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### **The Biomedical use of Silk: Past, Present, Future**

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**Abstract**

Humans have long appreciated silk for its lustrous appeal and remarkable physical properties yet as we unravel the mysteries of silk, it becomes clear that this outstanding biopolymer is more than a high-tech fiber. This review provides a critical but detailed insight into the biomedical use of silk. This journey begins with a historical perspective of silk and its uses, including the long desire to reverse engineer silk. Selected silk structure-function relationships are then examined to appreciate past and current silk challenges. From this, biocompatibility and biodegradation is reviewed with a specific focus of silk performance in humans. The current clinical uses of silk (e.g., sutures, surgical meshes and fabrics) are discussed, as well as clinical trials (e.g., wound healing, tissue engineering) and emerging biomedical applications of silk across selected formats, such as silk solution, films, scaffolds, electron spun materials, hydrogels, and particles. The journey finishes with a look at the roadmap of next-generation recombinant silks, especially the development pipeline of this new industry for clinical use.

**Keywords:** silk, fibroin, spider silk, clinical use, recombinant silk,

## 1. Prologue

Before we begin to define the current state of the art surrounding the field of silk-based biomaterials for (bio)medical use and look toward their future, we feel it is important to spend some time considering the motivation of the research and the history of the material that has led us to the present day.

The issue of motivation for studies in the field of silk research is generally divided into bottom-up, curiosity-driven fundamental research and top-down, challenge-based activities.

Fundamental silk research hinges on the question, “What can we learn from nature?” This is clearly a wider topic than silk itself, but the overall approach helps frame scholarly activities in the area. We certainly have more to learn beyond understanding the silk fiber itself, and dozens of cross-disciplinary researchers worldwide are using both simulation and experimentation <sup>[1]</sup> to make concerted efforts to understand the evolution <sup>[2]</sup>, processing <sup>[3]</sup>, and performance of silk <sup>[4]</sup>, from the molecule <sup>[5]</sup> to the material.<sup>[6]</sup> However, as we broaden our interpretive horizons, we must remember that silks are *biological* materials, and thus are defined by their *biology*, before we attempt to transfer this knowledge to *biomaterials*, which are defined through their *application*.

## 2. Introduction

For the purpose of this review, we use the term silk to refer to protein-based fiber-forming materials spun by living organisms. We also include in our terminology silk-inspired proteins produced by recombinant approaches.

When studying silks, one must always appreciate that the results derived from testing any naturally obtained biological material are a product of both Nature (its evolution) and nurture (its environment), with the latter typically constraining the property space of the former (although exceptions exist <sup>[4a, 7]</sup>).

Evolutionarily speaking, the biological definition of silks is that they are structural proteins that are spun into fibers for use outside the body <sup>[4a, 8]</sup>. In the wild, silks have undergone over 400 million years of “research and development” via natural selection, and they now present a plethora of solutions to biological challenges that range from predation (spider webs) to housing (honey bees and wasps) and protection (silkworm cocoons).<sup>[4a, 8]</sup> The ubiquity and widespread use of silk is a clear testament to its success, especially as it has arisen numerous times in independent convergent evolutionary events.<sup>[2c]</sup> Hence, looking at how silk materials have evolved can not only determine their performance in the present but can also unravel common design criteria and the molecular “blueprints” for high performance biological materials.<sup>[4a, 9]</sup>

In unraveling the properties of silk, we have also begun to address common misconceptions regarding biological materials and their potential for industrial application. These are often tarred with a brush of sample variability suggesting that they are unsuitable for engineering or medical applications where consistency is key. However, recent studies now show that the variation previously observed is typically a manifestation of a silk's exquisite responsiveness to its surroundings (making silks incredibly "smart" materials).<sup>[4d, 7a, 10]</sup> Yet for the uninitiated researcher this can sometimes become unwanted variation if they fail to ensure consistent sample preparation or testing environments. Hence, biological diversity and plasticity offer several important lessons for those wishing to make the best use of silk for their own applications.

At the other end of the spectrum is the widely held belief that biological materials automatically qualify as "biocompatible" materials. While these materials, including many silks, are often biocompatible, simply labeling silk as "biocompatible" without context specific biocompatibility testing and critical assessment of the available evidence is neither scientifically rigorous nor in the best interest of the field or, ultimately, patients. This mindset also permeates into the assumption that all natural materials are "green" which without appropriate and carefully considered environmental analysis, the ubiquitous use of the phrase ultimately detracts from the potential impact of these materials when such claims are shown to be valid.

Once we move past our prejudices, we can look forward and at the interface of fundamental and challenge-based activities sits biomimetics. This specifically looks to nature to reveal concepts, processes, and systems that can be applied to solve human challenges.<sup>[11]</sup> While the term “biomimetics” was only coined by American biophysicist Otto Schmitt in the latter half of the 20<sup>th</sup> century <sup>[11]</sup>, humans have been looking to translate silk’s natural utility for their own use for millennia.<sup>[12]</sup> The simplest, most primitive forms of mimicry are examples of imitation of the spider’s use of silk to catch prey, as seen in the Australian aborigines’ use of spider silk as fishing lines and New Guinean natives’ development of fishing nets and bags.<sup>[13]</sup> However, the biological diversity of silk soon inspired humans to adapt silk for their own needs (e.g. <sup>[14]</sup>), extending the silk phenotype beyond its natural remit. Some of the first examples were the use of silks medicinally by ancient Greeks and Romans, who bundled up spider silk to treat wounds (**Figure 1**).<sup>[13]</sup> This was even noted by Shakespeare’s character, Nick Bottom, in *A Midsummer Night’s Dream*, who says, “I shall desire you of more acquaintance, good master cobweb. If I cut my finger, I shall make bold of you.” (3.1.9.100-101).

However, the above examples describe the use of silk in its unprocessed, natural state. A step forward in the utilitarian evolution of silk came about with the realization that silk could be readily reprocessed into different forms. This was first performed at the macroscale by unwinding fibers from the non-woven composite cocoons of the silkworm *Bombyx mori* to create textiles. This skill originated in China, and direct archeological

evidence confirms human interactions with silkworm silk originating from the Neolithic period of the 4<sup>th</sup> millennia BC, with the discovery of examples of cut cocoons and rudimentary looms at numerous archeological sites.<sup>[15]</sup> Further archeological evidence suggests that the Indus Valley civilization (in what is now Northern Pakistan) was also developing silk materials based on *Antheraea* silk. Therefore, sericulture—the act of rearing silkworms specifically for their silk—can be estimated to have spread across South Asia from 5,000—2,000 BC.<sup>[16]</sup>

Textiles produced from silk were truly a disruptive product, as they required both a unique material and highly sophisticated processing (programmable looms for weaving that were, in essence, the progenitor of modern computing).<sup>[17]</sup> As such, silk textiles were sufficiently valuable to become a formal currency for Chinese soldiers at the edges of the empire and were used to barter with the locals for goods.<sup>[15]</sup> Nevertheless, silk production remained a closely guarded secret within the Chinese empire for several thousand years, and when asked, traders would say it was “derived from the wool of sheep sprinkled with water and exposed to sunshine”. However, this product monopoly could not go unchallenged for long, and the establishment of trade routes (the “silk roads”), and the apocryphal industrial espionage that ensued, made silk technology available throughout the world. As a result, *Bombyx mori* silk has developed hand in hand with humans, through domestication and artificial selection of the moths for over 4,000 years.<sup>[15]</sup> This extensive history is a testament to the success and suitability of this animal for large-scale industrial agricultural development, as ~980 billion animals are raised each year to produce ~400 megatonnes of commercial silk.<sup>[18]</sup>



Across millennia, silk has been a luxury item for the elite. However, Claudius Galenus of Pergamon (c. 131 – c. 211 AD) was the first to document a potential medical application of the silk thread. Galenus gained a reputation for treating gladiators whose tendons were severed in hand-to-hand combat and noted in his book *De Methodo Medendi* (150 AD) the use of several materials as sutures, including linen. He writes, “in many places under Roman rule you can obtain silk, especially in large cities where there are many wealthy women. If there is no such opportunity, choose from the material where you were living the least putrescible such as thin catgut.” Galenus’s teaching persisted for centuries after his death but was eventually lost.<sup>[19]</sup> The war surgeon Ambroise Paré (1510 – 1590) avoided cauterizing open wounds with boiling oil and reverted to using vascular ligatures made of silk or fine linen strips. However, only in 1869 did Joseph Lister introduce the first sterile silk suture into clinical practice.<sup>[19]</sup>

Throughout history, several alternative sources for textile silk beyond the domesticated silkworm have been sought, from the wild silkworms of India and Africa <sup>[20]</sup> to the more esoteric source represented by spider silk. The quest to commercialize spider silk, due to its favorable mechanical properties, seemingly began with the inventions of Abbé Ramon de Termeyer in the 18<sup>th</sup> Century for his reeling device.<sup>[21]</sup> Over the years, these inventions were followed by others, such as those of the Civil War surgeon Burt G. Wilder.<sup>[22]</sup> The most successful attempts are probably those made by the Madagascan spider silk industry, which has produced, to date, only a handful of items destined for the elite.<sup>[23]</sup>

Yet, while producing arguably mechanically superior materials compared to those made of silkworm silk, none of these endeavors were ultimately found to be scalable.

Hence, given the coveted nature of silk, the fact that industrialists wished to replicate it may come as no surprise. In fact, nearly every single industrial fiber produced in the latter half of the 19<sup>th</sup> and throughout the 20<sup>th</sup> century, from rayon to nylon to Kevlar®, has been developed in the hope that it would provide a suitable alternative to silk.<sup>[24]</sup> Nevertheless, even after 150 years of concerted research and development, and although replication of the properties and performance of silkworm silk is now possible, similar success with spider silk, and specifically dragline silk, remains elusive. A complete overview of the history, progress, and trends in artificial silk spinning from a fiber performance perspective is available in a recent review that comprehensively covers this topic.<sup>[24]</sup>

Fortunately, the attempts to replicate various silks resulted in several distinctly important innovations that led the biomaterials field to consider silk as more than just a fiber.<sup>[12]</sup> Akin to the Ancient Chinese realizing that a silkworm cocoon can be unspun, early attempts at creating artificial silk led to the conclusion that the silk fiber itself could be “unspun” back into a processable protein feedstock, which could then be solidified into a variety of forms. According to the original patents, this finding was largely motivated by a need to utilize the waste streams from the industry<sup>[25]</sup>, as the last tens to a hundred meters of silk from a cocoon could not be unraveled. (Today, this would be labelled an exercise in sustainability.)

To the best of our knowledge, the first attempt to create an artificial silk feedstock appeared at the turn of the 20<sup>th</sup> Century, 110 years ago. It began with the work of Baumann and Diesser, who proposed the dissolution of whole silk glands in formic acid.<sup>[26]</sup> In subsequent years, a notable race began between Japanese and German researchers in the 1920s, with patents granted in 1924-7<sup>[25a, 27]</sup> and 1928<sup>[28]</sup>, respectively, for the successful dissolution (and respinning) of artificial fibers using  $\text{ZnCl}_2$ ,  $\text{Mg}(\text{NO}_3)_2$ , and orthophosphoric acid as the main chaotropic agents. However, not until the 1930s did today's familiar degumming using  $\text{Na}_2\text{CO}_3$ <sup>[29]</sup> and dissolution in LiBr appear.<sup>[25b]</sup> The latter report clearly noted the potential of silk regeneration/reconstitution: *“These solutions containing, if at all, only a small amount of salt, may be used in the known manner to produce artificial articles, such as fibers, films, or plastic masses.”*

Beyond the replication of silk fibers for textile use, these feedstocks were originally intended for reprocessing into solid form to harness silk's excellent insulating properties.<sup>[25b]</sup> and enable the casting of films (to make fabrics water and air impermeable<sup>[30]</sup>). This was mainly because naturally derived materials were still superior in many aspects when compared to those arising from the burgeoning field of industrial polymers.<sup>[31]</sup>

Interestingly, nearly three decades passed before the first biomedical use for a regenerated silk was reported in the patent literature. In the 1960s, Boch and Messoris, of Ethicon Inc. (NJ, USA), were the first to propose the use of a LiSCN/LiBr reconstituted

silk as a replacement for the standard wax coating used on silk sutures to reduce their limpness, fraying, and unwanted capillary action.<sup>[32]</sup> In the following years, while developments continued in the suture field, another two decades passed before the first examples of non-fibrous silk-based biomaterial patents were reported. In 1986, a silk fibroin:fibrinogen glue, based on the “standard” LiBr reconstitution approach, was developed by a Japanese firm.<sup>[33]</sup> This was followed by the first patent for a silk based porous scaffold in 1987, again from Japan, produced from a freeze-dried native silk solution (i.e., silk extracted directly from the silk gland).<sup>[34]</sup> The 1990s saw more patent applications from Japan, including powdered silk for wound dressings<sup>[35]</sup>, reconstituted silk films and molded gels for skin, blood vessel, and corneal coatings<sup>[36]</sup>, and colloidal silk for consumption in medicine.<sup>[37]</sup> However, in the 2000s, an explosion occurred in the USA in research and commercialization activity around this area with the emergence of large patent families (>100) focused on the future medical exploitation of these materials.<sup>[38]</sup>

In summary, looking back, the ability to unspin silk, and thereby reconstitute it, has been a monumentally disruptive development in the field. It represents a platform technology for the development of biomimetic structures that are built *with* silk but are *not* built to *replicate* silk. While gaps still undoubtedly exist in our knowledge surrounding the process of reconstitution and how this affects the integrity and application of the silk proteins undergoing it<sup>[39]</sup>, the unspinning process has been widely adopted throughout the biomaterials field, as perhaps best evidenced by the impact of the landmark review of Altman<sup>[40]</sup> and the protocol of Rockwood<sup>[41]</sup>, which leads us in the present day.

### 3. Silk: Hierarchical and crystal structures

The fundamental building blocks of the silk biopolymer are amino acids that, through their sequence specificity and subsequent secondary, tertiary, and quaternary structures, govern the protein's overall function. In nature, silk and silk-like proteins are made by several organisms such as spiders, silkworms, scorpions, mussels bees and ants. However, the silk fibroins and silk-like proteins of each organism exhibit different physical and biological characters due to their different amino acid sequences, spinning conditions, and hierarchical structures.<sup>[7b, 42]</sup> The hierarchical structures of silk proteins vary among silk types.<sup>[42a, 43]</sup> Silk proteins produced by spiders and insects are referred to as silk spidroin and silk fibroin, respectively. The term “silk fibroin” is commonly used to differentiate “virgin” silk (silk filament still encased by sericin) or silk cocoons (i.e., the sericin-coated silk thread arranged into a cocoon) from purified silk (i.e., degummed; see section 6). For the purpose of this review, we will use the term silk fibroin to refer to degummed (*Bombyx mori*) silk unless otherwise stated.

As discussed in the previous section, the silk fibroin of the domesticated silkworm (*Bombyx mori*) is the most well studied silk for biomedical applications due to its established supply chain, abundance, and clinical track record. The *Bombyx mori* protein fiber is a composite material comprising a semi-crystalline silk core (i.e. silk fibroin), which is mainly responsible for the load-bearing capacity, and an outer layer of sericin, which functions as a gumming agent.<sup>[44]</sup> However, emerging evidence suggests that

sericin also inhibits the premature conversion of soluble silk (silk I) into  $\beta$ -sheet-rich silk.<sup>[45]</sup>

The *Bombyx mori* silk protein (i.e. silk fibroin) is very large and can be subdivided into light (approximately 26 kDa) and heavy (approximately 391 kDa) chains that are linked by a single disulfide bond at the C-terminus<sup>[46]</sup> (**Figure 2**). The C- and N-terminal capping sequences are completely non-repeating amino acid residues. The mechanical properties of silk fibroin arise due to the block copolymer-like arrangement of the silk heavy chain, which contains 11 short hydrophilic regions typically 31 amino acid long and 12 hydrophobic blocks that account for 94% of the silk heavy chain. These hydrophobic blocks contain predominately glycine-X (GX) repeats, where X is alanine (A) (65%), serine (S) (23%), or tyrosine (Y) (9%).<sup>[46a]</sup> These GX blocks can be broadly classified into three groups: (i) a highly repetitive GAGAGS sequence that contributes to the bulk of the crystalline regions and is typically found at the beginning of each motive, (ii) a relative less repetitive sequence containing hydrophobic and/or aromatic residues, namely, GAGAGY, GAGAGV, and GAGAGVGY, which form semi-crystalline regions, and (iii) motifs similar to (i) except for the presence of an AAS motif, which typically exists at the C-terminus of each motif and may play a role for sheet-breaking.<sup>[47]</sup> *Bombyx mori* silk fibroin lacks the tripeptide sequence arginine, glycine, and glutamic acid (RGD) that is typically exploited by cells to mediated cell-substrate attachment via integrin engagement; however, the N terminal of the silk heavy chain contains a fibroblast growth-promoting peptide.<sup>[48]</sup> Nevertheless, a sequence specificity exists between

different silkworm silks; for example, the Indian non-mulberry tasar silkworm (*Antheraea mylitta*) contains RGD sequences that are absent in *Bombyx mori* silk.

Spider dragline silk, one of the toughest material known to humankind, is composed of a skin layer and a bundle of microfibrils (Figure 1).<sup>[5b, 54]</sup> The microfibrils are composed of aligned granules, and their silk molecules form an amorphous phase and  $\beta$ -sheet-rich crystalline regions.<sup>[5b]</sup> In both spider and silkworm silk fibers, the aligned  $\beta$ -sheet structure provides crosslinks between the  $\beta$ -sheet domains embedded in an amorphous matrix that consists of less orderly structures in the form of random coils, helices, and  $\beta$ -turns.<sup>[55]</sup> These  $\beta$ -sheet crystals are critical structures in the hierarchical structures of silk fibers, because they play an essential role as crosslinking points and realize the stiffness, strength, toughness and characteristic deformation behaviors.<sup>[55a, 55b, 56]</sup>

The amino acid sequences that form the  $\beta$ -sheet are 7–9mer alanine sequences for *Nephila clavipes* dragline silk and GAGAGS for *Bombyx mori*<sup>[57]</sup>, whereas other silkworm silk species use polyalanine sequences to form the  $\beta$ -sheet structure.<sup>[6a]</sup> The influence of the number of alanine residues on the secondary structure and assembly behaviors of silk molecules has been studied using wide angle X-ray crystallography as well as solid-state NMR spectroscopy. Those data from X-ray and NMR analyses reveal that short poly(alanine) sequences, such as 6mers or shorter, form a packed rectangular

arrangement, while poly(alanine) sequences longer than 7mers pack in a staggered arrangement.<sup>[58]</sup>

The  $\beta$ -sheet is the most fundamental secondary structure in silk-based (bio)materials. The predominant  $\beta$ -sheet structure plays a key role in stabilizing silk materials via physical cross-links, as the  $\beta$ -sheet behaves as a crosslinking point. Crystal structures of silk  $\beta$ -sheets have been characterized using wide-angle X-ray analysis. The crystal structure of *Bombyx mori* silk fibre has a unit cell with the space group  $P2_1-C_2^2$ .<sup>[59]</sup> The crystal lattice of the *Bombyx mori* silk fiber reported by Marsh et al. had unit cell dimensions of  $a = 9.40 \text{ \AA}$ ,  $b = 9.20 \text{ \AA}$ , and  $c$  (fiber axis) =  $6.97 \text{ \AA}$ , while Takahashi et al. reported cell dimensions of  $a = 9.38 \text{ \AA}$ ,  $b = 9.49 \text{ \AA}$ , and  $c$  (fiber axis) =  $6.98 \text{ \AA}$ .<sup>[59]</sup> The lattice of other silks, such as *Antheraea yamamai* (Japanese silk moth), has been characterized and reported by many groups<sup>[56b]</sup>. The unit cells contain four molecular chains, a pair of which symmetrically forms a  $\beta$ -sheet structure via hydrogen bonds. The up- and down-molecular chains also alternate with each other in an antiparallel manner. Each silk has a different crystal lattice, which can be attributed to differences of the silk amino acid sequences. However, the relationship between the crystal lattices of different silks and the subsequent characteristics of silk fibroin as a biomaterial remains largely unexplored, despite the fact that crystallinity (i.e., the amount of crystalline region) affects the physical and biological properties of silk-based biomaterials.



