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Applications of silk-based biomaterials in biomedicine and biotechnology

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ABSTRACT

Silk-based biomaterials have gained significant importance making them a promising choice for the future of medical technology due to their versatility and biocompatibility. They can be fabricated and tailored through various processing methods such as electrospinning, freeze-drying, and 3D printing, to achieve specific properties and structures namely sponges, hydrogels, films, and scaffolds that can be utilized for different biomedical applications. Biocompatibility, a unique property of silk-based biomaterials, has been demonstrated through both *in vivo* and *in vitro* studies and to date many studies have reported the successful use of these silk-based biomaterials in different fields of medicine. In this review, we have elaborately discussed different types of silk, their structural composition, and biophysical properties. Also, the current review focuses on highlighting various biomedical applications of engineered and fabricated silk-based biomaterials which aid in the treatment of certain infections and diseases related to skin, eyes, teeth, bone, heart, nerves, and liver. Furthermore, we have consolidated the advancements of silk-based biomaterials in the different fields of biotechnology such as sensors, food coating and packaging, textiles, drug delivery, and cosmetics. However, the research in this field continues to expand and more significant observations must be generated with feasible results for their reliable use in different biomedical applications.

1. Introduction

The outstanding nature of silk has gained increased attention for its use in numerous biomedical and biotechnological applications. Silk is a proteinaceous biopolymer produced by several organisms such as silkworms, scorpions, spiders, fleas, and mites [1]. It is produced from the epithelial cells, and secreted in the lumen where they are stored before being spun into fibres. Depending on the source, a wide variety of silk with great variance in their structure, composition, and properties are available in nature [2]. The glycine-enriched silk is distinguished by its extensile nature, good strength, and unique processibility. The dragline spider silk possesses superior mechanical strength when compared with other kinds of silk. On the other hand, the silk obtained from silkworms displays exceptional water resistivity and accessibility [3]. Alongside its good biocompatibility, silkworm silk is proven to have outstanding mechanical properties with thermostability up to 250°C [4]. Different types of silk are explored to understand the fabrication mechanisms in order to harness their properties to produce various forms of biomaterials which can be employed in biomedical and biotechnological applications as illustrated in Fig. 1.

The fabrication process of biomaterials lies on the principles of engineering. Even though their primary use is seen in the medical field, they are also used in sensors, food coating and packaging, textiles, drug delivery, cell cultures, blood assays, even in cosmetics and other biotechnological applications [5]. The investigation of silk as a biomaterial has been a huge success since silk fibres of *Bombyx mori* are used as clinical sutures [6]. The isolation of silk protein and innovative fabrication techniques of 2-dimensional (2D) and 3-dimensional (3D) matrices like sponges, films, mats, hydrogels, and the most recent 3D printed scaffolds are leading the diverse applications of silk as a biomaterial. Important factors that are crucial for the clinical applicability of any biomaterial like biocompatibility, biodegradability, and reaction to immune response are being extensively studied with emphasis on less popular non-mulberry silk varieties to expand their use [7]. Although there is a wide acceptance and recognition of silk as a biocompatible material, there is still a possibility of eliciting an immune response when the silk-based product is exposed to the body for a long time and this persisting issue requires an in-depth investigation [8]. However, certain studies concentrating solely on the degradation of 3D silk fibroin (SF) scaffolds revealed that the scaffolds degraded within a week, demonstrating the biodegradability and bioresorbability of silk [9]. The high popularity of silk as a biomaterial can be attributed to its feasibility of functional modification and improvement in its load-bearing capacity. For manipulation of the structure and characteristics of regenerated silk, protic

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Table 1
Commercially available silk-based biomaterial products and their biomedical applications.

Product	Purpose	Company
Derma Silk Therapeutic Clothing	Therapeutic clothing for people suffering from skin diseases	AL.PRE.TEC. S.r.l.
Tympasil silk patch	Tympanic membrane perforation	Daewong Bio Inc
Silk Voice	Vocal fold medialization and vocal fold in sufficiency	Sofregen
Biosteel fibre	High Performance fibre	AM Silk
Silk Bridge	Peripheral nerve repair	KLIS Bio
BioSilk Silk Therapy Original	Hair Treatment	BioSilk
Biosilk	Moisturizing cream	Entirety
Skincare	Anti- aging skincare and skin health	Silk Therapeutics
Sidaiyi wound dressing	Wound dressing	Suzhou Soho Biomaterial Science and Technology Co., Ltd, China
D- Fibroheal Scarlite	Sterile surgical adhesive dressing	Fibroheal
Fibrovid	Topical antiseptic and wound healing ointment	Fibroheal
D- Fibroheal wound aid port dressing	Transparent wound dressing for surgical incision ports	Fibroheal
D- Fibroheal wound aid	Transparent dressing for low to moderate exuding wounds	Fibroheal
Silk and Soft	Moisturizing cream	Fibroheal
D- Fibroheal Ag Foam	Dressing around catheter at the tracheostomy site	Fibroheal
Fibromoist gel	Hydrogel wound dressing	Fibroheal
Fibromoist spray	Topical and surface antimicrobial antiseptic/ disinfectant spray	Fibroheal
D- Fibroheal Microgras	Non- adherent anti- microbial gauze dressing	Fibroheal
D- Fibroheal suture dress	Post - operative anti- microbial adhesive wound dressing	Fibroheal
D- Fibroheal	Surgical wound dressing	Fibroheal
D- Fibroheal PU Foam	Non- adhesive super absorbent PU Foam dressing	Fibroheal
D- Fibroheal Ag Meshed	Anti- microbial meshed surgical wound dressing	Fibroheal
D- Fibroheal Ag sprinkling powder	Anti-microbial surgical powder wound dressing	Fibroheal

