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REVIEW ARTICLE

Silk: A Promising Biomaterial Opening New Vistas Towards Affordable Healthcare Solutions

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Abstract | Substantial progress in biomaterial research over the years has culminated in revolutionary technological advancements in the healthcare domain. This has triggered the quest for affordable healthcare solutions with focus on sustainable biomaterials with versatile applications endowed with green fabrication strategies. Silk as a biopolymer has garnered special attention which can largely be attributed to the excellent material properties of silk in addition to its affordability and resource ability. Silk fibroin from various silkworm and spider species and sericin from various silkworm species have been researched for their potential applications in the healthcare industry such as tissue-engineered grafts, cancer therapeutics, high-throughput tissue-on-chip models, food preservatives, biomedical imaging, biosensing, biomedical textiles, implants, cosmetics and bioremediation products. The present review mainly focusses on the various sources of silk fibroin and its relevant properties that have been conferred to it by nature. Moreover, recent developments, progress and prevalent modalities of healthcare industry that involve the application of silk fibroin and sericin have been outlined in the present review.

Keywords: *Silkworm silk, Spider silk, Biomedical research, Biomaterials, Silk sericin, Silk fibroin, Spidroin*

1 Introduction

Biomaterial by definition is any material that is intended for use in the fabrication of medical device or implant/graft to replace function of defective body tissue in a safe, economic and reliable manner[1]. An ideal biomaterial endowed with biocompatibility, bioactivity, biodegradability, immuno-compatibility and mechanical resilience determines its suitability for use in various biomedical applications. In addition, the biomaterial must also possess appropriate material processing attributes to yield it into different formats. Advances in the field of material science have led to the discovery of novel biomaterials in the past few decades to fuel the revolutionary concept of bringing an ideal biomaterial to clinical reality. Among the various biomaterials, polymers have been in the forefront in biomaterial innovations[2], owing to their processing flexibility, superior physico-chemical attributes and bioactivity. However, not all shelved synthetic polymers are biocompatible and only handful of synthetic polymers have been approved for their application in various products by the Food and Drug Administration (FDA, USA)[3]. Moreover, the increasing mass volume usage of these polymeric-based composites for various biomedical applications has instigated concerns over the manufacturing processes and disposal issues after intended use of these polymeric biomaterials[4]. Hence, emphasis on greener synthesis and fabrication procedures for polymers and their alternatives is on the rise.

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Silk, a structural protein represents a distinct class of biocompatible and green polymers. It has been focussed upon in biomedical research pertaining to its biodegradability, low immunogenic response and easier processability[5]. Silk can be credited as one of the most ancient materials known to man which has been documented for its use as a medical suture, as early as 131–211 A.D by Greek physician Aelius Galenus[6]. The US pharmacopoeia (USP) classifies the conventional silk sutures which are still in practice today as non-degradable and non-adsorbent, primarily because of the wax coating which protects the silk fibroin (SF) from proteolytic digestion in vivo[7]. In addition to silk being a prime candidate for medical applications such as tissue engineering and drug delivery applications, silk has also been gaining prominence in new frontiers. The mild processing of silk fibres to obtain aqueous-derived regenerated silk fibroin aids in the feasibility of fabricating SF-based photonic devices or biosensors for various biomedical applications[8, 9]. Silk has also been proven to be an effective stabilizing agent extending the shelf life of fruits[10] and bio-pharmaceutical agents like vaccines and antibiotics[11]. For any material to be termed as bioresorbable, it must be physiologically acceptable by the body and broken down to get assimilated or safely eliminated from the body without eliciting any adverse reaction. Silk meets these criteria owing to its biocompatibility and biodegradability traits. Moreover, the greener approaches utilised to process the silk protein from the cocoon and silk glands further endow it as an ideal candidate for various biomedical applications. The present review of silk fibroin-based healthcare materials focusses on the diversity and sources of silk fibroin as well as on the components of silk fibroin that elicit the necessary properties for hitherto utilisation of silk as an ideal biomedical material. Following the exploration of silk as a biomedical material, the present review emphasizes on the real-world, potential, futuristic as well as the prototype technologies that comprise silk as a major component. These healthcare applications of silk have been broadly spanned into tissue engineering, cancer therapeutics, tissue-on-chip models, food technology, biomedical sensors, imaging and electronics, cosmetics, biomedical textiles and bioremediation.

Immunogenic: A commonly used term for describing the ability of a particular substance such as an antigen or epitope to provoke an immune response in the body.

Proteolytic: It refers to enzymes that are capable of breaking the protein chains into shorter fragments (peptides) and/or amino acids.

Bioremediation: It is the process of purification of contaminated water or soil using plant, microbial or natural enzymes and products.

2 Diversity and Components of Silk

2.1 Silkworm Silk

Lepidopterans larvae belonging to *Bombycidae* (mulberry silk) and *Saturniidae* (non-mulberry silk) have been exploited for commercial silk production, and their classification is based on the feeding habitat. The diversity of different types of silk, their lifecycle and feeding habitats have been exhaustively reviewed elsewhere[12, 13]. Silkworm silk protein biopolymer is made up of two components—silk fibroin (SF) and silk sericin (SS)[13]. In silk, SF forms the central core which imparts the toughness and load-bearing attributes, while SS acts a gumming agent. SS, a group of water-soluble glycoproteins accounts for 25–30% (w/w) of total silk worm cocoon[5]. The functional diversity observed among the different silks spun by silkworms depends upon the feeding habitat, nutritional indices and environmental factors such as temperature and humidity[14]. These determining factors play a crucial role in the compositional difference of amino acids of SF and differential presence of flavonoids or carotenoid in SS.

To understand the toughness and high strength exhibited by silk fibres, we need to explore the processing of these two proteins in the silk glands (Fig. 1A i–iii). SF is secreted by cells lining the lumen of posterior silk glands, particularly by fifth larval instar silkworms[15]. The SF secreted here exists as a complex α-helix-dominated structure referred to as silk-I form[16]. The secreted SF is pushed through the middle silk glands, where heterogeneous SS molecules are added, and the mixture passes through the anterior silk gland from where it is drawn through the spinnerets. The shear force and ionic gradient/pH differences experienced at the spinneret (Fig. 1A iv–v) causes the SF to attain antiparallel ß-sheet crystal conformation, referred to as silk-II form, rendering it as insoluble filament. This semi-crystalline nature of silk-II has been attributed to the toughness of silk, which is due to the unique crystal spinning process exhibited by the silkworms[17]. Silkworms (euarthropods) have evolved this process of folding and crystallization of SF for efficient fibre production and the spinning apparatus between *Bombycidae* and *Saturniidae* family has largely remained conserved[18]. However, the variability in between the different silks arises only due to, (i) differential presence and assembly of heavy chain (H-chain), light chain (L-chain) and glycoprotein; (ii) presence of repetitive polypeptide sequences which confer the unique bioactive, physico-chemical characteristics to the silk and (iii) divergent sericin fractions present in different silk varieties. The molecular weight of SF, SS and the repetitive sequence of most commercially important silk varieties are presented in Table 2. These include mulberry silk (*Bombyx mori, Bombyx mandarina*) and non-mulberry

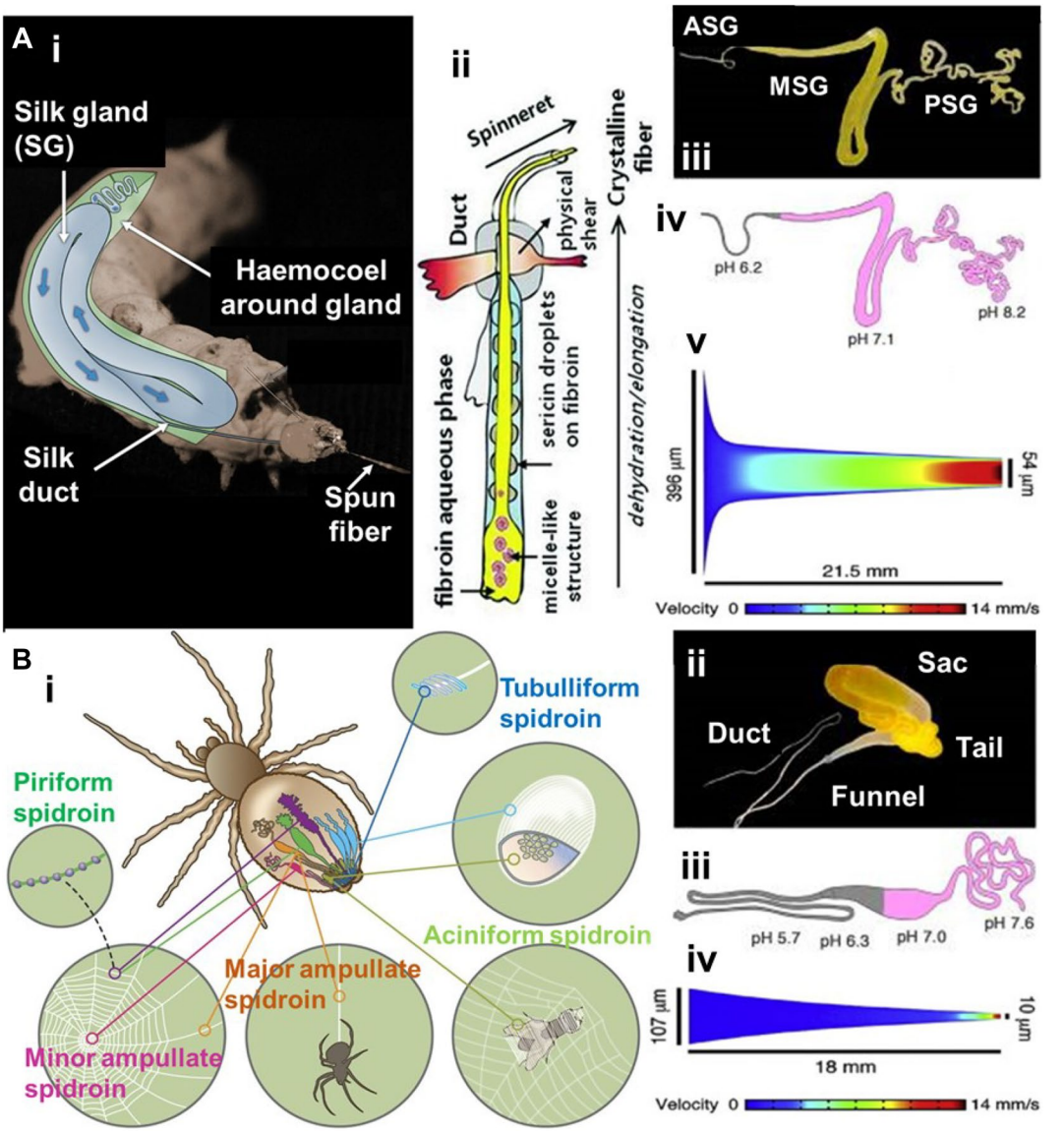


Figure 1: Spinning apparatus of **A** (**i**) *B. mori* silk gland (Copyright 2017, reproduced with permission from Springer Nature publication under creative common license CC-BY)[195] (**ii**) spinning duct (Copyright 2016, reproduced with permission from Elsevier)[194]; (**iii**) anterior silk gland (ASG), middle silk gland (MSG), posterior silk gland (PSG), (**iv**) different pH conditions seen in B. mori silk gland (Copyright 2016, reproduced with permission from MDPI publication under creative common license CC-BY), (**v**) velocity vector as seen in *B. mori* silk gland (Copyright 2016, reproduced with permission from Elsevier)[194] **B** spider, (**i**) depicting seven silk glands each producing its distinct spidroin (Copyright 2015, reproduced with permission from Springer Nature)[196]; (**ii**) major ampullate silk gland, (**iii**) different pH conditions (Copyright 2016, reproduced with permission from MDPI publication under creative common license CC-BY)[197]; (**iv**) velocity vector found along major ampullate silk gland (Copyright 2016, reproduced with permission from Elsevier)[194].

silk (*Antheraea assama*—Indian muga, *Antheraea mylitta*—Indian tropical tasar, *Antheraea pernyi*—Chinese temperate oak tasar, *Antheraea yamamai*—Japanese oak silk and *Philosamia ricini*—Indian eri silk).

2.1.1 Silk Fibroin

The Lepidoptera larvae of *Bombycidae* family spins twin delicate filaments of SF enwreathed by SS exhibiting a triangular smooth cross-section as observed at the microscopic level[19]. The mulberry SF consists of an H-chain, an L-chain and a glycoprotein (fibrohexamerin fhx/P25), assembled

in the molar ratio of 6:6:1. The H-chain and L-chain are linked by disulphide linkage and six such hetero-dimers get associated with a single P25 at the H-chain moiety through hydrophobic interactions[20]. The N-terminal has glycosylation containing mannose and glucosamine residues[21]. Poly-(glycine-alanine) repeats (GAGAGS, GAG AGY) forms the primary repeat sequences which accounts for the ß-sheet crystallite regions observed in the silk. The crystallite regions are embedded between amorphous α-helical regions, and these crystallite regions exhibit strong inter-chain interactions through hydrogen bonding, contributing to the silk's superior strength[22].

The silk spun by Lepidoptera larvae belonging to the *Saturniidae* family is very much distinct from the mulberry silk. The fibroin L-chain (flc) and fhx/P25 are absent in the non-mulberry silk varieties. Evolutionarily the gene encoding for fhx/P25 is thought to represent a paralog of gene(s) which might have adopted new functions other than the fibre formation as noticed in *Bombycidae* family[23]. Hence, the genes encoding for flc and fhx/P25 have been lost in the ancestors of *Saturniidae* family. The H-chain forms homodimers which constitutes the fibroin core in the silk fibre. Unlike the poly-(glycine-alanine) repeats which constitute the crystalline domains of mulberry silk, the non-mulberry silk possesses poly-(alanine) repeats. The silk fibroin arising from the *Saturniidae* larvae spinneret also consist of two fibroin monofilaments enveloped by SS, similar to *B. mori*[18]. However, the monofilaments of non-mulberry silk disorient during fibre formation, due to the inability of H-chains to pack closely owing to the higher percentage of bulky side chains present in the SF[24]. Thus, the non-mulberry cocoons appear more fibrous (flattened cross-section of SF) in nature which is evident from the density difference between the mulberry and non-mulberry silk fibres.

2.1.2 Silk Sericin

Silk sericin, an amorphous glycoprotein is produced in the middle silk gland and constituted primarily by serine (32–34%), aspartic acid (14–16%)[25] and other amino acids such as histidine, threonine, tyrosine and glutamic acid[26]. Sericin exists as random coil conformation and the molecular weight of sericin varies between 10 and 350 kDa. The fraction of sericin obtained solely depends upon the extraction protocol[26, 27]. For instance, hydrolysates of sericin obtained through plain water boiling are mostly low molecular weight (10–20 kDa), while other methods [usage of alkali or high temperature, high pressure) HTHP)] yields high-molecular-weight fractions[28]. Five different fractions of sericin from *B. mori* have been reported, namely ser-1, ser-2, ser-3, ser-4 and ser-5 ranging between 24 and 400 kDa[25]. The non-mulberry silk sericin varies from the mulberry silk varieties, for instance, the glycine content is lower in *A. mylitta* when compared to *B. mori*. Several sericin fractions have been reported from non-mulberry silk which is listed in Table 1. Though sericin is considered as a by-product and deemed as waste in sericulture industry, it has attracted immense interest from the cosmetic and pharmaceutical applications owing to its antibacterial, antioxidant, anticoagulant and wound healing properties[25, 28, 29].

2.2 Spider Silk

Unlike the euarthropods, spiders (order Araneae) rely on silk for the entire life span and their total dependence on it for their evolutionary success[30]. Based on the web weaving type, spiders could be classified as either orb web weavers or non-orb web weavers. Orb weavers capture their prey on the surface of the web, whereas the non-orb weavers entrap their prey inside intricate maze. Much of the current day research is focussed on orb-weaving spiders and interestingly these spiders use up to six different types of silks and a silk-like glue produced in seven distinct organs (Fig. 1Bi) to weave a single intact orb[31]. Each of these six types of structural silk protein is composed of repetitive monomers called spidroins comprised of a variable central core distinct for each silk flanked by non-repetitive amino and carboxy terminals[32]. The variable central core has modular repeat units which confer the variation in mechanical strength. The dragline silk secreted by major ampullate silk gland has a toughness of 180 MJ/m^3, whereas the auxiliary spiral thread secreted by minor ampullate silk gland has a toughness of 150 MJ/m^3[31]. The aciniform silk secreted by aciniform silk gland responsible for soft inner egg case wrapping has a toughness of 250 MJ/m^3[31].