#### 4. Hydration State

Silk and regenerated silk fibroin materials are expected to exhibit high toughness and ductility because of the excellent mechanical characters of spider (dragline) silks found in nature.<sup>[9, 60]</sup> However, in addition to sequence specificity, the hydration state of silk is critical for its performance.<sup>[61]</sup> For example, most native spider silks show significant fiber contraction when transitioned from a dry state to a high humidity environment. Exposure to humidity facilitates the rearrangement of the non-crystalline GPGXX sequence of orb web silks and the glycine-glycine-X<sub>310</sub> helices in non-orbicularian species (which lack the GPGXX sequence). This occurs due to disruption of hydrogen bonding by these sequences, which facilitates the transition from a parallel arrangement for the fiber axis to a lower energetic configuration that is accompanied by fiber shrinkage and thickening.<sup>[62]</sup> Thus, water is a key component that enables spiders to tailor the properties of their silks during spinning and for *in situ* web tightening (a phenomenon also known as “supercontraction”).<sup>[62]</sup>

In nature, silkworm cocoons and spider webs/draglines are tough structural materials that perform their function; for example, to capture prey in the spider’s web or to protect the developing moth from predators and infection<sup>[56b]</sup>. The mechanical robustness of the native silk fiber has been exploited by humans for biomedical applications both in pre-clinical (e.g.,<sup>[63]</sup>) and clinical trials (detailed below). For example, silk fibroin scaffolds proposed for bone repair have shown a high compressive strength of approximately 13 MPa when reinforced with *Bombyx mori* silk fibers.<sup>[63]</sup> A similar approach has been taken to enhance the mechanical properties of *Bombyx mori* silk hydrogels for cartilage tissue

engineering.<sup>[64]</sup> Recently, a high relative humidity of >97% was found to cause a dramatic increase in the toughness and crystallinity of silk films.<sup>[6a]</sup> This finding exemplifies how an appropriate hydration of silk molecules and materials can achieve crystallization and plasticization simultaneously, resulting in a high-strength and tough silk material.

## **5. Silk for tissue engineering and drug delivery – expectations, hopes, and the reality**

An excellent delivery system for bioactive molecules (e.g., small molecular weight drugs, peptides, proteins, etc.) must meet a number of requirements that include, but are not limited to: biocompatibility, biodegradability, mechanical robustness and durability, and amenability to processing under ambient aqueous conditions that preserve the bioactivity of the payload. Many of these requirements also apply for tissue engineering applications aimed at delivering or recruiting (endogenous) cells, although these silk constructs must also be able to provide cells with the necessary physical and biological cues to achieve the desired function.

### **5.1 Silk biocompatibility**

The exact set of biocompatibility requirements is application specific, although many pre-clinical studies simply cite that silk can meet all the necessary requirements, or they make reference to silk as a “clinically approved” biomaterial for use in humans. However, this ignores our appreciation that a universal biocompatibility does not exist: a material needs to be fit for its intended use <sup>[65]</sup> (as documented by dedicated biocompatibility studies); thus, its performance is context specific. The clinical approval of silk typically refers to

its load bearing applications; however, degummed *Bombyx mori* silk fibers processed into a knitted surgical mesh (SERI® Surgical Scaffold® manufactured by Sofregen Inc., Medford, MA, USA), silk sutures (coated with waxes, Ethicon Inc. and several other manufacturers), and silk garments to treat dermatological conditions are in wide use today in the clinical setting. Therefore, their performance in humans is becoming better documented in the literature <sup>[40, 66]</sup> and is accompanied by a cadre of clinicians with experience working with these silk materials.

Dedicated biocompatibility assessment is critical when generating novel silk formats (e.g., (nano)particles, hydrogels, scaffolds, films, coatings, etc.) to address areas of unmet clinical need or when applying existing silk technologies to new indications. Any non-autologous material will elicit an initial foreign body response that reflects the first steps of tissue repair.<sup>[67]</sup> Therefore, ensuring that the foreign body response is transient rather than chronic is a prerequisite to ensure that clinical endpoints can be met. Overall, biomaterial performance depends on the implantation site, size, geometry, surface topography, and physical characteristics.<sup>[67]</sup> A systematic literature review <sup>[68]</sup> examining the performance of silk constructs (e.g., vascular grafts, ligaments, and wound dressings for skin grafting) in small and large animal studies overwhelming showed that a variety of different *Bombyx mori* silk constructs performed well across the broad spectrum of indications and animal models.<sup>[68]</sup> Direct *in vivo* comparison of silk with commonly used natural (e.g., collagen) and synthetic (e.g., polycaprolactone, polylactic acid, poly[lactide-co-glycolic acid]) biomaterials indicates that *Bombyx mori* silk fibroin is typically at least as good as these synthetic materials and often superior than other natural biopolymers.<sup>[68]</sup>

As new applications for silk emerge, appropriate biocompatibility studies must be performed to support these developments. For example, silk nanoparticles for anticancer drug delivery are typically designed for intravenous administration <sup>[69]</sup> and thus require hemocompatibility assessments because biological performance cannot be deduced by extrapolating results from macroscopic films <sup>[70]</sup> to nano-scale particles <sup>[71]</sup>. An initial proof of biocompatibility is a first step to translate silk technologies from the bench to the clinical setting. For example, regulatory frameworks imposed by the Pharmaceuticals and Medical Devices Agency Japan, the Food and Drug Administration (FDA, USA), the Medicines and Healthcare Products Regulatory Agency (UK), and the European Medicine Regulatory Agency (EU) for medical devices (e.g., Regulation (EU) 2017/745 to obtain CE marking analogous to the Class III Premarket Approval/ 510(k) in the USA, and the Australian Register of Therapeutic Goods certificate of inclusion) stipulate that a biological safety assessment needs to be conducted first (by an ISO certified laboratory, in conjunction with a notified body) before progressing the device to first-in-man clinical assessment. Materials of animal or allogeneic origin need to fulfill additional safety requirements (e.g., absence of infectious agents such as retroviruses etc.) before use in humans. However, from a regulatory perspective, *Bombyx mori* silk is regarded as a non-animal product (EU Council Directive 93/42/EEC, rule 17).

Reports on the biocompatibility of silk in humans comes primarily from silk sutures (reviewed in <sup>[40]</sup>) that have been in use for several centuries <sup>[19]</sup> and from SERI Surgical Scaffold® that obtained 510(k) clearance by the FDA in 2008 and underwent a market

launch in 2013. Histological evidence of 69 breast tissue samples (by 60 patients) taken at stage 2 in patients undergoing two-stage breast reconstruction with SERI Surgical Scaffold® showed a mild inflammatory response in 59 patients, as confirmed by histology. This consisted of an infiltration of mostly macrophages and occasional multinucleated giant cells that phagocytosed the silk fibers, as well as occasional lymphocytes and, rarely, neutrophils or polymorphonuclear cells.<sup>[72]</sup> Ordered collagen deposition was observed, with minimal or no encapsulation of the silk surgical mesh. These clinical trial data <sup>[72]</sup> were similar to observations made in a sheep study.<sup>[73]</sup> However, one patient had a postoperative hematoma that led to mesh removal.<sup>[72]</sup>

Synthetics are now the most widely used suture material, but silk sutures are still in demand for specialized applications where exquisite handling is of paramount importance (e.g., eye surgery). Silk sutures are strong, are easy to handle, lie flat on the tissue surface, and allow for secure knots. Adverse reactions to silk sutures are typically reported for virgin silk, where the silk filaments are still coated with sericin (and often with additional waxes or silicones).<sup>[40]</sup> There is an ongoing debate about the potential role of sericin in these adverse reactions. However, emerging evidence suggests that sericin on its own shows a low allergenic and immunogenic profile in mice; in fact, this profile is similar to that seen for silk fibroin or alginate.<sup>[74]</sup> These observations are supported by *in vitro* data with macrophages: extracted sericin from *Bombyx mori* silk cocoons showed no significant release of the inflammatory marker TNF- $\alpha$ ; similar observations were made with silk fibroin.<sup>[75]</sup> However, extracted sericin in combination with bacterial lipopolysaccharide induced TNF- $\alpha$  release (but not for the silk fibroin group).

Furthermore, re-coating of silk fibroin with sericin showed no macrophage response, while virgin silk induced a high level of TNF- $\alpha$  release.<sup>[75]</sup> These data suggest that other leachable compound(s), or these compounds combined with sericin, may be responsible for the adverse clinical reactions reported for silk.<sup>[68, 75]</sup> For example, patients subjected to bilateral cataract surgery showed no suture reaction on the first eye but a severe reaction on the second eye when it was treated 6 to 3 months later. This suggested that these patients had undergone a sensitization toward virgin silk. Prompt removal of the offending silk suture resulted in significant clinical improvement.<sup>[76]</sup> Examining the clinical literature regarding silk sutures and identifying the exact cause of the adverse reaction is challenging because often little (or no) information is provided about the exact nature of the silk suture (e.g., virgin silk, type of coatings, etc.). Nonetheless, an allergic response to *Bombyx mori* virgin silk is documented for the occupational and domestic setting (reviewed in <sup>[77]</sup>). For example, exposure to virgin silk fibers and re-purposed silk waste (e.g., silk floss incorporated into rugs and bedding) has been linked to the development of asthma in silk weavers<sup>[78]</sup> and children<sup>[79]</sup>, mounted by an IgG and IgE immune response.<sup>[80]</sup> Textile workers have an increased risk of developing chronic obstructive pulmonary disease, and this risk is highest in silk workers.<sup>[81]</sup>

Complete and reproducible sericin removal from *Bombyx mori* silk (a.k.a. degumming) is an essential step in silk utilization. Clinically acceptable limits for residual sericin levels for marketed silk products have not been released into the public domain (note that SERI Surgical Scaffold® is described by the manufacturer as highly purified silk with  $\geq 95\%$  purity). Current evidence from both preclinical *in vivo* studies and clinical experience in

humans across a range of applications indicates that *Bombyx mori* silk fibroin is biocompatible, provided that all other contaminants are successfully removed.

Sericin has traditionally been linked to the adverse effects reported for virgin silk (reviewed in <sup>[77]</sup>). However, over the past decade, sericin has emerged as an interesting biopolymer (reviewed in <sup>[82]</sup>), and dedicated biocompatibility studies are now showing encouraging results in relation to the allergenic and immunogenic profile of sericin (e.g., <sup>[74]</sup>). An increasing number of studies report the biomedical use of the biopolymer sericin. For example, the development of composite sericin/silicone nerve guides<sup>[83]</sup> or sericin/polyacrylamide hydrogels proposed for dermal repair<sup>[84]</sup>. Preliminary Phase I clinical trials using sericin composite wound dressings for split-thickness skin grafting are on going and the results are eagerly awaited (NCT01539980 and NCT02643680 reported at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov)).

## 5.2 Silk biodegradation

Silk sutures are classified by regulatory agencies as non-biodegradable because regulatory guidelines expect a loss of most tensile strength within 60 days post implantation. Over this time scale, silk sutures do not lose their mechanical performance, as they require longer time frames to degrade in humans.<sup>[40]</sup> In patients undergoing two-stage breast reconstruction, histological evidence of breast tissue samples taken from 60 patients at stage 2 (median 152 days after initial scaffold implantation, range 74 to 357 days) showed consistent SERI Surgical Scaffold® degradation (although this was not

quantified). The one exception was a patient that had a postoperative hematoma, which was accompanied by an apparent lack of silk degradation.<sup>[72]</sup>

The silk protein is known to degrade *in vitro* and *in vivo* in response to proteolytic enzymes<sup>[68]</sup>, as exemplified by studies with silk films (e.g.,<sup>[85]</sup>) and porous silk scaffolds (e.g.,<sup>[86]</sup>). Experience with SERI Surgical Scaffold® in a sheep model of two-stage breast reconstruction showed progressive degradation and vascularization of the silk mesh: at 1 month post implantation, tissue ingrowth and marked vascularization was evident; at 4 months, the mesh was no longer felt through the skin; and at 12 months, the mesh degradation and vascularization was scored as mild but with substantial silk loss that precluded mechanical testing of the remaining SERI Surgical Scaffold®.<sup>[73]</sup> At 12 months, the SERI Surgical Scaffold® had stimulated extensive type I collagen deposition and the resulting tissue was mechanically strong.<sup>[73]</sup> Clinical hernia repair in a horse showed incomplete SERI Surgical Scaffold® degradation at 2 years post implantation, but no hernia relapse.<sup>[87]</sup>

The time scale for silk degradation depends on a number of factors, including, but not limited to: (i) the amount of material, (ii) gross morphology, (iii) silk secondary structure, (iv) silk treatment history, (v) mechanical environment, and (vi) implantation site (or final destination). The implantation site directly impacts the type of proteolytic enzyme encountered by the silk, because these enzymes vary between tissues, cells, and subcellular location.



Silk fibroin sequence alignment indicates a susceptibility to a number of proteases (e.g., protease XIV,  $\alpha$ -chymotrypsin, proteinase K, papain, matrix metalloproteinases, collagenase etc.)<sup>[50, 88]</sup>). Nonetheless, predicting silk fibroin degradation simply based on the primary sequence is unreliable; for example, chymotrypsin has 434 cleavage sites in the silk heavy chain and 81 in the light chain, while protease XIV has 348 in the heavy chain and 41 in the light chain. Despite numerous cleavage sites, chymotrypsin treatment for 20 days had no quantifiable effect on silk fibroin, while protease XIV significantly degraded silk fibroin *in vitro*.<sup>[50]</sup> Papain, a cysteine protease enzyme that mimics the activity of lysosomal enzymes, has 26 cleavage sites in the silk heavy chain (albeit exclusively in the amorphous regions) and 15 in the light chain, and it caused significant silk fibroin degradation over 20 days but at a slower rate than protease XIV. Similar observations were made with isolated lysosomal enzyme preparations.<sup>[50]</sup>

Overall, these studies exemplify that the structure beyond the primary sequence is of critical importance for silk degradation. The current working model supports the notion that, for *Bombyx mori* silk, degradation begins with the 11 hydrophilic amorphous segments in the silk heavy chain, as well as the C- and N-terminal and the silk light chain, which consist of completely non repeating amino acid sequences; this is then followed by degradation of the more crystalline sequences.<sup>[50, 60c, 88]</sup> The tightly packed crystalline domains are degraded last.<sup>[89]</sup> Furthermore, the silk format is a critical factor in determining degradation rates, as *in vivo* studies in rodent models indicated faster degradation for open silk structures than for tightly packed monolithic silk fibroin films (rank order: hydrogel > silk scaffold > monolithic film).<sup>[68]</sup>

Protease XIV is a useful model enzyme for studying silk degradation and for comparing with earlier studies. However, protease XIV is a non-mammalian enzyme cocktail, so it must not be used to deduce or predict biocompatibility performance. Silk, as a protein-based biopolymer, is commonly considered to yield harmless biodegradation products; however, a more critical inspection of silk and its degradation products is timely. For example, silk fibrils have molecular-level similarity to amyloid fibrils<sup>[90]</sup>, and they were also reported to enhance amyloidosis of amyloid protein through a mechanism based on cross-seeding effects.<sup>[91]</sup> However, when silk nano- and micro-fibrils were composed of  $\beta$ -sheets, which are known to affect various properties of silk fibers, they demonstrated no significant cytotoxicity toward *in vitro* neuronal cells. When the silk fibroin was degraded with chymotrypsin to yield mainly unordered soluble fragments with a low  $\beta$ -strand content, the degradation products caused no significant amyloidosis. By contrast, significant cytotoxicity was observed when silk fibroin was degraded with protease XIV due to the formation of soluble  $\beta$ -sheet rich fragments<sup>[60c]</sup>.

Formation of  $\beta$ -amyloid structures is a concern because amyloid beta fibrils are a hallmark of Alzheimer's disease.<sup>[60c, 92]</sup> Preliminary studies in mice injected with self-assembling silk fibroin hydrogels into the caudate putamen (striatum) showed no decline in cognitive function or animal behavior over the 6 week study period.<sup>[93]</sup>

## 6. Processing of silk cocoons – generating silk for biomedical use

Unspinning the *Bombyx mori* silk cocoon and degumming to remove sericin, are two crucial steps that yield silk suitable for biomedical use. Sericin can be removed by enzymatic methods (i.e., digesting sericin but not silk) or chemical processing (e.g., alkaline treatment). The latter approach is widely used and typically involves boiling silk in sodium bicarbonate for 20 to 60 minutes.<sup>[41]</sup> Degumming times as short as 5 minutes are also sufficient to remove sericin while minimizing silk damage, which usually occurs due to cleavage of the disulfide bond between the silk heavy and light chain and fragmentation of the amorphous silk sequences in the silk heavy chain, which results in polydispersed silk.<sup>[94]</sup>

The degummed silk fibers can be fully reverse engineered by dissolving them in a high concentration chaotropic agent (for example, 9.3M lithium bromide) at 60 °C over several hours to disassemble the higher order silk structure. The resulting silk fibroin solution is then dialyzed extensively against water to yield an aqueous silk solution that is stable at room temperature for weeks and at 4°C for several months.<sup>[41]</sup> When compared to native silk feedstock, this reverse engineered silk fibroin solution has a reduced solution conformation<sup>[95]</sup> and changed rheological properties.<sup>[39d]</sup>

The reverse engineered aqueous silk fibroin solution is commonly used to generate novel silk formats; for example films, fibers, scaffolds, and (self-assembling) silk hydrogels, as well as (nano)particles and (nano)coatings, and these formats are often achieved using an

all aqueous processing under ambient conditions. These mild processing conditions are ideal for preserving the activity of biologics.

### **7. Present routine clinical use of silk**

The silk surgical mesh SERI Surgical Scaffold®, silk sutures, and silk clothing to treat dermatological conditions are the only available products in routine clinical use today. All these products are manufactured by unwinding *Bombyx mori* silk cocoons and working with the silk thread. The clinical performance of silk sutures, their adverse effects, and the developments and potential solutions to improve suture performance have been reviewed previously.<sup>[40]</sup>

The SERI Surgical Scaffold® technology is based on work conducted by David Kaplan and colleagues at Tufts University, Medford, MA, USA.<sup>[96]</sup> The resulting patent portfolio and proprietary silk processing technologies formed the basis of the spinoff company, Serica Technologies Inc. (Medford, MA, USA). Serica Technologies Inc. was able to prove to the FDA that SERI Surgical Scaffold® was “substantially equivalent” to existing surgical meshes and thus received 510(k) clearance to market the device. Serica Technologies Inc. was subsequently acquired by Allergan Inc., and the SERI Surgical Scaffold® became commercially available for soft tissue support and repair in 2013 and was since then acquired by Sofregen Medical Inc. (Medford, MA, USA).

The current SERI Surgical Scaffold® indications are for abdominal wall reconstruction<sup>[97]</sup> and investigational plastic surgery applications, including total body contouring, brachioplasty, abdominoplasty, mastopexy, and breast reconstruction (**Table 1**).

The clinical performance of SERI Surgical Scaffold® has been reported in the literature, which includes open label clinical trials and case reports (Table 1). Many of these encouraging clinical studies have been sponsored by Allergan Inc. A few independent retrospective clinical reports of small patient cohorts are reporting side effects (e.g., poor scaffold integration, see Table 1, **Figure 3**), often requiring surgical removal of the mesh.<sup>[66b, 104-105]</sup> Therefore, some clinicians are abandoning the use of SERI Surgical Scaffold® in their clinical practices<sup>[104]</sup>, and caution has been raised by others.<sup>[107]</sup> In 2013, Allergan Inc. voluntarily withdraw several SERI Surgical Scaffold® batches due to concerns about product sterility. How this might have affected the reported adverse events is not known.

We are familiar with silk for the textile industry, although silk garments are also used clinically to treat dermatological conditions, especially atopic dermatitis<sup>[108]</sup> and acne vulgaris<sup>[109]</sup> (**Table 2**). Mechanical skin irritation by harsh, rough (e.g., wool) and short (e.g., cotton) textile fibers are thought to contribute to atopic dermatitis. Furthermore, the skin of atopic dermatitis patients is often colonized with *Staphylococcus aureus* and the extent of colonization correlates with the severity of the disease. Silk fibers are very long (up to 1,500 m) and smooth, so they minimize mechanical irritation when knitted into clothing. This silk clothing has been chemically modified to achieve antibacterial

properties with the aim of reducing *Staphylococcus aureus* colonization of the skin. Sericin-free silk has also been covalently functionalized with 3-trimethylsilylpropyl-dimethyloctadecyl ammonium chloride (AEM 5700/5772; AEGIS™), resulting in commercial products (e.g., DermaSilk®) for the treatment of atopic dermatitis. These silk garments use highly purified silk to minimize the risk of contact dermatitis.<sup>[110]</sup>

The silk garments are also knitted in a specific fashion to improve transpiration of sweat through the fabric (unlike everyday silk, which can worsen atopic dermatitis by trapping moisture). A randomized double-blind study in 30 patients with atopic dermatitis on both arms received an AEM 5700/5772 functionalized silk sleeve and a silk-only sleeve. Patients treated with the silk sleeve showed a rapid improvement within 2 weeks but remained similar until the end of the study. The contralateral arm treated with the AEM 5700/5772 functionalized silk showed similar results at 2 weeks but reached a greater level of improvement over 4 weeks<sup>[108d]</sup>. Other clinical trials using AEM 5700/5772 functionalized silk garments in small patient cohorts reported substantial improvements in skin conditions (Table 2). By contrast, a randomized, controlled, observer-blinded clinical trial in 300 children showed only a 3% reduction in skin infection compared to control and was therefore not regarded as providing a significant clinical benefit.<sup>[108e]</sup> Overall, these clinical trials are difficult to conduct, and the use of different silk garments (DermaSilk® and DreamSkin®, see Table 2 for details) undermines the power of the study; therefore, these difficulties might result in underestimation of treatment effects due to objective primary outcome measures. Overall, improving these silk garments to maximize their clinical performance requires more research.