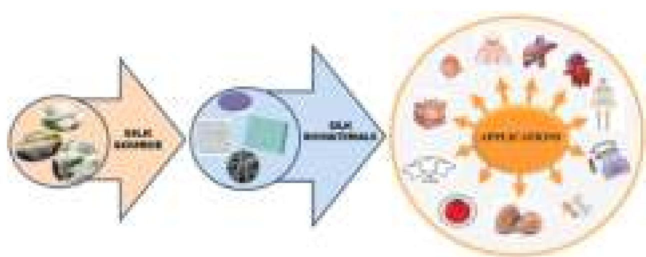

Fig. 1. Silk and its (silk-based biomaterials) various applications in the field of biomedicine and biotechnology.

ionic liquid system can be employed as well [10]. Apart from improving the load-bearing capacity, such modifications can affect the drug release kinetics, cell interaction, and biodegradability of the silk-based biomaterial [11]. The morphology of the different kinds of silk-based biomaterials can be altered to obtain the desired product (biomaterials) required for the target tissue. They can be processed into non-woven silk fibres, hydrogels, aqueous and solvent-based porous sponges and films [12]. Commercially available silk-based biomaterials are listed in Table 1, and the majority of the products are found to be mostly focused on wound healing and cosmetic applications.

Even though many studies are carried out using other polymers, the ideal characteristics found in silk have made it stand out from the rest, allowing its use as a suitable material for biomaterial fabrication. In this review, we discussed the various types of silk-based biomaterials and their properties to employ their use in various biotechnological and biomedical fields.

2. Types of silk

Large varieties of silk produced from different organisms are present throughout the globe. The classification of silk is based on the feeding plant source of the silkworms. Silk obtained from species that feed solely on mulberry plants are termed as mulberry silk and the remaining varieties of silk produced from species which do not feed on mulberry plants are termed as non-mulberry silk [13]. Due to its high availability, majority of the work carried out in the area of silk-based biomaterials involves the use of mulberry silk, particularly obtained from *Bombyx mori*.

2.1. Mulberry silk

Mulberry silk is obtained from the silkworm *Bombyx mori*. This silkworm feeds mainly on the leaves of mulberry plant and is solely domesticated and nurtured indoors. The origin of this species is believed to be of a native wild ancestor species, *Bombyx mandarina* through chromosomal fusions and gene duplication mechanisms [14]. This silkworm has been domesticated and reared for more than 2000 years. As a result of this long history, mutations have occurred with time, giving rise to a combination of a wide variety of genes that have produced many known races of silkworms. Based on the geographical origin, the silkworms are divided into Indian races, Japanese races, Chinese races, and European races. Depending on the number of generations that are produced in a year, they are classified as multivoltine, bivoltine, and univoltine as well [15].

2.2. Non-mulberry silk

Non–mulberry silk is largely associated with Indo-Australian origin [16]. Around 95 % of non-mulberry silk production is claimed to come from the tasar strains [17]. The principal silkworms in this category are Eri (*Philosamia ricini/Samia ricini*), Fagaria (*Attacus atlas*), Muga (*Antheraea assamensis*), Shashe (*Gonometa postica*) and tropical tasar (*Antheraea mylitta*) and temperate tasar (*A. pernyi, A. roylei, A. proylei* and *A. frithi*). Spider silk is a non-insect variety of silk obtained from the Madagascan species (*Nephila madagascarensis*) [18]. Most of these silkworms arise from wild habitats and are found on a wide range of plant hosts in many geographic regions. Because of this extensive distribution, the silk that is produced varies greatly with regards to their colour, lustre, and properties [19].