Much similar to silkworm sericin, spiders too coat their capture silk with a special glue-like substance secreted from the aggregate gland[33]. This adhesive coating is primarily comprised of lipids, phosphorylated glycoproteins and organic low-molecular-weight components such as γ-aminobutyramide, choline, betaine and isethionic acid[34]. Listed in Table 1 are the different structural silks secreted by orb-weaving spider's abdominal glands namely major and minor

Glycosylation: It refers to the reaction of a carbohydrate, a glycosyl donor, and hydroxyl or other functional groups of another molecule, a glycosyl acceptor. In biological sciences, it is commonly used to refer to the enzymatic reaction that leads to the attachment of glycans to proteins and other organic molecules

Anticoagulant: Anticoagulants refer to the group of compounds or molecules that prevent or reduce coagulation of blood thereby prolonging the clotting time. They are also known as blood thinners.

Antioxidant: Antioxidants are compounds that prevent oxidation, especially of stored organic and food products, thereby limiting their spoilage. Some examples include Vitamins C and E, thiols, etc.

Table 1: Molecular weight of silk fibroin, silk sericin and repetitive sequences observed in different silk varieties.

Family	Species	Structural protein	Glue protein	Structural protein repetitive sequence(s)	References
Silkworm silk					
Bombycidae	*Bombyx mori* *Bombyx mandarina*	Fibroin H-chain—350 kDa L-chain—26 kDa P25 (glycoprotein)—30 kDa	Sericin Four polypeptide fractions of 400 kDa, 250 kDa, 150 kDa and 24 kDa	GAGAGS GAGAGY	13, 93–95
Saturniidae	*Antheraea assama*	Fibroin H-chain—230 kDa	Sericin High-molecular -weight fractions of 100 kDa and 66 kDa Low-molecular-weight fractions of 36–50 kDa	$AAA(A)_{5–15}$	25, 59, 96, 97
	Antheraea mylitta	Fibroin H-chain—197 kDa	Sericin High-molecular-weight fractions of > 200 kDa, 200 kDa and 70 kDa	AAAAAAAAAAAASS	52, 59, 96
	Antheraea pernyi	Fibroin H-chain—220 kDa	Sericin High-molecular-weight fraction between 200 and 70 kDa	AAAAAAAAAAAAAGS	97–99
	Antheraea yamamai	Fibroin H-chain—218 kDa	Sericin High-molecular-weight fractions of 240 kDa, 200 kDa and 180 kDa	AAAAAAAAAAAASS	59, 100, 101
	Philosamia ricini	Fibroin H-chain—230 kDa	Sericin Single fraction of 66 kDa	$YGGDGG(A)_{12}GGAG$	102–104
Spider silk					
Araneidae	*Nephila clavipes*	Spidroin Major ampullate spidroin (MaSp1 and MaSp2) secreted by major ampullate silk gland serves as the structural and dragline silk 250–350 kDa	Aqueous glue coat secreted by aggregate silk gland Complex mixture of glycoproteins, organic compounds and inorganic salts	$(A)_{4–15}$ and GGX (X = A, Q or Y) repeats	105, 106
		Spidroin Flagelliform spidroin (FSP) secreted by flagelliform silk gland, serves as capture spiral silk thread 195–266 kDa	Spidroin Piriform spidroin secreted by piriform silk gland, serves as attachment glue/cement	$(GPGGX)_{43–63}$ (X = A, V, Y or S) and GGX (X = A, Q or Y) repeats	107–109

Table 1: continued

Family	Species	Structural protein	Glue protein	Structural protein repetitive sequence(s)	References
	Nephila antipodiana	Spidroin Minor ampullate spidroin (MiSp) secreted minor ampullate silk gland, serves as auxiliary spiral silk thread (partial sequence consisting of repetitive domain, linker domain and C-terminal domain) 33 kDa		$(GA)_{4-6}$ and GGX (X = A, Q or Y) repeats	32, 110
	Nephila antipodiana *N. clavipes*	Spidroin Tubuliform spidroin (TuSp1) secreted by tubuliform silk gland, serves as tough outer egg casing ~366 kDa		$(GPGGX)_{41}$ and $(GGX)_7$	35, 111
	Latrodectus hesperus *Argiope trifasciata*	Spidroin Aciniform spidroin (AcSp1) secreted by tubuliform silk gland, serves as tough outer egg casing ~300 kDa		S_n and TGPSG	112

ampullate, tubuliform, flagelliform, aciniform, piriform and aggregate silk glands. Major research focus has been attributed to the major ampullate spidroins (MAS) which constitute the dragline silk of orb weavers (Fig. 1B ii–iv). The MAS complex is majorly constituted by two proteins namely, major ampullate dragline silk proteins 1 (MaSp1) and 2 (MaSp2)[35]. Both MaSp1 and MaSp2 proteins comprise poly-alanine domains and poly-glycine rich domains, but the main difference is that MaSp2 accounts for 15% proline in the total amino acid content, while MaSp1 is proline free[36]. Each orb weaver spider's silk varies based on the ratio of MaSp1 and MaSp2 content, for instance, *Nephila clavipes* has 81% MaSp1 and 19% MaSp2, while *Argiope aurantia* has 41% MaSp1 and 59% MaSp2.

3 Features of Silk Befitting for Biomedical Research

3.1 Silkworm Silk

The amino acid compositional diversity and differential presence of repetitive units [poly-(alanine) or poly-(glycine-alanine)] govern the distinct physico-chemical properties noticed in the different silk varieties which are listed in Table 2. The relationship between the physical properties and chemical constituency of fourteen types of silk, inclusive of the commercially important mulberry and non-mulberry silk highlighted in the current section, has been reported elsewhere[19]. As stated earlier, the fibre packing density of mulberry silk (1.35–1.37 g/cm^3) is higher in comparison to the non-mulberry silk (1.30–1.31 g/cm^3)[37]. This high order of packing may be attributed to the compact assembly of fibroin H-chain, L-chain and fhx/P25 in the ratio 6:6:1, contributing to the mulberry silk's high tensile strength in comparison to non-mulberry silk, as noticed in Table 2. However, the lack of orientation in H-chain homodimers and the inability to form a close association due to presence of bulky side chains of hydrophobic amino acids, contributes to higher yield rates (higher extension percent while breaking). The disoriented entangled H-chains open up resulting in higher breaking extension[24]. Additionally, the poly-(glycine-alanine) repeats namely GAGAGS and GAGAGY are responsible for forming the crystalline regions in the ß-sheet domains confer heterogeneity along the SF peptide. On the contrary, the homogenous poly-alanine repeats in the non-mulberry silk result in uniform transitions during tensile deformations, thus contributing to the mechanical resilience of non-mulberry silk.

Table 2: Physico-chemical properties of degummed fibres of different silk varieties.

Family	Species	Extension (%)	Tensile strength (GPa)	Toughness (GJ/m^3)	Crystallinity (%)	References
Silkworm silk						
Bombycidae	*Bombyx mori* *Bombyx mandarina*	24.5 ± 10.1	0.57 ± 0.12	0.103 ± 0.057	30.5–33.7	19, 41, 113
Saturniidae	*Antheraea assama*	29.2 ± 10.7	0.36 ± 0.10	0.068 ± 0.031	34.7	19, 113
	Antheraea mylitta	26–39	0.25–0.45	0.13	39.5	24, 114, 115
	Antheraea pernyi	29.6 ± 10	0.43 ± 0.08	0.079 ± 0.027	30.9	19, 114, 116
	Antheraea yamamai	35.6 ± 13.7	0.39 ± 0.07	0.092 ± 0.043	32.5	19, 117
	Philosamia ricini	29.4 ± 8.0	0.47 ± 0.11	0.086 ± 0.030	25.8	19, 41, 113
Spider silk (orb weavers)						
Araneidae	*Araneus diadematus* *Dragline spidroin*	27	1.1	0.180	~	31
	Argiope trifasciata Dragline spidroin	~ 30	6.9 ± 0.4	~ 0.09	17–29	118, 119
	Nephila clavipes Dragline spidroin	12–37	6.9–11	0.08	10–15	120, 121

Silk films and scaffolds fabricated from the regenerated non-mulberry silk fibroin also exhibited similar strain hardening behaviour[38]. Moreover, silk possesses a strength-to-density ratio that is ten times more than that of the steel[39], affirming their suitability for load-bearing applications.

As discussed earlier, SF's main structural component is the H-chain which is amphiphilic, consisting of hydrophobic crystallite domains (made up of ß-sheets) interspaced between hydrophilic amorphous domains (made up of α-helices). Hence, SF can be considered a co-block polymer, which exists in an anionic (pI = 4) form in neutral solution[40]. The basic/acidic amino acid ratio of the non-mulberry silk is slightly higher than the mulberry counterpart. This in turn contributes to the SF's varied surface property in terms of hydrophilicity and hydrophobicity and subsequently plays a crucial role in surface wettability of biomaterials derived from the regenerated silk solution. Adding to the notion, it was found that the grand average of hydropathicity (GRAVY) indices of mulberry silk is higher than the non-mulberry silk[41]. Crystallinity in silk-II conformation is conferred by the ß-sheet structure that is packed in an asymmetrical fashion. If the crystallinity of the crystallite domains is considered, the non-mulberry silk varieties are more crystalline than the mulberry silk (as seen in Table 2). This is attributed to the poly-alanine repeats found in the crystallite domains of non-mulberry silk which constitute tight ß-sheet crystals in comparison to the poly-(glycine-alanine) repeats found in the mulberry silk. Crystallinity also remains to be a crucial factor for biodegradation of regenerated SF-based materials in vivo. By controlling the degree of crystallinity of ß-sheets through various physical and chemical treatments, the biodegradation rate and the water stability of the SF-based materials can be modulated[16, 42]. Owing to the extraordinary physico-chemical properties and high thermal stability (for fibres, T_g = 190–200 °C[40]; for films obtained from regenerated SF from mulberry and non-mulberry silks, T_g = 224–290 °C[43]), silk-based biomaterials are compatible to most of the sterilization methods. This is very advantageous, as in most polymer-based matrices sterilization through autoclaving or any heat-based method, denatures the polymer network, however, silk overcomes this drawback.

Sterilization: It is the process of elimination, removal, killing or deactivation of life forms and biological agents (e.g., fundi, bacteria, viruses, spores, and prions) in a specified surface or volume. Some commons methods used include heat, chemicals, irradiation, high pressure and filtration.

3.2 Spider Silk

Spider silk unlike silkworm silk has not been explored to a great extent largely due to the difficulty in rearing and collection in large quantities for commercial upscaling. Though instances

of spider silk being used for wound healing by the ancient Greek[44] and as fishing nets by the Australian aborigines[45] have been found, but it was not until the recently that a full-scale spider silk has been materialized[44]. Howbeit, the mechanical superiority of spider silk has been documented to match the strength of Kevlar (4×10^9 N/m^2) while being six times more flexible than Kevlar. This uniqueness of the spidroin has pushed it to be recombinantly engineered and produced in bacterial systems for biomedical applications, particularly MaSp1 and MaSp2 which have been well studied[30]. Interestingly, the elasticity of spider silk has been attributed to the phenomenon of 'supercontraction' when hydrated or under humid conditions, causing it to contract to half of its dry length. This could be attributed to the entropy-driven rearrangement of GPGGX motifs of MaSp spidroins giving rise to a recoiling effect of dragline silk[46, 47]. The mechanical properties bestowed by spider silk may deem it to be an ideal candidate for biomedical textile or biosensing applications. Additionally, the recent reports of its applications in tissue engineering owing to its biodegradability, presence of inherent cell adhesion motifs and possibility to tailor new properties have given a new lease of life for this ancient material[48].

3.3 Affordability and Resourceability

One of the utmost advantageous features of silk fibroin as a biopolymer over other biopolymers is the sheer commercial scale in which silkworm silk is produced (Fig. 2A) in tonnes per annum. Notably, more than 70% of world silk production is from the Asian subcontinent with China and India being the major players. Mulberry silk (*B. mori*) accounts for 90% of global silk production[12]. Sericulture is mainly concentrated in the tropical and sub-tropical regions, with the prevalence of bivoltinism (two generations per year) and multivoltinism (multiple generations per year) in silkworms, there is year round availability of silk[49]. On the other hand, biopolymers like collagen-I are primarily extracted from animal sources (porcine, bovine skin, bovine tendon) and human cadavers. The major drawbacks associated with animal-sourced collagen are contamination with pathogens, disease transmission through prions, immunogenicity and antigenicity related with telopeptides[50]. To overcome these drawbacks, there is very critical and stringent quality control associated with downstream processing of collagen deemed for biomedical use. This ultimately results in increase of production cost. The extraction of silk fibroin from cocoons or from silk glands is relatively easy and the greener aqueous processing has rendered US FDA approval of silk fibroin for drug delivery, surgical suture and applications pertaining to tissue engineering[51]. The facile processing and purification strategies have resulted in cost effectiveness in production of silk fibroin solution[5, 52]. For instance, 1 mL of 0.3% (w/v) collagen-I (from bovine skin) costs ~ $12 (USD), while a mL of 0.3% (w/v) silk fibroin solution (mulberry *B. mori*) costs just ~ $ 1 (USD) (Sigma, USA, cell culture reagent grade; information retrieved from https://www.sigmaaldrich.com). Though synthetic polymers, for instance, poly(D,L-lactic acid) (PLA), polycaprolactone (PCL) and poly(D,L-lactic-co-glycolic acid) (PLGA) are relatively inexpensive than biopolymers, they are plagued with drawbacks of little or no bioactivity and biodegradability, toxic degradation products and non-resorbability. Moreover, these synthetic polymers do not meet the requirements of natural polymers (Fig. 2B).

3.4 Processing Feasibility and Ease in Modification

Mulberry silk cocoons can be subjected to aqueous processing which is a major advantage when compared to other biopolymers. For instance, biopolymers such as collagen, chitosan need to be processed in acidic conditions, which is a major concern when dealing with delivery of bioactive molecules or cells for therapeutic applications. On the contrary, regeneration of silk fibroin from mulberry silk cocoons involves two steps. (i) degumming or process of removal of silk sericin from raw silk using 0.02 M sodium carbonate, (ii) dissolution of obtained silk fibroin fibres in 9.3 M lithium bromide (chaotropic agent) to obtain regenerated silk fibroin solution after thorough dialysis in water to remove LiBr[5]. Few protocols also use a ternary system, namely Ajisawa's method (ethanol/water/$CaCl_2$ in 92/144/111 w/w ratio) for dissolution of degummed silk fibres, but the yield of the regenerated silk fibroin is relatively less than the former protocol[53, 54]. The obtained aqueous regenerated silk fibroin solution can be concentrated and stored in liquid form in cold storage (1 month or lesser) and in lyophilized powder form at 25 °C for longer durations. Non-mulberry degummed silk fibres dissolution with the help of chaotropic reagents such as lithium bromide, lithium thiocyanate do not result in good yield of the protein[12, 13]. This is partly because of the amino acid composition,

Lyophilized: Freeze drying or lyophilization refers to the low temperature dehydration procedure that is accomplished by freezing the samples, lowering the temperature and then removal of the ice using sublimation.

Antigenicity: It is synonymous to the term immunogenicity as it refers to the capacity of a chemical structure to bind to T-cell receptors and antibodies to produce immune reaction.

Chaotropic: It refers to an aqueous solution of an agent that can be employed to disrupt hydrogen bonding networks in macro-molecules such as proteins and nucleic acids and thereby destabilizes them.

