericin).
[b] *Bombyx mori* silkworm silk—determined from single brins (individual fibroin filaments following extraction of sericin).
[c] *Bombyx mori* silkworm silk—average calculated from data in Ref. [13].
[d] *Nephila clavipes* silk produced naturally and through controlled silking.
[e] Rat-tail collagen Type I extruded fibers tested after stretching from 0% to 50%.
[f] Rat-tail collagen dehydrothermally cross-linked and tested after stretching from 0% to 50%.
[g] Polylactic acid with molecular weights ranging from 50,000 to 300,000.

2. Utility in biomedical applications

B. mori silkworm silk fibers have been the primary silk-like material used in biomedical applications particularly as sutures. During decades of use, silk fibers have proven to be effective in many clinical applications. At the same time, some biological responses to the protein have raised questions about biocompatibility. One of the major difficulties in assessing the biological responses reported to these silk fibers is the absence of detailed characterization of the fibers used including, extent of extraction of the sericin, the chemical nature of wax-like coatings sometimes used, and many related processing factors. This variability in source material has resulted in confusion in the literature and in clinical settings concerning the benefits or potential concerns with this class of fibrous protein. For example, of greatest importance is that based on many studies it is clear that the sericin glue-like proteins are the major cause of adverse problems with biocompatibility and hypersensitivity to silk (Table 2). If sericin is removed, the biological responses to the core fibroin fibers appear to be comparable to most other commonly used biomaterials (Table 3). An additional major misconception revolves around silk's classification as non-degradable. There is clear evidence in the literature that silk, as a protein, is susceptible to proteolytic degradation in vivo and over longer time periods in vivo will slowly be absorbed.

3. In vivo biocompatibility of virgin silk

Despite centuries of use as sutures [25], silk matrices are now being rediscovered and reconsidered as potentially useful biomaterials for a range of applications in clinical repairs and in vitro as scaffolds for tissue engineering [26]; some benefits and concerns of silk are detailed in Table 4. Silk has been used most extensively as sutures for wound ligation and became the most common natural suture surpassing collagen (Catgut™ or Chromic Catgut™, cross-linked collagen) used in the biomedical industry over the past 100 years [25]. During the past 20 years, a variety of degradable synthetic materials including polyglycolic acid (PGA)(Dexon™), co-polymers of PGA (polyglyconate)(Maxon™ or gylcolide and trimethylene carbonate), and co-polymers of PGA and polylactic acid (Vicryl™ or polyglactin 910, a co-polymer of 90% glycolide and 10% L-lactide), polydioxanone (PDS), and non-degradable synthetics including braided polyester (Ethibond™ or Mersilene™), nylon (Ethilon™), Teflon coated polyester (Tevdek™) and polypropylene (Prolene™ or Surgilene™) have dominated the suture market. In general, sutures should be strong, handle easily, and form secure knots [25]. Sutures require the following characteristics for general surgical applications [27]:

1. Tensile strength—to match the clinical repair.
2. Knot strength—the amount of force required to cause a knot to slip.
3. Elasticity—the ability to conform to the current stage of wound repair.
4. Memory—change in stiffness over time; the better the suture, the less memory.
5. Degradability—ability to be metabolized by the host once its repair function has been completed.
6. Tissue Reactivity—non-irritant.

Table 2
Biological responses to virgin silk fibers

Type of silk[a]	Response	In vivo or in vitro model/tissue site	Authors
?	Mucosal edema was greatest around silk and chromic collagen compared to PGA	Dog/genitourinary tract	Morrow et al. [38]
?	Chronic inflammation including granulosous and fibrosis	Rabbit/median or sciatic nerve ligation	Nebel et al. [39]
Virgin	(a) Acute and chronic inflammation initially including conjunctival and episcleral hyperemia progressing to chemosis and nodular episcleritis, peripheral corneal ulceration and wound necrosis (b) Sensitization to virgin silk during bilateral cataract surgery following the use of virgin silk on the first eye	Human/ocular cataract surgery	Soong and Kenyon [34]
Black braided	No comparable suture reactions	Human/ocular cataract surgery	Soong and Kenyon [34]
Virgin	IgE obtain from sera isolated from 8 of 9 patients with allergy to silk bound specifically to sericin and was negative towards fibroin	In vitro immunoblot of IgE and IgG isolated from sera of 9 persons allergic to silk	Dewair et al. [32]
?	(a) Acute and chronic inflammation (b) However, the response was not significantly different from Vicryl™, Chromic Catgut, Tevdek™ and polypropylene	Rabbit/tracheal anastomosis	Peleg et al. [40]
?	Severe delayed chronic inflammatory reaction to suture	Three human pediatric patients/ neurosurgery	Rossitch et al. [31]
Virgin	Delayed hypersensitivity to virgin silk resulting in asthma	Children <15 yr old/skin tests	Wen et al. [29]
Virgin	(a) 90% of patient population developed an allergic reaction to silk extract (sericin); none of the control group was allergic (b) IgE from 41% of the patient population sera bound specifically to sericin in in vitro testing	41 humans with asthma and a clinical history of silk allergy; 4 control patients with no allergy to silk/sera and skin tests were used.	Zaoming et al. [33]
?	(a) Type I allergy response to black braided silk (b) Patient sensitized to silk 7 years prior in an independent surgical procedure	Single case in a human/trachea and throat region	Kurosaki et al. [30]

[a] ? = Type of silk used was not specified, e.g., virgin silk or black braided; Virgin = virgin silk containing sericin; Black braided = black braided extracted silk fibroin (e.g., no sericin) coated in waxes or silicone.

7. Free from infection—related to the material's geometry, e.g., multifilament vs. monofilament.

Processing methods for virgin silk (fibroin containing sericin gum) were developed in industry to extract sericin from the inner silk fibroin fibers. As sutures, the silk fibroin fibers are usually coated with waxes or silicone to enhance material properties and reduce fraying; these sutures are commonly referred to as black braided silk (e.g., Perma-Hand™). The transition from virgin silk to black braided sutures occurred in the late 1970s and early 1980s; however, virgin silk is still commercially available today.