3. Silk protein structure

Majority of the silk produced is obtained from two groups of organisms which include the larvae of Lepidoptera and web spinners (spiders) from the arthropods [20]. Silk is a natural fibre that contains mainly protein polymers. Two distinct families of proteins, sericin and fibroin are present in all silks retrieved from different species of silkworms [21]. In spider silk, spidroin is the main protein that is present in the skin and core of the dragline silk [22].

3.1. Fibroin

Fibroin is a major component of the silk cocoon amounting to 72–81% while the remaining 19–28% comprises sericin proteins [23]. It is obtained from the posterior glands of the silkworms. The larvae secrete two fibroin strands from the exocrine silk glands through the spinnerets which are present on both sides of the body [24]. Large amounts of hydrogen bonds are observed in the protein and the orientation along with its molecular constituents give rise to its semi-crystalline structure. This structure contains two phases of a crystalline antiparallel β-sheet and a lower ordered β-sheet spacer. The toughness and strength of the fibre can be attributed to this crystalline structure and the flexibility arises from the presence of the non-crystalline parts [25].

3.2. Sericin

Sericin is secreted from the anterior and middle glands of silkworms [24]. It is an amorphous protein possessing glue-like nature. This protein binds fibroin together and maintains the structure of cocoons [25]. It consists of two-subunits, α-sericin and β-sericin, former is present in the external layer while latter is found in the internal layer of the cocoon. β-sericin has lesser solubility than α-sericin owing to its higher amounts of carbon, hydrogen and lesser amounts of nitrogen and oxygen atoms [26]. The hydrophilic nature of sericin allows its easy separation from fibroin by a method called 'degumming' [27].

3.3. Spidroin

Spidroin is a hydrophilic protein secreted from the silk glands of orb web spinning spiders. It contains glutamine residues in its amorphous regions and exhibits good elasticity and strength [28]. Spiders can produce a wide variety of silk that differ in properties and molecular weights (70–700 kDa) [1]. Glycine, glutamine, and alanine are found in dominance along with leucine and tyrosine residues. They also have a limited shared set of amino acid motifs [29] and the alanine-rich regions are responsible for the crystalline nature forming compact β-sheets [30].

4. Properties of silk protein

The properties exhibited by silk as shown in Fig. 3 is acknowledged in its wide usage for biomaterial fabrication in comparison to other polymers as it opens a route of applications that is not attainable by other polymers. The biocompatible and biodegradable nature are explored in a variety of tissue engineering applications and the feasible mechanical properties has allowed its use as a base for developing different silk-based sensors, textiles and coatings [31].

4.1. Biological properties

4.1.1. Biodegradability

One of the most essential requirements of any biomaterial is the ability to match the degradation time with tissue regeneration [32]. Evidence indicates that silk fibres are vulnerable to *in vivo* proteolytic degradation and gradual absorption over long durations [1]. The biodegradability of silk sericin allows its use in regenerative studies wherein sericin is either blended or copolymerized with different materials [33]. When utilized as an implanted construct, regenerated silk fibroin biomaterials have a degradation rate much higher than normal fibers since the degradation relies on the secondary structure which is obtained through the preparation of regenerated silk materials [34]. *In vivo* and *in vitro* experiments conducted on silk biomaterials have revealed that silk fibroin degrades slowly because of β-sheet protection as the structural state plays a more important role than the presence of cleavage sites in the biomaterial [35]. The presence of more β-sheets slows down the degradation and the porosity, pore size, molecular weight distribution, and sites of implantation also play a role in the degradation rates [36].

4.1.2. Biocompatibility

The low immunogenicity of silk fibroin corroborates its biocompatible nature as verified by the US Food and Drug Administration [37]. The antigenic and cytocompatible nature of SF-based biomaterials are studied considerably and these studies assert the compatibility of the material for intended use in *in vitro* conditions and animal model study [38]. On comparison with polylactic acid (PLA) and collagen, upon *in vivo* implantation or *in vitro* coculturing with human mesenchymal stem cells, a lower inflammatory reaction was observed [39]. Another study has also highlighted that implantation of 3D SF scaffolds in Lewis rats activated a mild immune response after 1 year [9]. High compatibility has also been observed with many cell lines such as mesenchymal stem cells and fibroblast cells [40]. Silk sericin is also widely recognized as a biocompatible material with low immunogenicity, however in the past, there were reports of silk sericin inducing high immune response when compared with sericin free fibers [41]. Later on, it was confirmed that pure sericin alone did not elicit any immune response in *in vivo* studies and instead played a role in anti-inflammation by downregulation of chemokines and cytokines [42,43].