## 8. Clinical trials using silk

The renewed interest in silk for biomedical use over the past 20 years has resulted in a number of clinical trials; however, historically, these data sets have been difficult to source. Since 2007, the FDA has mandated that drug and device manufacturers register clinical trials ([www.ClinicTrials.gov](http://www.ClinicTrials.gov)) (Table 3).

Silk-based biomaterials show particular promise for skin wound healing due to their hemostatic properties, low inflammatory potential, and permeability to oxygen and water, as well as their ability to function as a barrier to bacterial colonization (Table 3). Sidaiyi, a silk fibroin sponge attached to a silicone membrane, is a first generation wound dressing currently approved by the China Food and Drug Administration for clinical use in that country.<sup>[112]</sup> The Sidaiyi platform was first compared to silk fibroin films for wound healing applications in preclinical animal models, followed by a randomized, single blinded Phase I clinical trial. Silk films were made by casting an aqueous *Bombyx mori* silk fibroin solution in a mold and treating it at 65°C and 90% relative humidity for 100 minutes. The resulting 64.9 µm films were water-resistant (albeit their ability to bind a small amount of water that acts as a plasticizer) and formed an effective barrier against bacterial infection *in vitro*. The films were found biocompatible for their intended use according to ISO 10993 tests for the biological evaluation of medical devices. In a rabbit full-thickness wound healing model, healing was three days faster in wounds treated with silk films than in wounds treated with Suprathel, a polyurethane-based synthetic wound dressing, and seven days faster than in wounds treated with the Sidaiyi wound dressing or PBS treated controls (Figure 4). Silk-film-treated wounds showed the development of an

organized epidermis by day 14 post-treatment and showed a mature and organized collagen matrix, hair follicles, and blood vessels histologically by day 21.<sup>[112]</sup> These results were further verified in a porcine full-thickness wound healing model prior to initiation of a Phase I clinical trial that ran from August 2013 to September 2014. This clinical trial enrolled 71 patients (36 randomly assigned to a silk film group and 35 to a Sidaiyi group). The silk-based wound dressings were used to cover donor sites following surgical harvesting of split-thickness skin grafts; healing was significantly faster in the silk film group than in the Sidaiyi wound dressing group and 100% of the wounds were healed by day 14 post injury in the silk film group. One case of inflammatory reaction to the silk film was noted but the exact etiology was not determined. No cases of wound exudation were observed, indicating that the silk films maintained a clean wound environment with suitable moisture levels. The films showed good adhesion to the wound surface and no changes of the wound dressing were required. As the wound healed, the silk films spontaneously detached from the regenerated skin areas. The exact mechanism underlying the improved clinical performance observed for the silk film group over the Sidaiyi group is currently unknown.

However, this study demonstrated the ability to manufacture silk films under Good Manufacturing Practice requirements and their successful use as wound dressings for skin repair and regeneration.<sup>[112]</sup> The potential for relatively easy modification of silk films for additional functionality, such as the incorporation of pores or the introduction of bioactive molecules<sup>[113]</sup>, makes silk films particularly attractive as wound dressings.



Thin silk films have also been used in prospective human clinical trials to repair acute and chronic tympanic membrane perforations.<sup>[114]</sup> These silk patches (Tympasil, Daewoong-Bio, Seoul, South Korea) were generated using reverse engineered *Bombyx mori* silk fibroin. The process leading to stabilization of these silk patches has not been established, but physical cross linking is most likely because the brittle patches were first wetted in PBS to plasticize them to facilitate their trimming to the required size and surgical placement.

The first clinical trial involved 52 patients with acute traumatic tympanic membrane perforation who were treated with either a silk film or a paper-based membrane.<sup>[114b]</sup> A number of conservative treatment modalities had been explored to support the (often spontaneous) healing of an acutely perforated tympanic membrane, including the placement of a “patch” on top of the damaged tympanic membrane. In this trial, the silk or paper patches were surgically placed and removed 7 days later, when the tympanic membrane appeared fully regenerated. The closure rate was similar for both the silk film and paper membrane (92.3 and 84.6%, respectively), but the silk patch significantly shortened the healing time from 16.7 days to 13.7 days.

A similar improved healing with a silk patch was also previously reported in animal studies.<sup>[115]</sup> A follow-up study of 40 patients with chronic traumatic tympanic membrane perforation showed that patients treated with a silk patch (Tympasil) had lower otorrhea, minor complication rates, and high patient satisfaction when compared with conventional perichondrium myringoplasty.<sup>[114a]</sup> The silk and autologous patches were removed one

week after placement, and the postoperative hearing outcomes were not significantly different between the two treatment groups. However, the surgical time for the silk patch was very short (13.7 versus 29.5 minutes) and no sourcing of connective tissue was required for the graft.<sup>[114a]</sup>

## 9. Preclinical use of silk – the future

As we move from the routine clinical use of silk fibers to human clinical trials, the line between a silk thread-based medical device and other forms of silk starts to blur. A very wide spectrum of silk materials and formats is now emerging in pre-clinical studies. We will first review silk solutions derived from reverse engineered *Bombyx mori* silk, followed by more complex formulations.

### 9.1 Silk solution

*Bombyx mori* silk in its solubilized aqueous form has been investigated for a range of therapeutic applications, including treatment of diabetes<sup>[116]</sup>, chronic wounds<sup>[117]</sup>, and inflammation<sup>[118]</sup>. Recent studies have investigated the utility of regenerated silk fibroin solution in preclinical animal models for the treatment of ocular conditions, including dry eye<sup>[119]</sup> and corneal injuries.<sup>[5b]</sup> Blindness from corneal disease affects over 50 million people worldwide, while another 337 million people suffer from dry eye disease, representing a significant healthcare burden.<sup>[5b, 120]</sup>

For example, silk fibroin treatment resulted in increased tear production and reduced the corneal irregularities observed in the absence of treatment in a mouse dry eye model

(consisting of NOD.B10.H2<sup>b</sup> mice exposed to desiccation stress and scopolamine hydrobromide treatment for 10 days). Silk fibroin treatment inhibited detachment of corneal epithelial cells, increased the number of conjunctival goblet cells, and inhibited the secretion of inflammatory factors in the lacrimal gland of the eye, resulting in recovery of the tear film and mucus layer of the eye, improved corneal health, and reduced dry eye symptoms. Other anti-inflammatory agents, such as cyclosporine and corticosteroids, are available on the market for the treatment of dry eye, but silk has demonstrated a potential multi-target therapeutic effect that lacks the common side effects, such as pain and irritation, and other complications associated with long-term corticosteroid use.<sup>[43]</sup> Silk fibroin was demonstrated to stabilize the tear film through anti-inflammatory effects in the lacrimal gland and increased number of conjunctival goblet cells, but the mechanisms underpinning these observations are unknown.

Clinical approaches to treat corneal injuries are relatively limited and predominantly involve topical application of anti-inflammatory or anti-microbial agents that do not promote tissue regeneration. A rabbit corneal injury model, which involved removal of a 7 mm diameter section of the central corneal epithelium, was used to study the effects of an aqueous *Bombyx mori* silk fibroin solution (deemed “silk-derived protein” due to an additional autoclaving step during solubilization in lithium bromide that results in a heterogeneous population of low molecular weight silk fragments) on corneal epithelial healing.<sup>[5b]</sup> All treatments showed corneal wound closure by 48 hours post-injury, as indicated by fluorescein staining; however, treatment with silk accelerated the rate of wound healing 3-fold in the first 6 hours post-injury. Relative to a PBS-treated control,

silk treatment resulted in a significant increase in the numbers of proliferating Ki-67 positive epithelial cells, a dose dependent increase in epithelial cell attachment to the underlying basement membrane (as indicated by focal adhesion kinase staining), and a dose-dependent reduction in MMP-9, a metalloprotease involved in matrix remodeling and corneal repair. Finally, compared to the PBS-treated control, the silk-treated group showed the formation of epithelial layers with tight junctions (ZO-1 staining) that more closely resembled those of healthy corneas.<sup>[5b]</sup> The potential of the silk solution in aiding wound healing is clearly demonstrated, but the exact mechanism of action is yet to be determined.

## 9.2 Silk films

Silk films are among the most extensively explored biomaterials due to the ease of their fabrication and characterization and their versatility. Silk films have been explored for their potential in drug delivery<sup>[60b]</sup>, wound healing<sup>[112-113, 121]</sup>, corneal replacement, and flexible electrode<sup>[122]</sup> applications, among others.

Due to their transparent nature, silk films have been particularly well explored for ocular applications, including corneal and retinal regeneration. Silk films cast from the Indian non-mulberry tasar silkworm *Antheraea mylitta* (which as mentioned previously, unlike *Bombyx mori* silk, contains a natural RGD sequence) displayed a transparency ( $94.4 \pm 0.006\%$ ) and a refractive index ( $1.44 \pm 0.03$ ) suitable for corneal repair<sup>[123]</sup>. These films supported the sprouting, migration, attachment, and proliferation of epithelial cells and keratocytes from rat corneal explants to form complete cell sheets. Further, the films

supported the growth of corneal limbal stem cells from the explants. Silk films cast from *Bombyx mori* silk have also been shown to support the adhesion and growth of human corneal epithelial cells as confluent epithelial sheets similar to those on the amniotic membranes used clinically.<sup>[124]</sup> Further, silk film topography<sup>[125]</sup> and biofunctionalization<sup>[126]</sup> can be optimized to enhance corneal epithelial cell interactions.

Following implantation into the corneal pockets of rabbits, acellular silk films made from *Antheraea mylitta* silk remained transparent and showed no signs of neovascularization.<sup>[123]</sup> The films remained intact for at least two months and had no adverse effect on tear production, intraocular pressure, electrophysiology of the eye, or the histology or ultrastructure of the cornea. Silk film degradation can be controlled through modification of its  $\beta$ -sheet content and this has been extensively used to optimize silk biomaterial properties, including those of silk films for corneal applications.<sup>[58, 127]</sup>

Silk films are also extensively explored for engineering corneal stroma<sup>[12, 128]</sup> and the development of *in vitro* corneal models incorporating epithelium, stroma, and innervation.<sup>[6c]</sup> In addition to corneal repair and regeneration, silk films are also being investigated as a substrate for the development of retinal prostheses. Recently reported retinal prostheses consisting of semiconductive poly(3-hexylthiophene) (P3HT) and conductive poly(3,4-ethylene-dioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) layers spin-coated onto silk films were shown to restore light sensitivity and visual acuity of the primary visual cortex in a well-established rat model of retinitis pigmentosa.<sup>[129]</sup>

Another promising application of silk films involves the introduction of various topographical features, such as microneedles. Microneedles are a minimally invasive and painless alternative to hypodermic needles for drug administration. Silk microneedles have been made using micromolding approaches and a variety of masters, including those made by thermal drawing <sup>[130]</sup>, micromilling followed by wet etching <sup>[131]</sup>, and laser micromachining <sup>[39a]</sup>. DeMuth and colleagues proposed an interesting approach to develop implantable-tip composite microneedles using poly(acrylic acid) (PAA) and *Bombyx mori* silk fibroin for sustained vaccine delivery (**Figure 5**).<sup>[39a]</sup> Recent studies have shown the importance of antigen and adjuvant delivery kinetics in developing an optimal immune response, and persistent antigenic and inflammatory signals have been shown to elicit stronger responses when compared with transient bolus vaccine exposure.<sup>[96, 132]</sup>

Silk has the advantage of controlled cargo release over time and can protect temperature-sensitive cargoes at elevated temperatures, potentially allowing elimination of the “cold chain” that limits the availability of vaccines in developing countries.<sup>[133]</sup> The composite microneedle consists of a silk fibroin tip loaded with the vaccine of interest and vaccine-loaded PAA microneedle pedestals.<sup>[39a]</sup> Upon exposure to the aqueous environment of the skin, the PAA rapidly dissolves within hours, releasing a bolus vaccine injection, while the silk fibroin tips remain implanted in the skin, releasing the vaccine over days. The microneedles were demonstrated to easily penetrate murine skin, with insertion occurring several hundred microns below the skin surface. The skin healed within a day of patch application. A fluorescently labeled model protein was used to demonstrate vaccine

release, and rapid PAA-delivered cargo clearance was observed from the treatment site within 24 hours, followed by a slow release of silk-encapsulated cargo at the treatment site between 4 days (when silk fibroin was not treated with methanol) and >16 days (when silk fibroin was treated with methanol), indicating the ability to tune the drug release. When used to deliver a model whole-protein vaccine, the composite platform resulted in over a 10-fold increase in antigen-specific T-cell and humoral immune responses when compared to traditional immunization approaches. Notably, the microneedles were stored at room temperature for two months prior to testing, demonstrating the potential of this technology to eliminate the cold chain.

### 9.3 Silk scaffolds

Three-dimensional porous silk scaffolds can be manufactured via a number of approaches<sup>[41]</sup>, including the use of sodium chloride as a porogen leached from aqueous or organic silk solutions or following freezing and lyophilization of aqueous silk solutions.<sup>[39c]</sup> Early applications of these biomaterials predominantly focused on bone replacement and regeneration<sup>[134]</sup> due to the high mechanical strength of silk scaffolds and the potential for further reinforcement using degummed silk fibers, which approached the mechanical properties required for load-bearing bones.<sup>[63]</sup> Silk scaffolds have since found applications in a range of tissue engineering procedures for replacement and regeneration of tissues<sup>[113, 135]</sup>, as well as in the development of 3D *in vitro* tissue models.<sup>[6c, 39c]</sup>

Recently, silk-based (*Bombyx mori*) fracture fixation devices have been developed using an approach that differs from the use of traditional highly porous silk scaffolds. Metal

alloys are the current gold standard for fracture-fixation devices, despite issues arising from the extreme mechanical mismatch with native bone, which can have profound effects on wound healing and long term viability of the devices, particularly in pediatric patients.<sup>[136]</sup> Resorbable fixation devices, such as those made of PLGA, PGA, and, recently, silk, have the potential to address the disadvantages of metallic fixation devices. Silk, in particular, has the advantage of maintaining high strength while eliciting a low inflammatory response. Furthermore, silk degradation does not generate an acidic microclimate, as is typically observed for solid PLGA and PGA devices.<sup>[137]</sup> *Bombyx mori* silk fibroin fixation devices, including bone plates and bone screws, were manufactured from lyophilized regenerated silk fibroin by casting in hexafluoroisopropanol, followed by machining into desired shapes (**Figure 6**).<sup>[138]</sup> Silk screws were successfully inserted into the hind limbs of rats (a non-functional pre-clinical model) to demonstrate the feasibility of the manufacturing and insertion process. The rats were mobile immediately following surgery and showed no visible signs of pain. The screws were well tolerated for up to 8 weeks, with early signs of resorption and formation of new bone evident around the threads of the screw. These devices are particularly appealing due to the potential for malleability when hydrated, which will allow shaping of the fixation plates for unique anatomical locations during surgery, as well as providing the potential to incorporate BMP-2 or antibiotics directly into the fixation devices to increase their functionality.



#### 9.4 Electrospun silk biomaterials

Electrospinning has emerged as a popular technique for the development of biomaterials due to the ECM-like fibrous nature of the non-woven matrices and the control over fiber properties that can be achieved by tuning the electrospinning parameters. Electrospun silk fibroin has been explored for a range of applications, including wound dressings<sup>[113, 139]</sup>, bone<sup>[140]</sup> and ligament<sup>[141]</sup> replacements, and vascular grafts.<sup>[142]</sup> Small diameter electrospun silk conduits have the potential to address the unmet need for off-the-shelf small diameter grafts with mechanical properties that match those of native vessels and support appropriate endothelial and blood cell interactions. In a comprehensive *in vivo* study of small diameter electrospun silk graft performance, Filipe and colleagues have demonstrated the production and performance of electrospun acellular silk fibroin grafts generated under aqueous conditions in the absence of chemical cross-linkers (**Figure 7**).<sup>[142c]</sup> The electrospun silk fibroin grafts were significantly more elastic when compared with the gold-standard synthetic polytetrafluoroethylene (ePTFE) grafts ( $4.2 \pm 0.5$  MPa vs  $31.9 \pm 1.3$  MPa) and more closely matched the elasticity of native rat vessels ( $2.1 \pm 1.0$  MPa), while demonstrating adequate burst pressure (849 mmHg) and suture pull-out strength (0.86 N). *In vitro*, the electrospun silk fibroin demonstrated excellent endothelial cell interactions and blood compatibility.

These characteristics are critical for the successful development of next-generation vascular grafts, because only grafts that have both outstanding hemocompatibility and support endothelialization will yield long-term performance. In an *in vivo* rat model of abdominal aortic replacement, electrospun silk fibroin scaffolds demonstrated excellent

surgical handling and patency for up to six months and outperformed the gold-standard ePTFE grafts. Silk fibroin grafts supported rapid endothelialization, with endothelial cells present as early as 3 weeks post-implantation and an almost complete monolayer forming by 6 weeks (Figure 7). By contrast, the ePTFE grafts remained largely uncovered by endothelial cells even at 24 weeks post-implantation. The silk fibroin grafts showed intimal hyperplasia stabilization by 6 weeks, with smooth muscle cells (SMCs) showing a phenotypic switch to the less proliferative SMC- $\alpha$  expressing cells and increases in collagen, elastin, and proteoglycan production.<sup>[142c]</sup> This study demonstrates the potential for the use of silk fibroin in small diameter vascular graft applications and the findings warrant further pre-clinical testing in large animal models.

## 9.5 Hydrogels

Silk fibroin hydrogels have emerged as promising platforms for the delivery of small molecular weight drugs, biologics, and cells (reviewed in <sup>[143]</sup>), as well as for mimicking of the ECM in three-dimensional *in vitro* tissue models (reviewed in <sup>[144]</sup>) and *in vivo* tissue fillers.<sup>[145]</sup> A classification of physically and chemically cross-linked *Bombyx mori* silk hydrogel is useful. The chemically cross-linked forms will not be discussed here (reviewed in <sup>[143b]</sup>), because these systems do not capitalize on the facile self-assembly process of silk fibroin, a feature that sets it apart from many other (bio)polymer-based hydrogels. This ability to self-assemble arises because silk I can undergo a transition to silk II, which is rich in  $\beta$ -sheets, by the exclusion of solvating water molecules from the hydrophobic domains of the silk block copolymer. The GY sequences are key drivers for the formation of  $\beta$ -sheets, while the exact molecular abundance and composition allows

fine tuning of the solution-gel transition process. Furthermore, pH responsive elements within the silk structure allow silk to adopt a more ordered state (reviewed in <sup>[146]</sup>).

Many different triggers have been used to control the solution-gel transition, including but not limited to (i) vortexing, (ii) ultrasound, (iii) temperature, (iv) osmotic stress, (v) pH, (vi) CO<sub>2</sub> acidification, (vii) non-solvent induced phase separation, and (viii) direct electric current (reviewed in <sup>[147]</sup>). Self-assembling silk fibroin hydrogels show shear thinning, making them ideal for injection and minimally invasive procedures. Treatment protocols are now available that yield self-assembling hydrogels in the absence of organic solvents, chemically cross linkers, or UV irradiation. However, a potential disadvantage of self-assembling silk fibroin hydrogels is that physically crosslinked systems (i.e., those with high  $\beta$ -sheet content) are opaque, due to the formation of nanocrystallites, and they are brittle, as they cannot undergo long range displacements, resulting in low elastic behavior and plastic deformation at strains >10%.

Electric fields yield silk fibroin hydrogels (also known as e-gels) that differ from most other physically cross-linked types.<sup>[148]</sup> First, the secondary silk structure of e-gels is dominated by helical conformations, whereas other (pH-induced) hydrogels are rich in  $\beta$ -sheets. Second, e-gel formation is reversible: a switch in the DC current induces a migration of silk to the new positive electrode (this is possible because of the absence of strong  $\beta$ -sheets). Third, e-gels have strong adhesive properties that are typically absent from other silk fibroin hydrogels. Fourth, e-gels have outstanding elastic properties and can withstand strains up to 2,500%.<sup>[148]</sup>

The assembly of silk micelles into an e-gel at the electrode is completed within minutes. The local drop in pH at the positive electrode below the isoelectric point of silk screens repulsive charges and enables hydrogel formation.<sup>[149]</sup> Overall, e-gels have been proposed to support a range of biomedical applications (e.g., adhesives for medical devices, sensors, etc.) and proof of principle studies are eagerly awaited.