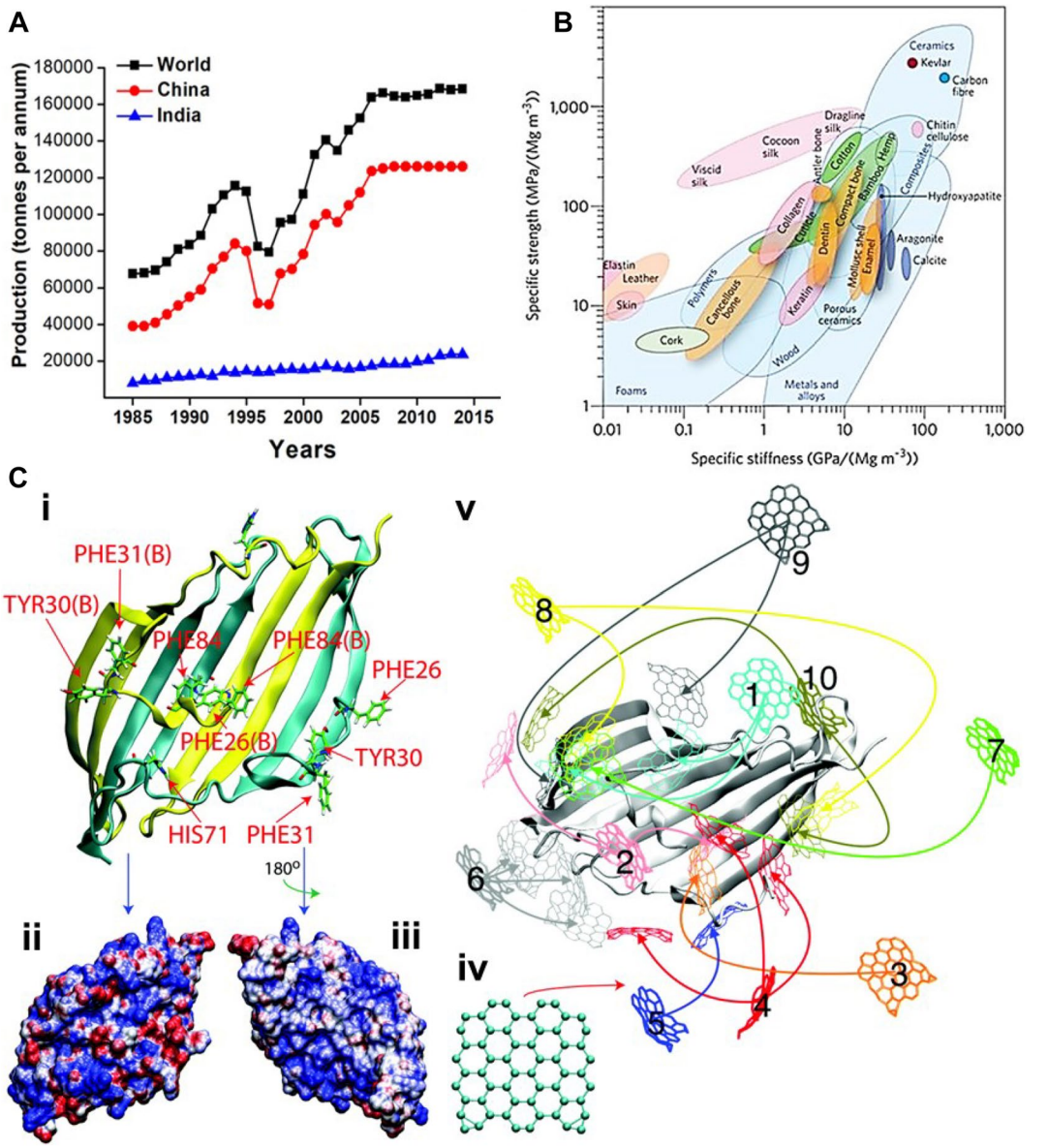


Figure 2: **A** Production quantity in tonnes per annum for raw silk. (data obtained from FAOSTAT—Food and Agriculture Organization of the United Nations Statistics Division http://www.fao.org/faostat/en/#data/QP, Assessed on 20th March 2019); **B** Ashby chart for strength vs. stiffness performance of natural and synthetic materials, silk outperforming some of the most commonly used synthetic polymers (Copyright 2014, reproduced with permission from Springer Nature)[198]; **C** simulated visualization of (**i**) *B. mori* heavy chain N-terminal domain whose electrostatic charge distributions are seen in **ii**, **iii** for docking (**iv**) graphene at (**v**) various docking positions (Copyright 2018, reproduced with permission from RSC)[80] for fabrication of composites with predictive physical properties.

where non-mulberry silk fibroin is constituted ~ 47% by alanine, which tend to take up α-helical conformation in aqueous solution[55, 56], getting packed closely thereby preventing chaotropic agents from disassociating the hydrogen bonding between chains. These non-mulberry silk fibroins, however, are endowed with unique physico-chemical properties such as the presence of poly(A) stretches which confer superior mechanical resilience[57, 58] and presence of RGD tripeptide which confer cell adhesion features[59] and thus exploiting it for tissue engineering applications is advantageous. To overcome this, silk fibroin from the silk glands of fifth instar silk worms (*A. mylitta*[52, 60, 61], *A. assama*[57, 58, 62–64], *P. ricini*[65–67]) was directly dissolved in anionic surfactant, sodium dodecyl sulphate (SDS) (1% w/v) to disrupt the hydrogen bonding in the native silk

fibroin, and dialysed to remove SDS to obtain the aqueous regenerated silk fibroin solution. The protein thus obtained maintains the biomechanical properties of SF (unlike chaotropic agents which shear the protein during isolation) and feasible to get high yields (1–1.5 g protein per silk gland), thus making the process amenable for scaling up in biomedical research[52]. Other harsher approaches for dissolving silk have been reported recently which mainly involve the application of ionic liquids such as 1-butyl-3-methyl imidazolium acetate (BMIAc) (*A. mylitta*[68, 69] and *A. assama*[70]), 1-butyl-3-methylimidazolium chloride (BMIMCl) (*B. mori*[71]). However, these harsher chemical agents irreversibly denature the isolated SF and the structural integrity of formed scaffolds or matrices is questionable[72].

The regenerated aqueous SF solution is amenable to be processed and formed into various formats such as 2D films, 3D silk sponges, electrospun membranes, microparticles, nanoparticles, hydrogels, 3D printed constructs, micropatterned surfaces, microfluidic and micromolded devices. Listed in Table 3 are few of the interesting instances of silk being used in different formats for myriad of applications in healthcare and other allied endeavours. In addition to the processing feasibility, silk structure is well explored and offers the opportunity to chemically modify it for more precise applications[73]. For instance, addition of another polymer chain such as polyethylene glycol or oligosaccharides or specific peptide chains (RGD tripeptide) could be done through coupling reactions (cyanuric chloride activated or carbodiimide)[74, 75]. Similarly, grafting of chemical moieties could also be done in silk fibroin. To achieve UV photo-crosslinking, poly-methacrylate grafting has been reported[76] for 3D printing applications. The versatility of silk matrices is also endowed with thermal stability, which allows them to be autoclaved to sterilize them post-fabrication (β-sheet-induced matrices)[77]. Silk matrices unlike other polymers such as PCL, PLGA which are thermosensitive, can withstand many sterilization techniques such as ethylene oxide treatment, 70% ethanol disinfection, γ-irradiation, UV irradiation and the mechanical properties are not drastically altered[78].

3.5 In Silico and Recombinant Engineering Strategies

With the age of machine-learning seeping into all aspects of study, biomaterial design is no exception. Silk as a biomaterial has been investigated at large in the last few decades and utilising predictive biomaterial design strategies enables material scientists to tailor the material more precisely to suit the need. In a pioneering study, Wong et al. describe an iterative material design process involving computation simulation, genetic engineering and mechanical characterization to obtain silk fibres with predictive mechanical properties[79]. Similarly, understanding the precise position of docking a molecule for composite fabrication would desirably help in improving its physico-chemical properties. In these lines, molecular dynamics simulation was utilised to determine the binding position of graphene in *B. mori* silk fibroin (Fig. 2C)[80, 81]. Multiscale modelling approaches also enables us to predict the structure–function properties in bottom-up approach[82]. For instance, silk hydrogels made through binary solvent-induced conformation transition approach rely solely on hydrogen bonding and atomistic modelling enabled to modulate the degree of bonding thus tailoring the hydrogel properties[83].

Spider silk, despite its phenomenal properties is difficult to obtain in large quantities, because rearing spiders is difficult owing to their cannibalistic nature. This has been overcome using recombinant engineering, where partial or full sequences of spider silk have been expressed in bacterial, yeast, mammalian, insect, transgenic plants and animal hosts[84]. Knowing the structural information of spider silk through sequencing has enabled it to be engineered with other useful domains such as for cell adhesion (fibronectin) or anti-microbial peptides, to obtain fusion spidroin peptides for tissue engineering applications[85, 86].

3.6 Biocompatibility of Silk Implants

The antigenicity and cytocompatibility of SF-based scaffolds (in different forms such as 3D porous matrices, 2D electrospun mats and films, gels, micro/nanoparticles) have been extensively studied over the years[38, 40]. The studies have affirmed the materials' compatibility for the intended use under in vitro conditions as well as in animal models. Implanted 3D SF scaffolds in a rat model showed lower expression of TNF-α IFN-ϒ, IL-4, IL-6 and IL-13 (M1 macrophage-specific genes) immune response genes[87] suggesting that SF activates the humoral-mediated immune response without C3 compliment activation. The host reaction post-implantation of the biomaterial determines the fate of the implant. The initial immune recognition, followed by subsidence of M1-specific inflammatory cytokines and finally the switch over to M2

Denature: Denaturation refers to the loss of quaternary or tertiary or secondary structure of biological molecules such as proteins and nucleic acids produced as a result of external stress (heat and pressure) or chemical (strong acid or base, organic/inorganic salts) treatment.

Electrospun: It refers to the product formed by electrospinning. Electrospinning is a method of nano-fibre production using an electric force to draw out charged threads of polymeric solutions or melts.

Transgenic: It refers to the production of genetically modified organisms that have been produced by the transfer of gene/genetic material from one organism to another by natural means or genetic engineering techniques. It brings about a change in the phenotype of the targeted organism.

Inflammatory cytokines: These are signalling molecules produced by immune cells (helper T cells and macrophages) that are involved in the promotion (pro-inflammatory) or suppression (anti-inflammatory) of inflammatory response.

Table 3: Insight into different processing formats feasible with silk.

S. No.	Material format	Silk type	Features	Applications	References
1.	Films	Silk fibroin (*B. mori* *A. mylitta, A. assama, A. pernyi, P. ricini*)	2D solvent casted, transparent films with nano-features ranging from 1 to 12 nm; patterned silk films by PDMS stamping	Bone, corneal, vascular tissue engineering, biomaterial design	41, 57, 122–125
		Silk sericin (*B. mori* *A. mylitta, A. assama, A. pernyi, P. ricini*)	2D solvent casted	Tissue engineering, drug delivery	126–128
		Spidroin (dragline silk from *A. diadematus*, recombinant and chimeric spidroins from *N. clavipes*)	2D solvent casted; targeted mineralization with fusion peptides; spin-coated free standing films	Biomaterial design, bone tissue engineering application, micro-electromechanical systems (MEMS), biosensors	129–132
2.	Sponge	Silk fibroin (*B. mori* *A. mylitta, A. assama, P. ricini*)	Porous silk sponges, with highly interconnected pores ranging from 60 to 300 µm; silk fibre-reinforced scaffolds with compressive strength up to 13 MPa; processed through freeze drying, salt-leaching techniques; ease in making composites with inorganic/organic additives	Bone, cartilage, intervertebral disc, meniscus liver, pancreas tissue engineering and drug delivery	58, 65, 133–144
		Silk sericin (*B. mori* *A. mylitta, A. assama, A. pernyi, P. ricini*)	Porous silk sponges	Tissue engineering and drug delivery	127, 128, 145
		Spidroin (partial dragline spidroin from *Euprosthenops australis*)	Porous silk sponges	Skin tissue engineering, wound dressings, biosensing	85, 86
3.	Fibres/sutures	Fibroin (*B. mori*, *A. assama*, *A. mylitta*)	Monofilament or multifilament degummed silk fibres (woven or non-woven); biospun silkworm filaments	Tendon tissue engineering, nerve guides, stents, suture material	146–150
		Spidroin (*N. clavipes* dragline silk)	Biospun spider filaments; braided or non-braided (stress failure 60–120 MPa yet lightweight 1.3 g/cm^3); wet spinning approaches for multitude of applications	Tendon repair, nerve guides	151–153
4.	Electrospun mats	Silk fibroin (*B. mori* *A. mylitta, A. assama, A. pernyi, P. ricini*)	Fibre diameter ranging from 50 to 500 nm; ease in making composites with other polymers, inorganic or organic additives	Bone, osteochondral, nerve, ocular skin, skeletal muscle tissue engineering, vascular conduits, wound dressings, drug delivery, biofilters	66, 67, 154–162
		Silk sericin (*B. mori*, *A. assama*)	Fibre diameter ranging from 100 nm to 300 nm	Tissue engineering and wound healing applications	163, 164
		Spidroin (MaSp4 dragline silk)	Piezoresponsive and electrically responsive nanofibres	Sensors and biosensing	86, 165

Table 3: continued

S. No.	Material format	Silk type	Features	Applications	References
5.	Microparticles	Fibroin (*B. mori*, *A. assama*)	Particle size ~ 3 μm prepared by solvation or mechanical comminution	Bioactive molecule/drug delivery	166, 167
		Sericin (*B. mori*)	Particle size ranging between 10 and 30 μm	Bioactive molecule delivery	168
		Spidroin (partial sequence of dragline silk from *A. diadematus; L. hesperus*)	Microspheres ranging between 0.5 and 2 μm through micro-mixing, emulsion based approaches	Targeted drug, vaccine delivery	169–171
6.	Nanoparticles	Silk fibroin (*B. mori* *A. mylitta, A. assama, A. pernyi, P. ricini*)	Nanoparticles ranging from 2 to 500 nm, fabricated via an array of methods such as capillary microdot desolvation, electrospraying, microemulsion, supercritical fluid application	Tissue engineering, drug, bioactive molecule delivery	172
		Sericin (*B. mori*)	Self-assembled nanoparticles ranging 100–200 nm	Drug delivery	173, 174
		Spidroin (partial sequence of dragline silk from *A. diadematus*, *N. clavipes*)	Nanoparticles ranging 500–800 nm, through micro-mixing or self-assembly	Drug delivery, triboelectric nanogenerators for energy harvesting	175, 176
7.	Hydrogels	Silk fibroin (*B. mori* *A. mylitta*, *A. assama*, *A. pernyi*, *P. ricini*)	Highly tuneable hydrogels with respect to elasticity, degradability developed through self-assembly, use of green crosslinkers, or physical crosslinkers	Drug delivery, tissue engineering	5, 62–64, 177, 178
		Spidroin (dragline silk from *A. diadematus*)	Self-assembly	Drug delivery	179, 180
8.	3D printed constructs	Silk fibroin (*B. mori*)	Methods used for 3D printing—direct-ink writing (DIW), microextrusion (self-curable bioinks by adding polyols for crosslinking, enzyme-mediated crosslinking (bioprinting)	Tissue engineering, optical wave guides and other applications	181–186
		Spidroin (dragline silk from *A. diadematus*) and other recombinant spider silks	Bioprinting through microextrusion-based nozzle	Tissue engineering	187–189
9.	Injection molding/ macro-molding	Fibroin (*B. mori*)	Injection molding, laminate reinforcements	Resorbable bone screws, high strength, flexural polymers for mechanical uses	190–193
10.	Micropatterned/microfluidic platforms	Fibroin and spidroin	Fabricated via lithography, micromolding, layer-by-layer deposition, dip coating, electro-dynamic processes	Sensors, blood typing, tissue-on-chip, biomaterial design	194

macrophage commitment is crucial for the biomaterial to be accepted. Assessing reports of the past 15 years, it has been found that, for the alkali heat purified SF-based materials exhibited promising biocompatibility mainly due to their cytocompatibility and lower immunogenic potential as compared to collagen, PLGA and many more other polymers[40]. However, careful removal of sericin from silk is essential to completely eliminate the chances of provoking immune response, as SF and SS when presented individually are relatively less immunogenic than when they are associated with each other[88]. The non-mulberry SF silk additionally benefits from the intrinsic presence of arginine–glycine–aspartate, i.e., RGD tripeptide in the N and C termini of G_C motifs of H-chain[59]. These tripeptides enhance the cell adhesion and proliferation via integrin-mediated pathway[43] and hence find immense potential in designing smart biomaterials for tissue engineering and varied biomedical applications.