4.2. Biophysical properties

4.2.1. Tensile strength

The tough nature of silk protein can be attributed to the presence of nanofibrils and the strong interactions taking place among themselves [44]. SF has an outstanding mechanical property which is a unique feature among all available natural materials. It is worthwhile to mention that silk fibres of *Bombyx mori* are proven to possess a tensile strength much higher than other fibers. The tensile strength of *Bombyx mori* silk fibers are reported at 500 MPa while other natural proteins like collagen and PLA exhibit 0.9–7.4 MPa and 28–50 MPa. After degumming the ultimate tensile strength of SF increased to 610–690 MPa which confirms its superior tensile strength. Similarly, the elastic modulus for SF was observed between 5 and 11 GPa whereas 0.0018–0.046 GPa was reported for collagen, and 1.2–3.0 GPa for PLA. [45]. The β-sheet structure allows its modification into different forms like films, hydrogels, nanofibres etc. The amino acids and carbons present can also be functionalized with other biomolecules to achieve biological activities [12]. Also, their reliability for use in load-bearing applications is confirmed through strength-to-density ratio that is ten times more than that of the steel [46]. The mechanical strength of silk sericin can be adjusted by developing it into different forms like films, hydrogels and scaffolds [32]. Crosslinking of sericin membranes gives rise to an improved and integrated structure which further stabilizes and improves the mechanical strength of the membranes [47]. The compression modulus of sericin hydrogels can be controlled by the addition of other polymers or through optimization of the sericin content. A study has reported that an increase in sericin concentration to 4% from 2% w/v leads to a relative increase in the compression modulus of a silk sericin hydrogel to 28 kPa from 1.6 kPa [33].

4.2.2. Extensibility

The structure of silk is refined and tuned during the regeneration or spinning process to acquire secondary structures which enable the manipulation of their properties [48]. The extensibility of silk can be credited to the ability of silk threads in the amorphous region to move and stretch into a β-strand configuration or an extended helix from its random coiled state [49]. A model was designed to describe the β-spiral secondary structure which has the ability to act as a spring and this structure is formed from the GPGXX motif in spidroin silk [50]. Some evidence suggested that with an increase in stress on the threads, the contraction of the fibres and nanofibrils create more alignment with respect to the thread's axis [51]. The nanofibril feature is also believed to contribute to the extensibility as the fibres get bent without any failure [52].

Fig. 2. Illustration depicting the different novel properties of silk.

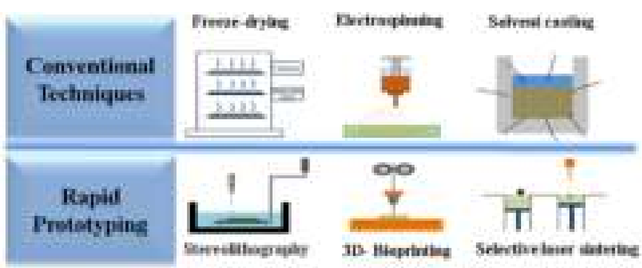

Fig. 3. Conventional and Rapid prototyping techniques used in fabrication of silk biomaterials.

5. Preparation and fabrication of silk material

Silk materials of sericin, SF, and spider silk are already utilized on a large scale due to their outstanding mechanical properties and cytocompatibility. However, they are fabricated into biomaterials through modifications of their native state to achieve enhanced functions using different techniques shown in Fig. 2 [53]. Fabrication techniques like solvent casting, freeze drying, electrospinning, thermal induced phase seperation (TIPS) and gas foaming are a few commonly used ones, but these conventional techniques are accompanied with minor drawbacks which led to the development of advanced techniques called rapid prototyping techniques. Rapid prototyping involves more advanced fabrication techniques using computer-aided design software and imaging modalities for rapid fabrication [54].

Poly (vinyl alcohol)/silk sericin membranes were fabricated using solvent casting to investigate their potential for removing remazol black B (RBB) from liquid solutions. Large amounts of RBB were removed using the membranes and the absorption of RBB by the membranes reached an equilibrium after 10 hrs. Since the procedure allowed easy synthesis for larger amounts, the results revealed good scope for the removal of charged pollutants from liquid solutions [55]. A robust SF scaffold was constructed through another conventional technique known as freeze drying. Using this method, the mechanical toughness of the silk fibers was preserved and this construct displayed improved toughness and strength providing a new alternative for producing SF materials [56]. Unlike other methods, electrospinning produces non-woven textiles with thin scales down till nanometer size range and this provides an advantage of largescale production [57]. Nerve guidance conduits fabricated through electrospinning of spider silk and collagen hybrid tubes showed good compatibility. Additionally, excellent cell growth and differentiation were observed in the peripheric tissue and the cells were able to carry out full neuronal functions [58]. Electrospun SF and silk sericin films were evaluated to study their effect at different mass ratios on macrophages. The addition of silk sericin activated macrophage and the highest vascularization was observed when the ratio reached 7:3 (SF: silk sericin) [59]. A biocompatible SF/sodium alginate composite was prepared using TIPS wherein the composite displayed a porosity of more than 90 % with MG63 cell attachment and growth on its surface [60].