Self-assembling *Bombyx mori* silk fibroin hydrogels with a high  $\beta$ -sheet content have also been explored for a broad range of biomedical applications. For example, self-assembling silk fibroin hydrogels were loaded with doxorubicin and administered locally to breast tumors in mice. Locally administered silk fibroin hydrogels loaded with doxorubicin provided a significant reduction in primary tumor growth and metastasis when compared to equivalent doses of doxorubicin administered systemically.<sup>[150]</sup> A parallel study that used the same animal model, drug, dosing schedule, and treatment strategy showed that the heparin-modified PEG hydrogels were outperformed by the self-assembling silk hydrogel.<sup>[151]</sup> Self-assembling silk fibroin hydrogels that use sonication as a trigger are ideal for the delivery of biologics. The payload is added following sonication and during the solution-gel transition time window. For example, self-assembling silk fibroin hydrogels have been examined for the delivery of bevacizumab (a monoclonal antibody of antivascular endothelial growth factor) for the treatment of age-related macular degeneration.<sup>[152]</sup>

Direct comparison of a silk fibroin hydrogel formulation with the current standard treatment showed that intravitreal injection in healthy rabbits significantly improved the drug levels in the vitreous and aqueous humor when compared to the commercial liquid formulation. The bioavailability of the commercial product and the silk fibroin hydrogel formulation were similar, but the terminal half-life for the silk hydrogel was 2 to 3 fold higher. This improvement would be expected to reduce the number of intravitreal injections required.<sup>[152]</sup> Although the results are encouraging, further optimization of the silk fibroin hydrogel is warranted to reduce the initial burst release of bevacizumab and the placement of the silk hydrogel, because vitreous administration has the potential to obstruct the light path into the eye and thereby limit vision.

Self-assembling silk hydrogels are emerging as useful tools for the therapeutic delivery of (stem) cells. For example, pancreatic islet transplantation is plagued by a functional decline and decreased viability of the islets during the peritransplantation period, so self-assembling silk fibroin hydrogels have been examined as a potential delivery system.<sup>[153]</sup> Silk self-assembly was initiated by vortexing, the islet cells were added prior to the completion of the solution-gel transition, and the mixture was injected into the epididymal fat pad of diabetic mice. Functional tests showed that silk fibroin hydrogels loaded with pancreatic islets were able to control glucose levels within 4 days, whereas this time was extended to 14 days in the absence of the silk carrier matrix. Intraperitoneal glucose tolerance tests showed that co-transplantation of mesenchymal stem cells (MSCs) with the islets improved the function of the graft through the production of trophic and angiogenic factors. Furthermore, transplantation of minimal islet cell grafts and MSCs

(serving as a supporting stromal cells) using self-assembling silk fibroin hydrogels resulted in euglycemia control in 75% of the transplanted mice at day 37, whereas no other treatment combinations successfully abolished diabetes.<sup>[153]</sup> The use of a silk fibroin hydrogel maximized the clinical performance; however, histological examination showed an unintended complication as the MSCs differentiated into osteoblasts at day 42. MSCs are responsive to environmental cues (e.g., mechanical forces, cytokines, etc.), which indicates that (silk) hydrogels still require fine-tuning to ensure the final desired outcome.

## 9.6 Particles

The pre-clinical development of silk (nano)particles is often aimed at the delivery of cytotoxic small-molecular-weight anticancer drugs (reviewed by <sup>[69]</sup>). Entrapment into a (nano)particle changes the pharmacokinetic and biodistribution characteristics of the payload, as these characteristics are now dictated by the carrier and not the physiochemical properties of the drug. Therefore, engineering the carriers opens up new possibilities for tuning the overall drug performance; for example, by altering the residence time in the blood or the uptake mechanism into target cells. Nanoparticles are often proposed for solid tumor targeting as they can exploit the leaky vasculature and reduced lymphatic drainage associated with tumors, which results in enhanced permeability and retention (EPR) at the target tissue.<sup>[154]</sup> Inclusion of a targeting ligand can further increase the specificity. The EPR effect exploits pathophysiology, but full clinical exploitation remains to be realized.<sup>[155]</sup> Nanoparticles designed for EPR-mediated tumor targeting are typically injected into the blood circulation and must therefore be compatible with blood. Silk fibroin nanoparticles showed very low plasmatic coagulation

and the observed response was significantly better when compared to silica nanoparticles. Furthermore, under simulated venous blood flow, the silk and PEGylated silk fibroin nanoparticles also showed low inflammation when compared to silica nanoparticles.<sup>[71]</sup> Overall, these initial studies on the hemocompatibility of silk fibroin nanoparticles are encouraging.

Early studies set out to explore techniques for the manufacture of silk fibroin nanoparticles and to establish their respective loading capabilities using (model) drugs (reviewed by <sup>[156]</sup>). Two of the early manufacturing techniques used to generate silk fibroin nanoparticles were nanoprecipitation <sup>[157]</sup> and capillary micro-dot printing.<sup>[158]</sup> Capillary-dot microprinted nanoparticles loaded with curcumin showed extended release profiles and a higher *in vitro* efficiency against breast cancer cells when compared to silk/chitosan composite nanoparticles.<sup>[158]</sup> Nanoparticles generated from *Bombyx mori* and *Antheraea mylitta* (the tropical tasar silkworm) silks were stable, spherical, negatively charged, and 150 to 170 nm in diameter, and they showed no cytotoxicity at the tested concentrations.<sup>[159]</sup>

The delivery of small molecular weight drugs with (silk) nanoparticles changes their uptake mechanisms (and their susceptibility to drug efflux pumps) from passive diffusion across the plasma membrane to an energy-dependent endocytic uptake (independent of drug efflux pumps). For these reasons, silk fibroin nanoparticles were able to improve anticancer drug delivery into drug resistance breast cancer cells.<sup>[160]</sup>

Once inside the cells, the payloads on silk fibroin nanoparticles can also be activated within lysosomes (i.e., lysosomotropic drug delivery) by the low pH and the proteolytic enzymes of lysosomes, given the correct intracellular trafficking of the nanoparticles.<sup>[161]</sup>

The lysosomal environment not only triggers drug release but is also the site of silk fibroin nanoparticle degradation.<sup>[50]</sup> Silk fibroin (nanoparticles) can stabilize a broad spectrum of payloads by tailoring the water content, locking the payload into place, buffering the microenvironment, and restricting the access of degradative enzymes (reviewed in <sup>[133]</sup>). For example, entrapment of L-asparaginase into *Bombyx mori* silk fibroin nanoparticles resulted in an increased resistance to enzymatic degradation, better stability in serum, prolonged storage stability in solution, and minimal leakage of the enzyme from the carrier.<sup>[162]</sup> PEGylation of silk fibroin nanoparticles has been exploited to improve colloidal stability and to tailor drug release and carrier degradation.<sup>[163]</sup> Magnetically responsive, drug-loaded silk fibroin nanoparticles have also been developed by seeding silk with Fe<sub>3</sub>O<sub>4</sub> nanoparticles.<sup>[164]</sup> Subjecting these silk nanoparticles to a magnetic field in the tumor area allowed their enrichment in the tumor, thereby promoting drug accumulation and an improved antitumor response <sup>[164]</sup>. Silk fibroin nanoparticles have also been combined with other silk formats (for example, silk fibroin hydrogels) to yield first generation all-silk dual-drug delivery systems.<sup>[165]</sup>

## 10. Bioengineered silks

Recent efforts have focused on developing recombinant forms of silks that can be altered at the sequence level to achieve specific modalities, for example, for biomedical use.<sup>[166]</sup>

Recombinant approaches are unique because they allow the design and manufacture of



bespoke “silks”.<sup>[167]</sup> For example, key elements of the spider silk sequence can be lifted and combined with polylysine to develop novel biopolymers for the delivery of genetic material (reviewed in <sup>[168]</sup>). Chimeric proteins, such as silk-collagen-like proteins<sup>[169]</sup> or silk-elastin-like proteins (SELPs), are exciting new materials (although the SELPs have been extensively studied over the past 3 decades; reviewed in<sup>[170]</sup>). SELPs are facile biopolymers that can be fine-tuned to achieve a desired form and function; for example, SELP micelles have been designed for anticancer drug delivery that exploits both passive and active tumor targeting. SELPs have also been used to generate hydrogels intended for local drug release.<sup>[170]</sup> Many other studies have examined the performance of “biopolymer alloys” by blending silk with another biopolymer, such as tropoelastin<sup>[171]</sup>, collagen<sup>[172]</sup>, and fibronectin<sup>[173]</sup> or have included inorganic ceramics (reviewed in <sup>[174]</sup>) to generate new material systems with expanded function. One of the hallmarks of silk is its inherent ability to organize structures at the nanometer scale; these structures then assemble, grow, and ultimately produce macroscale constructs with defined function. The ability of silk to work seamlessly across several orders of magnitude is exciting and has motivated the development of engineered nanoscale systems. For example, atomic force microscopy has been used to drive and direct the self-assembly of SELP nanofibers<sup>[175]</sup>, while macroscopic silk constructs have been patterned using ion beam lithography to yield diverse silk-like constructs with defined shapes at the nanometer scale.<sup>[176]</sup> Other examples include engineered silk oligonucleotide conjugates that direct silk assembly into a parallel, antiparallel, and branched configurations (reviewed in<sup>[177]</sup>). The timely review by Aigner *et al.* provides extensive insight into this important branch of silk

research.<sup>[166]</sup> Here, we only provide a selection of a few examples of recombinant silks (**Table 4**), with a specific reference to silk (nano)particles.

Recombinant silk proteins have been inspired by *Araneus diadematus* fibroin 4 (ADF4); ADF4 is from the common European garden spider. ADF4 is one of the most widely studied spidroins<sup>[5a]</sup> and has been used to prepare microcapsules for drug delivery by exploiting its self-assembly at an emulsion interface.<sup>[186]</sup> The resulting spider silk-based carriers are useful for encapsulating low molecular weight drugs under mild conditions, which maintains the activity of the payload.<sup>[186]</sup> Exposure of ADF4-like silks to potassium chloride (>400  $\mu\text{M}$ ) generates particles<sup>[187]</sup>, which have been extensively characterized for their ability to entrap and release (small molecular weight) payloads.<sup>[188]</sup> Silk sequence modifications are now yielding cationic proteins that can be loaded with low and high molecular anionic payloads (e.g., nucleic acids).<sup>[189]</sup> Libraries of silk proteins containing modifications, such as the RGD integrin binding domain and the Tat cell penetrating peptide, have been designed to enhance cellular uptake and drug delivery.<sup>[190]</sup> The first inroads have been made to unravel the mechanisms for endocytic (e.g., caveolae, clathrin-mediated) uptake of nanoparticles into cells using putative chemical inhibitors.<sup>[191]</sup>

Recombinant technologies have also been exploited to generate silks inspired by major ampullate spidroin 1 (MaSp1) from the Gold Orb-web spider (*Nephila clavipes*). For example, MaSp1-like proteins have been modified with poly(L-lysine) cationic sequences to allow complexation with nucleic acids via electrostatic interactions for use in gene

delivery. These engineered protein vectors demonstrated excellent DNase resistance and gave transfection efficiencies similar to those achieved with the commercial reagent Lipofectamine 2000. The silk sequence has also been fused with tumor-homing peptides (e.g., F3, Lyp1 CGKRK) and attachment ligands (e.g., RGD to exploit cell binding and receptor-mediated endocytosis) to enhance their targeting capabilities.<sup>[60c, 92, 165]</sup> These recombinant silks formed nanometer-sized globular complexes with plasmid DNA (150 to 250 nm in diameter), and they demonstrated significantly improved target specificity for melanoma and highly metastatic human breast cancer cells.<sup>[92, 165]</sup> A silk vector harboring the tumor-homing peptide F3 showed minimal toxicity in healthy cells, the best tumor specificity, and a capability to deliver its payload in a human orthotopic breast cancer model.<sup>[192]</sup> Overall, these studies demonstrate the potential of silk-based delivery systems as non-viral gene delivery vectors.

## **11. (Old) New silk industries: opportunities and challenges for the road ahead**

Current production routes for *Bombyx mori* silk are well established and are the foundation of several products in routine clinical use today. Following a period of decline, global silk production is growing once again and has the capacity to keep up with a growing silk demand for (bio)medical use.<sup>[18]</sup> However, sericulture is an agricultural process that depends on several factors, including, but not limited to, climate, seasonal variations, disease and pest control, and the susceptibility of silkworms to common pesticides used in other agriculture sectors (within geographical proximity, with fatal consequences for the silkworm). The silk community should make a concentrated effort to work with well-controlled silk cocoon stocks with a known process history in order to

propel the silk research community into working practices that align with Good Laboratory Practice. This type of approach would be invaluable for improving comparisons across different studies and accelerating clinical translation. While public guidelines exist for CE-marked organic sericulture, the production of *Bombyx mori* silk for medical use is shrouded in secrecy and proprietary protocols. For example, the domesticated *Bombyx mori* silkworm line used for silk sutures and surgical meshes is a well-kept secret.

The optimal environmental condition during cocoon spinning is another unknown. Laboratory experiments indicate that silk cocoons spun in a low humidity environment do not require degumming by boiling in an aqueous alkaline solution; instead, physical manipulation is sufficient for quantitative removal of sericin.<sup>[193]</sup> Of course, adequate silk cleaning is critical for producing hypoallergenic silk. Eliminating the boiling step also preserves the silk structure, thereby yielding a mono-dispersed biopolymer (rather than polydispersed silk fibroin fragments). This, in turn, is likely to simplify the regulatory requirements that currently mandate a well-defined material stock (although these regulations vary depending on the specific classification of the final product as “medical device”, “excipient”, “advanced therapy medicinal product”, “novel chemical entity” etc.).

Full reverse engineering of the silk cocoon is complex from both industrial and regulatory perspectives. At present, all licensed (*Bombyx mori*) silk products in the USA and EU are considered medical devices based on the nascent, but degummed, silk fiber

(Table 1, 2 and 5). In contrast reverse engineered silk is marketed as cosmetics to navigate regulatory challenges. Irrespective of the medical or cosmetic use, the current silk production technologies are batch processes, which go against the current industrial efforts aimed at continuous manufacturing to ultimately improve product reliability and cut costs.

Manipulating (the germ line of) silkworms by genetic engineering opens up new possibilities to generate improved silks, for example, by inserting spider silk sequences into *Bombyx mori* silk to improve the mechanical properties of the *in vivo* spun fiber.<sup>[194]</sup> Another possibility is to exploit the silkworm as a biosynthetic host to synthesize xenogenic proteins or functional silks (e.g., insertion of green fluorescent protein, RGD sequences, etc.<sup>[195]</sup>). Improvements in genome editing are likely to increase the protein yields, ultimately leading to new silkworm lines (e.g., that produce modified silk sequences etc.) of appreciable economic value.

Recombinant proteins are routinely used in the clinic and are manufactured on a large scale by the pharmaceutical industry. Therefore, the necessary expertise exists within the healthcare sector to manufacture these complex products to clinical specifications. Already, today, recombinant DNA technology, in combination with *Escherichia coli* expression systems, has revolutionized silk research<sup>[166]</sup> and is opening up new commercial avenues (Table 5). However, silk presents a number of specific challenges; for example, the silk molecules cannot be post-translationally modified, but this is required to faithfully mimic the silk protein.<sup>[196]</sup> The expression of native-sized silk

proteins is also not possible using standard *E. coli* expression systems because of the highly repetitive nature of the gene constructs, the very high glycine content of the protein, and the high molecular weight of the product (250–320 kDa). These challenges can be addressed by using genetically modified *E. coli* with an elevated glycyl-tRNA pool, which give native-like silk proteins, albeit with a low protein yield.<sup>[197]</sup> Another complication is that silk inspired proteins with repeated domains containing Ala-rich sequences are poorly soluble in water and buffers. Inclusion of a cationic histidine tag with the hydrophobic crystallizable silk sequences improves the aqueous solubility of the resulting protein.<sup>[60c]</sup> The economic issues and greenhouse gas emissions associated with *E. coli* cultures are other critical aspects that must be considered for the development of a sustainable recombinant silk industry.<sup>[198]</sup>

### 11.1 Staying on the “silk road”

Perhaps one of the most important aspects to consider regarding the future of the biomedical use of silk is the safeguarding of the silk development pipeline. Silk is a truly amazing biopolymer that has inspired generations of scientists and is likely to continue to do so. However, researchers must not get carried away or over promise. In a globally connected world, with many clinically unmet needs, the news of promising biomedical research has the potential to make headlines simply by the fact that the material is “silk”. We must not exploit the familiarity of the general population with common silk for the purpose of short term gains. The road to the clinical translation of basic biomedical research is a long and tortuous one – this also applies to silk research. The path is difficult and requires a carefully measured balance of optimism (to inspire people) and realism (to

avoid a silk bubble). It is important to learn from past failures as well as from the challenges experienced in allied fields.<sup>[199]</sup> Clearly, silk has its limitations, as detailed in this review; for example, (i) it is an expensive biopolymer when compared to mass-produced fully synthetic polymers, (ii) sericulture is a labor-intensive agricultural process and highly responsive to its environment, which ultimately impacts silk quality, and (iii) the durability of recombinant spider silks might be challenging based on the simple premise that the material has evolved in nature as a short-term high-performance material (e.g., orb-weaver spiders repair or build a new web often daily). It is therefore important to consider alternative materials as well that are able to perform, and perhaps surpass, the function of silk. The emergence of fully synthetic sutures is a testament to this type of development. However, silk fibroin sutures still remain the first choice for specialized surgeries, indicating that silk should serve as a blueprint for next generation sutures.

### **11.2 The current silk drivers**

Silk continues to inspire and serves as the thread for exploration new avenues: curiosity-driven research and learning from nature are key elements for innovation. Newly emerging silk industries are now translating research findings that go beyond fiber technology<sup>[200]</sup> (Table 5). As these technologies, which often have their origins in the academic setting, move from the public domain into the industrial space, tracking their progress becomes more difficult. However, the first products have entered clinical trials and products are emerging on the market (Table 5 and detailed below).

Recombinant (spider) silks are the lifelines of many small to medium sized enterprises. Spider silk is a prime focus, because this remarkable biopolymer cannot be obtained at an industrial scale by “farming.” Instead, genetic engineering and recombinant expression systems are essential, and they also provide greater flexibility and rapid production of novel silk-inspired proteins. The (pharmaceutical) industry is accustomed to working with recombinant proteins, and these provide many opportunities for patterning and proprietary knowledge. Many companies currently working with recombinant silks are generating fibers. The remarkable mechanical properties of spider silk, coupled with its processing under ambient conditions in an all aqueous environment, might serve as an inspiration for this new industry.

The Synthetic Bioproducts Center, under the leadership of Randy Lewis (Utah State University, Logan, UT, USA), produces synthetic spider silks for biomedical engineering applications using a range of expression hosts, including genetically modified goats. These genetically engineered dairy goats carry the dragline silk genes of the *Nephila clavipes* (identified by the Lewis lab <sup>[201]</sup>) in mammary gland cells and excrete soluble silk in their milk. This silk can be extracted and subsequently spun into fibers. The genetically modified goats were developed by Nexia Biotechnologies Inc. (Quebec, Canada) in the early 2000s and subsequently acquired by Randy Lewis. Nexia Biotechnologies and the Materials Science Team of the U.S. Army Soldier Biological Chemical Command (Natick, MA, USA) expressed the silk proteins ADF-3/MaSp2 and MaSp1 for the first time in mammalian cells <sup>[202]</sup>, thereby laying the foundation for subsequent work in goats.



AMSilk GmbH (Planegg/Munich, Germany) is a spinoff company based on seminal work by Thomas Scheibel and co-workers and is the world's first industrial supplier of recombinant silk biopolymers across a range of applications. AMSilk exploits ADF4-inspired silks to generate high quality materials for apparel, cosmetic, industrial, and structural applications. Biosteel® fiber is their leading product, and it is at various developmental stages for application in the footwear and automotive industries. AMSilk received ISO 13485 accreditation in 2017—a prerequisite for initiating clinical studies in humans. In February 2018, AMSilk announced the launch of the POSIS Phase I clinical trial, in partnership with POLYTECH Health & Aesthetics GmbH. The trial examines the performance of silicone breast implants coated with ADF4-based silk (BioShield-S1) (SILKline®) in several human subjects at a number of University hospitals in Austria (with the option to expand to other centers). This trial has been underpinned by both proprietary product development efforts, as well as animal studies available in the public domain. For example, ADF4 coated silicone implants showed no acute toxicity or immunogenicity and they reduced post-operative inflammation and minimized implant-induced capsule thickness and contraction when compared to uncoated control implants.<sup>[203]</sup>

Founded in 2008, Spiber Technologies AB (Stockholm, Sweden) produces Spiber® silk biomaterials, which can be processed into a range of material formats (e.g., fibers, films, foams, coatings, etc.). Their work is underpinned by academia-industry collaborations that support the pre-clinical Research and Development pipeline exploiting spider silk for

biomedical applications (e.g., cell-matrix interactions, culture matrices, surface modifications, and electrochemical meshes etc.).<sup>[204]</sup> Scientists affiliated with Spiber Technologies AB have also unraveled the molecular mechanisms of spider silk spinning<sup>[205]</sup> in order to develop better biomimetic fibers.<sup>[206]</sup>

The pH-sensitive N-terminal of silk enables the silk protein to stay in solution at very high protein concentrations; thus, recombinant fusion of the N-terminal domain converted an aggregation-prone therapeutic protein to its hyper-soluble counterpart (this technology is now marketed as SolvNT®).<sup>[207]</sup> This strategy also simplified protein purification, improved yields, and allowed the expression of non-transmembrane proteins that are otherwise refractory to recombinant production. SolvNT® exemplifies how silk continues to amaze us, and goes beyond applications we are accustomed to.