3.7 Biodegradability of Silk Implants

Silk being a protein polymer is prone to undergo proteolytic digestion owing to the presence of proteolytic cleavage sites[89]. The intrinsic physicochemical attributes possessed by the SF such as ß-crystal polymorphism, strong intermolecular and hydrophobic interactions governs the rate of degradation of silk[40]. The self-assembled regenerated SF-based matrices also exhibit similar biodegradation kinetics. The degraded products include soluble amino acids and shorter peptide fragments which are resorbed into the system. Unlike other FDA-approved synthetic polymers such as PLGA, PLA which undergo rapid dissolution in vivo and whose degraded products increases local pH, silk as a polymeric matrix mitigates these drawbacks. Moreover, depending upon the degree of crystallinity conferred on the regenerated SF-based matrices, the degradation rate can be fine tuned to meet the requirement. A high content of ß-sheet content results in slow degradation and vice versa[90]. The mechanism of degradation studied in vitro studied using a model enzyme protease XIV, revealed that the enzyme acted upon the amorphous regions reducing the α-helical and random coil structures. The ß-sheet crystallite remained largely non-degraded, nevertheless the breakage of random coils linking the ß-sheet led to the progressive but slow removal of sheets from the structure[91]. Also, the rate of degradation of regenerated SF-based matrices was found to be dependent on the concentration of SF and molecular weight of SF used in the fabrication procedure[40]. The rate of degradation under in vivo conditions mirrored the results obtained under in vitro conditions. 3D scaffolds prepared from regenerated SF when implanted inside Lewis rats, started to get remodelled within few weeks and totally got resorbed by the system after 1 year[92]. These results are promising, as any biomaterial used for tissue engineering must be resorbed shunning the need for another secondary correctional surgery to remove the graft from the site of application.

4 Applications of Silk in Healthcare Industry

4.1 Silk-Based Tissue Engineering

Tissue engineering involves the in vitro formation of a functional three-dimensional natural tissue by utilising cells, scaffold and biomolecules to improve the damaged tissue and organs. The choice of biomaterial and formulation strategy decides whether a scaffold will be functional or not. The slower rates of degradation of silk fibroin and low inflammatory response render it useful for biodegradable scaffolds where slow tissue growth is imperative[199]. However, the sericin protein present in the silk is responsible for eliciting immune response and thus should be separated properly from the silk fibroin before the processing of scaffolds[61]. Mulberry silk fibroin from *B. mori* has been used predominantly in tissue engineering applications followed by the non-mulberry ones such as *A. mylitta*, *A. pernyi*, *A. assama* and *P. ricini* silk fibroin[13, 64, 133, 137, 200]. With the rapid advancement in tissue engineering, various scaffold formulation strategies are available (Fig. 3).

Scaffolds: These refer to temporary structures that are used to support the proliferation, retention and growth of cells. These may fabricated using natural or artificial polymers and materials that are biocompatible in nature.

4.1.1 Fabrication Strategies for Tissue Engineering Scaffolds and Constructs

A three-dimensional tissue-engineered scaffold should support cell recruitment, adhesion, proliferation and differentiation as under in vivo conditions[61, 133]. These scaffolds can be fabricated using conventional techniques such as freeze drying, fibre bonding, self-assembly, solvent casting, gas foaming, electrospinning and porogen leaching[133, 199, 201] as listed in Table 4.

Differentiation: Differentiation generally refers to the change of one cell type to another cell type that is more specialized in terms of function. This process allows the progenitor cells to mature into various terminally differentiated cell types exhibiting different phenotypes and specialized function despite possessing the same genome.

Though in practice, traditional scaffold fabrication techniques possess drawbacks such as uncontrolled pore size, pore distribution and non-uniform seeding and proliferation of cells and other biologics. This can be overcome by rapid prototyping (RP) techniques. These are advanced computer-aided scaffold fabrication

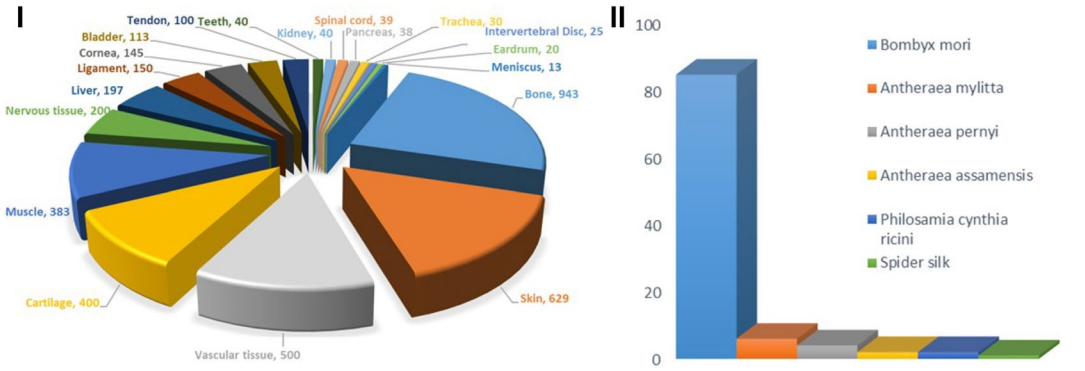


Figure 3: **I** Reports published on various tissue engineering applications by utilising silk fibroin. (Source: An extensive PubMed search till April 2019). **II** Percentage of reports published exhibiting the application of various silk types in tissue engineering. (Source: PubMed).

techniques used to construct three-dimensional structures in a layer-by-layer fashion[202]. RP involves designing of a 3D model from a scanned target tissue by computer-aided design (CAD) software and biomedical imaging modalities for rapid reconstruction of the native tissue structure. Thereafter, the 3D model is replicated using techniques such as selective lase sintering (SLS)[203], fused deposition modelling[204], pressure-assisted micro-syringe[205] and 3D bioprinting. Bioprinting has gained immense prominence in tissue/organ engineering and can be performed using several types of printers like Inkjet-based, microextrusion-based and laser-based bioprinters[206]. Some of the multitude of tissue constructs that have been fabricated by bioprinting technology are listed below in Table 5.

Microextrusion: It is the process of extrusion of polymers and other substances through a micrometre size orifice to microform structures during additive manufacturing.

Autograft: Autografts are tissue grafts that are taken from one part and grafted to another part of the same individual's body.

Allograft: Allografts are tissue grafts that are taken from one donor and grafted to recipient that are different organisms and genetically unidentical but hail to the same species.

Osteoinductive: Any biomaterial or molecule which induces the formation of osteoprogenitor cells.

4.1.1.1 Bone Grafts The field of bone tissue engineering has gained immense interest ever since people tried to find alternatives for autograft and allograft in repairing bone defects[221]. Autografts are currently the gold standard as they are histocompatible, but are expensive and difficult to procure, while allografts may lead to immune rejection[221, 222]. Currently, a number of biomaterials are available for scaffold-based bone tissue engineering[223], but silk fibroin acts as an appropriate biomaterial for constructing osteoinductive functional bone grafts on account of its remarkable physico-chemical and biological properties while possessing a close resemblance collagen I[58]. Porous scaffolds of *A. mylitta* silk fibroin have been shown to mimic native bone tissue using bone marrow mesenchymal stromal cells (BMSCs)[60]. *B. mori* has been extensively used to make functional bone grafts[224] by inducing osteogenic differentiation of human mesenchymal stromal cells. Apatite overlayed silk fibroin scaffolds have successfully repaired mandibular border defects by means of BMSCs. Premineralization of these scaffolds aided in closely mimicking the native extracellular matrix, thereby leading to rapid formation of new bone tissue from the BMSCs[225]. A combination of *B. mori* silk fibroin and hydroxyapatite (HA) has also been explored widely for treating bone defects[226, 227]. Such scaffolds made of silk fibroin/HA completely repaired segmental bone defects in Sprague–Dawley rats after 12 weeks of implantation[228]. Recombinant human bone morphogenetic protein-2 (rBMP2)-laden silk fibroin scaffolds induced faster osteoblast differentiation as compared to the non-loaded silk fibroin scaffolds[229]. Tricomposite scaffolds made by blending hydroxyapatite (HA), *A. assama* silk fibroin fibres and its solution revealed high osteogenic potential along with enhanced proliferation rates of hBMSCs and MG63 cell line[135]. Incorporation of HA into the silk scaffold enhances the compressive modulus to a greater extent as compared to pure silk scaffold[230]. Another study demonstrates that a blend of 70S bioactive glass, *B. mori* and *A. assama* silk fibroin made by electrospinning provides a good scaffold matrix for repairing osteochondral tissue defects[208]. Repairing large volume bone defects was exhibited by copper-doped bioactive glass silk composite matrices[58]. Such porous composite matrices enhanced the resorption, maturation of stem cells and endothelial cells within the scaffold, thereby exhibiting its potential for clinical translation as seen in Fig. 4I.

4.1.1.2 Skin Grafts and Wound Dressings Tissue engineering strategies for skin have been widely explored due to its high susceptibility to damage

Table 4: List of conventional strategies reported for silk-based tissue engineering.

Fabrication technique	Tissue	Scaffold material	Cell tested	Conclusion	References
Electrospinning	Skin	Mats (*B. mori*/*A. assama* + PVA) coated with 4RepCT spider silk	HDF, HaCaT	Improved cell adhesion, antimicrobial activity	85
Electrospinning	Skin	Mats (silk sericin + PVA)	Murine fibroblasts, HaCaT	No inflammatory response; better healing rates	164
Electrospinning	Skin	Mats (*A. assama* + *P. ricini*) with EGF, ciprofloxacin HCl	HDF, HaCaT	Scar-less wound healing	66
Electrospinning	Skin	Silk + collagen solution + 1,1,1,3,3,3-hexafluoro-2-propanol	HEK, HEF	Functional wound dressing matrix	207
Electrospinning	Bone	Biphasic scaffold (bioactive glass + silk)	MG63	Increased ALP, OPN, sGAG and collagen deposition	208
Freeze drying	Skin	Sponge (silk + alginate sol.)	Used in rat wound model	Increased wound healing rate	209
Freeze drying	Bone	Scaffold (silk solution + fibres + HA)	MG63, hBMSCs	Functional bone graft substitute	135
Freeze drying	Bone	Scaffold (Cu-doped bioactive glass + silk fibres + silk sol.)	hMSCs	Enhanced vascular and fibrous tissue growth in scaffolds	58
Freeze drying	Bone	Scaffold (Si/Zn-doped brushite cement + silk sol.)	MG63, THP1, pECs	Affordable and viable bone graft substitute	144
Freeze drying	Liver	Scaffold (*B. mori* + *A. assama* silk sol.)	HepG2, rat hepatocytes	Functional bioartificial liver	137
Freeze drying	IVD	Lamellar scaffold (*B. mori* silk sol.)	pAF, hMSCs	AF cell alignment, proliferation, ECM deposition	200
Solvent casting	Vascular tissue	Films (silk fibroin sol. + water vapour annealing)	pECs, SMCs, Fibroblasts	Closely mimics native vessel	57
Solvent casting	Vascular tissue	Patterned films (silk sol.)	pECs, SMCs	Functional due to aligned vascular cells	125
Solvent casting	Vascular tissue	Films (silk fibroin + pectin + glycerol)	ADSCs	Useful for cutaneous wound healing	210
Solvent casting	Muscle	Films (silk sol. + melanin)	C2C12 myoblasts	Promoted myogenesis	161
Self-assembly	Pancreas	Hydrogel (*B. mori* + *A. assama* silk sol.)	RIN-5, rat islet cells	Functional hydrogel encapsulating islets	64
Self-assembly	Cartilage	Hydrogel (DMEM powder + silk fibroin sol.)	Chondrocytes	Completely degradable construct	211
Self-assembly	Cartilage	Hydrogel (silk sol. + silk fibres)	Bovine chondrocytes	Functional cartilage constructs	212

and wear[66, 232, 233]. Various biomaterials such as chitosan, collagen, cellulose, alginate, silk fibroin, dextran, polylactic acid (PLA), elastin, polyethylene glycol(PEG), polycaprolactone (PCL), and silicone have been utilised to make acellular scaffolds for wound healing till date[234–236]. Amongst them, B. mori silk fibroin has been extensively accepted as a wound dressing material owing to its remarkable properties such as biodegradability, biocompatibility, cost-effective and low immune response[199]. Recent studies demonstrated the use of electrospun silk fibroin (A. assama and P.

Table 4: continued

Fabrication technique	Tissue	Scaffold material	Cell tested	Conclusion	References
Self-assembly	Bone	Hydrogel (Silk sol. + nano HA)	MG63	Properties identical to native tissue	213
Self-assembly	Skin	Hydrogel (*B. mori* + *A. assama* silk sols.)	HDF, HaCaT	Enhanced healing in full thickness burn wounds	63
Salt leaching	Bone	Scaffold (silk sol. + HFIP + NaCl)	HMSCs	Enhanced mineralization	214

HFIP 1,1,1,3,3,3,-hexafluoroisopropanol, ***HDF*** human dermal fibroblast, ***HaCaT*** human keratinocyte, ***EGF*** epidermal growth factor, ***HEK*** human embryonic kidney, ***HEF*** human esophageal fibroblast, ***MG63*** osteosarcoma cells, ***THP1*** human monocytic cells, ***HepG2*** human hepatocellular carcinoma cell, ***pECs*** porcine endothelial cells, ***SMCs*** smooth muscle cells, ***ADSCs*** adipose-derived stem cells, ***RIN-5*** rat insulin-producing cells

Table 5: Reports of tissue constructs 3D bioprinted using silk as a biomaterial.

Bioprinting technique	Bioink	Engineered Tissue	Features	References
Microextrusion	Silk fibroin–gelatin	MSCs differentiated to chondrocytes, osteocytes	Successful differentiation of encapsulated cells	215
Microextrusion	Silk fibroin–gelatin	Cartilage	Chondrogenic differentiation leading to hypertrophy	183
Microextrusion	Silk fibroin–gelatin	Soft tissue reconstruction	Promoted cellular infiltration	216
Microextrusion	Silk fibroin–collagen	Knee cartilage	Cell growth supported	217
Microextrusion	Silk fibroin + cells + PEG	hMSC	Functional construct post-implantation	184
Microextrusion	Silk fibroin + chitosan	BMSC	Cell growth supported	217
Microextrusion	Silk + gelatin + sulfonic acid + FGF	Skin	Visible skin regeneration	218
Inkjet	Silk fibroin–alginate	3T3 fibroblast	Rapid cell proliferation	219
Inkjet	Silk fibroin + PEG	Cartilage	High shape fidelity	220
Digital light processing	Silk fibroin–glycidyl methacrylate	Bone	Cell growth maintained	76

ricini) mats as a potential wound dressing material[66]. Similarly, electrospun blends of polyvinyl alcohol (PVA) and silk nanofibrous mats promoted faster wound healing via granulation tissue formation when activated by combining growth factors[67]. An amalgamation of recombinant spider silk along with silkworm silk fibroin has also shown to do wonders as wound dressing[85] as shown in Fig. 4III.

Chondrogenesis: It is a process of cartilage formation which results from the condensation of mesenchymal cells and differentiation of chondroprogenitor cells.