Virgin silk, like most proteins, is a potential allergen [28,29] causing a Type I allergic response in some cases [30]. Delayed allergic responses to silk (an average of 10 months after initial exposure) induced some patient complications including asthma and specific upregulation in IgE levels [29,31]. Wen et al. [29] showed the extracted sericin was responsible for sensitization (the development of a T-cell mediated allergic response) by skin testing of 64 children with asthma. Further biochemical analysis by Dewair et al. [32] and Zaoming et al. [33] concluded that upregulated IgEs were produced in response to the sericin, clarifying the role of these fibroin contaminants as the main allergenic agent in silk and not the core fibroin fibers. Fibroin degradation products could also potentially be involved in adverse biological responses. For example, one case was reported where fibroin (black braided silk) may have induced a Type I allergic reaction [30]. However, the authors clarified later that there were no cases in the literature that implicated black braided silk as inducer of IgE hypersensitivity reactions. In addition, in the preparation of this review no references were found in the literature that implicated black braided silk in inducing hypersensitivity and allergic reactions. Kurosaki et al. [30] concluded that the hypersensitivity in their single case may have been the result of patient sensitization to silk from a prior surgical procedure 7 years earlier. The type of silk used in this earlier exposure was unknown (e.g., virgin vs. black braided).

Table 3
Common sutures that elicit a foreign body response following implantation in vivo

Suture trade name/material	Model/implantation site	Absorbable[a]	Foreign body response	Filament type[b]	Authors
Surgical control	Rat/	—	8.2% (7.3–10.6)	—	[c]Foschi et al. [43]
Vicryl™ (polyglactin 910)	Abdominal cavity	Yes; <60 days	8.7% (7.1–9.9)	Multi	
Catgut™ (Collagen)		Yes; <60 days	9.48% (8.3–15)	Multi	
Polyglycolic acid salt (PGA)		Yes; <60 days	11.25% (9.5–12.7)	Multi	
Silk (assumed black braided)		*Yes; >60 days*	*14.2% (10.9–22.3)*	*Multi*	
Titanium (metal clip)		No	15.8% (12.1–19.1)	Mono	
Extruded Teflon™	Rabbit/	No	Mild (1)	Mono	[d]Setzen et al. [44]
Prolene™ (polypropylene)	Subcutaneous	No	Mild (1)	Mono	
Surgilene™ (polypropylene)		No	Mild (1)	Mono	
PDS™ (polydioxanone)		Yes; <60 days	Moderate (2)	Multi	
Vicryl™		Yes; <60 days	Moderate (2)	Multi	
Silk (black braided)		*Yes; >60 days*	*Moderate (2)*	*Multi*	
Nylon		No	Moderate (2)	Mono	
Ethibond™ (braided polyester)		No	Moderate (2)	Multi	
Mersiline™ (braided polyester)		No	Moderate (2)	Multi	
Tevdek™ (Teflon coated polyester)		No	Moderate (2)	Multi	
Chromic Catgut™ (cross linked collagen)		Yes; <60 days	Extensive (3)	Multi	
Nylon	Rat/	No	Minimal	Mono	[e]Bucknall et al. [45]
Nylon	Abdominal incision	No	Minimal	Multi	
PGA		Yes; <60 days	Minimal	Multi	
Silk (black braided)		*Yes; >60 days*	*Moderate*	*Multi*	

[a]Absorbable is defined as the loss of tensile strength within 60 days in vivo, but does not reflect the persistence of a foreign body response (FBR) for greater than 60 days.

[b]Monofilament sutures contain a single fiber and multifilament sutures contain multiple fibers increasing material surface area in vivo and the potential risk of infection (i.e., more places for contamination to hide in multifilament sutures).

[c]FRB was characterized by the percent growth of neovessels (angiogenesis) around the suture 7 days post-implantation.

[d]FBR was characterized on a 3 level scale 30 days post-implantation: Mild (1) included the presence of histiocytes and foreign-body giant cells, Moderate (2) indicates histiocytes and foreign-body giant cells, and cluster of lymphocytes and a few plasmacytes, and Extensive (3) includes histiocytes, foreign-body giant cells, and diffuse, scattered lymphocytes and plasmacytes.

[e]FBR was characterized histologically 10 days in vivo; however, the authors do not provide a definition of 'minimal' or 'moderate' inflammation and provide no means by which they systematically characterize the response histologically.

Table 4
Benefits and concerns with the use of silks for biomedical applications

Benefits
Novel mechanical properties of some silks that are superior to any other natural fiber and rival many high performance fibers
Natural fiber with a long standing history of use in clinical applications
The ability to process silks in aqueous solutions for subsequent formation of films and other material formats, with relatively simple insolubilization via exposure to alcohols and other environmental factors
Easily chemically modified with surface decorations, such as adhesion sites or cytokines, due to the availability of amine and acid side chains on some of the amino acids
Genetically tailorable composition and sequence to moderate specific features, such as molecular weight, crystallinity, solubility
Slow rates of degradation in vitro and in vivo, this is particularly useful in biodegradable scaffolds in which slow tissue ingrowth is desirable
No known risk of bioburden
Concerns
Adequate removal of contaminating sericin from silkworm silk to avoid biocompatibility problems
Slow degradation of crystalline (β-sheet) regions
Aborted proteolytic attack by macrophages and giant cells leading to encapsulation and the formation of a granuloma
Potential sensitization to silk fibroin resulting in an allergic response upon exposure to the biomaterial

In other cases where the type of silk suture was unknown [31], silk induced a delayed allergic reaction that necessitated the removal of the suture. The authors did not distinguish between the use of virgin silk containing sericin versus black braided silk. However, based on the time frame of the surgical procedures (1970s) and the severity of the reaction, sericin can be considered as the most likely cause of the response. Soong and Kenyon [34] detailed 12 patients from 1980 to 1983 that reported severe reactions to virgin silk sutures following cataract surgery. No comparable reactions were noted when black-braided silk sutures were used. Further, they provided evidence of patient sensitization to virgin silk in bilateral surgical procedures; an allergic reaction developed against virgin silk following its use in the first eye procedure.

The use of virgin silk during the 1960s to the early 1980s negatively impacted the general acceptance of this biomaterial from the surgical practitioner perspective [35–37]. For example, Morrow et al. [38] observed silk and collagen (catgut) to be moderately more reactive than polyglycolic acid (PGA) in the genitourinary tract following 3 weeks of implantation in vivo, although again the type of silk used in the study was not identified. Nebel et al. [39] observed cellular infiltration, granulosous and fibrosis for silk sutures that persisted for more than 4 weeks compared to autologous collagen sutures used for peripheral nerve ligation in the rabbit. Again the specific type of silk was not described. Of note in the study was that inflammation was comparable to chromic collagen sutures. Peleg et al. [40] drew a similar conclusion regarding the inflammatory potential of silk used in tracheal anastomosis in the rabbit without identifying the type of silk used. Of interest was a similar acute and chronic inflammatory response to Vicryl^{TM} and Teflon^{TM} coated polyester sutures in the study.