Spiber Inc. (Yamagata, Japan), founded by Kazuhide Sekiyama in 2007 as a start-up at Keio University, produces various types of recombinant proteins, especially spider silk-like proteins. (Note that Spiber Inc. is an independent company from Spiber Technologies AB). The synthetic spider thread QMONOS™ (based on the Japanese word *kumonosu* for spider web) is a technology fiber that has been fed into several proof of concept items, such as a child's dress, and The North Face branded MOON PARKA®. The current target markets are the apparel and automotive segments, with healthcare materials serving as an emerging future area.

Another contender in the recombinant silk market space is Bolt Threads Inc. (Emeryville, CA, USA), which produces silk fibers by wet spinning (Engineered Silk™) using advanced yeast expression systems. The recombinant silk is produced on a scale that makes it a viable contender for broad applications. To date, Bolt Threads has already introduced several engineered Silk™ fiber and Bolt Microsilk™ products (e.g., ties, clothing etc). Whether these materials will also enter the healthcare arena is not known at present.

## **12. Conclusions**

In this review, we have unraveled some of the mysteries of silk and critically examined the current and emerging clinical uses of silk. We eagerly await further clinical reports, especially on engineered second-generation silk materials, such as “silk”-coated implants. For the past 5,000 years, silk has captivated humans, and it continues to amaze us as we explore new applications. The silk biopolymer represents a pioneer material for medical applications today, and yet, even after many centuries, it continues to be a valued suture material. As we continue to extract secrets from silk, we will be able to develop new “old materials” to address current and future biomedical needs.

## **Competing interests**

The authors (CH, KN, JRK, FPS) declare no potential conflicts of interest with respect to the research, authorship, and publication of this article. Furthermore, the authors disclose that they do not have any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript. FPS is supervising a M Res

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### References

- [1] a) J. Sparkes, C. Holland, *Nat. Commun.* **2017**, *8*, 594; b) D. Porter J. Guan, F. Vollrath, *Adv. Mater.* **2013**, *25*, 1275; c) D. Porter F. Vollrath, Z. Shao, *Eur. Phys. J. E. Soft Matter.* **2005**, *16*, 199; d) N. G. Rim E. G. Roberts D. Ebrahimi N. Dinjaski M. M. Jacobsen Z. Martin-Moldes M. J. Buehler D. L. Kaplan, J. Y. Wong, *ACS Biomater. Sci. Eng.* **2017**, *3*, 1542; e) D. Ebrahimi O. Tokareva N. G. Rim J. Y. Wong D. L. Kaplan, M. J. Buehler, *ACS Biomater. Sci. Eng.* **2015**, *1*, 864.
- [2] a) C. Craig, *Annu. Rev. Entomol.* **1997**, *42*, 231; b) C. L. Craig, *Spiderwebs and silks: tracing evolution from molecules to genes to phenotypes*, Oxford University Press, New York, **2003**; c) T. D. Sutherland J. H. Young S. Weisman C. Y. Hayashi, D. J. Merritt, in *Annu. Rev. Entomol.*, *Vol. 55*, **2010**, pp. 171; d) Q. Xia S. Li, Q. Feng, *Annu. Rev. Entomol.* **2014**, *59*, 513; e) P. L. Babb N. F. Lahens S. M. Correa-Garhwal D. N. Nicholson E. J. Kim J. B. Hogenesch M. Kuntner L. Higgins C. Y. Hayashi I. Agnarsson, B. F. Voight, *Nat. Genet.* **2017**, *49*, 895; f) N. Yonemura, F. Sehnal, *J. Mol. Evol.* **2006**, *63*, 42.

- [3] a) P. R. Laity S. E. Gilks, C. Holland, *Polymer* **2015**, *67*, 28; b) P. Laity, C. Holland, *Int. J. Mol. Sci.* **2016**, *17*, 1812; c) M. Andersson L. Holm Y. Ridderstråle J. Johansson, A. Rising, *Biomacromolecules* **2013**; d) A. Rising, J. Johansson, *Nat. Chem. Biol.* **2015**, *11*, 309.
- [4] a) B. Mortimer C. Holland, F. Vollrath, *Biomacromolecules* **2013**, *14*, 3653; b) B. Mortimer S. D. Gordon C. Holland C. R. Siviour F. Vollrath, J. F. C. Windmill, *Adv. Mater.* **2014**, *26*, 5179; c) G. R. Plaza P. Corsini E. Marsano J. Perez-Rigueiro M. Elices C. Riekkel C. Vendrely, G. V. Guinea, *J Polym. Sci. B* **2012**, *50*, 455; d) G. B. Perea C. Solanas N. Marí-Buyé R. Madurga F. Agulló-Rueda A. Muínelo C. Riekkel M. Burghammer I. Jorge J. Vázquez G. R. Plaza A. L. Torres F. del Pozo G. V. Guinea M. Elices J. L. Cenis, J. Pérez-Rigueiro, *Eur. Polym. J.* **2016**, *78*, 129.
- [5] a) T. Y. Lin H. Masunaga R. Sato A. D. Malay K. Toyooka T. Hikima, K. Numata, *Biomacromolecules* **2017**, *18*, 1350; b) W. Abdel-Naby B. Cole A. Liu J. Liu P. Wan R. Schreiner D. W. Infanger N. B. Paulson B. D. Lawrence, M. I. Rosenblatt, *PLoS One* **2017**, *12*, e0188154.
- [6] a) A. D. Malay R. Sato K. Yazawa H. Watanabe N. Ifuku H. Masunaga T. Hikima J. Guan B. B. Mandal S. Damrongsakkul, K. Numata, *Sci. Rep.* **2016**, *6*, 27573; b) M. Humenik T. Scheibel, A. Smith, *Prog. Mol. Biol. Transl. Sci.* **2011**, *103*, 131; c) J. Guan Y. Wang B. Mortimer C. Holland Z. Shao D. Porter, F. Vollrath, *Soft Matter* **2016**, *12*, 5926.

- [7] a) B. Madsen Z. Z. Shao, F. Vollrath, *Int. J. Biol. Macromol.* **1999**, *24*, 301; b) Z. Shao, F. Vollrath, *Nature* **2002**, *418*, 741; c) J. Perez-Rigueiro M. Elices G. Plaza J. I. Real, G. V. Guinea, *J. Exp. Biol.* **2005**, *208*, 2633.
- [8] A. A. Walker C. Holland, T. D. Sutherland, *Proc. Biol. Sci.* **2015**, *282*, 20150259.
- [9] C. A. Holland A. E. Terry D. Porter, F. Vollrath, *Nat. Mater.* **2006**, *5*, 870.
- [10] a) M. A. Garrido M. Elices C. Viney, J. Perez-Rigueiro, *Polymer* **2002**, *43*, 4495; b) T. A. Blackledge R. A. Cardullo, C. Y. Hayashi, *Invert. Biol.* **2005**, *124*, 165; c) H.-P. Zhao X.-Q. Feng, H.-J. Shi, *Mater. Sci. Eng. C.* **2007**, *27*, 675; d) C. Viney, in *Conference Proceedings of the Society for Experimental Mechanics Series, Vol. 5*, **2013**, pp. 127.
- [11] J. F. V. Vincent O. A. Bogatyreva N. R. Bogatyrev A. Bowyer, A.-K. Pahl, *J. Royal Soc. Interface* **2006**, *3*, 471.
- [12] E. S. Gil B. B. Mandal S. H. Park J. K. Marchant F. G. Omenetto, D. L. Kaplan, *Biomaterials* **2010**, *31*, 8953.
- [13] E. Morgan, *Gossamer Days: Spiders, Humans and Their Threads*, Strange Attractor, London, UK, **2016**.
- [14] Bon, *Phil. Trans.* **1710**, *27*, 2.
- [15] M. Cartwright, *Ancient History Encyclopedia*, <https://www.ancient.eu/Silk/>, accessed: February, 2018.
- [16] I. L. Good J. M. Kenoyer, R. H. Meadow, *Archaeometry* **2009**, *51*, 457.

- [17] A. Hyman, *Science and Reform: Selected Works of Charles Babbage*, Cambridge University Press, Cambridge, England, **1989**.
- [18] FAOSTAT Statistics Division, Food and Agriculture Organization of the United Nations. <http://faostat3.fao.org/download/Q/QP/E>, accessed: October, 2016.
- [19] T. M. Muffly A. P. Tizzano, M. D. Walters, *J. R. Soc. Med.* **2011**, *104*, 107.
- [20] a) A. Teshome S. K. Raina, F. Vollrath, *J. Insect Sci.* **2014**, *14*, 36; b) M. F. Astudillo G. Thalwitz, F. Vollrath, *J. Clean. Prod.* **2014**, *81*, 158.
- [21] H. C. McCook, *American spiders and their spinningwork. A natural history of the orbweaving spiders of the United States, with special regard to their industry and habits, Vol. 3*, Academy of Natural Sciences of Philadelphia, Philadelphia, U.S.A., **1893**.
- [22] R. Lewis, *BioScience* **1996**, *46*, 636.
- [23] S. Peers, *Golden Spider Silk*, V&A Publishing, **2012**.
- [24] A. Koeppel, C. Holland, *ACS Biomater. Sci. Eng.* **2017**, *3*, 226.
- [25] a) M. Tomenosuke, H. Saburo (Kanebo Ltd), Type US1714039A **1927**; b) I. Wilhelm, M. Herbert (IG Farbenindustrie AG ), Type US1913487A **1930**.
- [26] C. R. Baumann, G. G. Diesser (Carl Rudolf BaumannGottlieb Gottfried Diesser), Type US976977A **1908**.
- [27] H. Masaru (SHOZABURO HOSHINO), Type US1603080A **1924**.

- [28] F. Heinrich, R. Ernst Type US1990588A **1928**.
- [29] L. D. Myers, L. A. Stegemeyer (TWITCHELL PROCESS Co ), Type US1896494A **1929**.
- [30] A. P. Furman (Ira Furman ), Type US1992202A **1932**.
- [31] I. Meisel, R. Mülhaupt, *Macromol. Chem. Phys.* **2003**, 204, 199.
- [32] A. Bloch, A. S. Messores (Ethicon Inc ), Type US3424164A **1966**.
- [33] M. Iwatsuki, T. Hayashi (Ajinomoto Co Inc ), Type US4818291A **1986**
- [34] Y. Tsukada, N. Minoura (National Institute of Advanced Industrial Science and Technology Minister of Agriculture, Forestry and Fisheries), Type JPH01118544A **1987**.
- [35] T. Ninagawa, K. Tsubouchi (National Institute of Sericultural and Entomological Science), Type JPH11104228A **1997**.
- [36] a) M. Murase (Marie Murase ), Type JPH06166850A **1992**; b) S. Yoshida K. Yamaura S. Matsuzawa, M. Suzuki Type JPH0669485B2 **1988**.
- [37] H. Akai T. Nagashima, S. Terauchi (Takayuki Nagashima), Type JPH08268905A **1995**.
- [38] a) J. Huang C. Wong A. George, D. L. Kaplan, *Biomaterials* **2007**, 28, 2358; b) D. L. Kaplan R. Nazarov G. Vunjak-Novakovic, L. Meinel (Tufts University, Massachusetts Institute of Technology), Type US7842780B2 **2003**



- [39] a) P. C. DeMuth Y. Min D. J. Irvine, P. T. Hammond, *Adv. Healthc. Mater.* **2014**, *3*, 47; b) H.-Y. Wang, Y.-Q. Zhang, *Soft Matter* **2013**, *9*, 138; c) Y. Chen Y. Lin K. M. Davis Q. Wang J. Rnjak-Kovacina C. Li R. R. Isberg C. A. Kumamoto J. Mecsas, D. L. Kaplan, *Sci. Rep.* **2015**, *5*, 13708; d) C. Holland A. E. Terry D. Porter, F. Vollrath, *Polymer* **2007**, *48*, 3388.
- [40] G. H. Altman F. Diaz C. Jakuba T. Calabro R. L. Horan J. S. Chen H. Lu J. Richmond, D. L. Kaplan, *Biomaterials* **2003**, *24*, 401.
- [41] D. N. Rockwood R. C. Preda T. Yucel X. Wang M. L. Lovett, D. L. Kaplan, *Nat. Protocols* **2011**, *6*, 1612.
- [42] a) Y. Liu Z. Z. Shao, F. Vollrath, *Nat. Mater.* **2005**, *4*, 901; b) F. Vollrath, *Nature* **2010**, *466*, 319.
- [43] S. Barabino Y. Chen S. Chauhan, R. Dana, *Prog. Retin. Eye Res.* **2012**, *31*, 271.
- [44] J. Perez-Rigueiro C. Viney J. Llorca, M. Elices, *J. Appl. Polym. Sci.* **2000**, *75*, 1270.
- [45] H. W. Kwak J. E. Ju M. Shin C. Holland, K. H. Lee, *Biomacromolecules* **2017**, *18*, 2343.
- [46] a) C. Z. Zhou F. Confalonieri M. Jacquet R. Perasso Z. G. Li, J. Janin, *Proteins* **2001**, *44*, 119; b) C. Z. Zhou F. Confalonieri N. Medina Y. Zivanovic C. Esnault T. Yang M. Jacquet J. Janin M. Duguet R. Perasso, Z. G. Li, *Nucleic Acids Res.* **2000**, *28*, 2413.

- [47] a) T. Asakura K. Okushita, M. P. Williamson, *Macromolecules* **2015**, *48*, 2345; b) S. W. Ha H. S. Gracz A. E. Tonelli, S. M. Hudson, *Biomacromolecules* **2005**, *6*, 2563.
- [48] H. Yamada Y. Igarashi Y. Takasu H. Saito, K. Tsubouchi, *Biomaterials* **2004**, *25*, 467.
- [49] H. J. Jin, D. L. Kaplan, *Nature* **2003**, *424*, 1057.
- [50] T. Wongpinyochit B. Johnston, F. P. Seib, *ACS Biomater. Sci. Eng.* **2018**, *4*, 942.
- [51] T. Asakura K. Ohgo T. Ishida P. Taddei P. Monti, R. Kishore, *Biomacromolecules* **2005**, *6*, 468.
- [52] E. Pennisi, R. F. Service, *Science* **2017**, *358*, 292.
- [53] L. Eisoldt A. Smith, T. Scheibel, *Mater. Today* **2011**, *14*, 80.
- [54] A. Spohner W. Vater S. Monajembashi E. Unger F. Grosse, K. Weisshart, *PLoS One* **2007**, *2*, e998.
- [55] a) T. Lefevre M. E. Rousseau, M. Pezolet, *Biophys. J.* **2007**, *92*, 2885; b) J. D. van Beek S. Hess F. Vollrath, B. H. Meier, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10266; c) Q. Wan K. J. Abrams R. C. Masters A. C. S. Talari I. U. Rehman F. Claeysens C. Holland, C. Rodenburg, *Adv. Mater.* **2017**, *29*.
- [56] a) S. Keten Z. Xu B. Ihle, M. J. Buehler, *Nat. Mater.* **2010**, *9*, 359; b) K. Numata, *Polym. J.* **2015**, *47*, 537.

- [57] a) C. Y. Hayashi, R. V. Lewis, *J. Mol. Biol.* **1998**, *275*, 773; b) O. N. Tretinnikov, Y. Tamada, *Langmuir* **2001**, *17*, 7406.
- [58] T. Asakura M. Okonogi K. Horiguchi A. Aoki H. Saito D. P. Knight, M. P. Williamson, *Angew. Chem. Int. Ed. Engl.* **2012**, *51*, 1212; T. Asakura M. Okonogi K. Horiguchi A. Aoki H. Saito D. P. Knight, M. P. Williamson, *Angew. Chem.* **2012**, *124*, 1238
- [59] a) R. E. Marsh R. B. Corey, L. Pauling, *B.B.A.* **1955**, *16*, 1; b) Y. Takahashi M. Gehoh, K. Yuzuriha, *Int. J. Biol. Macromol.* **1999**, *24*, 127.
- [60] a) D. L. Kaplan C. M. Mello S. Arcidiacono S. Fossey K. Senecal, W. Muller, in *Protein-based materials*, Springer, **1997**, pp. 103; b) E. M. Pritchard, D. L. Kaplan, *Expert Opin. Drug Deliv.* **2011**, *8*, 797; c) K. Numata P. Cebe, D. L. Kaplan, *Biomaterials* **2010**, *31*, 2926.
- [61] a) J. Guan F. Vollrath, D. Porter, *Biomacromolecules* **2011**, *12*, 4030; b) D. Porter, F. Vollrath, *Biochim. Biophys. Acta* **2012**, *1824*, 785.
- [62] C. Boutry, T. A. Blackledge, *J. Exp. Biol.* **2010**, *213*, 3505.
- [63] B. B. Mandal A. Grinberg E. S. Gil B. Panilaitis, D. L. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 7699.
- [64] S. Yodmuang S. L. McNamara A. B. Nover B. B. Mandal M. Agarwal T. A. Kelly P. H. Chao C. Hung D. L. Kaplan, G. Vunjak-Novakovic, *Acta Biomater.* **2015**, *11*, 27.

- [65] D. F. Williams, *Biomaterials* **2008**, *29*, 2941.
- [66] a) M. Jewell W. Daunch B. Bengtson, E. Mortarino, *Ann. N.Y. Acad. Sci.* **2015**, *1358*, 44; b) A. van Turnhout C. J. J. Franke E. J. C. Vriens-Nieuwenhuis, W. B. van der Sluis, *J. Plast. Reconstr. Aesthet. Surg.* **2018**, *71*, 644.
- [67] S. Franz S. Rammelt D. Scharnweber, J. C. Simon, *Biomaterials* **2011**, *32*, 6692.
- [68] A. E. Thurber F. G. Omenetto, D. L. Kaplan, *Biomaterials* **2015**, *71*, 145.
- [69] F. P. Seib, *AIMS Bioeng.* **2017**, *4*, 239.
- [70] a) F. P. Seib M. Herklotz K. A. Burke M. F. Maitz C. Werner, D. L. Kaplan, *Biomaterials* **2014**, *35*, 83; b) F. P. Seib M. F. Maitz X. Hu C. Werner, D. L. Kaplan, *Biomaterials* **2012**, *33*, 1017.
- [71] M. F. Maitz C. Sperling T. Wongpinyochit M. Herklotz C. Werner, F. P. Seib, *Nanomedicine* **2017**, *13*, 2633.
- [72] N. A. Fine M. Lehfeldt J. E. Gross S. Downey G. M. Kind G. Duda D. Kulber R. Horan J. Ippolito, M. Jewell, *Plast. Reconstr. Surg.* **2015**, *135*, 339.
- [73] J. E. Gross R. L. Horan M. Gaylord R. E. Olsen L. D. McGill J. M. Garcia-Lopez K. Biber K. Barnico I. Toponarski, G. Altman, *Plast. Reconstr. Surg.* **2014**, *134*, 700e.
- [74] Z. Jiao Y. Song Y. Jin C. Zhang D. Peng Z. Chen P. Chang S. C. Kundu G. Wang Z. Wang, L. Wang, *Macromol. Biosci.* **2017**, *17*.

- [75] B. Panilaitis G. H. Altman J. Chen H. J. Jin V. Karageorgiou, D. L. Kaplan, *Biomaterials* **2003**, *24*, 3079.
- [76] H. K. Soong, K. R. Kenyon, *Ophthalmology* **1984**, *91*, 479.
- [77] C. Pecquet, *Eur. J. Dermatol.* **2013**, *23*, 767.
- [78] E. Fuchs, *Dtsch. Med. Wochenshr.* **1955**, *80*, 36.
- [79] a) J. C. Celedon L. J. Palmer X. Xu B. Wang Z. Fang, S. T. Weiss, *Pediatrics* **2001**, *107*, E80; b) C. M. Wen S. T. Ye L. X. Zhou, Y. Yu, *Ann. Allergy* **1990**, *65*, 375.
- [80] a) M. Dewair X. Baur, K. Ziegler, *J. Allergy Clin. Immunol.* **1985**, *76*, 537; b) W. Zaoming R. Codina E. Fernandez-Caldas, R. F. Lockey, *J. Investig. Allergol. Clin. Immunol.* **1996**, *6*, 237.
- [81] P. S. Lai, D. C. Christiani, *Curr. Opin. Pulm. Med.* **2013**, *19*, 152.
- [82] R. I. Kunz R. M. Brancalhão L. F. Ribeiro, M. R. Natali, *Biomed. Res. Int.* **2016**, *2016*, 8175701.
- [83] H. Xie W. Yang J. Chen J. Zhang X. Lu X. Zhao K. Huang H. Li P. Chang Z. Wang, L. Wang, *Adv. Healthc. Mater.* **2015**, *4*, 2195.
- [84] B. Kundu, S. C. Kundu, *Biomaterials* **2012**, *33*, 7456.