Rapid prototyping provides a new outlook into the fabrication of silk materials with the assistance of a computer-aided design. 3D printed organ models is an increasing area of attention that helps in better understanding of different cell-signalling pathways [7]. SF is utilized as a viscosity moderator in bioinks for printing 3D constructs employed for kidney dialysis systems, vascular prosthesis, wound dressings, artificial skin, implants, and contact lenses [61]. Blended bioinks of SF and gelatin were used for printing 3D constructs for cartilage repair. The construct showed superior reliability for repairing the cartilage as it acts as a physical barrier while retaining adequate bone marrow stem cells [62]. Admane and team utilized silk-based bioinks for bioprinting of a skin construct having full thickness. Excellent migration of keratinocytes was observed within the construct along with recapitulation of the extracellular matrix organization of the skin, collagen fibril organization and keratinization. This bioprinted construct could offer good potential for better understanding of the physiological mechanism related to human skin [63]. A chemically modified SF known as Methcrylated silk (Sil-MA) was crosslinlked to produce a thin grid structure using a new technique of stereolithography known as Digital Light Processing UV projector along with another technique known as pneumatic extrusion. Since Sil-MA was proven to have good biocompatibility and osteoconductivity, its constructs can be applicable for bone regeneration [64].

6. Silk-based biomaterials

The utilization of silk-based biomaterials and their applications can be summarized in the field of biomedicine and biotechnology. Their applications are seen in skin, eyes, teeth, bones, heart, nerves, and liver for treatment of various ailments. In the area of biotechnology, silk-based biomaterials are used for developing smart textiles, biosensors, food packaging, cosmetics. Although many clinical trials have been completed in the past years as shown in Table 2, commercial availability of silk-based biomaterials is still very low compared to the amount of work that has been carried out.

7. Applications in the biomedical field

7.1. Skin

Many studies in the past have attempted to mimic the human skin using different silk-based biomaterials [65]. The results obtained from experiments conducted on these silk biomaterials revealed that silk supports the growth and proliferation of fibroblast and keratinocytes [66]. The synergistic effect ofSF spuns embedded with silver oxide nanoparticles (NPs) were reported to display excellent antibacterial activity against both pathogenic and non-pathogenic bacteria. Wound healing test performed using scratch assay showed efficient migration of T3T fibroblast cells with full coverage of the treated scratch area over a period of 24 hrs [67]. For the treatment of atopic dermatitis, an inflammatory skin disorder, Senti and team showed that commercially available silk fabric undergarments named Derma Silk displayed higher superiority to cotton because of the antimicrobial nature of the silk fabrics. The effectiveness of the fabric was found to be comparable to a topical ointment (corticosteroid), commonly used for the treatment of the same disease [68]. In combination with chitosan, SF blended films were developed

Table 2
List of various silk-based biomaterials completed clinical trials (Source: Data retrieved from www.ClinicTrials.gov).