4. Foreign body response to silk

All biomaterials derived from a non-autologous source will elicit some level of foreign body response (FBR) following implantation in vivo. Absorbable and non-degradable biomaterials generally do not induce a T-cell mediated hypersensitivity immune response (as was observed with sericin) [30]. However, while rare, sensitization to fibroin due to pre-exposure or a failed phagocytic response can result in the formation of a granuloma years after the implantation of black braided silk [30]. Some common materials that have been shown to induce a FBR are detailed in Table 3.

Biomaterial characteristics, including implantation site, size, geometry [41], and surface topography can influence the level of the foreign body response [42]. This response can be predicted in part based on the surface to volume ratio of the biomaterial [42]. This relationship is frequently not addressed in the relative comparison between different suture types in vivo. Several studies have shown black braided silk to elicit a greater FBR when compared to other commonly used sutures [43–45]. However, a single fiber of native silk is composed of several individual fibroin filaments and a single black braided suture comprises several extracted silk fibers. No studies have attempted to normalize the FBR to the suture surface area, rather equating sutures based on their final diameter. Thus studies that report silk as inducing a heightened FBR may not be reflective of the inflammatory potential of the silk but rather a manifestation of the overall suture structure and geometry.

Silk sutures induced angiogenesis, a limiting step in mounting a foreign body response, in the rat's mesenteric window 7 days post-implantation to an lesser extent as titanium and to a greater extent than collagen, polyglactin, and PGA; however the differences

were not statistically significant ($p > 0.05$) (Table 3) [43]. The type of silk suture (virgin versus black braided) was not described but it is assumed to be black braided based on the year of study. A curiosity about the study was the choice to close all the wounds of all study groups with silk suture, potentially sensitizing the animals to the silk suture under investigation. Bucknall et al. [45] examined four sutures in abdominal wound closure in a rat model 10, 30, and 70 days post-implantation. Histological examination revealed silk elicited a 'moderate' zone of inflammation compared to a 'minimal response' induced by multi- and monofilament nylon and multifilament PGA (Table 3). No histological criteria or ranking system was provided. However, the authors noted that even monofilament nylon, thought to be the most biocompatible of the materials evaluated, induced fibrous encapsulation 10 days post-implantation. Of interest was the finding that PGA, despite a 92% decrease in tensile strength 30 days post-implantation continued to elicit a FBR over 70 days in vivo, the final time point of the study.

Setzen et al. [44] in a comprehensive blind study, statistically analyzed tissue responses to 11 types of sutures subcutaneously implanted in the rabbit (Table 3). Responses were based on the number of giant cells present in the fibrous capsule and within the individual filaments of the sutures, and the thickness of the fibrous capsule surrounding the suture. Evaluations were performed 30, 60 and 120 days post-implantation. The inflammatory response surrounding the encapsulation was graded on a scale of 1–3 based on the type of cells and the number of vessels found. Silk elicited an equivalent response to absorbable sutures and multifilament non-absorbable sutures when evaluating the number of foreign body cells surrounding each suture 30 days post-implantation. Non-degradable Ethibond™ (braided polyester) and Tevdek™(Teflon coated polyester) induced a significantly greater accumulation of cells at the wound site compared to the group of sutures under investigation. However, when assessing the thickness of the fibrous capsule, black braided silk as well as Ethibond™ induced the formation of significantly thicker capsules compared to the mean response of the group. Histological analysis 30 days after implantation showed the tissue response to the silk was equivalent to Vicryl™ and PDS™ (two common synthetic absorbables), nylon (monofilament), and Ethibond™, Mersilene™ and Tevdek™ (three common multifilament non-degradables), and less than cross-linked collagen (Chromic Catgut™). The foreign body response to Vicryl™ lasted in excess of 120 days in vivo, long after the loss of tensile integrity. In conclusion, Setzen et al. [44] confirmed that the inflammatory response induced by silk was no greater than that in response to common absorbable sutures 30 days post-implantation in a subcutaneous rabbit in vivo model (Table 3). Furthermore, they found a significantly higher response to multifilament sutures than monofilament, confirming the influence of higher surface area to volume ratio as indicative of induction of a greater inflammatory response.

As a suture, silk is still popular in ocular, neural and cardiovascular surgery, but has also been used in a variety of other tissues in the body. Silk's knot strength, handling characteristics and ability to lay low to the tissue surface make it a popular suture in cardiovascular applications where bland tissue reactions are desirable for the coherence of the sutured structures [36]. However, black braided silk is thrombotic following implantation within ateriovessels [46,47]. Using a rat model, Dahike et al. [47] showed black braided silk binds fibrin and platelets within 3 days in vivo following implantation into arteriovessels. Within 7 days, silk was encapsulated by a dense thrombus consisting of platelets and lymphocytes, erythrocytes, and granulocytes. From 14 to 28 days, the gradual decline of the thrombus and the development of a new endothelium layer was observed. By 28 days in vivo, silk was completely endothelialized, indicating the presence of regenerated endothelial tissue. While silk was initially thrombotic (e.g, 3 and 7 days post-implantation) when compared to Prolene™, Ethilon™, Vicryl™ and Mersilene™, by 28 days in vivo, silk was less so when compared to both Ethilon™ and Vicryl™. At 56 days post-implantation only traces of thrombogenicity were detected with black braided silk by the presence of loosely bound platelets to the neoendothelium.

The ability of black braided silk to induce thrombosis immediately following implantation in blood flow may be due to the surface properties and ability to bind proteins in the clotting cascade such as fibrin. Altering the surface properties of extracted fibroin silk (i.e., eliminating the wax coating) significantly diminished the initially high thrombotic response to silk [48]. Sakabe et al. [49] coated polyester suture with solubilized fibroin and demonstrated that the reconstituted fibroin protein did not induce significant thrombogenicity in vivo. In vitro studies performed by Santin et al. [50] confirmed the ability of silk fibroin films (extracted sericin-free fibroin dissolved in LiBr salt, dialyzed in dIH_2O, cast and made insoluble with methanol) to bind fibrinogen as a mechanism by which the alternative complement pathway is activated in vivo. However, compared to two reference materials, polystyrene and poly(2-hydroxyethyl methacrylate), fibroin films bound fibrinogen to a lesser extent [50]. C3 and IgG, components of the human plasma complement system bound similarly when the silk was compared to the reference materials. The authors concluded that C3 binding may be the result of specific binding to hydrophobic patches (e.g., β-sheets) of fibroin. When compared to the reference materials after 24 h in vitro, diminished interleukin-1β

production from mononuclear cells isolated from human plasma incubated with fibroin was observed [50].