4.1.1.3 Repair Strategies for Cartilage Tissue Chondroblasts and chondrocytes form an integral part of the cartilage tissue and hence should be able to proliferate, differentiate and mature over the engineered tissue constructs that are meant to mimic the native cartilage. Various biomaterials have been evaluated to construct artificial cartilage tissue[237]. Silk fibroin scaffolds have been shown to enhance the maturation of cartilage cells when used in conjunction with dexamethasone and transforming growth factor (TGF-β3)[238]. Other silk blends have also been shown to positively influence chondrogenesis[239, 240]. Silk fibroin sponge scaffolds provided mechanical stimulation to the chondrocytes when cultured inside a bioreactor[241] and such cartilage grafts could repair knee joint defects. Agarose–silk fibroin blended hydrogels have been evaluated for cartilage regeneration and found to support chondrogenesis and cartilage-like native extracellular matrix deposition[242]. Moreover, the non-mulberry hydrogel blends exhibited higher sulphated glycosaminoglycans (sGAGs) and collagen content as compared to the mulberry ones. Porous scaffolds made from non-mulberry *A. assama* silk fibroin have also been

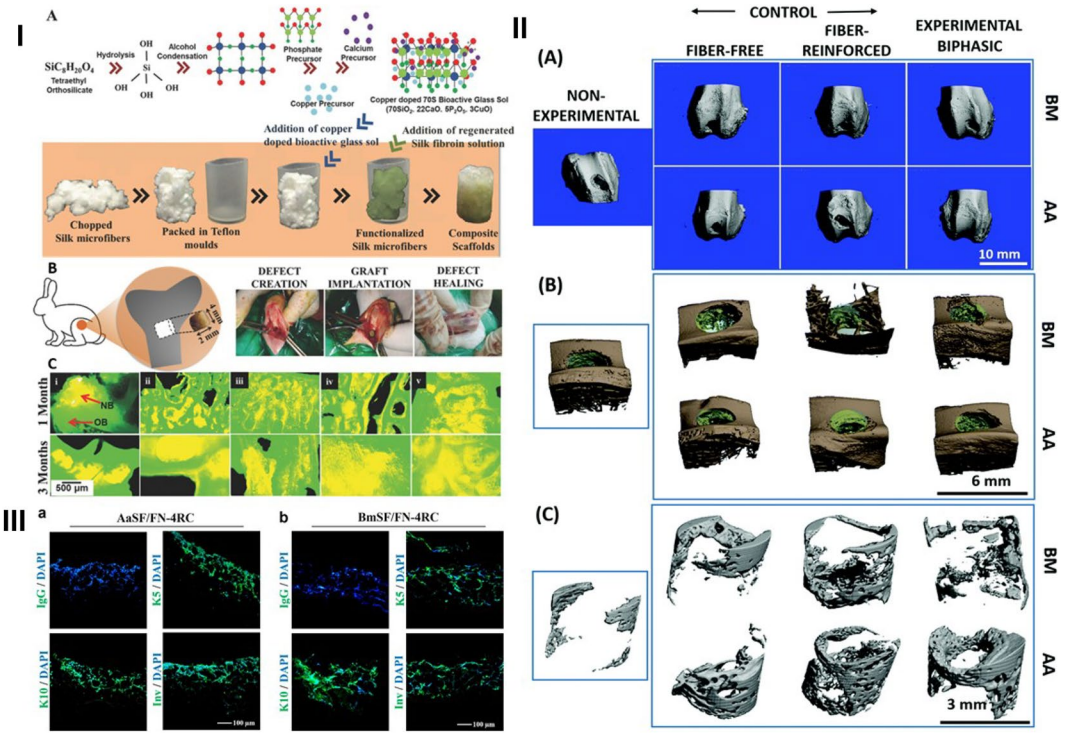


Figure 4: **I** (**A**) Fabrication of bioactive glass silk composite scaffolds by sol–gel coating of the silk microfibres. (**B**) In vivo studies using the matrices to treat volumetric bone defect created in rabbit. (**C**) Fluorochrome (oxytetracycline) labelling after 1–3 months of implantation showing a bright yellow new bone (NB) formation region and a green old bone (OB) region. (adapted with permission from[58] © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim) **II** Micro-CT images showing formation of subchondral bone after 8 weeks of implantation. (**A**) Morphological analysis of the implant site; (**B**) 3D reconstructed images depicting the volume of interest (VOI); (**C**) CT images showing the actual bone volume formed. (Adapted from[231] with permission from The Royal Society of Chemistry). **III** SF scaffolds cross-sections coated with FN-4RC peptide and co-cultured along with HDF and HDMEC before seeding HaCaTs on top, followed by culturing at air–liquid interface conditions. (**a**) FN-4RC-peptide-coated A. assamensis SF (AaSF) scaffolds showed better keratinization in contrast to, (**b**) the *B. mori* SF (BmSF) counterpart. Cytokeratin markers: K5, K10, and Inv (in green) in the FN-4RC coated AaSF scaffolds. IgG depicts isotype control. DAPI was used for counterstaining of nuclei (in blue). Scale bar = 100 µm. (Adapted with permission from[85], Copyright © 2016 American Chemical Society).

reported to support cartilage tissue growth[136]. Silk-based biphasic osteochondral interface tissue constructs showed excellent regeneration of knee osteochondral joints in rabbits[231].

4.1.1.4 Vascular Grafts Vascularization and endothelialisation are two important factors determining the long-term functionality of any bioengineered tissue to be used an substitute for autologous grafts[243]. Most of the biomaterials used earlier faced the drawback of mechanical stability and incomplete vascularization[243]. Non-woven silk fibroin mesh showed incomplete endothelialisation when human endothelial cells were cultured on them[244], but coating the meshwork with extracellular matrix components improvised the endothelial cellular response. Gel-spinning technique has been used by Lovett et al. to fabricate silk fibroin microtubes[245], which exhibited rapid endothelialisation. Tri-layered vascular grafts have been made using silk, elastin, collagen and polycaprolactone (PCL) to replace the traditional autografts[246]. *B. mori* silk fibroin has been comprehensively explored in vascular tissue engineering[247]. Multi-layered vascular grafts made by rolling patterned mulberry and non-mulberry silk films[57] mimicked the native vessels as shown in Fig. 5I.

4.1.1.5 Cardiac Tissue Patches The biggest challenge in cardiac tissue engineering is to efficiently mimic the native extracellular matrix so that it can be used to replace damaged heart muscles. Silk can be used as an effective natural biomaterial for such purposes, as its matrix stiffness can be tuned accurately to match the native

Vascularization: It is a process of capillary tube formation by the assembly of differentiated de novo endothelial progenitor cells (EPCs).

Endothelialisation: It refers to the process of endothelial tissue formation.

muscle rigidity[199, 249]. The prime focus of the moment is proper vascularization and maturation of the cardiac cell-laden constructs. Both *B. mori* and *A. mylitta* silk fibroin scaffolds have been shown to treat myocardial infarction[250, 251] with the latter demonstrating better maturation of myocytes. Three-dimensional cardiac constructs fabricated by stacking of cell-laden patterned silk films have been pitched as an excellent platform for cardiac tissue regeneration[248] as depicted in Fig. 5II.

Myocardial infarction: It is defined as the necrosis of cardiac myocytes due to prolonged ischemia.

Cirrhosis: It is defined as a late stage of liver fibrosis indicated by regenerative nodules formation that are separated and enclosed by fibrotic septa.

4.1.1.6 Liver Modules In recent times, hepatic disorders especially liver cirrhosis has posed a grave danger to the population due to their adoption of abnormal lifestyle and unhealthy eating habits. Several bioartificial liver

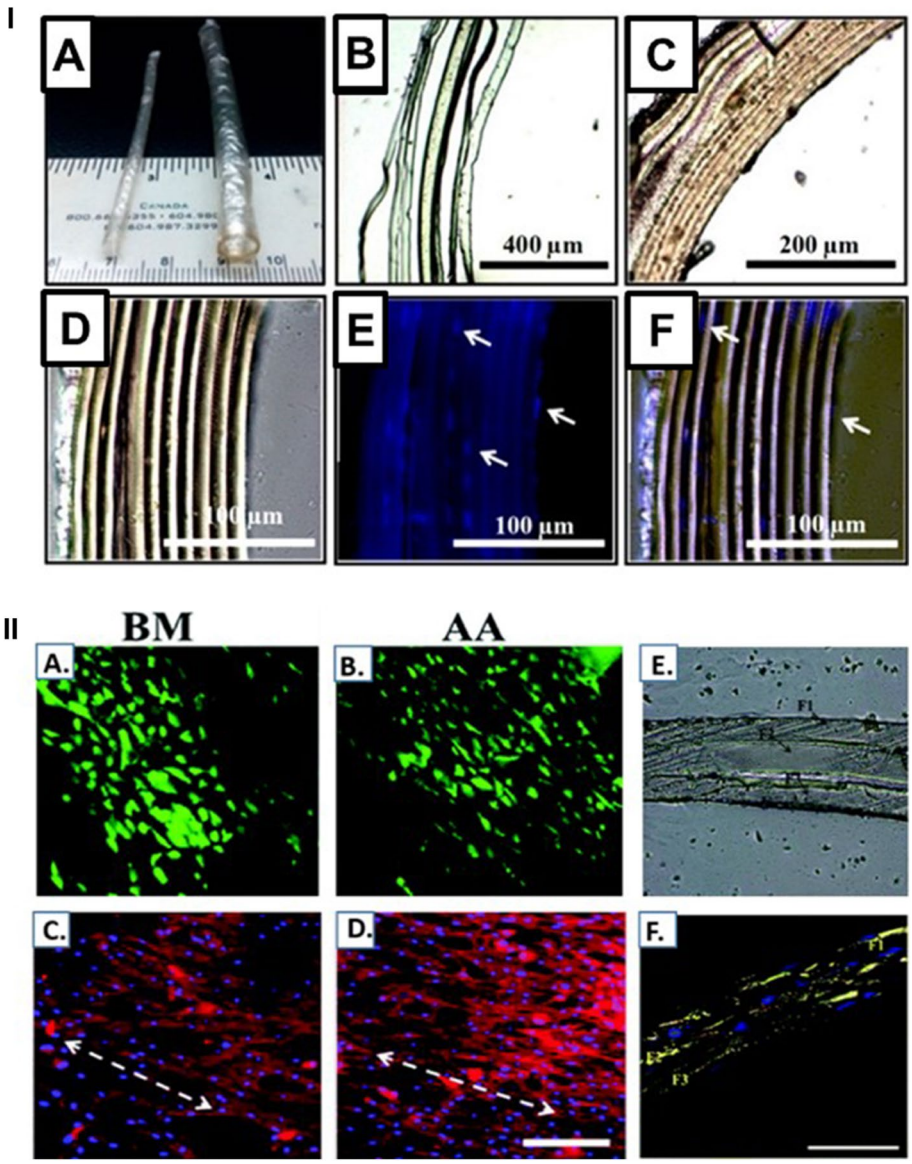


Figure 5: **I** Evaluation of the small diameter vascular grafts fabricated by rolling vascular cell sheets grown on patterned silk films. (**A**) Appearance of the graft. Cross-section of the graft after (**B**) 1 day of cell seeding, (**C**) 14 days of cell seeding showing enhanced structural integrity. Hoechst 33,342 (blue) staining of the constructs to show presence of vascular cells: (**D**) Unstained, (**E**) stained and (**F**) merged constructs. (Adapted with permission from[57]. Copyright © 2016 American Chemical Society). **II** Live/dead assay of primary rat cardiomyocytes on patterned (**A**) *Bombyx mori*, (**B**) *Antheraea assama* silk films. (**C**), (**D**) Rhodamine phalloidin staining was done for the same. (**E**) Cross-section of the cardiac tissue construct made by stacking of cell-laden patterned silk films. (**F**) Cell distribution analysis using Hoechst 33,342 dye (scale bar—100 µm). (Adapted with permission from[248], Copyright © The Royal Society of Chemistry).

devices[252] and cell therapies[253, 254] have been developed in the past several years to treat liver disorders. Implantable hepatic tissues have been developed by loading hepatocytes on 3D scaffolds made up of polymers like polylactide-co-glycolide, polycaprolactone, polyethylene glycol, polyethylene, alginates and cellulose[255, 256]. Silk fibroin–collagen blended films have been successfully demonstrated to enhance proliferation of rat hepatocytes[257]. Silk fibroin–chitosan–heparin blended scaffolds also exhibited hepatocyte regeneration[258]. PLA–silk fibroin scaffolds were shown to increase hepatocyte attachment, proliferation and differentiation compared to only PLA scaffolds[259]. Janani et al. demonstrated the fabrication of functional liver scaffolds by blending mulberry (*B. mori*) and non-mulberry (*A. assama*) silk fibroin[137].

4.1.1.7 Muscle Tissue Repair Strategies Tissue engineering of muscle requires robust biomaterials with desired mechanical strength. Scaffolds seeded with satellite cells have been used widely for skeletal muscle tissue engineering (SMTE)[260–262]. Manchineella et al. demonstrated that electrospun silk fibroin/melanin composite films promoted myogenesis and myotube formation of C2C12 cells[161]. Recently, conducting polymers have been in focus as they can influence the electrical activity of the muscles. Silk fibroin and a conducting polymer, poly(aniline-co-*N*-(4-sulfophenyl) aniline) (PASA) have been blended together to devise functional muscle constructs[263]. These scaffolds illustrated the rapid proliferation of C2C12 cells in vitro. Moreover, electrospun nanofibrous scaffolds made using a blend of silk fibroin/PLA/collagen revealed enhanced adherence, proliferation and maturation of myoblasts[264].

4.1.1.8 Tendon and Ligament Grafts Tendon and ligament tissue damage becomes more prevalent during sports injury leading to hindrance in movement. Tissue engineering is much needed for restoring the function of such tissues as they have poor regeneration capacity[265]. The tensile strength of silk makes it an ideal candidate for tendon/ligament tissue engineering scaffolds[199, 266]. A silk fibroin matrix has been braided to resemble the human anterior cruciate ligaments (ACL)[267]. It had mechanical strength similar to the native ACL and supported the proliferation of human bone marrow mesenchymal stromal cells. Hennecke et al. demonstrated that spider silk bundle sutures possess the appropriate tensile strength and elastic modulus to restore tendons[152]. A three-dimensional composite scaffold was made by electrospinning a mixture of polyurethane and collagen on knitted silk to mimic the native tendon tissue[268]. A hybrid knitted silk–collagen sponge scaffold seeded with human embryonic stem cell-derived mesenchymal stem cells restored tendon regeneration when in vitro mechanical stimulation was given[269]. The alignment of tenocytes was mimicking the native tissue due to the stimulation. Scleraxis overexpressed hESC-MSCs were seeded onto the composite sponge scaffolds in the next consecutive study[270]. Several other silk fibroin scaffolds have been designed to mimic the natural medial collateral ligament, rotator cuff and Achilles tendon[271].

4.1.1.9 Engineered Intervertebral Disc Intervertebral disc (IVD) degeneration in the form of lower back pain, spinal stenosis and radiculopathy[272, 273] affects posture and stability of the backbone. None of the current therapies available can restore the function of the IVD[274]. An ideal biomaterial for IVD scaffold should have biocompatibility, high tensile strength and mimic the native extracellular matrix[275]. Silk fibroin owing to its wondrous properties[199] have been used in this regard. The successful tissue-engineered IVD should resemble the morphology and function of both the components of IVD: nucleus pulposus (NP) and annulus fibrosus (AF). A biphasic hybrid scaffold was made using silk fibroin/fibrin/hyaluronic acid to mimic both the NP and AF[276]. The lamellar silk fibroin scaffold part seeded with porcine AF cells resembled the native AF, whereas the fibrin/hyaluronic acid scaffold part seeded with porcine chondrocytes resembled the native NP. Another 3D biphasic silk fibroin scaffold has been fabricated by paraffin sphere-leaching technique to resemble the AF phase and phase separation technique for the NP phase[277]. Rabbit AF and NP cells were shown to adhere and proliferate on these porous scaffolds. Bhunia et al. fabricated a bioartificial AF construct comprising of concentric rings of lamellar silk scaffold made utilising directional freezing technique[200] and demonstrated the proliferation of primary porcine AF cells and hMSCs over it as depicted in Fig. 6I. Furthermore, this angle-ply construct was designed using a gradient of two different silk combinations—mulberry (*B. mori*) and non-mulberry (*A. assama, P. ricini*)[65]. This aided in successful cellular alignment, maturation and extracellular matrix deposition.

4.1.1.10 Repair and Replacement of Meniscus Tissue *The main function of the meniscus is shock absorption[279] and providing mechanical stability*

Spinal stenosis: It is a condition described by the osteoarthritis of the intervertebral discs and facet joints.

Radiculopathy: It is a condition characterized by pain, weakness, motor loss and sensory changes in a specific nerve root distribution.