Sl.No	Clinical Trial Identifier	Study Title	Condition	Intervention	Completion date	Number of participants
1.	NCT04315415	A Histological Study Evaluating Silk Voice and Cross-linked Hyaluronic Acid	• Vocal Fold Palsy • Vocal Cord Paralysis • Vocal Cord Atrophy	• Silk Voice	18April 2022	14
2.	NCT02091076	Efficacy and Safety of Silk Fibroin with Bioactive Coating Layer Dressing	• Late Complication from Skin Graft • Infection of Skin Donor Site • Impaired Wound Healing • Pain, Intractable	• Silk fibroin with bioactive coating layer dressing • Bactigras wound dressing	May 2015	29
3.	NCT02636894	Evaluation of the Efficacy of Restylane Silk in the Treatment of Cheek Fold (Radial Smile Lines)	• Smile Lines	• Restylane Silk	August 2016	30
4.	NCT05534204	Evaluation of the Effects of Silk and Polyester Suture on Postoperative Complications	• Impacted Third Molar Tooth	• Silk suture • Polyester suture	June 1, 2022	30
5.	NCT02689947	Evaluation of the Efficacy of Restylane Silk in the Correction of Tear Trough Deformity	• Tear Trough Deformity	• Restylane Silk	March 2017	30
6.	NCT02780258	Restylane Silk for Photoaged Thinned Hands	• Photoaged Thinning of the Hands	• Restylane Silk	October 28, 2016	25
7.	NCT02925741	Silk-Like Bed Linens for Prevention of Unit-Acquired Ulcers	• Pressure Ulcer	• Silk-Like Linens	March 2015	3343
8.	NCT03763981	Comparison of Prolene Thread Seton Vs Silk Thread Seton for the Treatment of Perianal Fistula	• Perianal Fistula	• prolene seton treatment • Silk seton treatment	August 30, 2018	100
9.	NCT03241862	Assess the Impact of Lip Rejuvenation on Projected First Impressions and Mood Perceptions	• Lip Rejuvenation	• Restylane® Silk	June 16, 2017	20
10.	NCT02703948	Injection Technique Assessment of Restylane Silk with Lidocaine for Lip Augmentation	• Lip Augmentation and Correction of Perioral Rhytids	• Restylane Silk with Lidocaine	August 2016	60
11.	NCT05064072	Pain Felt During Removal of the Products from Infant's Skin Used in Nasogastric Tube Fixation	• Pain, Acute	• Silk tape	September 15, 2021	120
12.	NCT04032977	Safety and Effectiveness of PN40082 for Lip Augmentation	• Lip Augmentation	• PN40082 • Restylane • Silk	May 3, 2019	158
13.	NCT04898816	Comparing the Effectiveness of Cyanoacrylate Tissue Adhesives and Conventional Sutures	• Wound Heal	• Closure of wound using *n*- butyl 2-octyl cyanoacrylate tissue adhesive • Closure of wound using braided silk suture	April 25, 2021	20
14.	NCT01914653	SERI® Surgical Scaffold Postmarket Study of Soft Tissue Support and Repair in Breast Reconstruction	• Breast Reconstruction	• Silk surgical mesh	March 2016	17
15.	NCT02293798	Circumferential Periareolar Mastopexy Using SERI Surgical Scaffold	• Mastopexy	• Silk surgical scaffold	June 2016	13
16.	NCT02016612	Seri Surgical Scaffold Support of the Lower Pole of the Breast (SeriSupport)	• Recurrent Ptosis of the Breast	• Seri Surgical Scaffold	November 30, 2016	76

and examined for their use in skin tissue engineering. Good biocompatibility was observed upon co-culturing with fibroblast cells and the film showed no signs of toxicity by XTT assay. Fibroblast cells proliferated via dendritic extensions and cell-to-cell interactions were also observed. These findings suggested the promising use of chitosan and SF films as a supporting substrate in skin tissue engineering [69]. Another combination of recombinant spider silk with SF was analysed for its potential in wound healing applications. Microporous scaffolds and nanofibrous mats were top-coated with a layer of recombinant spider silk protein (4RepCT), antimicrobial peptides, growth factors and a cell binding motif. The functionalized silk mats demonstrated enhanced antimicrobial activity, cell adhesion, and stimulation of growth factors. A bi-layered tissue structure with a keratin epidermal layer was observed upon co-culturing with human dermal fibroblasts and human keratinocytes. The positive results supported a promising use in wound healing and application of the fabrication method for skin substitutes and wound dressings in the future [70]. Wendt and team also demonstrated the feasibility of spider silk for enhancing skin regeneration by constructing a

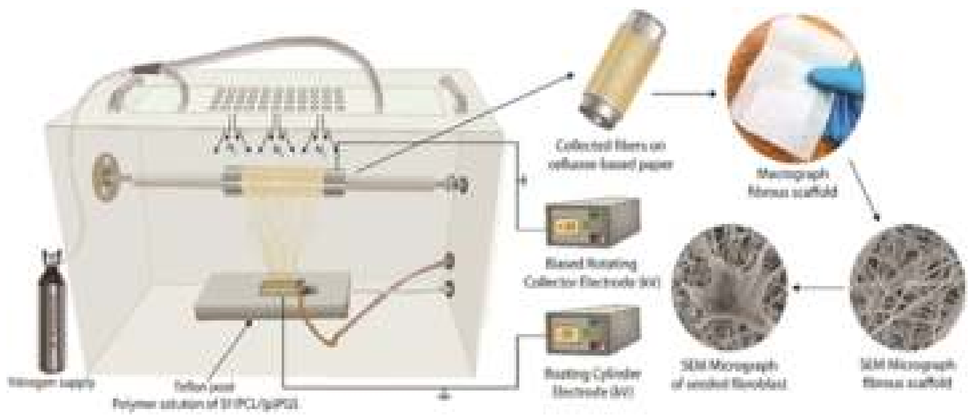

Fig. 4. Diagrammatic presentation of a nozzle free electrospinning process presented with a micrograph of the obtained fibrous scaffold and SEM analysed micrograph of the fibres present with fibroblast. Reproduced from Keirouz et al., (2020).