- [85] L. Meinel S. Hofmann V. Karageorgiou C. Kirker-Head J. McCool G. Gronowicz L. Zichner R. Langer G. Vunjak-Novakovic, D. L. Kaplan, *Biomaterials* **2005**, *26*, 147.
- [86] Y. Wang D. D. Rudym A. Walsh L. Abrahamsen H. J. Kim H. S. Kim C. Kirker-Head, D. L. Kaplan, *Biomaterials* **2008**, *29*, 3415.
- [87] J. Haupt J. M. Garcia-Lopez, K. Chope, *BMC Vet. Res.* **2015**, *11*, 58.
- [88] J. Brown C. L. Lu J. Coburn, D. L. Kaplan, *Acta Biomater.* **2015**, *11*, 212.
- [89] K. Numata, D. L. Kaplan, *Biochemistry* **2010**, *49*, 3254.
- [90] U. Slotta S. Hess K. Spiess T. Stromer L. Serpell, T. Scheibel, *Macromol. Biosci.* **2007**, *7*, 183.
- [91] K. Lundmark G. T. Westermark A. Olsen, P. Westermark, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6098.
- [92] S. Gomes K. Numata I. B. Leonor J. F. Mano R. L. Reis, D. L. Kapan, *Biomacromolecules* **2011**, *12*, 1675.
- [93] L. Fernandez-Garcia N. Mari-Buye J. A. Barios R. Madurga M. Elices J. Perez-Rigueiro M. Ramos G. V. Guinea, D. Gonzalez-Nieto, *Acta Biomater.* **2016**, *45*, 262.
- [94] L. S. Wray X. Hu J. Gallego I. Georgakoudi F. G. Omenetto D. Schmidt, D. L. Kaplan, *J. Biomed. Mater. Res. B Appl. Biomater.* **2011**, *99*, 89.

- [95] I. Greving C. Dicko A. Terry P. Callowc, F. Vollrath, *Soft Matter* **2010**, *6*, 4389.
- [96] C. M. Jewell S. C. B. Lopez, D. J. Irvine, *Proc. Natl. Acad. Sci. U.S.A* **2011**, *108*, 15745.
- [97] M. W. Clemens S. Downey F. Agullo M. R. Lehfeldt G. M. Kind H. Palladino D. Marshall M. L. Jewell A. B. Mathur, B. P. Bengtson, *Plast. Reconstr. Surg. Glob. Open* **2014**, *2*, e246.
- [98] J. E. Gross, *Plast. Reconstr. Surg. Glob. Open* **2013**, *1*, e86.
- [99] B. Bengtson R. A. Baxter M. W. Clemens, D. Bates, *Plast. Reconstr. Surg. Glob. Open* **2014**, *2*, e182.
- [100] N. Karp M. Choi J. Ippolito M. Lehfeldt M. Jewell, N. Fine, *Eur. J. Surg. Oncol.* **2014**, *40*, 621.
- [101] A. Kornstein, *Plast. Reconstr. Surg. Glob. Open* **2014**, *2*, e244.
- [102] A. N. Kornstein, *Plast. Reconstr. Surg. Glob. Open* **2014**, *2*, e190.
- [103] R. De Vita E. M. Buccheri M. Pozzi, G. Zoccali, *J. Exp. Clin. Cancer. Res.* **2014**, *33*, 78.
- [104] T. P. Crowley, T. Collin, *J. Plast. Reconstr. Aesthet. Surg.* **2015**, *68*, 1629.
- [105] D. Almesberger N. Zingaretti C. Di Loreto S. Massarut A. Pasqualucci, P. C. Parodi, *J. Plast. Reconstr. Aesthet. Surg.* **2015**, *68*, 870.

- [106] N. Karp M. Choi D. A. Kulber S. Downey G. Duda G. M. Kind M. L. Jewell D. K. Murphy M. R. Lehfelddt, N. Fine, *Plast. Reconstr. Surg. Glob. Open* **2017**, *5*, e1327.
- [107] M. F. Freshwater, *J. Plast. Reconstr. Aesthet. Surg.* **2014**, *67*, 1457.
- [108] a) C. Fontanini I. Berti L. Monasta, G. Longo, *G. Ital. Dermatol. Venereol.* **2013**, *148*, 293; b) D. Y. Koller G. Halmerbauer A. Bock, G. Engstler, *Pediatr. Allergy Immunol.* **2007**, *18*, 335; c) G. Ricci A. Patrizi B. Bendandi G. Menna E. Varotti, M. Masi, *Br. J. Dermatol.* **2004**, *150*, 127; d) G. Stinco F. Piccirillo, F. Valent, *Dermatology* **2008**, *217*, 191; e) K. S. Thomas L. E. Bradshaw T. H. Sach J. M. Batchelor S. Lawton E. F. Harrison R. H. Haines A. Ahmed H. C. Williams T. Dean N. P. Burrows I. Pollock J. Llewellyn C. Crang J. D. Grundy J. Guinness A. Gribbin E. J. Mitchell F. Cowdell S. J. Brown A. A. Montgomery, U. K. D. C. T. N. s. C. T. Team, *PLoS Med* **2017**, *14*, e1002280.
- [109] C. Schaunig, D. Kopera, *Int. J. Dermatol.* **2017**, *56*, 589.
- [110] A. Inoue I. Ishido A. Shoji, H. Yamada, *Contact Dermatitis* **1997**, *37*, 185.
- [111] G. Senti L. S. Steinmann B. Fischer R. Kurmann T. Storni P. Johansen P. Schmid-Grendelmeier B. Wuthrich, T. M. Kundig, *Dermatology* **2006**, *213*, 228.
- [112] W. Zhang L. Chen J. Chen L. Wang X. Gui J. Ran G. Xu H. Zhao M. Zeng J. Ji L. Qian J. Zhou H. Ouyang, X. Zou, *Adv. Healthc. Mater.* **2017**, *6*.
- [113] E. S. Gil B. Panilaitis E. Bellas, D. L. Kaplan, *Adv. Healthc. Mater.* **2013**, *2*, 206.



- [114] a) J. H. Lee D. K. Kim H. S. Park J. Y. Jeong Y. K. Yeon V. Kumar S. H. Bae J. M. Lee B. M. Moon, C. H. Park, *Laryngoscope* **2016**, *126*, 2798; b) J. H. Lee J. S. Lee D. K. Kim C. H. Park, H. R. Lee, *Clin. Exp. Otorhinolaryngol.* **2015**, *8*, 117.
- [115] J. Kim C. H. Kim C. H. Park J. N. Seo H. Kweon S. W. Kang, K. G. Lee, *Wound Repair Regen.* **2010**, *18*, 132.
- [116] C. L. Hu J. Y. Cui F. Z. Ren, C. Peng, *Int. J. Food Eng.* **2008**, *4*.
- [117] C. Martinez-Mora A. Mrowiec E. M. Garcia-Vizcaino A. Alcaraz J. L. Cenis, F. J. Nicolas, *Plos One* **2012**, *7*.
- [118] D. W. Kim H. S. Hwang D. S. Kim S. H. Sheen D. H. Heo G. Hwang S. H. Kang H. Kweon Y. Y. Jo S. W. Kang K. G. Lee J. Park W. S. Eum Y. J. Cho, S. Y. Choi, *J. Micro. Biotech.* **2012**, *22*, 494.
- [119] C. E. Kim J. H. Lee Y. K. Yeon C. H. Park, J. Yang, *Sci. Rep.* **2017**, *7*, 44364.
- [120] J. P. Whitcher M. Srinivasan, M. P. Upadhyay, *Bull. World Health Organ.* **2001**, *79*, 214.
- [121] K. Jaudzems G. Askarieh M. Landreh K. Nordling M. Hedhammar H. Jornvall A. Rising S. D. Knight, J. Johansson, *J. Mol. Biol.* **2012**, *422*, 477.
- [122] a) D. H. Kim J. Viventi J. J. Amsden J. Xiao L. Vigeland Y. S. Kim J. A. Blanco B. Panilaitis E. S. Frechette D. Contreras D. L. Kaplan F. G. Omenetto Y. Huang K. C. Hwang M. R. Zakin B. Litt, J. A. Rogers, *Nat. Mater.* **2010**, *9*, 511; b) H. Tao S. W. Hwang B. Marelli B. An J. E. Moreau M. Yang M. A. Brenckle S. Kim

- D. L. Kaplan J. A. Rogers, F. G. Omenetto, *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 17385.
- [123] S. Hazra S. Nandi D. Naskar R. Guha S. Chowdhury N. Pradhan S. C. Kundu, A. Konar, *Sci. Rep.* **2016**, *6*.
- [124] L. J. Bray K. A. George S. L. Ainscough D. W. Hutmacher T. V. Chirila, D. G. Harkin, *Biomaterials* **2011**, *32*, 5086.
- [125] B. D. Lawrence Z. Pan A. H. Liu D. L. Kaplan, M. I. Rosenblatt, *Acta Biomater.* **2012**, *8*, 3732.
- [126] L. Jia C. E. Ghezzi, D. L. Kaplan, *J. Biomed. Mater. Res. B* **2016**, *104*, 431.
- [127] C. E. Ghezzi L. Q. Wang I. Behlau J. Rnjak-Kovacina S. R. Wang M. H. Goldstein J. B. Liu J. K. Marchant M. I. Rosenblatt, D. L. Kaplan, *J. Appl. Biomater. Funct. Mater.* **2016**, *14*, E266.
- [128] J. Wu J. Rnjak-Kovacina Y. Q. Du M. L. Funderburgh D. L. Kaplan, J. L. Funderburgh, *Biomaterials* **2014**, *35*, 3744.
- [129] J. F. Maya-Vetencourt D. Ghezzi M. R. Antognazza E. Colombo M. Mete P. Feyen A. Desii A. Buschiazzo M. Di Paolo S. Di Marco F. Ticconi L. Emionite D. Shmal C. Marini I. Donelli G. Freddi R. Maccarone S. Bisti G. Sambuceti G. Pertile G. Lanzani, F. Benfenati, *Nat. Mater.* **2017**, *16*, 681.
- [130] J. Lee S. H. Park I. H. Seo K. J. Lee, W. Ryu, *Eur. J. Pharm. Biopharm.* **2015**, *94*, 11.

- [131] K. Tsioris W. K. Raja E. M. Pritchard B. Panilaitis D. L. Kaplan, F. G. Omenetto, *Adv. Funct. Mater.* **2012**, *22*, 330.
- [132] P. Johansen T. Storni L. Rettig Z. Y. Qiu A. Der-Sarkissian K. A. Smith V. Manolova K. S. Lang G. Senti B. Mullhaupt T. Gerlach R. F. Speck A. Bot, T. M. Kundig, *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 5189.
- [133] A. B. Li J. A. Kluge N. A. Guzewicz F. G. Omenetto, D. L. Kaplan, *J. Control. Release* **2015**, *219*, 416.
- [134] X. Jiang J. Zhao S. Wang X. Sun X. Zhang J. Chen D. L. Kaplan, Z. Zhang, *Biomaterials* **2009**, *30*, 4522.
- [135] a) J. Rnjak-Kovacina L. S. Wray J. M. Golinski, D. L. Kaplan, *Adv. Funct. Mater.* **2014**, *24*, 2188; b) M. Zamani M. Khafaji M. Najji M. Vossoughi I. Alemzadeh, N. Haghighipour, *Sci. Rep.* **2017**, *7*, 4455.
- [136] a) M. J. Imola D. D. Hamlar W. Shao K. Chowdhury, S. Tatum, *Arch. Facial Plast. Surg.* **2001**, *3*, 79; b) S. J. Min X. Gao C. M. Han Y. Chen M. Y. Yang L. J. Zhu H. P. Zhang L. Liu, J. M. Yao, *J. Biomater. Sci. Polym. Ed* **2012**, *23*, 97.
- [137] J. C. Middleton, A. J. Tipton, *Biomaterials* **2000**, *21*, 2335.
- [138] G. S. Perrone G. G. Leisk T. J. Lo J. E. Moreau D. S. Haas B. J. Papenburg E. B. Golden B. P. Partlow S. E. Fox A. M. Ibrahim S. J. Lin, D. L. Kaplan, *Nat. Commun.* **2014**, *5*, 3385.

- [139] A. Schneider X. Y. Wang D. L. Kaplan J. A. Garlick, C. Egles, *Acta Biomater.* **2009**, *5*, 2570.
- [140] K. H. Kim L. Jeong H. N. Park S. Y. Shin W. H. Park S. C. Lee T. I. Kim Y. J. Park Y. J. Seol Y. M. Lee Y. Ku I. C. Rhyu S. B. Han, C. P. Chung, *J. Biotechnol.* **2005**, *120*, 327.
- [141] H. Fan H. Liu S. L. Toh, J. C. Goh, *Biomaterials* **2009**, *30*, 4967.
- [142] a) V. Catto S. Fare I. Cattaneo M. Figliuzzi A. Alessandrino G. Freddi A. Remuzzi, M. C. Tanzi, *Mater. Sci. Eng. C Mater. Biol. Appl.* **2015**, *54*, 101; b) B. Marelli A. Alessandrino S. Fare G. Freddi D. Mantovani, M. C. Tanzi, *Acta Biomater.* **2010**, *6*, 4019; c) E. C. Filipe M. Santos J. Hung B. S. L. Lee N. Yang A. H. P. Chan M. K. C. Ng J. Rnjak-Kovacina, S. G. Wise, *J. A. C. C.* **2018**, *3*, 38.
- [143] a) S. Kapoor, S. C. Kundu, *Acta Biomater.* **2016**, *31*, 17; b) F. P. Seib, *Ther. Del.* **2018**, *9*, 469.
- [144] R. D. Abbott E. P. Kimmerling D. M. Cairns, D. L. Kaplan, *ACS Appl. Mater. Interfaces* **2016**, *8*, 21861.
- [145] E. Bellas T. J. Lo E. P. Fournier J. E. Brown R. D. Abbott E. S. Gil K. G. Marra J. P. Rubin G. G. Leisk, D. L. Kaplan, *Adv. Healthc. Mater.* **2015**, *4*, 452.

- [146] F. P. Seib, in *Self-assembling Biomaterials. Molecular Design, Characterization and Application in Biology and Medicine*, 1 ed. (Eds.: H. S. Azevedo, R. M. P. da Silva), Woodhead Publishing 2018, **2018**, pp. 27.
- [147] M. Floren C. Migliaresi, A. Motta, *J. Funct. Biomater.* **2016**, *7*.
- [148] G. G. Leisk T. J. Lo T. Yucel Q. Lu, D. L. Kaplan, *Adv. Mater.* **2010**, *22*, 711.
- [149] N. Kojic M. J. Panzer G. G. Leisk W. K. Raja M. Kojic, D. L. Kapan, *Soft Matter* **2012**, *8*, 6897.
- [150] F. P. Seib E. M. Pritchard, D. L. Kaplan, *Adv. Funct. Mater.* **2013**, *23*, 58.
- [151] F. P. Seib M. Tsurkan U. Freudenberg D. L. Kapan, C. Werner, *ACS Biomater. Sci. Eng.* **2016**, *2*.
- [152] M. L. Lovett X. Wang T. Yucel L. York M. Keirstead L. Haggerty, D. L. Kaplan, *Eur. J. Pharm. Biopharm.* **2015**, *95*, 271.
- [153] D. C. Hamilton H. H. Shih R. A. Schubert S. A. Michie P. N. Staats D. L. Kaplan, M. J. Fontaine, *J. Tissue Eng. Regen. Med.* **2017**, *11*, 887.
- [154] H. Nakamura J. Fang, H. Maeda, *Expert Opin. Drug Deliv.* **2015**, *12*, 53.
- [155] S. Wilhelm A. J. Tavares Q. Dai S. Ohta J. Audet H. F. Dvorak, W. C. W. Chan, *Nat. Rev. Mater.* **2016**, *1*, 16014.
- [156] E. Wenk H. P. Merkle, L. Meinel, *J. Control. Release* **2011**, *150*, 128.

- [157] Y.-Q. Zhang W.-D. Shen R.-L. Xiang L.-J. Zhuge W.-J. Gao, W.-B. Wang, *J. Nanoparticle Res.* **2007**, *9*, 885.
- [158] V. Gupta A. Aseh C. N. Rios B. B. Aggarwal, A. B. Mathur, *Int. J. Nanomedicine* **2009**, *4*, 115.
- [159] J. Kundu Y. I. Chung Y. H. Kim G. Tae, S. C. Kundu, *Int. J. Pharm.* **2010**, *388*, 242.
- [160] F. P. Seib G. T. Jones J. Rnjak-Kovacina Y. Lin, D. L. Kaplan, *Adv. Healthc. Mater.* **2013**, *2*, 1606.
- [161] J. D. Totten T. Wongpinyochit, F. P. Seib, *J. Drug Target.* **2017**, *25*, 865.
- [162] Y. Q. Zhang Y. J. Wang H. Y. Wang L. Zhu, Z. Z. Zhou, *Soft Matter* **2011**, *7*, 9728.
- [163] T. Wongpinyochit P. Uhlmann A. J. Urquhart, F. P. Seib, *Biomacromolecules* **2015**, *16*, 3712.
- [164] Y. Tian X. Jiang X. Chen Z. Shao, W. Yang, *Adv. Mater.* **2014**, *26*, 7393.
- [165] K. Numata A. J. Mieszawska-Czajkowska L. A. Kvenvold, D. L. Kaplan, *Macromol. Biosci.* **2012**, *12*, 75.
- [166] T. B. Aigner E. DeSimone, T. Scheibel, *Adv. Mater.* **2018**, *30*, 1704636.
- [167] N. Dinjaski, D. L. Kaplan, *Curr. Opin. Biotechnol.* **2016**, *39*, 1.
- [168] K. Numata, D. L. Kaplan, *Adv. Drug. Deliv. Rev.* **2010**, *62*, 1497.

- [169] B. An T. M. DesRochers G. Qin X. Xia G. Thiagarajan B. Brodsky, D. L. Kaplan, *Biomaterials* **2013**, *34*, 402.
- [170] R. Price A. Poursaid, H. Ghandehari, *J. Control. Release* **2014**, *190*, 304.
- [171] X. Hu X. Wang J. Rnjak A. S. Weiss, D. L. Kaplan, *Biomaterials* **2010**, *31*, 8121.
- [172] J. O. Buitrago K. D. Patel A. El-Fiqi J. H. Lee B. Kundu H. H. Lee, H. W. Kim, *Acta Biomater.* **2018**, *69*, 218.
- [173] M. M. Jacobsen D. Li N. Gyune Rim D. Backman M. L. Smith, J. Y. Wong, *Sci. Rep.* **2017**, *7*, 45653.
- [174] M. Farokhi F. Mottaghtalab S. Samani M. A. Shokrgozar S. C. Kundu R. L. Reis Y. Fatahi, D. L. Kaplan, *Biotechnol. Adv.* **2018**, *36*, 68.
- [175] J. Chang X. F. Peng K. Hijji J. Cappello H. Ghandehari S. D. Solares, J. Seog, *J. Am. Chem. Soc.* **2011**, *133*, 1745.
- [176] J. Jiang S. Zhang Z. Qian N. Qin W. Song L. Sun Z. Zhou Z. Shi L. Chen X. Li Y. Mao D. L. Kaplan S. N. Gilbert Corder X. Chen M. Liu F. G. Omenetto X. Xia, T. H. Tao, *Adv. Mater.* **2018**, *30*, e1705919.
- [177] M. Humenik, T. Scheibel, *J. Phys. Condens. Matter.* **2014**, *26*, 503102.
- [178] J. T. Prince K. P. McGrath C. M. DiGirolamo, D. L. Kaplan, *Biochemistry* **1995**, *34*, 10879.
- [179] O. S. Rabotyagova P. Cebe, D. L. Kaplan, *Macromol. Biosci.* **2010**, *10*, 49.