Satellite cells: These are adult muscle stem cells that are lineage committed and are localized between the muscle sarcolemma and the basal lamina.

Sutures: Materials used to stitch body tissues together after an injury or surgery.

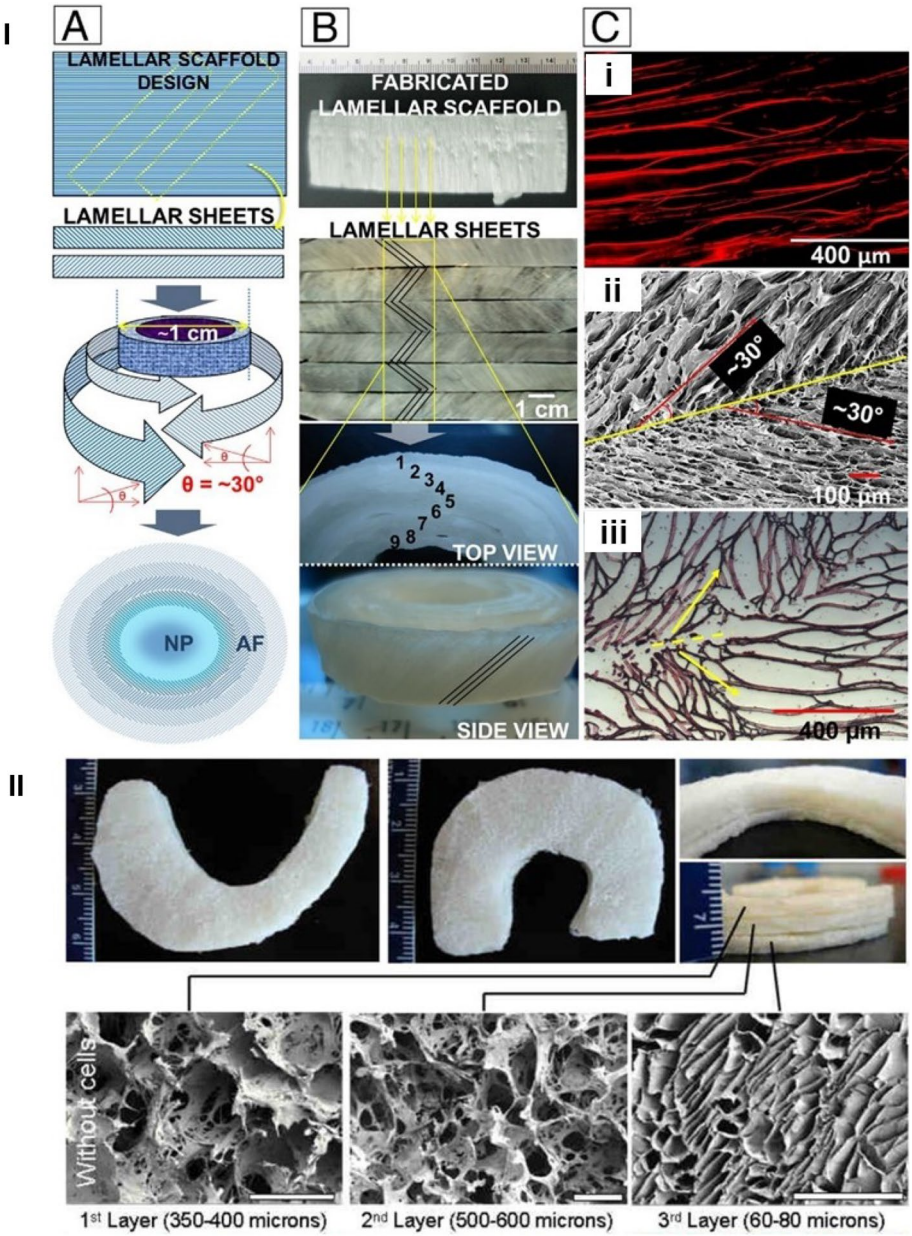


Figure 6: **I** (**A**) Fabrication plan of the silk fibroin angle-ply construct. (**B**) Step-wise construction of the scaffold. (**C**) (i) Lamellar pattern is seen in the fluorescent image. (ii) SEM and (iii) histology of the cross-aligned section. (Reproduced with permission from Proceedings of the National Academy of Sciences of the United States of America under creative common license CC-BY[200]) **II** Triple layered scaffold resembling meniscus. SEM images representing porosity of each layer. (Adapted with permission [278], Copyright © Elsevier).

to the knee for aiding in locomotion. A damaged meniscus due to any traumatic injury or degeneration has been repaired using different implants like the collagen meniscus implant (Menaflex)[280]. Several biomaterials like collagen[281], polyurethane[282], polycaprolactone[283], polyvinyl alcohol[284] and silk[285] have been explored for meniscal tissue engineering. Mandal et al. designed a multilamellar silk fibroin scaffold and seeded it with human BMSCs to closely mimic a functional meniscus[285]. A three-layered silk fibroin scaffold seeded with fibroblasts on the outside and chondrocytes on the inside resembled the meniscus both structurally and functionally[278] as shown in Fig. 6II.

4.1.1.11 Neural Conduits Large nerve injuries are difficult to treat completely without utilising neural grafts[286]. The only tentative solution to treat spinal cord injury is via neural tissue engineering. Silk-based scaffolds have been proved to be a boon in this context[287, 288]. Electrospun silk fibroin–nerve guidance conduits have been shown to be effective for peripheral nerve repair[289] by Wang et al. Mulberry and non-mulberry silk fibroin scaffolds seeded with human neural progenitor cells were evaluated for neural tissue regeneration and the non-mulberry ones demonstrated an increase in cell proliferation and deposition of extracellular matrix[288]. Conducting polymers like polypyrrole have been blended with silk fibroin to form functional neural tissue scaffolds[290]. In a different study, silk fibroin/polycaprolactone scaffolds fabricated by electrospinning were found suitable for peripheral nerve regeneration[291].

4.1.1.12 Bioartificial Pancreas With the rapid increase in patients suffering from diabetes, there is an increasing need to focus on new treatment strategies other than the traditional insulin therapy, drug therapy and islet transplantation[292]. Several hydrogels, nanoparticles and microspheres have been fabricated for ensuring sustained release of insulin[293]. Encapsulation of islets with biomaterials before transplantation ensures minimal immune response and controlled insulin release[294]. Pancreatic islets encapsulated in silk hydrogel along with laminin, collagen and mesenchymal stromal cells exhibited enhanced graft survival rate[295]. Encapsulation of islets in silk hydrogel along with MSCs improvised the in vivo function of the islets post-transplantation[296]. In another study, Kumar et al. fabricated bioartificial pancreas by encapsulating insulin-secreting cells in silk alginate/agarose scaffolds[138].

4.2 Cancer Therapeutics and Models for Drug Screening

Silk serves as an excellent biomaterial for cancer therapy due to its biocompatibility, biodegradability and no immune rejection[199]. The biggest obstacle an anti-cancer drug faces is sustained release leading to its target destination, which can be overcome using silk formulations like films, hydrogels, capsules, silk-coated liposomes and nanoparticles as the drug carrier[297]. *B. mori* silk films have been shown to deliver doxorubicin when administered intratumorally in a human orthotopic breast cancer model[298]. Moreover, the drug delivery rate could be controlled by altering the crystallinity of the films. These silk films also exhibited sustained release of doxorubicin in a neuroblastoma mouse model[299]. Sieb et al. reported that *B. mori* silk hydrogels can be fine tuned for controlled release of doxorubicin and the metastatic spread was reduced to a great extent in mice breast cancer model[300]. Wu et al. demonstrated that silk fibroin nanoparticles encapsulated with the drug paclitaxel were non-cytotoxic and potential drug carriers in gastric cancer model[301]. Xia et al. employed thermally induced silk elastin-like protein polymers (SELPs) to form nanoparticles for the efficient delivery of doxorubicin in cervical cancers[302]. Silk fibroin-coated liposomes have lately been used to coat emodin, a receptor tyrosine kinase inhibitor for its efficient delivery to breast cancer cells[303]. These coated liposomes could easily target the specific cancer cells via enhanced drug diffusion rates. Gupta et al. showed that curcumin-loaded silk–chitosan nanoparticles act as biodegradable delivery systems to target breast cancer cells[304]. In another study, a hybrid injectable hydrogel comprising silk fibroin blend (*B. mori* and *A. assama*) and folic acid functionalized single-walled carbon nanotubes was used for targeted delivery of doxorubicin[62] to the folic acid receptor-positive cancer cells.

Silk fibroin can also be used for making 3D cancer models to mimic the in vivo conditions much better than in a 2D culture dish and for drug discovery applications. Ewelina et al. designed a 3D heterotypic breast cancer model using silk scaffolds made by salt leaching[305]. These scaffolds showed successful EMT6 and NIH3T3 cellular proliferation and expression of cancer cell markers. An osteosarcoma model made by Tan et al. using freeze-dried *Bombyx mori* scaffolds[306] closely resembled the in vivo expression profile and behaviour of the cancer cells. Similarly, mammary adenocarcinoma and hepatocarcinoma models were also made by freeze drying non-mulberry *A. mylitta* silk fibroin scaffolds[307, 308] with the latter clearly amplifying the mechanism of hyaluronan synthase inhibitor (4-MU). Reagan et al. developed *B. mori* silk fibroin scaffolds to effectively target breast cancer cells using tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) expressing mesenchymal stem cells[309]. These models helped in a better understanding of the cellular behaviour in vivo.

4.3 Tissue-On-Chip for High-Throughput Screening

The growing need for newer technologies to reduce failures in pre-clinical trials of drug

Neuroblastoma: Neuroblastoma refers to the cancer of sympathetic nervous system which originates from neural precursor cells (neuroblasts) and affects the infants mostly.

Liposomes: They are small spherical vesicles containing lipid bilayers of phospholipids mostly. Due to their size, hydrophilicity and hydrophobicity, liposomes are preferred for enhanced drug delivery.

Intratumorally: It is an adverb which refers to an activity directed inside the tumour.

Orthotopic: Orthotopic describes the occurrence of anything in its original place. In medical science, it refers to the restoration or implantation of a tissue or organ to its original position within the body.

discovery have resulted in the birth of a state-of-the-art technique called tissue-on-chip or organ-on-chip (TOC/OOC), which is an innovative approach towards three-dimensional microfluidic devices that mimic a functional tissue/organ and can replace animal models for drug screening and drug development applications[310]. Several such chips have already been devised to engineer heart[311], skin[312], lung[313], kidney[314] and arteries[315]. Advancements in technology have even lead to development of human-on-chip model[316]. Recently, silk hydrogel microfluidics has been in the limelight for exhibiting enhanced biological activities resulting in replication of human tissues[317]. Silk bypasses the limitations of polydimethylsiloxane (PDMS) and other traditional materials used to make microfluidic devices owing to its amazing properties of biocompatibility, biodegradability and enhanced cell proliferation support[199, 317]. A silk-based microfluidic device was fabricated utilising gelatin sacrificial molding and construction of three-dimensional microchannel networks within horseradish peroxidase–hydrogen peroxide ($HRP–H_2O_2$) crosslinked silk hydrogel[317]. Such devices can be easily functionalized for regenerative medicine applications. In another instance, silk fibroin-based microfluidic device was designed to replicate a functional liver[51]. The hepatocytes cultured on this device exhibited morphology and functions similar to in vivo conditions. These micro-engineered devices have been the latest paradigm in the field of biomedical science leading to a better understanding of cell physiology, behaviour and function (Fig. 7).

Waveguides: They are the structures that transport electromagnetic waves from one point to another by limiting loss of energy.

4.4 Advances in Silk-Based Biosensing and Biomedical Imaging

Silk fibroin has been subjected to different fabrication techniques that controllably influence its property as a biomaterial, to reconfigure its purpose for different end uses. Silk fibroin has been used in formulations to develop bioinks that can be doped with components to develop inkjet printable functional devices for sensing, therapeutics and regenerative medicine. Silk-based Au NP-doped inks can be applied for photonics and thermal sensing applications, while those doped with enzymes can be used for extending their lifetime as opposed to traditional loading techniques. Similarly, silk-based inks can be doped with therapeutically active molecules such as antibiotics and topographically deposited onto anti-microbial assay kits[318]. Silk fibroin has also been employed in the fabrication of conventional biosensors for amperometric detection of pesticides[319, 320]. Moreover, silk fibroin-based edible food sensors were created to detect their spoilage[321]. Development of silk-based optical waveguides[322, 323] and diffractive elements[324] has opened new avenues towards development of optical sensing devices. Moreover, silk has shown improved resonance at terahertz frequencies and paved a promising path towards the fabrication of hybrid metamaterial-inspired bioelectric and

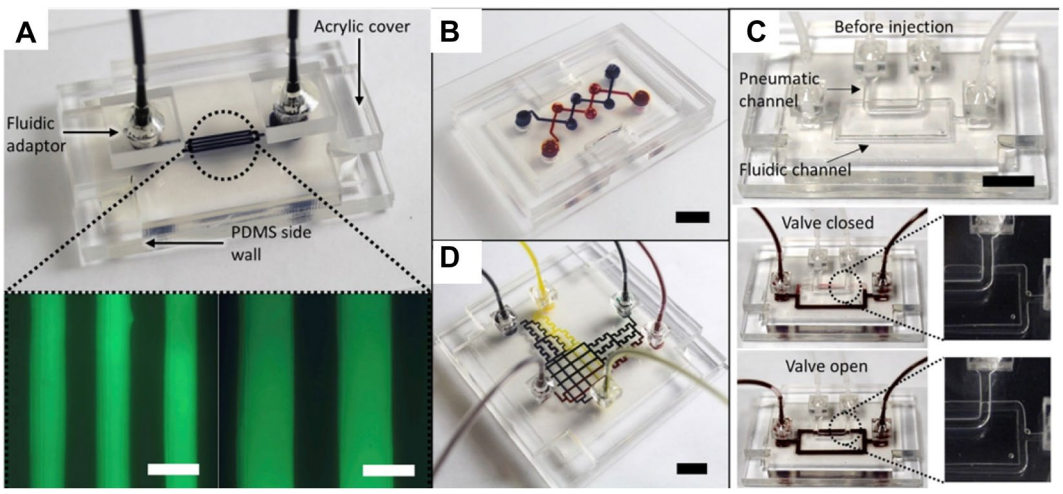
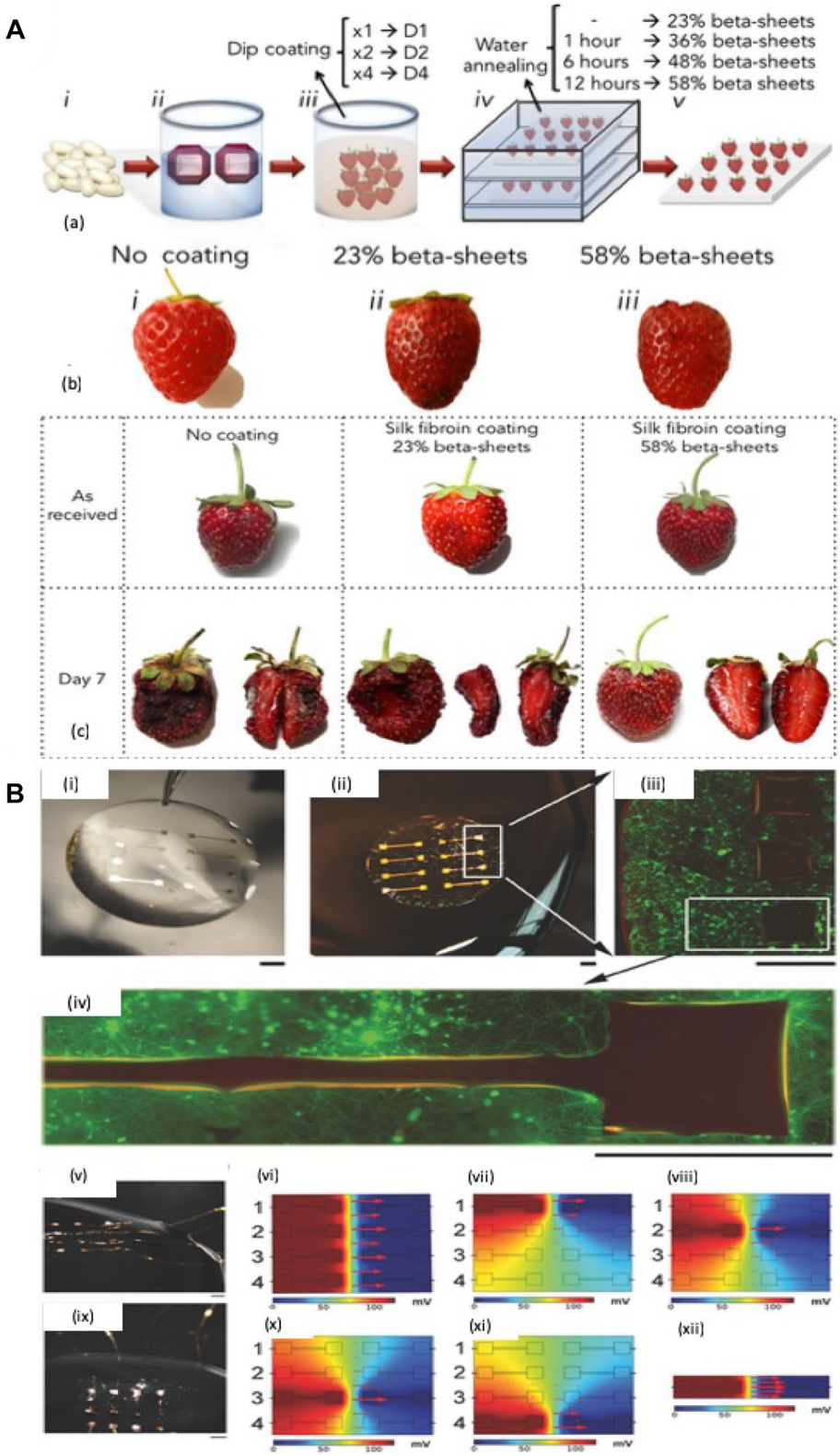


Figure 7: **a** Silk hydrogel microfluidic device showing the minimum fluid channel thickness. (scale: 200 μm), **b** A bi-layered microfluidic system exhibiting serpentine channels. **c** Silk hydrogel microfluidic system attached to a pneumatic valve. **d** 2D bi-layered chemical gradient generator chip. (scale: 1 cm). (Adapted with permission from[317]. Copyright © 2019 Elsevier).