Uff et al. [51] examined the effects of soluble factors from sutures on macrophage activation (e.g., attachment, phagacytosis and the production of lysozyme and proinflammatory tumor necrosis factor (TNF)) in vitro. Soluble factors were acquired by subjecting sterile sutures to ultrasound for 1 h in detergent. Soluble factors from silk were found to elicit the highest phagocytic response from macrophages when compared to catgut, PDS™, steel, nylon and Vicryl™. However, Vicryl™ induced the highest response when characterizing macrophage adherence and TNF production. PDS™ induced the highest production of lysozyme of the group of sutures extracted. The authors failed to characterize the soluble factors produced by sonification of the suture materials. In the case of silk, the resulting response from macrophages could be due to a variety of factors including residual sericin, waxes or silicones used in manufacture of the sutures, or the specific size of fibroin particles or crystals potentially generated. Thus, the response may be completely independent of actual silk fibroin material properties and rather a function of the coating and/or geometry and size of particles produced.

5. Silk degradation

According to the US Pharmacopeia an absorbable (suture) biomaterial is defined as one that 'loses most of its tensile strength within 60 days' post-implantation in vivo. Within this definition, silk is correctly classified as non-degradable. However, according to the literature, silk is degradable but over longer time periods due to proteolytic degradation usually mediated by a foreign body response [31,34,41,52]. Several studies detail variable rates of silk absorption in vivo dependent on the animal model and tissue implantation site (Table 5). In general, silk fibers lose the majority of their tensile strength within 1 year in vivo, and fail to be recognized at the site within 2 years [53].

Silk studies in vitro have demonstrated that proteases such as chymotrypsin will cleave the less-crystalline regions of the protein to peptides which are then capable of being phagocytosed for further metabolism by the cell. Furthermore, protease cocktails [54] and chymotrypsin (known to be produced by macrophages) are capable of enzymatically degrading silk [55]. Of interest, the silkworm *B. mori* produces a protease inhibitor in the silk gland embedding it within the silk cocoon for protection against premature proteolytic degradation [56].

In a comparative study of six absorbable and four non-degradable sutures implanted circumferentially under the skin of rats, silk lost 55% of its tensile

Table 5
Evidence of silk degradation in vitro and in vivo

Type of silk[a]	In vivo/in vitro	Mechanism	Degree and measure of degradation	Author
Extracted fibroin film	In vitro	Proteolytic degradation	~10% weight loss 5 days following enzymatic digestion	Minoura et al. [54]
Unknown/assumed black braided	Rat/subcutaneous	Unknown/assumed foreign body response	55% loss in tensile strength 6 weeks in vivo	Greenwald et al. [57]
Black braided	Rat/subcutaneous	Unknown/assumed foreign body response	83% loss in tensile strength 10 weeks in vivo	Bucknall et al. [45]
Unknown/assumed black braided	Rat/abdominal wall muscle	Foreign body response (proteolytic degradation)	Fragmentation at 6 weeks; not detected at 24 weeks	Lam et al. [41]
Black braided	Rabbit/cornea, sclera and ocular muscle	Foreign body response (proteolytic degradation)	Reduced number of filaments and diameter at 42 days; absorption at 90 days in vivo	Salthouse et al. [52]
Unknown/assumed virgin silk	Rabbit/abdominal wall muscle	Foreign body response (proteolytic degradation)	80% decrease in tensile strength at 12 weeks; 0% strength at 2 years; decrease in the number of fibers observed histologically; fragmentation following 4 weeks in vivo	Prostlethwait [36]

[a] Type of silk: virgin = raw silk containing sericin; black braided = extracted silk fibroin fibers (e.g., no sericin) coated in waxes or silicone; fibroin film = extracted and solubilized fibroin cast and insolubilized with methanol.

Table 6
Material properties of 10 common sutures following 6 weeks of subcutaneous implantation in a rat model. Values at time 0–6 weeks taken from Table 1 of Ref. [57]

Suture name	Strength (N/m^2)[a]			Tangent elastic modulus (N/m^2)[b]		
	0 Weeks	6 Weeks	% Decrease	0 Weeks	6 Weeks	% Decrease
Vicryl™ (polyglactin 910)	1.42 E14	Fragmented	~100	3.00 E8	—	100
Dexon™ (polyglycolic acid)	3.46 E14	Fragmented	~100	2.62 E8	—	100
Catgut™ (collagen)	2.30 E14	Fragmented	~100	1.41 E8	—	100
Chromic Catgut™ (cross-linked collagen)	2.28 E14	Fragmented	~100	1.56 E8	—	100
PDS™ (polydioxanone)	7.20 E14	9.72 E13	86.5	9.87 E7	7.13 E7	85.8
Maxon™(polyglyconate)	5.96 E14	8.29 E13	86.1	1.00 E8	7.4 E7	26^{TM}
Silk[c]	*8.48 E13*	*3.80 E13*	*55.2*	*2.42 E8*	*2.03 E8*	*16.1*
Ethilon™ (nylon)	4.57 E14	2.54 E14	44.4	1.04 E8	1.07 E8	0
Prolene™ (polypropylene)	4.02 E14	2.99 E14	25.6	1.00 E8	1.01 E8	0
Ethibond™ (polyester)	2.49 E14	2.32 E14	6.8	2.47 E8	2.30 E8	6.9

[a]Strength of initial suture prior to implantation (0 weeks) and at 6 weeks following in vivo circumferential subcutaneous implantation in a rat model. Fragmented sutures that were unable to be harvested following implantation at 6 weeks were assumed to have lost 100% of their tensile integrity.
[b]Elastic modulus of sutures (N/m^2) prior to implantation and following 6 weeks in vivo.
[c]Type of silk (e.g., virgin or black braided) was not described. It is assumed to be black-braided silk.

Table 7
Percent loss in suture mechanical tensile strength over time subcutaneously implanted in a rat model. Durability data was taken from Table 3 of Ref. [45]

Suture	Time post-implantation		
	10 days	30 days	70 days
Polyglycolic acid	65%	92%	99%
Silk, black braided	*29%*	*73%*	*83%*
Multifilament nylon	16%	19%	22%
Monofilament nylon	3%	7%	16%

strength and 16% of its elastic modulus 6 weeks post-implantation [57] (Table 6). In a rat model with subcutaneous implantation, silk fibers lost 29% of their tensile strength at 10 days, 73% at 30 days and 83% 70 days post-implantation (Table 7) [45]. In abdominal wound closures in the rat, silk promoted a moderate foreign body response compared to mono- and multi-filament nylon and polyglycolic acid (PGA) sutures [45]. However, common to all sutures, inflammation was required as the main mechanism for degradation.