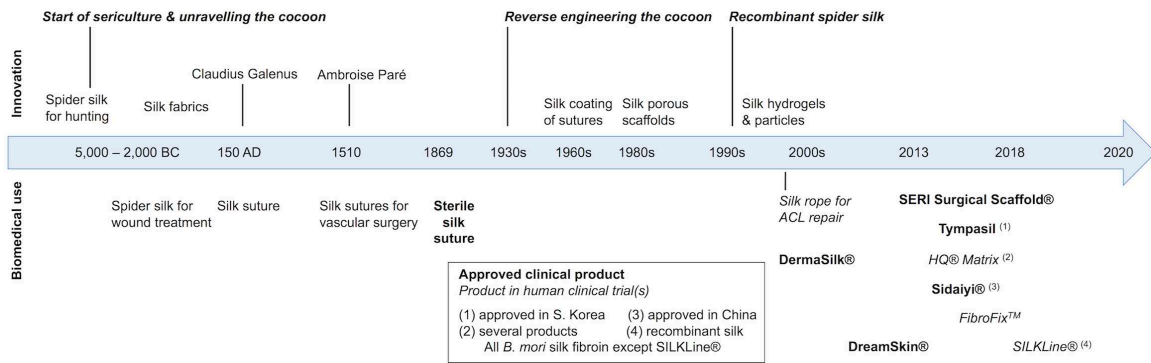
- [180] D. Huemmerich T. Scheibel F. Vollrath S. Cohen U. Gat, S. Ittah, *Curr. Biol.* **2004**, *14*, 2070.
- [181] Z. Megeed M. Haider D. Li B. W. O'Malley, Jr. J. Cappello, H. Ghandehari, *J. Control. Release* **2004**, *94*, 433.
- [182] S. Yanagisawa Z. Zhu I. Kobayashi K. Uchino Y. Tamada T. Tamura, T. Asakura, *Biomacromolecules* **2007**, *8*, 3487.
- [183] a) C. Wong Po Foo S. V. Patwardhan D. J. Belton B. Kitchel D. Anastasiades J. Huang R. R. Naik C. C. Perry, D. L. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 9428; b) L. L. Brott R. R. Naik D. J. Pikas S. M. Kirkpatrick D. W. Tomlin P. W. Whitlock S. J. Clarson, M. O. Stone, *Nature* **2001**, *413*, 291.
- [184] K. Rittner A. Benavente A. Bompard-Sorlet F. Heitz G. Divita R. Brasseur, E. Jacobs, *Mol. Ther.* **2002**, *5*, 104.
- [185] S. Christian J. Pilch M. E. Akerman K. Porkka P. Laakkonen, E. Ruoslahti, *J. Cell Biol.* **2003**, *163*, 871.
- [186] K. D. Hermanson D. Huemmerich T. Scheibel, A. R. Bausch, *Adv. Mater.* **2007**, *19*, 1810.
- [187] U. K. Slotta S. Rammensee S. Gorb, T. Scheibel, *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 4592; U. K. Slotta S. Rammensee S. Gorb, T. Scheibel, *Angew. Chem.* **2008**, *120*, 4668.



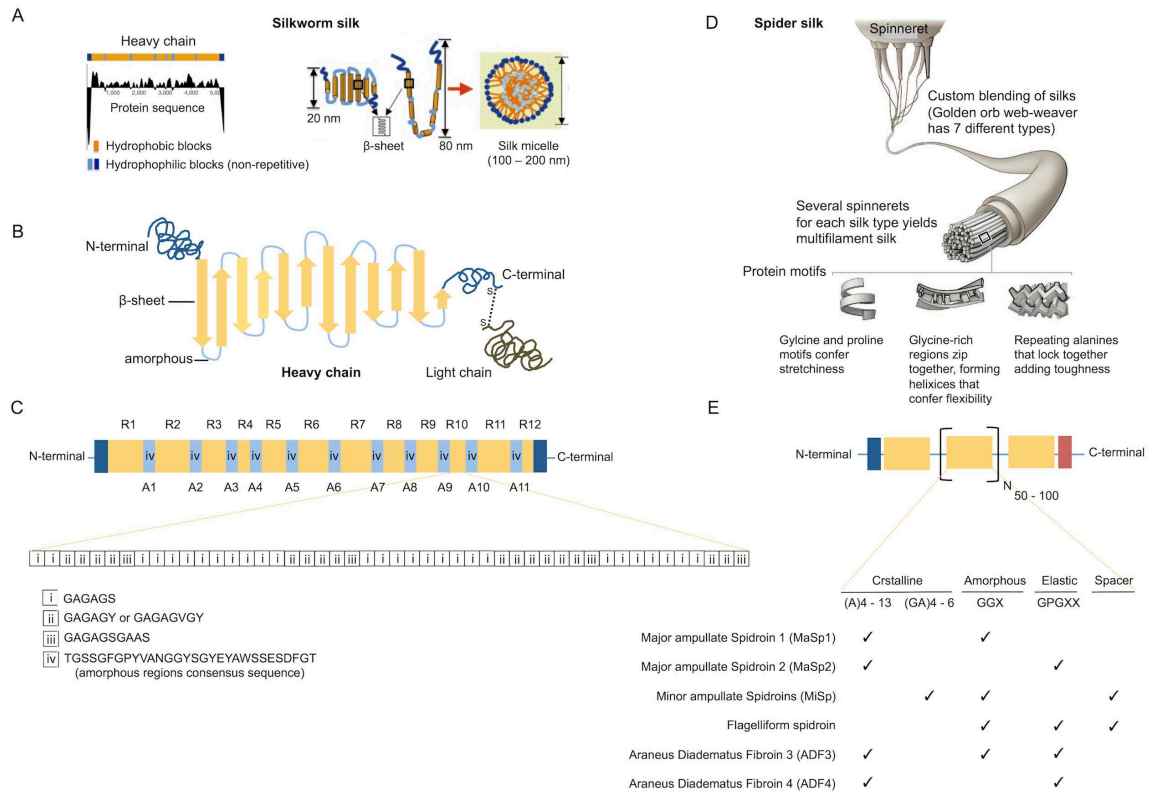
- [188] A. Lammel M. Schwab U. Slotta G. Winter, T. Scheibel, *Chem. Sus. Chem* **2008**, *1*, 413.
- [189] E. Doblhofer, T. Scheibel, *J. Pharm. Sci.* **2015**, *104*, 988.
- [190] M. B. Elsner H. M. Herold S. Muller-Herrmann H. Barga, T. Scheibel, *Biomater. Sci.* **2015**, *3*, 543.
- [191] M. B. Schierling E. Doblhofer, T. Scheibel, *Biomater. Sci.* **2016**, *4*, 1515.
- [192] K. Numata M. R. Reagan R. H. Goldstein M. Rosenblatt, D. L. Kaplan, *Bioconjug. Chem.* **2011**, *22*, 1605.
- [193] M. Boulet-Audet C. Holland T. Gheysens, F. Vollrath, *Biomacromolecules* **2016**, *17*, 3198.
- [194] F. Teule Y. G. Miao B. H. Sohn Y. S. Kim J. J. Hull M. J. Fraser, Jr. R. V. Lewis, D. L. Jarvis, *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 923.
- [195] a) Y. Kambe K. Yamamoto K. Kojima Y. Tamada, N. Tomita, *Biomaterials* **2010**, *31*, 7503; b) T. Tamura C. Thibert C. Royer T. Kanda E. Abraham M. Kamba N. Komoto J. L. Thomas B. Mauchamp G. Chavancy P. Shirk M. Fraser J. C. Prudhomme, P. Couble, *Nat. Biotechnol.* **2000**, *18*, 81.
- [196] J. R. dos Santos-Pinto G. Lamprecht W. Q. Chen S. Heo J. G. Hardy H. Priewalder T. R. Scheibel M. S. Palma, G. Lubec, *J. Proteomics* **2014**, *105*, 174.
- [197] X. X. Xia Z. G. Qian C. S. Ki Y. H. Park D. L. Kaplan, S. Y. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 14059.

- [198] A. M. Edlund J. Jones R. Lewis, J. C. Quinn, *Nat. Biotechnol.* **2018**, *42*, 12.
- [199] V. J. Venditto, F. C. Szoka, Jr., *Adv. Drug Deliv. Rev.* **2013**, *65*, 80.
- [200] R. F. Service, *Science* **2017**, *358*, 293.
- [201] M. Xu, R. V. Lewis, *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7120.
- [202] A. Lazaris S. Arcidiacono Y. Huang J. F. Zhou F. Duguay N. Chretien E. A. Welsh J. W. Soares, C. N. Karatzas, *Science* **2002**, *295*, 472.
- [203] P. H. Zeplin N. C. Maksimovikj M. C. Jordan J. Nickel G. Lang A. H. Leimer L. Römer, T. Scheibel, *Adv. Funct. Mater.* **2014**, *24*, 2658.
- [204] a) C. Muller M. Hamedi R. Karlsson R. Jansson R. Marcilla M. Hedhammar, O. Inganas, *Adv. Mater.* **2011**, *23*, 898; b) L. Nileback J. Hedin M. Widhe L. S. Floderus A. Krona H. Bysell, M. Hedhammar, *Biomacromolecules* **2017**, *18*, 846; c) N. D. Shalaly M. Ria U. Johansson K. Avall P. O. Berggren, M. Hedhammar, *Biomaterials* **2016**, *90*, 50; d) M. Widhe N. D. Shalaly, M. Hedhammar, *Biomaterials* **2016**, *74*, 256.
- [205] G. Askarieh M. Hedhammar K. Nordling A. Saenz C. Casals A. Rising J. Johansson, S. D. Knight, *Nature* **2010**, *465*, 236.
- [206] M. Andersson Q. Jia A. Abella X. Y. Lee M. Landreh P. Purhonen H. Hebert M. Tenje C. V. Robinson Q. Meng G. R. Plaza J. Johansson, A. Rising, *Nat. Chem. Biol.* **2017**, *13*, 262.

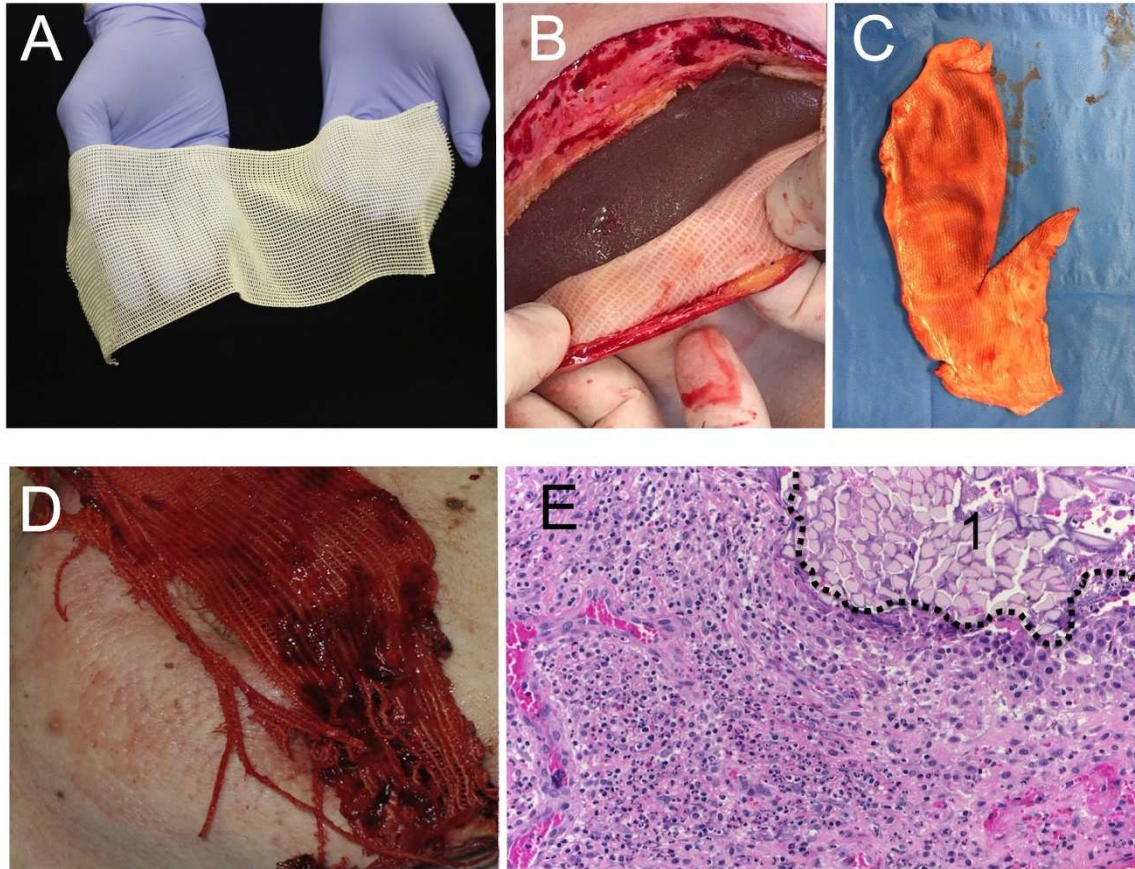
[207] N. Kronqvist M. Sarr A. Lindqvist K. Nordling M. Otikovs L. Venturi B. Pioselli  
 P. Purhonen M. Landreh H. Biverstal Z. Toleikis L. Sjöberg C. V. Robinson N.  
 Pelizzi H. Jörnvall H. Hebert K. Jaudzems T. Curstedt A. Rising, J. Johansson,  
*Nat. Commun.* **2017**, *8*, 15504.



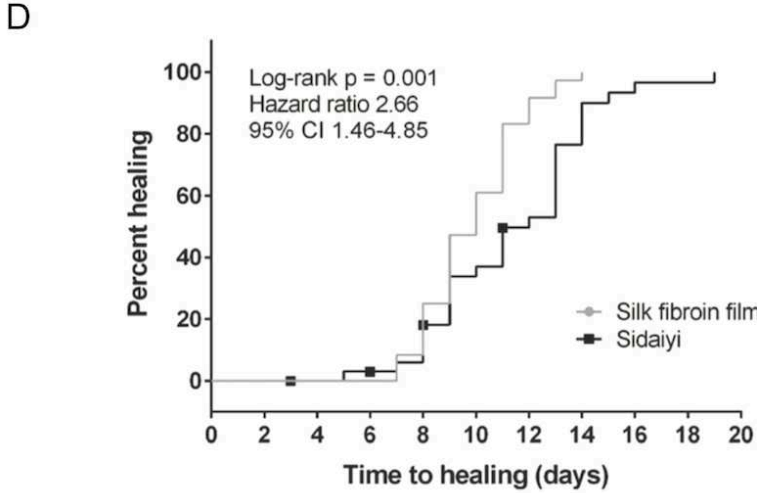
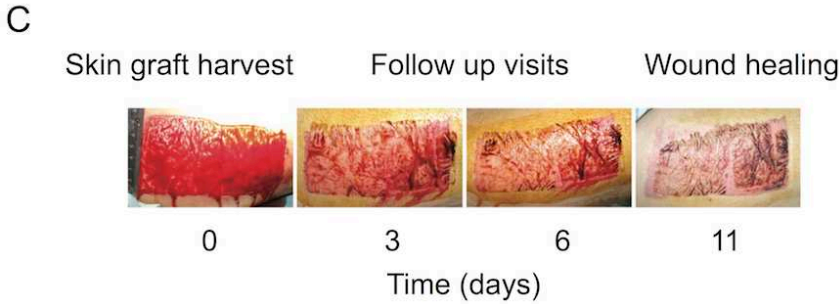
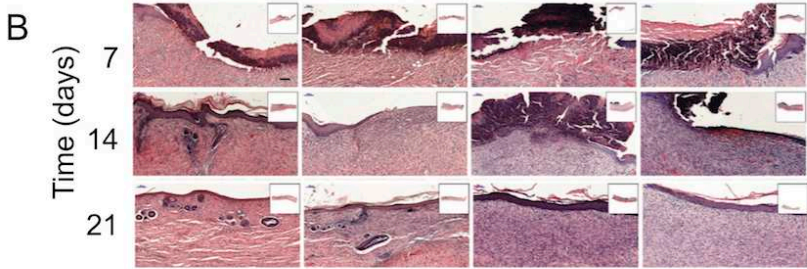
**Figure 1.** Timeline: Milestones in the emergence of silk for biomedical applications.



**Figure 2. Silk structure.** (A) Solution conformation of *Bombyx mori* silk. Hydrophobicity pattern of the heavy chain with possible chain folding and micelle assembly of silk fibroin in water. (B) Two dimensional silk fibroin schematic. (C) Primary structure of the *Bombyx mori* silk heavy chain. R01 to R12 and A01 to A11 represent the arrangement of 12 repetitive and 11 amorphous regions, respectively. The approximate amino acid sequence of the R10 is shown by combination of sequences of i, ii and iii. (D) Hierarchical structure of spider silk. (E) Primary structure of spider silks. (Panel A adapted with permission from.<sup>[49]</sup> Copyright 2003 Macmillan Publishers. Panel B adapted with permission from<sup>[50]</sup> and panel C adapted with permission from<sup>[51]</sup>, Copyright 2018 and 2005 American Chemical Society. Panel D adapted with permission from<sup>[52]</sup> and panel E adapted from<sup>[53]</sup>, Copyright 2011 Elsevier and Copyright 2017 American Association for the Advancement of Science).



**Figure 3. Examples of SERI Surgical Scaffold® implant loss in humans. (A)** Silk fibroin surgical mesh prior to implantation. **(B)** Intraoperative view showing a free lying scaffold in the breast pocket. **(C)** Retrieved scaffold surrounded with seroma. **(D)** Intraoperative view of surgically removed scaffold with interpenetrated granulation tissue/scar plate (at >5 months), and **(E)** histology of retrieved sample showing granulation tissue with neutrophils and giant cells at the material (1) interface (dotted line). Images reproduced with permission (panel B, C) from <sup>[66b]</sup> and (panel D and E) from <sup>[105]</sup>. Copyright 2018 and 2015, Elsevier.



**Figure 4. Silk fibroin films for wound healing applications.** (A) Healing of rabbit full-thickness wounds over a 21-day period following application of *Bombyx mori* silk fibroin films, a polyurethane based wound dressing Suprathel, a silk-silicone wound dressing Sidaiyi, and a blank control treated with PBS. Silk-film-treated wounds healed 3 days faster than Suprathel-treated wounds and 7 days faster than Sidaiyi-treated and untreated wounds. (B) Histological evaluation of the wounds: (A) moderate to complete epidermal organization in silk film treated wounds by day 14 and mature regenerated tissue with well-formed collagen matrix by day 21. (C) Photographs of representative silk-film-treated partial thickness wounds in a human Phase I clinical trial, showing complete healing at day 11, with an average time for complete wound healing of  $9.86 \pm 1.97$  days (n=36). (D) Kaplan–Meier curves comparing cumulative healing by treatment group. Images reproduce with permission from <sup>[112]</sup>. Copyright 2017 Wiley.