bi-layered skin embedded with keratinocytes and fibroblasts on a woven dragline silk. This provided an appropriate matrix for culturing skin cells and both the keratinocytes and fibroblasts cell lines showed significant adherence and proliferation which confirmed the skin regeneration activity [71]. SF film developed by Zhang and team was examined for its translational potential in skin repair. The film displayed outstanding features of transmittance, moisture and vapour permeability, fluid handling capacity, waterproofness, biocompatibility, and anti-bacterial properties. *In vivo* studies performed on a rabbit with full thickness skin defect showed remarkable skin regeneration and efficient reduction on average healing time compared to commercially available wound dressings. A randomized clinical trial with 71 patients further confirmed the significant reduction in wound by the SF film providing a systematic clinical and pre-clinical evidence of promotion of skin regeneration and repair [72]. In another study, electrospun mats produced through a nozzle free electrospinning process as shown in Fig. 4 were fabricated for use in skin regeneration. These mats were produced from regenerated SF and Poly (caprolactone) blended with different forms of Poly (glycerol sebacate) (PGS) to develop an electrospun fibre mat having tuneable hydrophilicity or hydrophobicity. Upon altering the surface properties of the electrospun membranes, the composite biomaterial revealed excellent behaviour for fibroblast attachment and optimal growth, presenting the possibilities of developing artificial skin like substrates that can support skin regeneration and wound healing [73].

7.2. Ocular

The cornea of the eye is the transparent front portion which plays a major role in visualization. Any injury to the cornea due to physical damage, illness or ailment leads to disruption in vision [74]. The probable use of SF membranes for rejuvenation of cornea was demonstrated through implantation of corneal epithelial cells cultured on a SF membrane into the stroma of rabbits. The membrane showed transparency for 4 weeks with observed neovascularization in the limbus and new vessels were seen growing to their peak after a period of 8 weeks [75]. Another study performed by Higa and Shimazaki verified the significant use of SF as a biological carrier for cultivated epithelial cells used in corneal epithelial restoration. The SF films provided a clear medium when compared to other membranes like amniotic membrane or fibrin sealers [76]. Using *Antheraea mylitta*, a non-mulberry silk, a SF film having thin transparency was developed to offer a promising alternative for its use in corneal regeneration. The film promoted the attachment, growth, sprouting, and migration of keratinocytes and epithelial cells of corneal explants from rats. It supported the growth of limbal stem cells confirmed by ABCG2 expression and upon implantation onto the rabbit cornea, the implanted film remained transparent and stable. Observations from clinical examinations and histology revealed that *Antheraea mylitta* based non-mulberry silk showed insignificant inflammatory response. The surface integrity of the cornea was maintained with no adverse change in tear formation, electroretinography, and intraocular pressure of implanted eyes. These results confirmed the potential of the SF film as a notable biomaterial for use as a corneal scaffold [77].

7.3. Dental

Tooth regeneration is an area studied by researchers for decades with investigations conducted in a variety of approaches. Till date, silk-based biomaterials are extensively used in soft and hard tissue engineering [78]. The effectiveness of four kinds of hexa fluoro isopropanol (HFIP) SF scaffolds was determined for use in mineralized dental tissue engineering. These scaffolds seeded with a cultured 4-day post-natal bud cells from rat tooth were grown for 2 weeks in a rat omentum. Observations from the harvested implants demonstrated the formation of a robust bioengineered mineralized tissue. The shapes and sizes of the pores on the silk scaffold guided the mineralized formation of tissues and since the study was the first characterization of bioengineered tissues from tooth bud cells that were seeded into the silk scaffolds, it signified the use of silk scaffold in the formation of mineralized osteodentin [79]. In another report, Yang and team assessed the probability of pulp regeneration with basic fibroblast growth factor (bFGF) and dental pulp stem cells (DPSCs) using a SF scaffold. The porous SF scaffold was fabricated via freeze drying followed by seeding with DPSCs and insertion into the root fragments of the tooth. The presence of bFGF promoted the viability of DPSCs in the scaffolds/tooth fragments between 7 and 28 days. A pulp like tissue formation was generated and this generated tissue had good vascularity, formation of a dentin like tissue, and a new matrix deposition on both the host-derived and transplanted cells. The data generated from this study supported the use of this bFGF inserted silk-based scaffold as a promising candidate for treating regenerative endodontics [80].