Lam et al. [41] described silk as biodegradable due to its susceptibility to proteolytic enzymes. The study compared dry-spun hot-drawn poly(L-lactic acid) (PLLA) fibers to several absorbable and non-degradable sutures in the muscle layer surrounding the abdomen of rats. PLLA, PDS™, Vicryl™, and black braided silk showed signs of degradation after 2 weeks in vivo as determined by scanning electron microscopy. All sutures, in addition to monofilament nylon, provoked a chronic inflammatory response at 2 weeks. At 6 and 12 weeks in vivo, the authors were unable to retrieve silk due to fragmentation. At 24 weeks, neither silk nor inflammation was observed in vivo most likely due the rapid degradation of silk by proteolytic enzymatic digestion. Inflammation subsided by 6 weeks in vivo for Vicryl; a chronic inflammatory response was present surrounding PLLA at 80 weeks in vivo, the final time point of the study.

In general, silk is slowly absorbed in vivo. The rate of absorption is dependent upon the implantation site, mechanical environment, and variables related to the health and physiological status of the patient, the type (virgin silk versus extracted black braided fibroin), and the diameter of the silk fiber [31,34,52]. Furthermore, alterations in silk processing may cause conformational changes in the protein structure potentially increasing or decreasing susceptibility to degradation. None of these variables has been studied in detail; therefore, it is difficult to gain a clear understanding of the relationships between structure, processing and degradability. Regardless, silk protein fibers will degrade in vivo; rates are variable depending on the factors listed above.

It should be recognized that amyloid-like deposits in Alzheimer's and related diseases are often associated with β-sheet structures, particularly cross-beta crystals [58]. Similar structures are also implicated in membrane-associated cytotoxicity [58]. It is unknown if silks, such as those from silkworm, spiders or genetic variants thereof, will negatively influence biological function in a similar mode as these amyloid systems. This issue should be addressed over time as tissue-engineering studies move forward. The outcomes of these studies would influence the types of tissue applications that should be addressed with silk. It is encouraging that no amyloid-related problems have been reported throughout the literature to date associated with silk fibers, despite their extensive use in the clinical setting for many centuries. As the use of silk-based biomaterials broadens, presumably the ASTM would be involved to establish standard guidelines for this type of assessment.

6. Scaffolds for tissue engineering

The emergence of tissue engineering has increased the demand for a diverse portfolio of biomaterials to support the development of tissues in vitro prior to implantation in vivo. The biomaterial or matrix plays a key role in communicating or transducing environmental cues to cells seeded within or on the matrix. In essence, the matrix acts as the translator between the local (e.g., mechanical) environment (either in vitro or in vivo) and the developing tissue, aiding in the development of biologically viable functional tissue. The matrix must support cell attachment, spreading, growth and differentiation. In most instances it is advantageous if the matrix degrades into biocompatible fragments or monomers capable of being metabolized by host cells; however, the rate of degradation must match or be less than the rate of tissue ingrowth and development. This balance assures appropriate mechanical and physiological compatibility during integration of the host and/or implanted tissue in vivo. A mismatch in these rates can lead to premature failure of the tissue. Silk fibroin offers versatility in matrix scaffold design for a number of tissue engineering needs (Table 8) in which mechanical performance and biological interactions are major factors for success, including bone, ligaments, tendons, blood vessels and cartilage. Silk fibroin can be processed into foams, films, fibers and meshes.

Inouye et al. [59] demonstrated the utility of a silk fibroin film in culturing animal cells (SE1116, human colon adenocarcinoma; KB, human mouth epidermoid carcinoma; Colo201, human colon adenocarcinoma; QG56, human lung carcinoma) in comparison to a collagen matrix. Films of both fibroin and collagen supported equivalent cell growth after 5 days in cell culture conditions. Minoura et al. [26] compared the ability of fibroin and sericin films, as well as a control, collagen (cast bovine acid soluble) film, in supporting attachment, spreading and growth of the L-929 fibroblast cell line. The results indicated that fibroin and collagen films were equivalent in their ability to support cell attachment, physiological morphology and growth when compared to the sericin film. These results support the concept that extracted fibroin free of sericin is a suitable matrix for cell and tissue culture.

In our own studies, fibroin films induced bone tissue growth in vitro when seeded with osteoblasts [60]. When the films were chemically decorated with the peptide RGD to promote integrin interactions for adhesion, the induction of bone formation in vitro was significantly enhanced. The response was determined based on increased alkaline phosphatase levels, upregulation of bone-specific transcripts, and calcification levels over 4 weeks. Similar responses were not observed when parathyroid hormone was immobilized and also assayed with osteoblasts. The utility of a protein matrix offering

Table 8
Examples of silk's utility as a matrix material in tissue engineering

Silk form	Supported cell type in vitro	Comments	Authors
Fibroin film	L-929 mouse fibroblast	Comparable growth rates to collagen films	Minoura et al. [26]
Fibroin film	SE1116 (human colon adenocarcinoma); KB (human mouth epidermoid carcinoma); Colo201 (human colon adenocarcinoma); QG56 (human lung carcinoma)	Comparable growth rates to collagen films as well as rates of protein production of carcinoembryonic antigen (CEA)	Inouye et al. [59]
Fibroin film	Saos-2 (human osteoblast-like cells)	Bone formation was evident on fibroin films, but was enhanced on RGD-coupled matrices	Sofia et al. [60]
Fibroin film	hBMSC (human bone marrow stromal cells)	Supports bone nodule formation from adult stem cells	(Unpublished data)
Fibroin fibers	hBMSC; human adult anterior cruciate ligament fibroblasts	Supports ligament specific development in vitro	Altman et al. [61]

a diversity of sites (e.g., amino acids) for selective chemical couplings for tissue engineering is a benefit of the silk system. Bone tissue formation was a logical target tissue for silk due to the unique mechanical properties of the protein in fiber and film forms.

Recent research with silk has focused on the development of a wire rope matrix for the development of autologous tissue engineered anterior cruciate ligaments (ACL) using a patient's own adult stem cells [61]. Biologically compatible and mechanically robust biomaterials are critical based on the stringent requirements of ligaments and the dynamic and demanding mechanical environment of the knee. Human bone marrow stromal cells (BMSCs) cultured in collagen gels were grown in a mechanically dynamic environment relevant to that present in vivo leading to the formation of ligament fibroblasts (from the BMSCs) and mature tissue development in vitro [62]. However, the poor integrity of the collagen gels and the demanding and dynamic intra-articular mechanical and biochemical environment of the knee have prompted renewed interest in silk as a long term absorbable material with good mechanical integrity and biocompatibility.