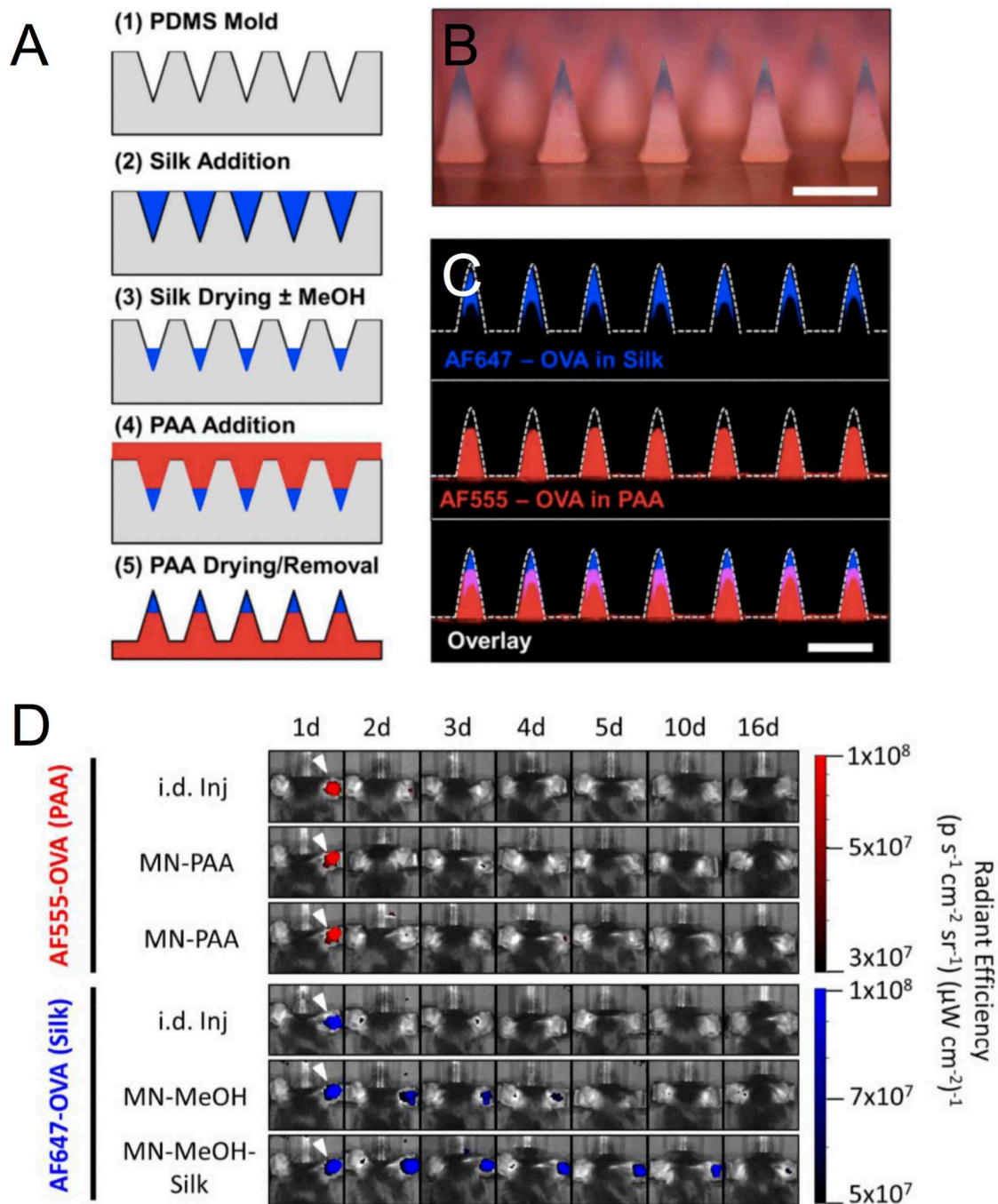
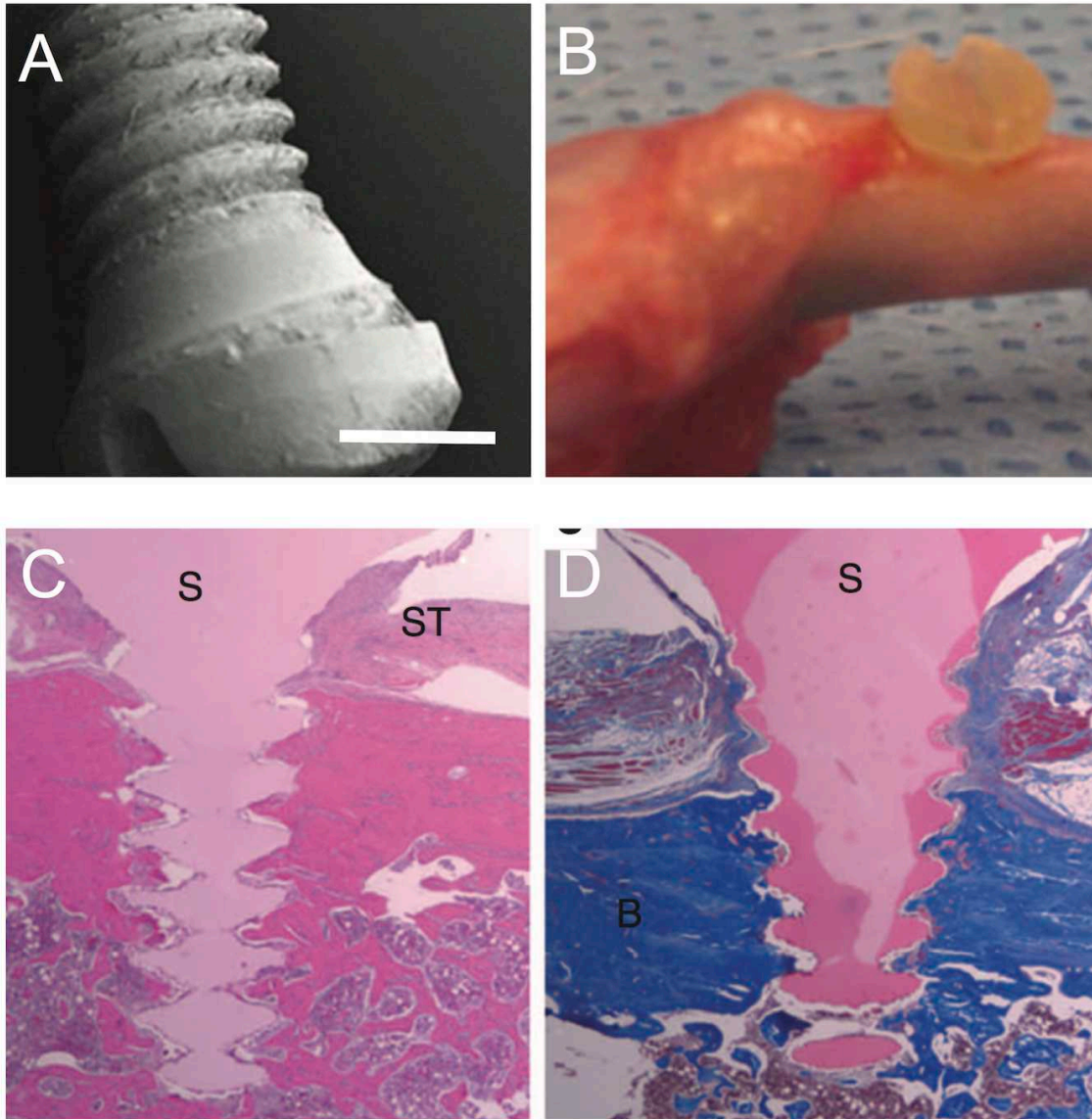


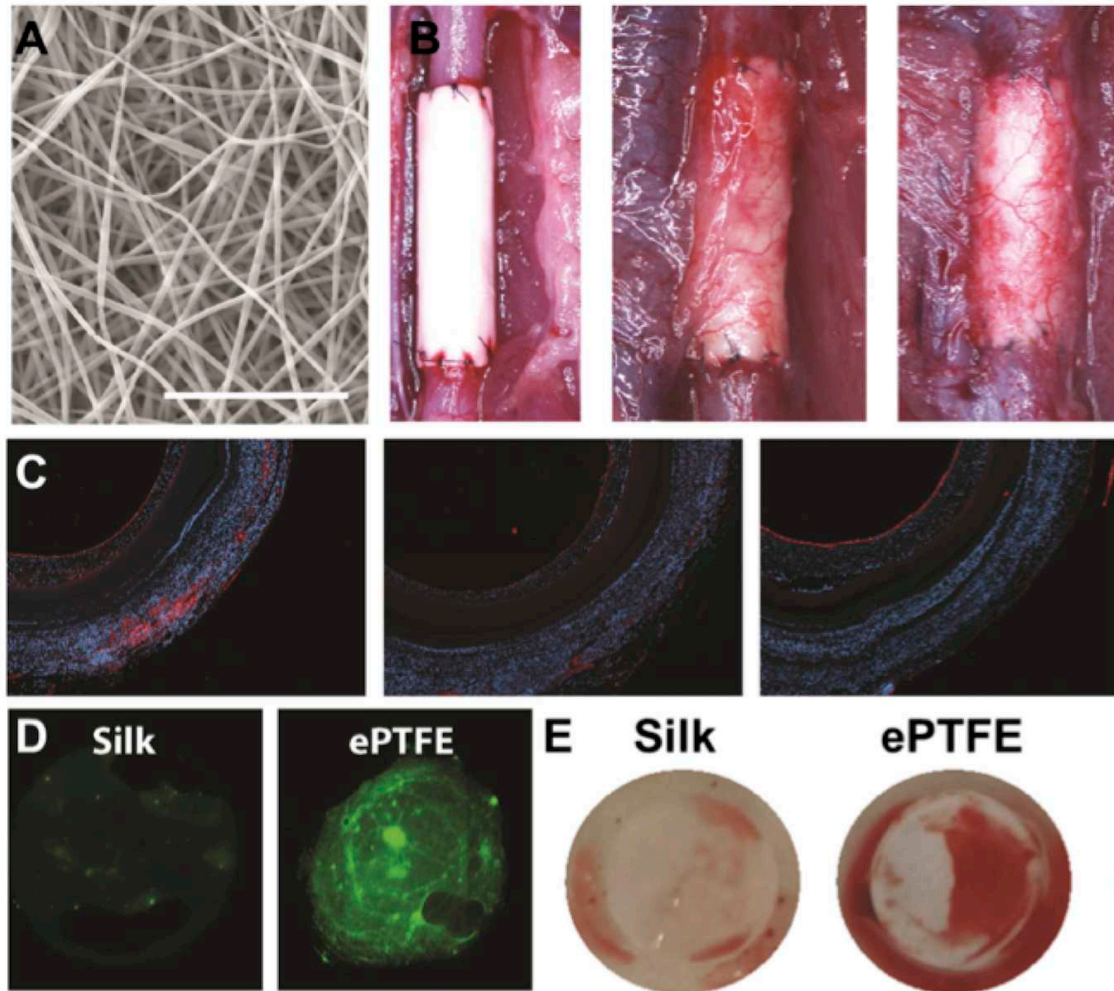
Figure 5. Implantable poly(acrylic acid)-silk fibroin microneedles for controllable vaccine release kinetics and enhanced immunogenicity. (A) Schematic representation



of the composite microneedle fabrication process, showing fabrication of the silk fibroin tips followed by poly(acrylic acid) (PAA) pedestals. **(B)** Photograph of the composite microneedles, **(C)** Confocal microscopy images of composite microneedles loaded with fluorescently labeled model proteins in the silk tip (blue) or PAA pedestals (red) and **(D)** Delivery of fluorescently labeled model proteins to murine skin showing burst release and fast clearing from the PAA pedestal and slow, sustained release from the silk tip over 4 days (non-methanol-treated silk) or 16 days (methanol-treated silk). i.d. inj refers to the intradermal injection control. Adapted from <sup>[39a]</sup>. Copyright 2014 Wiley.



**Figure 6. Silk-based devices for fracture fixation.** (A) SEM image of a silk fibroin screw. Scale bar is 1 mm. (B) Silk fibroin screw inserted into a rat femur at 4 weeks post-surgery. (C-D) Cross-sections of the silk fibroin screw inserted into a rat femur at 4 weeks post-surgery; sections stained with H&E and Masson's trichrome, respectively. Adapted from <sup>[138]</sup>. Copyright 2014 Macmillan Publishers.



**Figure 7. Electrospun silk fibroin biomaterials for vascular applications.** (A) Electrospun silk fibroin graft morphology. Scale bar is 10  $\mu\text{m}$ . (B) Electrospun silk fibroin grafts implanted in a rat model of abdominal aortic replacement: at the time of implantation (left), 6 weeks (middle), and 24 weeks (right) post-implantation. (C) Endothelialization of implanted silk fibroin grafts as demonstrated by vWF+ staining (red) at 6 weeks post-implantation in distal (left), middle (middle), and proximal (right) regions. (D-E) In vitro assessment of blood compatibility of electrospun silk fibroin biomaterials, compared to ePTFE, as indicated by formation of a fluorescent fibrinogen

network (D) and whole blood incubation (E). Adapted from <sup>[142c]</sup>. Copyright 2018 Elsevier.

**Table 1.** Published data reporting the clinical use of SERI Surgical Scaffold® in humans

Year	Article type	Patient number	Study sponsor	Intervention	Clinical follow up (months)	Reported outcome	Reference
2013	Retrospective case report	1	Allergan	Abdominoplasty and use of scaffold to provide soft-tissue support to the abdominal fascia in patient with massive weight loss	24	Contour and flatness of the anterior abdominal wall was maintained.	[98]
2014	Retrospective study Multi center	141	Allergan	Revision of breast augmentation (n=40); revision of breast reconstruction (n=24); mastopexy augmentation (n=20); mastopexy augmentation-revision (n=16); hernia repair (n=11); other (n=30)	0 to 12	Adverse side effect reporting voluntary. Surgeons rated the ease of use a mean of 2.86 (scale 0–3) Surgeons rated their satisfaction a mean of 9.31 (scale 0–10)	[99]
2014	Prospective study Multi center	139	Allergan	2-stage implant-based breast reconstruction	6	75 subjects undergone stage 2, subject satisfaction score $4.3 \pm 0.91$ (5 best). Investigator satisfaction score was $9.4 \pm 0.84$ (10 best). Adverse effects in 214 breasts: tissue necrosis (6.1%), seroma (6.1%), hematoma (2.8%), breast infection (1.9%), cellulitis (1.9%), implant loss (1.9%), capsular contracture (0%).	[100]
2014	Retrospective case report	1	Allergan	abdominoplasty and lower body lift of in patient with massive weight loss. Scaffold implantation on left lower body only.	7	No complications reported, Improve patient satisfaction	[101]

2014	Retrospective case report	1	Allergan	brachioplasty	6	No complications reported, perceived faster maturation process and a better-quality scar	[102]
2014	Retrospective study Multi center	172	Not disclosed	77 patients (71 women, 6 men) underwent abdominal wall fascial repair or reinforcement. The remaining 95 patients not reported on.	18.4 ± 7.5	The overall complication rate (N77) was 6.5%, consisting of 2 wound dehiscences, 1 with device exposure, 1 seroma, 1 infection with explantation, and a perioperative bulge requiring reoperation	[97]
2014	Retrospective study Single center	15	No	Direct-to-implant after skin-sparing mastectomy	6 to 13	Capsular contraction (35%); loss of scaffold due to necrosis (n=1); seroma (n=1); hematoma self-limiting (n=1); patient satisfaction (5.77 out of 10)	[103]
2015	Correspondence Retrospective study Single center	5	No	Unilateral skin-sparing mastectomy and immediate reconstruction	Not reported	Late infection (6 weeks and 3.5 months post-surgery) in 2 breasts leading to scaffold and implant removal. In 2 patients successful completion with tissue integration and vascularization.	[104]
2015	Prospective study Multi center	139	Allergan	71 patients undergoing 2-stage breast reconstruction	12	Investigator satisfaction score was 9.4 ± 0.91 (10 best) and patient scores was 4.5 ± 0.82 (5 best). Complication rates in 105 breasts were tissue necrosis (6.7 %), seroma (5.7 %), hematoma (4.8 percent), implant loss (3.8 %), capsular contracture (1.9 %), breast infection (1.0%).	[72]
2015	Correspondence Retrospective study. Single center	4	No	Breast reconstruction	12	Late infection with <i>Ps. aeruginosa</i> in 2 patients at 5 months resulting in implant replacement. Lack of mesh integration (or degradation) in all 4 patients	[105]
2017	Prospective study Multi center	103	Allergan	2-stage implant-based breast reconstruction	24	Investigator satisfaction high. Adverse effects in 161 breasts:	[106]

						necrosis and wrinkling (8.1% each) and seroma, wound dehiscence, and breast redness (5.0% each). Over 2 years, 4 breasts in 4 subjects underwent reoperation with explantation of any device; 2 breasts required SERI explantation.	
2018	Prospective study Single center	16	No	direct-to-implant reconstruction with surgical scaffold after skin-sparing mastectomy	24 to 37	No intraoperative complications. Adverse effects in 22 breasts: Postoperative bleeding, that required reoperation occurred in 5% breast, postoperative seroma in 45% and surgical site infection in 9%. Scaffold-related complications occurred in 14% breasts, lack of scaffold integration in all, resulting in skin ulceration in 2 and the scaffold lying free in the breast pocket surrounded with seroma in one.	[66b]

**Table 2.** Published data reporting the clinical use of silk garments for the treatment of atopic dermatitis in humans

Year	Article type	Patient number	Study sponsor	Patients and intervention	Clinical follow up	Reported outcome	Reference
2004	Retrospective study Single center	46	ND	Children 4 months to 10 years. DermaSilk® treatment or cotton clothing, topical moisturizing cream or emulsion.	1 week	Local score of treated and untreated area of same child (SCORAD index). Overall DermaSilk® decreased atopic dermatitis severity and local improvement of silk treated areas.	[108c]
2006	Retrospective study Single center	15	ND	Children 0.6 to 9.2 years, body suits made out of DermaSilk® and cotton (50:50). Cotton side also received topical corticosteroid	3 weeks	Significant improvement of atopic dermatitis by assessing eczema area and severity (EASI index) irrespective of intervention. No significant difference between DermaSilk® and corticosteroid treatment.	[111]
2007	Retrospective study Single center, Randomized, single-blinded	22	ND	Children 5 to 12 years old. DermaSilk®, silk fabric and cotton. Control on contralateral arm (silk for first for 2 weeks followed by cotton for rest of study period), topical moisturizing cream or emulsion, antihistamines prn	3 months	DermaSilk® arm significantly improved over study period when compared to cotton. Silk control treatment showed significant improvement of SCORAD index.	[108b]
2008	Retrospective , randomized, double-blinded	30	ND	Age 3 to 31 years (mean 14.2). DermaSilk® sleeve versus equivalent silk only fabric.	1 month	Significant decrease in SCORAD index for both groups. Silk only fabric rapid reduction over 2 weeks only while DermaSilk® over 4 weeks; decrease in pruritus values similar during first 2 weeks but further decrease for DermaSilk® group until end of study	[108d]



2013	Retrospective, single center, double blind randomized controlled trial	22	ND	Age 4 to 18 months. Acute phases treated as per international guidelines. DermaSilk® body and tights, or wear clothes in pure cotton; expect May to September	24 months	Significant reduction in topical steroid use for DermaSilk® group; subjective pruritus also reduced significantly	[108a]
2017	Retrospective, multi-center parallel-group, randomised, controlled, observer-blind trial	300	University of Nottingham, National Institute for Health Research Clinical Research Network	Children aged 1 to 15 years. Standard care or standard care plus silk garment (either DermaSilk® or DreamSkin®). Eczema outcome and skin infections	24 weeks	No statistical difference between groups for eczema area and severity (EASI index). Less frequent skin infections in silk group. Data not stratified for different silk garments. Included cost-benefit analysis rejects garments.	[108e]

SCORAD: An atopic dermatitis scoring system combining extent, severity, and subjective symptoms

EASI: Eczema area and severity index

DermaSilk®: AL.PRE.TEC. Italy, Silk is covalently functionalized with the antimicrobial 3-trimethylsilylpropyl-dimethyloctadecyl ammonium chloride (AEM 5700/5772; AEGIS™)

DreamSkin®: DreamSkin Health Ltd UK. The silk fabric contains zinc-based antimicrobial and is coated with 2-(methacryloyloxy)ethyl-2-(trimethylammonio)ethylphosphate-stearylmethacrylate copolymer to form multilayer lamellar structures. The amphiphilic copolymer is proposed to serve as both a barrier and moisturizer.

**Table 3.** Reported human clinical trials using silk

<b>Title</b>	<b>Status</b>	<b>Number of patients</b>	<b>Study completion date</b>	<b>Study results</b>	<b>Primary outcome measure</b>	<b>Identifier</b>
SeriACL™ device trial for anterior cruciate ligament (ACL) reconstruction	Unknown. Status was active not recruiting	30	October 2008	Not posted	Safety - measured by device related serious adverse events (Time frame: 12 months)	<a href="#">NCT00490594</a>
Coated VICRYL Plus suture compared to Chinese silk in scheduled breast cancer surgery	Completed	101	May 2009	Posted	Mean score on cosmetic outcome visual analog scale (Time frame: 30 days (+/- 5) post-operative)	<a href="#">NCT00768222</a>
Clinical and economic outcomes with the use of SERI Surgical Scaffold® in direct-to-implant breast reconstruction	Withdrawn (strategic priorities impacted study)	ND	August 2014	Not posted	Incidence rate of implant loss (SERI Surgical Scaffold® and breast implant) (Time frame: 52 weeks)	<a href="#">NCT02033590</a>
Evaluation of HQ® Matrix medical wound dressing for healing of donor site wounds	Completed	71	September 2014	Posted	Time to wound healing (Time frame: Days 0, 3±1, 7±1, 10±2, 14±3, and 21±3 post-operation)	<a href="#">NCT01993030</a>
SERI Surgical Scaffold® use in reconstruction post market study for tissue support and repair in breast reconstruction surgery in Europe	Completed	100	February 2015	Posted	Investigator satisfaction following use of SERI Surgical Scaffold® evaluated using an 11-point scale questionnaire (Time frame: Six months)	<a href="#">NCT01389232</a>
Efficacy and safety of silk fibroin with bioactive coating layer dressing	Completed	29	May 2015	Not posted	Clinical efficacy of wound dressing containing silk fibroin with a sericin bioactive coating layer dressing in the treatment of split-thickness skin graft donor sites (Time frame: Within 14 days)	<a href="#">NCT02091076</a>

SERI Surgical Scaffold® postmarket study of soft tissue support and repair in breast reconstruction	Completed	17	March 2016	Not posted	Incidence of Implant Loss (Time frame: 24 months postoperatively)	<a href="#">NCT01914653</a>
A SERI Surgical Scaffold® postmarket study of soft tissue support in ventral hernia repair	Completed	1	June 2016	Not posted	Rate of hernia recurrence	<a href="#">NCT01981044</a>
Circumferential periareolar mastopexy using SERI Surgical Scaffold®	Completed	14	June 2016	Not posted	Size of the areola at 12 months as measured by physical mammometry	<a href="#">NCT02293798</a>
Suture materials: an evaluation	Completed	36	November 2016	Not posted	Accumulation of soft deposits (Time frame: 7 to 14 days)	<a href="#">NCT03410433</a>
SERI Surgical Scaffold® Support of the Lower Pole of the Breast (SeriSupport)	Completed	76	November 2016	Posted	Nipple to Fold Measurement on Stretch (Time frame: 1 year post op)	<a href="#">NCT02016612</a>
Evaluation of HQ® Matrix soft tissue mesh for the treatment of inguinal hernia	Unknown	144	December 2016	Not posted	Postoperative recurrent rate (Time frame: Day 1 post-operation)	<a href="#">NCT02487628</a>
The comparison of microbial adherence to various sutures in patients undergoing oral surgery	Unknown. Status was not yet recruiting	30	May 2017	Not posted	Bacterial counts on blood agar plates from each suture will be quantified in CFU (colony-forming units) and expressed as total bacteria/suture (Time frame: Outcome measure will be assessed 10 days after sample incubation for the different sutures obtained from each study participant)	<a href="#">NCT02653924</a>
Initial safety evaluation of FibroFix™ Meniscus	Terminated (Safety). Devices explanted. 12 m post-explant	4	July 2017	Not posted	Safety (Time frame: 12 months)	<a href="#">NCT02205645</a>