A
Dip coating
x1 → D1
x2 → D2
x4 → D4
Water annealing
- → 23% beta-sheets
1 hour → 36% beta-sheets
6 hours → 48% beta-sheets
12 hours → 58% beta sheets
i
ii
iii
iv
v
(a)
No coating
23% beta-sheets
58% beta-sheets
(b)
No coating
Silk fibroin coating 23% beta-sheets
Silk fibroin coating 58% beta-sheets
As received
Day 7
(c)
B
(i)
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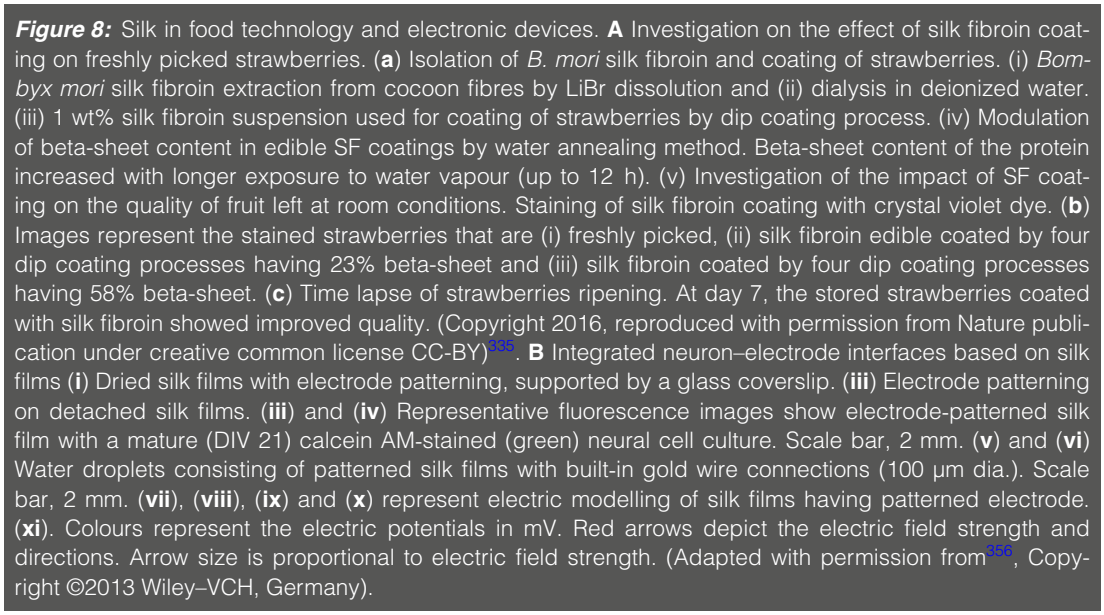

Figure 8: Silk in food technology and electronic devices. **A** Investigation on the effect of silk fibroin coating on freshly picked strawberries. (**a**) Isolation of *B. mori* silk fibroin and coating of strawberries. (i) *Bombyx mori* silk fibroin extraction from cocoon fibres by LiBr dissolution and (ii) dialysis in deionized water. (iii) 1 wt% silk fibroin suspension used for coating of strawberries by dip coating process. (iv) Modulation of beta-sheet content in edible SF coatings by water annealing method. Beta-sheet content of the protein increased with longer exposure to water vapour (up to 12 h). (v) Investigation of the impact of SF coating on the quality of fruit left at room conditions. Staining of silk fibroin coating with crystal violet dye. (**b**) Images represent the stained strawberries that are (i) freshly picked, (ii) silk fibroin edible coated by four dip coating processes having 23% beta-sheet and (iii) silk fibroin coated by four dip coating processes having 58% beta-sheet. (**c**) Time lapse of strawberries ripening. At day 7, the stored strawberries coated with silk fibroin showed improved quality. (Copyright 2016, reproduced with permission from Nature publication under creative common license CC-BY)[335]. **B** Integrated neuron–electrode interfaces based on silk films (**i**) Dried silk films with electrode patterning, supported by a glass coverslip. (**iii**) Electrode patterning on detached silk films. (**iii**) and (**iv**) Representative fluorescence images show electrode-patterned silk film with a mature (DIV 21) calcein AM-stained (green) neural cell culture. Scale bar, 2 mm. (**v**) and (**vi**) Water droplets consisting of patterned silk films with built-in gold wire connections (100 μm dia.). Scale bar, 2 mm. (**vii**), (**viii**), (**ix**) and (**x**) represent electric modelling of silk films having patterned electrode. (**xi**). Colours represent the electric potentials in mV. Red arrows depict the electric field strength and directions. Arrow size is proportional to electric field strength. (Adapted with permission from[356], Copyright ©2013 Wiley–VCH, Germany).

Biophotonic: Biophotonics is the application of photonics in the field of biology. It involves the development of novel imaging techniques to visualize biological materials like cells and tissues.

Microresonators: They are the microcavities formed by the two reflecting surfaces of an optical medium.

Fluorophore: It is a fluorescent substance that can re-emit light upon excitation by an external light source. It helps in the imaging of various biological components like cellular vesicles.

biophotonic devices[325]. Also, silk-based microresonators have been developed for the biophotonic devices[326]. Silk-based bioimaging modalities have been advanced by research in the area of fabrication of fluorescent silk fibroin nanospheres[327], luminescent silk-based carbon dots[328, 329] and silk-derived graphene oxide magnetic fluorophore[330] .

5 Applications of Silk in Allied Healthcare Applications

5.1 Food Technology

Food and Agricultural Organization (FAO) of the United Nations estimated that a lot of (approximately one-third) food produced annually for human consumption worldwide is lost or wasted[331]. It has been reported that wastage of food not only has an impact on food quality and safety but also is a loss of both economic value and resources. Silk fibroin as a biomaterial has been extensively investigated in textiles, biomedical, photonic, electronic applications and in food technology[9, 62, 323, 332]. It has been used as edible sensors to monitor cheese ageing as well as fruit ripening[323]. Silk cocoon materials have been reported to have gas diffusion properties. Tailoring of these materials into regenerated protein can be regulated by silk fibroin polymorphism[333, 334].

Marelli et al. has developed a water-based silk fibroin protein suspension. These suspensions coat on the surface of the food when dipped in it. It has self-assembling property and helps to enhance the shelf life of the food at room conditions by decreasing cellular respiration rate and moisture loss. As reported, protein polymorphism and post-processing are the key parameters in maintaining food freshness by enabling it to modulate the diffusion of gases through the thin membrane of silk fibroin. Dip coating of bananas and strawberries have been used in the study as a proof of principle to depict that a silk fibroin membrane of micrometer thickness around the fruit helps in maintaining the physiology after harvesting (Fig. 8A)[335].

Pritchard et al. reported silk micro- and macro-particles (diameter ranging from 300 μm to 4 mm) that have potential applications in food, nutritional and medicinal products[336]. Flavourless and odourless are some of the characteristics of silk fibroin, which are the key parameters for food coating and packaging applications[335]. Veletini et al. developed a living hybrid composite of a single cell fungi and regenerated silk (RS) nanofibrils based on yeast fermentation. The activated metabolic activity of microorganisms with regenerated silk has been reported to not only reduce water permeability but also increases the shelf life of the food over a period of 7 days. Another method of smart food packaging which transfer prints a free standing RS and RS/yeast layer onto an adherent parafilm substrate has been reported. This method can potentially be used to manage food storage conditions by its temperature-sensing mechanism[337].

Baycin et al. explored the adsorbent property of silk fibroin. It was used to adsorb olive leaf antioxidants, which increased the antioxidant property of silk and showed antimicrobial activity against *S. aureus* and *K. pneumonia*. Silk fibroin adsorbed with olive leaf antioxidants has been proposed as a biopolymer to produce antioxidant and antimicrobial functional food and dietary supplements[338]. *Bombyx mori* silk sericin in food is reported to relieve constipation, suppress development of bowel cancer and also increase absorption of minerals[339]. There are several advantages, reported, of using silk sericin in food industry which include its ready availability, non-toxicity, excellent moisture-retaining capacity, antioxidant and good emulsifying and antifrosting agent[340–344]. Silk sericin added in calculated amount in bread is reported to be an ideal processed food that affects digestion and absorption[345]. Silk protein has also been used for the production of baby food which is claimed to prevent and reduce skin disease such as atopic asthma and atopy[346]. Silk protein has also been used for producing health functional food that is claimed to prevent or treat Parkinson's disease[347].

5.2 Electronics

Flexible, stretchable and wearable have been some of the key features of next-generation electronics. Recently, implantable medical devices having electronic components are being developed for therapeutics or functions like cardiovascular regulation, delivery of drugs as well as for biological structure enhancement[348]. Beside these functions, electronic components also increase the sophistication of the medical devices[349]. These electronic devices have been developed to operate while inserted in the living tissue, that might lead to several complications and restrictions on the constituents of the material. Implantable devices should have regulated degradation while functioning reliably and integrate into the biological milieu without inflammation or rejection. Several natural and synthetic polymers such as silk, collagen, gelatin, thermoplastic polyesters have been used to fabricate biodegradable and biocompatible electronic devices[350–354]. Silk due to its unique structure and properties have exhibited several advantages which include robust mechanical properties, tunable degradation and fabrication into several forms. Food and Drug Administration (FDA) approval has made silk suitable for electronic devices with implantable biomedical and healthcare applications (Fig. 8B)[350, 355–358].

Tao et al. recently developed a silk-based fully degradable, remote controlled implantable therapeutic device that can counter *Staphylococcus aureus* infection and be fully resorbed once its function is complete. The device consisted of a serpentine resistor and a coil for receiving power, on a silk substrate. Both the serpentine resistor and the coils were made of magnesium. The device was developed for thermal treatment in infection area[355]. Kim et al. fabricated thin polyimide films, embedded with PDMS stamp and silicon-based transistors, onto free standing silk fibroin films which showed good mechanical property and bendability without adhesive failure[351]. Silk fibroin-based electrodes have been fabricated for application in supercapacitors composed of carbon-based microporous nanoplates containing heteroatoms-N[359]. Silk fibroin has also been used as a dielectric material for organic field-effect transistors (OFET), because of its dielectric properties, favourable mechanical flexibility and processability. Wang et al. has developed a flexible pentacene OFET on poly (ethylene terephthalate) by utilising silk fibroin thin films as the gate dielectric[360].

Silk has also been used for biosensing application. For instance, a flexible and biocompatible graphene field emission effect transistor have been developed by applying silk fibroin as both gate dielectric and substrate for glucose sensing applications[361]. Silk sensors have also been developed for monitoring food quality. A radio-frequency identification (RFID) like silk sensor was developed, for monitoring food quality, by integrating silk substrates with wireless antennas[362]. Despite the evolution, these silk-based sensor platforms are still in a rudimentary stage and more research is needed to address technical challenges.

5.3 Biomedical Textiles

For nearly 5 decades, biomedical textiles have been manufactured and used for first aid, clinical and hygiene needs. Synthetic and natural materials are used for engineering fibrous textile structures which are utilised as medical devices to improve the quality of life of the patients[363–365]. Non-implantable fibrous textile structures include wound dressings while implantable ones include vascular grafts, heart valves, polymer sensors and sutures used for medical implants. Advancement in biomedical textiles has given solution for several clinical applications which include tissue engineering, bariatric surgery,

Antimicrobial: Antimicrobials are the chemical compounds that kills (microbicidal) or prevents the growth of microbes (biostatic).

Inflammation: Inflammation is the body's natural protective response arising due to damaging stimuli like pathogenic attack or cellular damage. It can be acute (instant response) or chronic (prolonged response).

Thermoplastic: Thermoplastics are those polymers which gains plasticity (softening) on heating to a high temperature and solidifies (hardens) on reducing the temperature.

Bariatric: Bariatric relates to the pathology or treatment of adipose (fat) tissue-related disorders, majorly obesity.

orthopaedic, cardiovascular and cosmetic surgeries and veterinary needs[134, 149, 201, 366–371].

Silk-based biomaterials are clinically employed for several years and are now being recognized as a potential alternative material for biomedical textiles. Silkworms have been domesticated by humans for centuries, while spider silk proteins are produced by recombinant DNA methods[372, 373]. There are several other modifications that have been introduced into silk-based biomaterials to make it suitable for biomedical textile applications. The various silk-based biomaterials for biomedical textiles can be categorized into non-implantable materials, implantable materials, extracorporeal implants and healthcare materials.

Recombinant: It is the use of exogenous DNA engineered into host genome (the complete genetic material in an organism) at very specific sites.

Extracorporeal: It refers to any procedure or biomaterial performing bodily or physiological function outside the host.

Peritoneal: Peritoneal is the abdominal cavity where organs such as stomach, intestines are housed.

Prosthetic: Prosthetic refers to any artificial implant to augment the function of a lost body part.

5.3.1 Silk-Based Non-Implantable Materials

In biomedical textiles, non-implantable materials include wound dressings, pressure garments, orthopaedic bandages, prosthetic socks and many others. Silk fabrics have been used very often for wound dressings. It has been used for the fabrication of fibrous mats by non-weaving and electrospinning techniques[374]. It has been reported that silver nanoparticles when blended with silk fibroin gave rise to antibacterial wound dressings. Bacterial growth was inhibited by the combination of *Bombyx mori* fibroin films and titanium dioxide nanoparticles[375].

In recent times, a two-layered wound dressing has been developed where wax-coated SF woven fabric was introduced along with a sericin sponge and a bioactive layer of glutaraldehyde crosslinked silk fibroin gelatin. Such wound dressings showed reduction in wound size, epithelialization and collagen formation[376, 377]. Chouhan et al. (2018) fabricated nanofibrous mats with silkworm SF coated with recombinant spider silk peptide, cell binding antimicrobial peptides and growth factors showed enhanced cell adhesion as well as antimicrobial activity[85].

Atopic dermatitis: Also known as eczema, atopic dermatitis is a chronic skin disease which is characterized by dry and itchy skin, mainly occurring due to different allergies.