Collagen fibers have been used as a matrix biomaterial to support *de novo* ligament formation in vivo with limited success. In the early 1990s Dunn et al. [63] reported initial work on the development of a ligament prosthesis involving a collagenous ACL prosthesis [63]. Results showed inconsistent neoligament formation and significant weakening of the prosthesis in a rabbit model. Enhanced scaffolds were examined including a collagen fiber-PLA composite to further maintain mechanical integrity allowing for neoligament tissue ingrowth [64]. In both studies, only half of the structures remained intact 4 weeks post-reconstruction suggesting that this composition (collagen and PLA) was inadequate for the rigorous in vivo environment of the ACL.

Silk's unique mechanical properties, coupled with the ability to weave the fibers into a wire-rope geometry provides control over the matrix's final mechanical properties (i.e., matrix stiffness can be altered by adjusting the pitch angle of a wire rope) to mimic the mechanical properties of the native ACL and support host tissue ingrowth offering new options in ACL tissue engineering. Pilot scale manufacturing equipment has been developed for the fabrication of ACL wire-rope

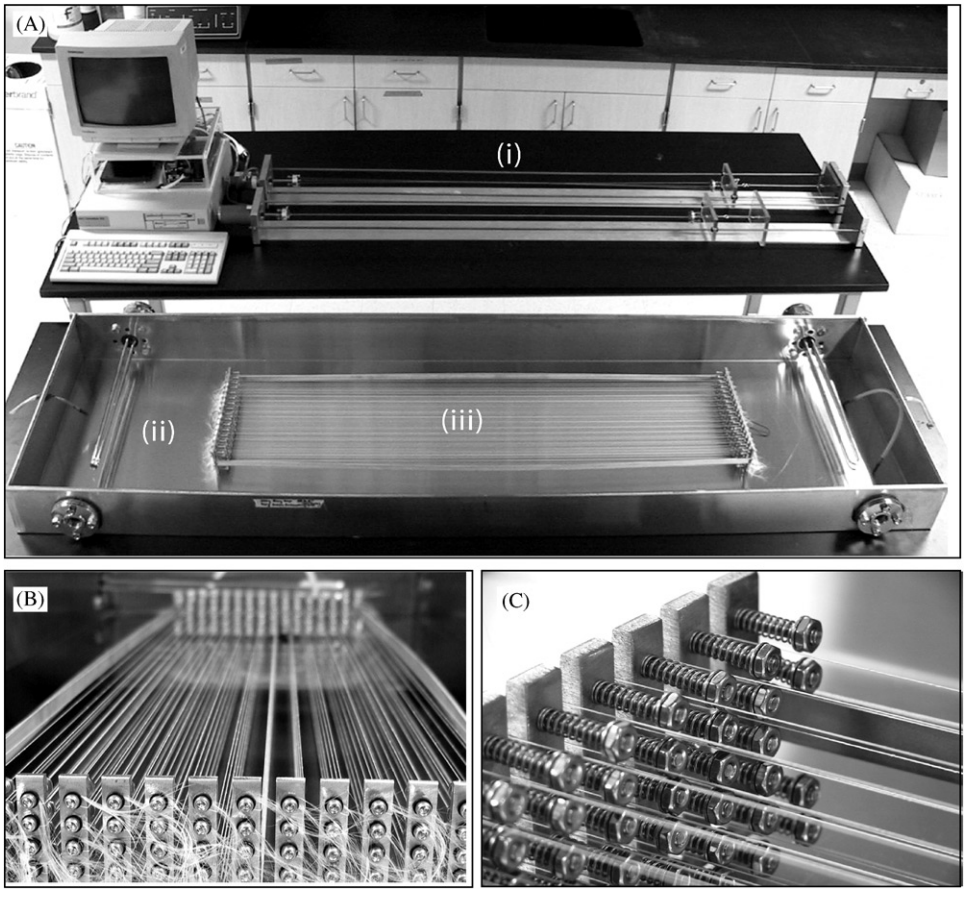

Fig. 1. (A) Pilot-scale manufacturing equipment for the fabrication of silk wire-rope matrices showing (i) two computer controlled twisting machines, (ii) a 160 l stainless steel water bath with immersion heaters, and (iii) a custom designed fiber extraction rack holding 94 individual groups of fibers to be extracted free of sericin; (B) a close up of the extraction rack containing 94 individually anchored groups of fibers; (C) spring-loaded clamps used to keep fibers or groups of fibers in equal tension during extraction, rinsing and drying.

fibroin matrices (Fig. 1). A 160 l stainless steel water bath (Fig. 1A) and custom designed fiber extraction racks to keep individual fibers or groups of fibers in equal tension were developed. The extraction rack was designed to anchor up to 94 individual fibers or groups of fibers (Fig. 1B) with spring-loaded clamps (Fig. 1C). The water bath (214 cm × 61 cm × 15 cm) combined with two immersion heaters and an external pump allow recirculating flow of the extraction broth at 90°C over the rack containing the silk. Following sericin extraction, two computer controlled twisting machines (Figs. 1A and 2) were developed to fabricate wire-rope matrices with desired geometries (e.g., material properties). One of many fabricated matrices is shown in Fig. 3. Confirming silks utility as a biocompatible material for in vitro ligament tissue engineering, BMSCs were shown to attach, spread and proliferate on the silk fiber matrices [61]. Scanning electron microscopy (SEM) images of virgin silk and extracted silk (Fig. 4) as well as BMSC seeded fibroin wire-rope matrices are shown (Fig. 5). BMSC are able to attach within 5 min (Fig. 5A), initially spread at 1 h (Fig. 5B), and form confluent cell sheets with possible extracellular matrix formation 7 days and 14 days post-seeding (Figs. 5C and D).

It is generally accepted in the literature that silk will slowly degrade in vivo as a function of proteolytic attack. However, in vitro in immune system deficient tissue culture conditions, sericin-extracted fibroin silk fibers retained their initial tensile integrity over 21 days [61]. In vitro studies of the extracted silk fibroin exhibited a negligible response from macrophages as assessed by cytokine release in vitro (unpublished data). As a result, appropriate, directed and rigorous mechanical stimuli can be communicated to adhered stem cells in vitro without concern for premature graft mechanical failure; however, the effects of mechanical fatigue on graft integrity will need to be characterized for each culture condition and variant matrix geometry.

We have shown that the application of mechanical stimuli will induce BMSC ligament specific differentiation and matrix formation in vitro [62]. The utility of this tissue engineering approach in combination with a mechanical robust silk matrix is the potential inhibition of a FBR following implantation in vivo due to the

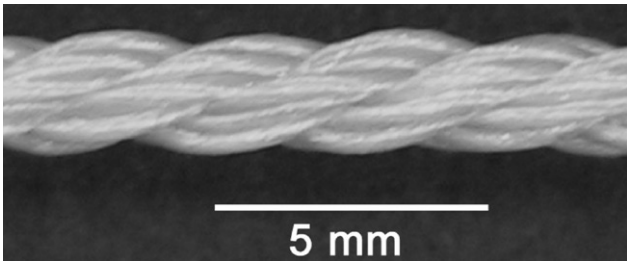

Fig. 3. One of many silk cords manufactured by the twisting equipment. The silk cord shown contains 5 levels of twisting hierarchy and 540 individual fibers twisted to approximate the stiffness of the human ACL.

Fig. 2. A close-up view of the twisting machines showing the motor controlled spring-loaded clamps. The clamps can anchor from 2 to 6 fibers or groups of fibers for twisting.

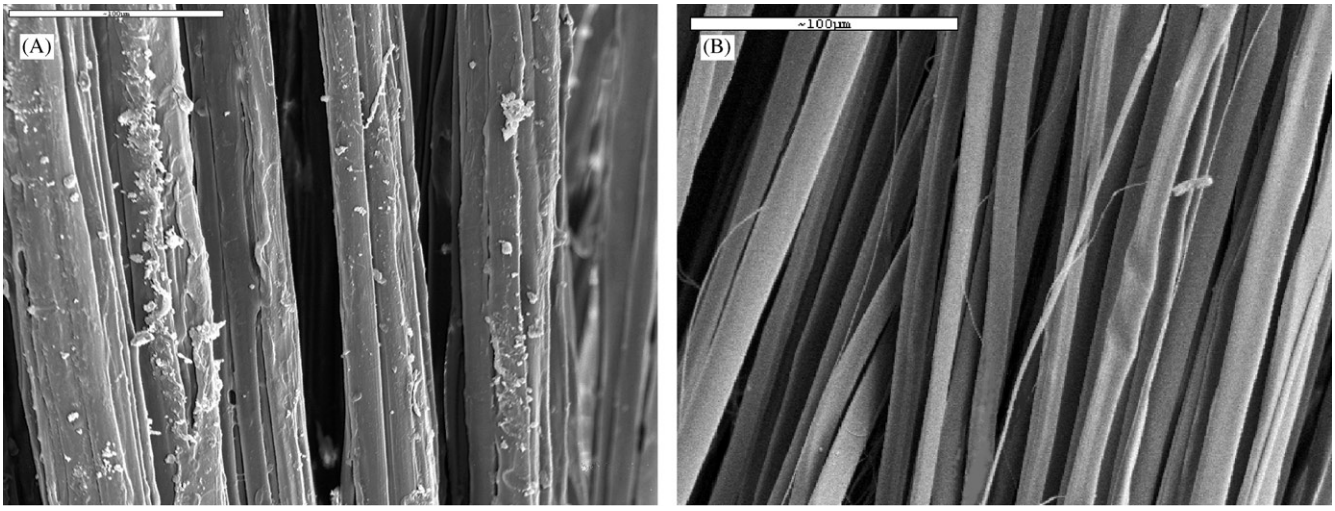

Fig. 4. (A) SEM of raw virgin *B. mori* silk fibers prior to extraction showing the gum-like sericin proteins coating the core fibroin and (B) following extraction at 90°C for 60 min in conditions previously described [59] showing the individual smooth fibroin filaments following the removal of the covering sericin.