5.3.2 Silk-Based Implantable Materials

Implantable materials in biomedical textiles are used for wound closures during surgeries of skin, vascular implants, artificial tendons/ligaments, artificial heart valves and several others. Sutures made out of natural silk fibres have been used for several years. Addition of 50 wt% PVA into silk fibroin enhanced the tenacity and elongation at break of the fibres as well as increased their knot strength, rendering the mats suitable for suturing[378]. Antimicrobial sutures have been developed by coating *Bombyx mori* fibres with silver or tetracycline[379, 380]. The other silk-based implantable materials for replacement of various tissues post-fabrication into various formats have already been thoroughly explored in the previous section dedicated to tissue engineering applications of silk.

Lesions: Lesions are any abnormal damage to healthy tissue.

Thrombogenicity: It is the property of any material to form a blood clot when it comes into contact with blood.

5.3.3 Silk-Based Extracorporeal Implants

Artificial organs which are involved in the purification of blood are defined as extracorporeal organs. Artificial kidney, artificial liver and mechanical lung are referred to as extracorporeal organs. Silk-based wearable artificial kidney system has been developed recently using urease-immobilized SF membrane and polymer-based spherical carbonaceous adsorbent for peritoneal dialysis. In vivo results showed that the filtering system had good efficiency in toxins removal (Fig. 9A)[381]. Janani et al. fabricated a functional liver construct that can potentially be used in an extracorporeal device, using a blend of mulberry and non-mulberry silk fibroin. These scaffolds not only enhanced biological activity but also influenced the spheroidal growth of hepatocytes[137].

5.3.4 Other Silk-Based Healthcare Materials

5.3.4.1 Silk-Based Healthcare/Hygiene Biomedical Textiles Silk-based healthcare/hygiene biomedical textiles have been in clinical applications in the operating theatre which include surgeon's gowns, masks, caps, patient drapes and cover cloths as well. The several advantages of these substances include mechanical property, softness and several antibacterial properties of the silk fabric. Special silk fibre (MICROAIR DermaSilk®) has been evaluated for its effectivity in the treatment of atopic dermatitis with acute lesions in young children[382]. The results depicted a significant decrease in the severity of atopic dermatitis. Silk mask papers have also been developed by combining silk fibres and nano-TiO_2 which has properties like degradation of volatile organic compounds[383, 384].

5.3.4.2 Physical and Chemical Modification of Silk for Biomedical Textiles The surface properties of a biomaterial are very important for its use in biomedical textiles. Surface properties such as topography, hydrophobicity and electrostatics are to be taken into consideration as these properties affect thrombogenicity, antimicrobial behaviour as well as biocompatibility[385]. Coating of *B. mori* silk on poly(propylene) and poly(amide) films has been reported to exhibit antibacterial properties and inhibit the

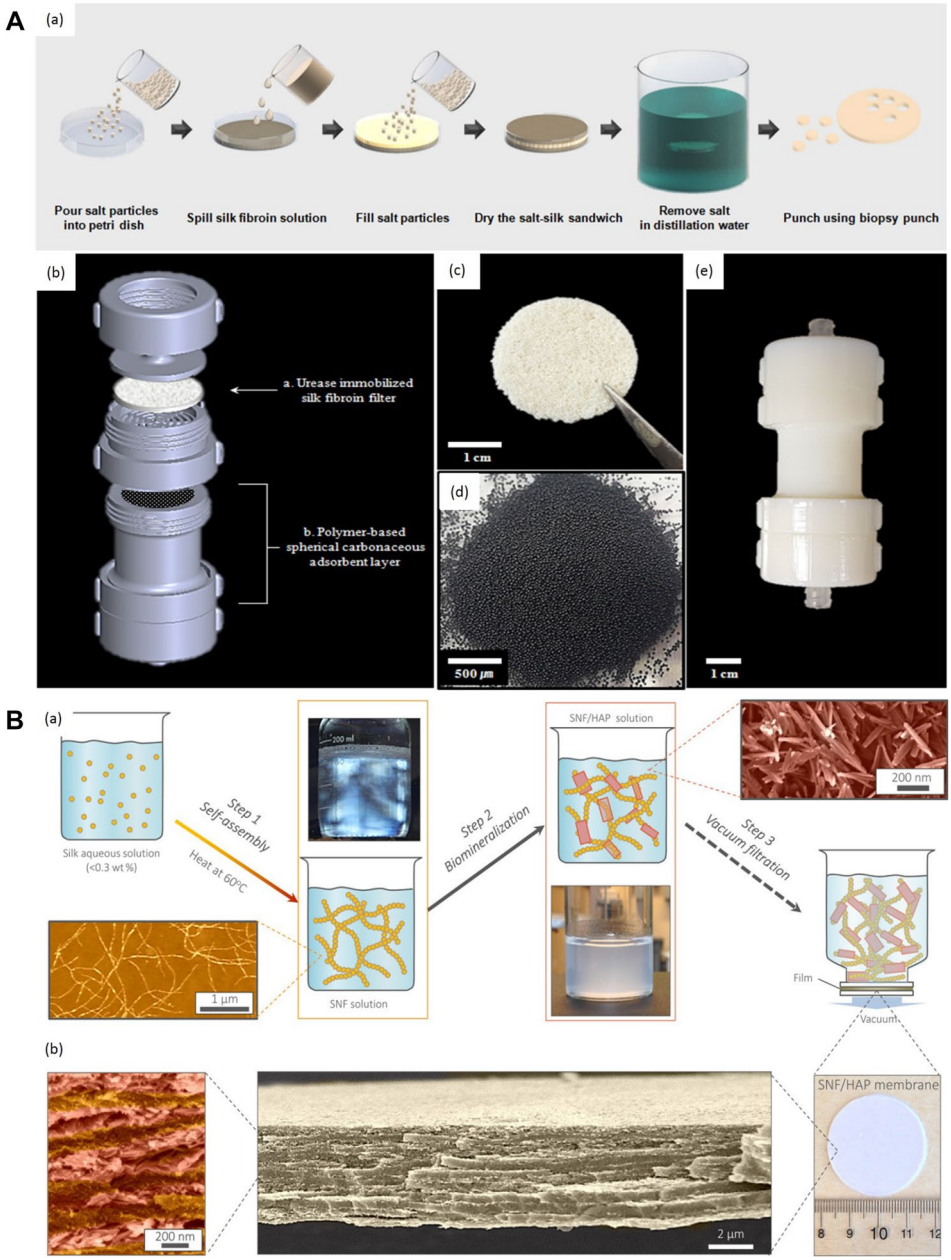

Figure 9: Silk in biomedical devices and bioremediation. **A** Schematic fabrication procedure of porous silk fibroin (SF) filters membrane and illustration of the filter used for wearable artificial kidney system. (**a**) Salt-leaching method for the preparation of three-dimensional porous SF membrane. (**b**) Image of the filter components, (**c**) Urease-immobilized silk fibroin filter membrane, (**d**) polymer-based spherical carbonaceous adsorbent and (**e**) illustration of the filter for wearable artificial kidney system. Figure reproduced with permission from (Sultan et al. 2019)[381]. ©2019 Elsevier. **B** Fabrication procedure of the silk nanofibril (SNF)/hydroxyapatite (HAP) membranes and visualization of the structures formed. (**a**) Schematic process of the SNF/HAP membranes preparation. Step 1: Assembly of silk into SNFs in aqueous solution. The bottom image in the first row depicts SNFs in an atomic force microscopy (AFM); the top image in the second row indicates the presence of a nematic phase of SNFs, when the SNF solution was under polarized light. Step 2: HAP nanocrystals were grown on the SNFs. The bottom image in the third row is an image of SNF/HAP solution; the top image in the fourth row illustrates biomineralized HAP nanocrystals in scanning electron microscopy (SEM). Step 3: Vacuum filtration was used to assemble the SNF/HAP dispersions into membranes. (**b**) Illustration of the multilayer structures of the membrane. The first high-resolution cross-sectional image depicts SEM image of SNF/HAP membrane. The second SEM image shows nacre-like, highly ordered multilayer structures of the SNF/HAP membrane. The third image is a 4-μm-thick SNF/HAP membrane. False colour has been shown in AFM and SEM images. (Copyright 2019, Reproduced with permission from Science Advances under creative common license CC-BY)[416].

attachment of *Staphylococcus epidermis* in vitro[386]. There are several biomedical implants that require anticoagulant coatings. Antithrombic features have been reported by a blend of *B. mori* silk and tetramethylpyrazine or carboxymethyl keratin-modified poly(acrylic acid)[387]. Gogoi et al. studied the surface modification of muga silk fibres using argon plasma treatment. It enhanced the tensile strength and hydrophobicity of the muga silk fibres[388]. Several other studies have been conducted on radio-frequency plasma treatment of silk fibres to alter properties like wetting, flame resistance, antibacterial properties, hydrophobicity, hydrophilicity, shrinkage resistance and tensile properties[389–393]. Chemical modification on silk protein enables site-specific addition of unique chemical moieties. Vepari et al. demonstrated that pH, hydrophobicity and pI influenced the attraction or repulsion of proteins by the silk surface[394].

Antithrombic: Antithrombic agents are substances which prevent the formation of blood clots.

5.4 Cosmetics

Silk has been used in cosmetics for several years. Sericin and its combination with SF have been used for skin, hair and nail cosmetics. Lotion, cream and ointment have been developed based on silk sericin. These have been reported to show skin elasticity, antiwrinkle and antiaging effects[395–397]. Sheng et al. showed that amino acid composition of sericin is responsible for its moisture absorption and retention capacity, which makes it an ideal substance for maintaining moisture content in the skin and make it more elastic, smooth and soft. It is also reported that sericin inhibits tyrosinase activity and melanochrome formation, which makes it a suitable component for skin whitening cosmetics. Through scanning electron microscopy (SEM), they also displayed that sericin provides excellent hair care and repair effect[398].

Padamwar et al. demonstrated the moisturizing property of sericin gel. It was reported to enhance hydroxyproline content in the stratum corneum and decrease skin impedance. SEM images revealed the decrease in skin cracking and flaking as compared to the normal skin replicas[399]. Anti-staticity and moisture absorbability has been shown by a powder that contains 5–30% sericin (average molecular weight 7000–3,00000) and 70–95% SF when applied as films[400]. Miyashita reported that cosmetics containing cellulose fibres soaked with fibroin dispersion and aqueous sericin solution absorb sweat and sebum[401]. Moisturizing and conditioning lotions have been developed with 1% w/w sericin and 4% w/w D-glucose[402]. Creams have also been developed for enhanced cleansing property with less skin irritation, containing 0.001–30% w/w of sericin[403]. Foundation creams and eyeliners have been formulated by coating sericin hydrolysate on talc, titanic, mica, nylon and iron oxide[404].

Anti-staticity: Anti-staticity is the property of a material or surface which helps in reducing the static charge build up.

Sunscreen consisting of sericin has been reported to enhance the light screening effect of UV filter like triazines and cinnamic acids ester[405]. It has been reported that nail cosmetics consisting of 0.02–20% sericin prevent nail from becoming brittle, chapping and imparts inherent gloss to nails[406]. Hoppe et al. reported reduction in hair surface damage by hair and bath preparations consisting of 0.02–2% sericin and 0.01–1% olive oil, fatty acid or their salts[407]. Conditioners for skin and hair have also been developed consisting of sericin hydrolysates (average molecular weight 300–3000)[408]. Shampoos suitable for care and cleaning of hair have been reported to consist of sericin and pelarogenic acid[409].

5.5 Bioremediation

Remediation of polluted land, air and groundwater is a major environmental issue having worldwide significance[410]. Silk has been extensively used singly or in combination with other polymers for the removal of heavy metals from aqueous solution, purification of water, as an adsorbent for toxic dyes and in air filtration[411–417]. Xiao et al. developed ultrafine silk fibroin powder which could be used as low-cost adsorbents for the removal of dyes from printing wastewater[418].

Gao et al. developed a modified SF membrane (water insoluble) by blending fibroin and silane coupling agent and used it for the adsorption of six metal ions, Cu(II), Co(II), Ni(II), Cr(III), Pb(II) and Cd(II)[419]. Ajitha et al. reported a practical and highly efficient method for treatment of water containing toxic heavy metals at a moderate concentration. A novel chitosan oligosaccharide–graft–maleic anhydride(COS-g-MAH)/polyvinyl alcohol (PVA)/silk fibroin (SF) composite was fabricated for removal of toxic heavy metal lead (II) ion from aqueous solution[420]. Pelit et al. reported the potential use of natural spider silk as a biosorbent for Cu(II) and Pb(II) ions from aqueous solutions[410]. Kwak et al. fabricated silk sericin beads modified by polyethylenimine (PEI) for potential Cr(VI) adsorption and detoxification of aqueous solutions[421]. Also, Kwak et al. fabricated sericin-derived activated carbon/alginate beads that served as economical adsorbents for the removal of contaminants from water[422].

Ling et al. fabricated a low-cost and highly efficient silk nanofibril and hydroxyapatite-based multilayer membrane with nanoporous features that can be used for purification of water for various applications (Fig. 9B)[416]. Min et al. developed a highly efficient and multifunctional silk nanofibrous air filter that not only has chemosensing functions but also air-filtering capacity. It can be used as a translucent window screen which provides viewability and controls room temperature[423]. Silk fibroin fibres have also been modified to prepare a superhydrophobic natural sorbent that can adsorb oil efficiently from water surfaces, while repelling water. The adsorbed oil can be removed from the fibres by squeezing and the fibres can be reused. This approach promotes silk fibres for repeated usage in oil spill clean-up applications[424].

6 Opportunities and Challenges

With increase in the population size, there is an increase in the demand for biomedical and healthcare needs. Silk-based biomaterials are being used in conjunction with other polymeric materials for various healthcare and clinical applications. Owing to its biodegradability, biocompatibility, easy processability and immense tensile strength, silk has paved its way into the healthcare industry[199]. Low immunogenicity and high tunability also add up to its set of marvellous properties in tandem, thereby proving the importance of silk in healthcare applications such as tissue engineering, drug delivery, biomedical imaging and cancer therapeutics[199, 201]. Many natural and synthetic polymers have been investigated in the past few years for tissue engineering, but the latter poses disadvantages in biocompatibility and immune rejection in the long run[425]. Silk is an ideal biomaterial for tissue engineering due to its tuneability to mimic the native extracellular matrix of various tissues[201]. Though some of the products have achieved clinical translation over the past few decades, but there are many avenues that are yet to be forwarded by regulatory authorities for application in the real world. Though silk-based healthcare products have shown tremendous potential as ideal healthcare materials in tissue engineering domain, yet the translation remains a demand of the future. Similarly, the field of silk-based biomedical imaging, sensing and electronics is at its nascent stage and is advancing exponentially to reach the efficacy of the conventional counterparts as silk is biocompatible and non-toxic. Also, the field of cosmetics and food technology is poised to benefit tremendously with the translation of silk-based modalities with little to no side effects of the same. Also, silk can offer cost-effective bioremediation solutions for areas where the drinking water is contaminated with heavy metals and other pollutants leading to health hazards.

7 Conclusion

Silk is one of the most promising biopolymers bestowed with excellent biocompatibility, low immunogenicity, tuneable mechanical strength and regulated degradability with non-toxic by-products. It is one of the prime sources of livelihood for many families in the Asian countries and remains popular among researchers for its easy availability and marvellous processability. For instance, exploitation of endemic non-mulberry silk varieties would open avenues for utilisation of this bioresource for prospective biomedical research more extensively and in turn help the local dwindling sericulture industry by creating new job opportunities and help in preserving the biodiversity. The inherent characteristics confer silk with the potential to be employed in multitude of applications across the wide spectrum of healthcare products and modalities. Silk has been found its purpose in the facile fabrication of wound dressings, textiles and sutures since the olden days. It continues to fascinate researchers all over the globe with its immense potential for applications spanning cosmetics, cancer therapeutics, tissue-on-chip screening platforms, tissue-engineered artificial grafts and organs, food preservation and bioremediation. Though a few bottlenecks still exist in the regulatory aspects of the various silk-based technologies and products, the advantages of using silk-based solutions far outweigh the hurdles being faced in the development of finished marketable products for the future.

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