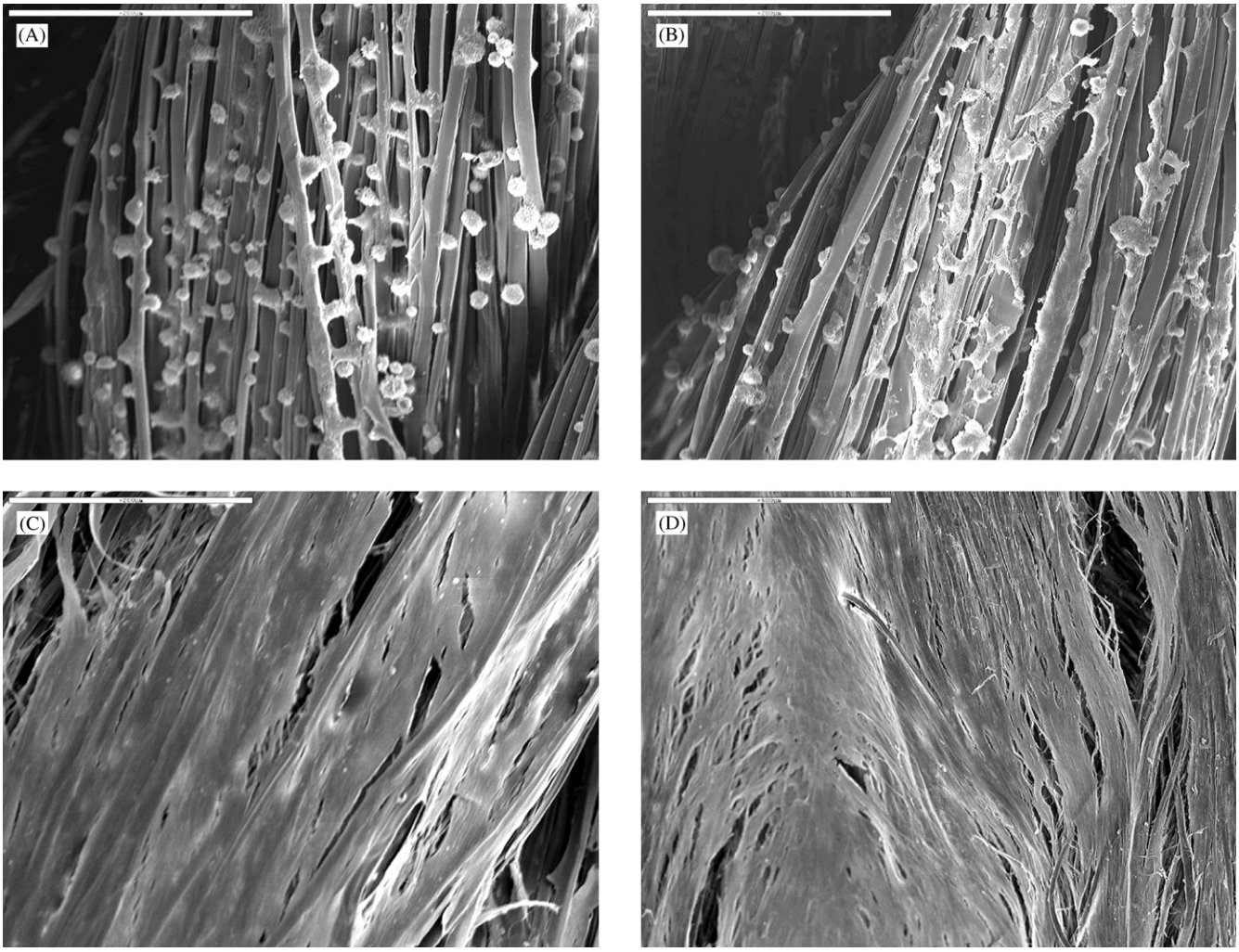

Fig. 5. (A) Initially attached BMSCs on the fibroin cord shown in Fig. 3, 5 min following seeding of the fibers with 2 million cells/ml (1 ml total per 3 cm of silk cord). (B) Initial cell spreading on the fibroin 1 h after seeding. (C) Seven days post-seeding with a cell and ECM sheet evident. (D) Fourteen days following seeding, thick encapsulation of the matrix by cells and ECM.

presentation of autologous ligament tissue (derived from the BMSC) to the host. This approach should allow both infiltration of host tissue and the in vitro developed ligament tissue incorporated into and surrounding the matrix to form mature functional, biologically viable autologous ligament tissue. Studies are currently ongoing to identify an optimal matrix geometry and stiffness to best support tissue ingrowth both in vitro and in vivo. Furthermore, silk's high ultimate tensile stress (N/mm^2) provides significant void volume in vivo occupying only $\sim$12% of the space of a human ACL (given a human ACL 27 mm in length and 8 mm in diameter). We suspect, following angiogenesis and revascularization of the tissue engineered ligament within 8–12 weeks in vivo, the core original silk matrix will be proteolytically degraded and metabolized in vivo. The effects of the rigorous mechanical knee joint environment on matrix fatigue life over the long term remain to be determined but will clearly guarantee the eventual failure of the silk matrix in vivo, a desired goal to ensure biologically viable, host ligamentous tissue eventually sustains the functional roles of the ACL over the life of the patient.

In terms of matrices for tissue engineering, the novel mechanical properties of silk in film, fiber (Table 1) or sponge forms, coupled with the facile chemical decoration, the potential to form complex rope designs to match functional requirements for specific tissues, and the slow rate of degradation in vivo to provide adequate robust support, suggest that silks offer many benefits when compared to other types of natural or synthetic degradable fibers, films and foams. For example, in the case of bone, biomaterials that can provide robust support during compression would be advantageous, and silks are known to function well under compression with little evidence for cracking or crazing under conditions in which synthetic high performance fibers fail [13]. Furthermore, in the case of ligaments, the high tensile strength of silks provides a strong advantage in matching ligament function to immediately restore knee function, a feature difficult to achieve with collagen [61].

Silks fibers and films are often prepared from resolubilized protein after dissolution in high concentrations of lithium salts or calcium nitrate. Once soluble, the proteins can be dialyzed, lyophilized and resolubilized in organic solvents such as hexafluoroisopropanol or in water for limited periods of time depending on concentration. In the case of fiber formation, silks [65,66] and collagens [67] have been electrospun to form nanometer-scale diameter fibers. Thus, modulation in fiber diameters from the native 10's of microns in the case of *B. mori* to submicron by electrospinning offers a range of morphologies for cell interactions. It is also interesting to note that unrestrained dragline silk from *N. clavipes* will supercontract. However, when embedded in a material or restrained, this unusual feature is lost. Silkworm silk fibers from *B. mori* do not supercontract.

7. Conclusions

B. mori silk fibers are composed primarily of two types of proteins: (1) sericin, the antigenic gum-like protein surrounding the fibers and (2) fibroin, the core filaments of silk comprised of highly organized β-sheet crystal regions and semi-crystalline regions responsible for silk's elasticity compared to fibers of similar tensile integrity. Silk has been used in biomedical applications for centuries primarily for the ligation of wounds. Virgin silk suture (containing sericin) induces hypersensitivity in patients, causing a Type I allergic reaction. Exposure to silk debris (e.g., broken virgin silk fibers used in bedding and fabrics) may sensitize patients to silk causing adverse allergic reactions when silk is used as a suture material. Sericin, identified as the antigenic agent of silk, is removed and replaced with a wax or silicone coating in commercial black braided silk sutures. As a result, T-cell mediated hypersensitivity has not been observed in response to black braided silk in the literature. Unfortunately, poor documentation of the exact type of silk suture used when describing host responses to silk in the literature has led to confusion regarding the utility of silk in biomedical applications. As a result, the use of silk over the past 20 years has declined since the arrival of commonly used absorbable and non-degradable synthetics such as Vicryl™ and Ethibond™.

Another misconception about silk revolves around its ability to degrade in vivo. Defined as non-degradable by the USP because it retains the majority of its tensile strength beyond 60 days in vivo, silk is commonly thought of as a permanent suture once implanted into the body. As a protein, silk is susceptible to proteolytic degradation, but over a longer time periods. Based on the literature, it is apparent that silk fibroin will lose the majority of its tensile strength within 1 year in vivo. In some cases, the silk or the features associated with inflammation cannot be found at the implantation site within months following surgery. Therefore, silk is a long-term absorbable suture.

Silk fibroin elicits a foreign body response following implantation in vivo. Yet the response is comparable to the most popular synthetic materials in use today as biomaterials, and is dependent upon the implantation site and model used for investigation. In rare cases a granuloma may form as a result of an abandoned phagocytic response to silk by macrophages and giant body cells. Again, the response is dependent on the implantation site of the silk. It is known that within blood, silk is highly thrombic. Clearly, this results from a unique feature of the silk fibroin protein to bind

components of the clotting cascade such as fibrin and fibrinogen. However, in most cases, the response is moderate and subsides with time.

To examine the material properties of silk fibroin in relation to other common degradable and non-degradable biomaterials in vivo, comparisons should be made by equating surface area of the materials under investigation. Individual silk fibroin filaments, due to their small diameter ($\sim 5\,\mu m$) increase the surface area to volume ratio of silk sutures when compared to other biomaterials where the equating factor is typically final suture diameter. As a result, in cases that demonstrate a greater foreign body response to silk in vivo, the comparison may not be equitable to the inherent material properties of silk fibroin. Therefore, silk fibroin when utilized as films, foams and fibers, may offer a 'new' alternative biomaterial for use as matrices in tissue engineering where mechanically robust, long-term degradable materials are needed.

Acknowledgements

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