7.4. Bone

Tissue engineering of bones aims at the regeneration of a natural bone using artificial methods [81]. Recombinant spider silk proteins obtained from *E. coli* can be used in combination with other biodegradable polymers to produce tuneable scaffolds applicable for bone tissue engineering [82]. Composites of hydroxyapatite (HA) and silk have shown outstanding toughness and mechanical strength with the integration of a mineralized macroporous SF scaffold. The biomimetic bone like composites have compressive strength, toughness, are resorbable and comparable to the mechanical resilience of cancellous bone [83]. In a research work carried out by P. He, a hybrid scaffold of knitted silk sponges and silk fibres was developed and incorporated with an osteoinductive phase on the ends of a ligament scaffold. The scaffold had the ability to generate a bone-ligament-bone graft which improves graft osteointegration with the host bone. In this hybrid scaffold, a HA coating was applied

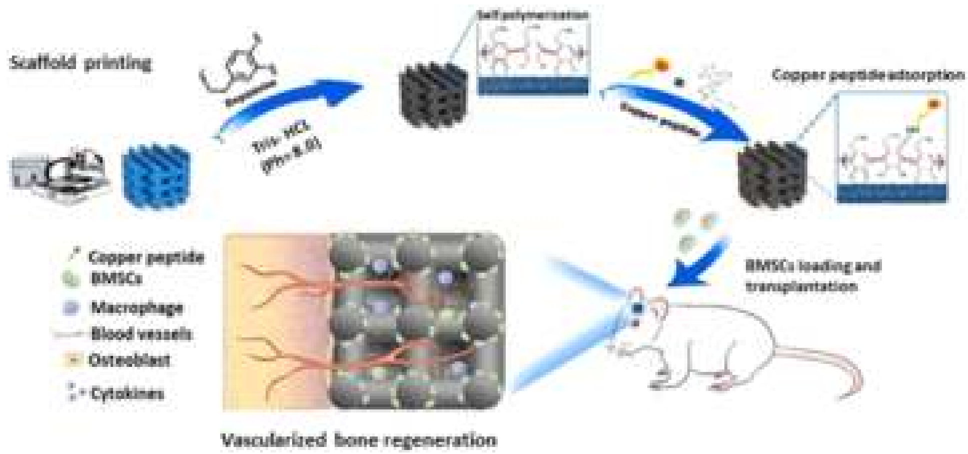

Fig. 5. Diagrammatic illustration representing the fabrication of a 3D Scaffold embedded with GHK and Cu for bone regeneration. Reproduced from Zhou et al., (2021).

on the osteoinductive ends and its effects on bone related cells were observed. From the alkaline phosphatase assay and real-time polymerase chain reaction, the osteoinductivity of the silk scaffold coated with HA exhibited osteogenic differentiation of the bone marrow mesenchymal stem cells. The osteoconductivity of the HA-coated silk scaffold also revealed significant osteoblast growth and maintenance of mature osteoblasts. These results demonstrated the applicability of the scaffold in fabricating silk-based grafts that can enhance the integration of graft to bone host [84]. 3D printed silk-based scaffold incorporated with copper peptide, a copper ion-specific binding tripeptide (tripeptide glycyl-L-histidyl-L-lysine (GHK)) having a sustained release ability was fabricated as shown in Fig. 5. The therapeutic effects were revealed upon control of the dissemination of copper ions with decreased toxicity. The copper peptide enhanced the M2 macrophage polarization and aided in the tissue repair of bone at an early stage. In addition, the released copper peptide regulated a microenvironment that stimulated cytokine secretion and bone marrow derived stem cell proliferation (BMSC). *In vivo* experiments on calvarial defect regeneration revealed that the scaffold embedded with BMSC enhanced the vascularized bone tissue regeneration [85]. A porous SF/cellulose nanowhiskers chitosan (SF/CNW-CS) scaffold produced using freeze-drying method showed good biocompatibility and mechanical properties. The scaffold was assembled to form a lamellar structure in which the porosity decreased with an increase in the number of assembled layers. In order to estimate its reliability for use as a bone scaffold, it was cultured with Human MG-63 osteosarcoma cells and the results revealed good promotion of cell proliferation and improved expression of osteocalcin [86].

7.5. Cardiac

Non-functional cardiac tissue leads to morbidity and mortality, so in cardiac tissue regeneration, one of the first objective would be fabrication of a scaffold capable of providing an adequate microenvironment that allows proper cell and tissue functionality [11]. A successful *in vivo* study has reported the use of an injectable silk sericin hydrogel for the treatment of ischemic heart disease. This silk sericin hydrogel assisted in functional heart recovery along with a reduction in inflammatory response, improved micro vessel density, and attenuated apoptosis in fracted hearts [43]. In a similar study, an electrospun scaffold obtained from a carbon nanotube/ SF was developed using a solvent free fabrication method for improving cardiomyocyte functionalities. The uniform dispersion of the carbon nanotubes on the nanofibre allowed an increased conductivity on the scaffold along with an outstanding biocompatibility for culturing neonatal rat cardiomyocytes. Furthermore, the scaffold exhibited good guiding ability to the oriented organization of the cardiac tissues. All the findings obtained from the study revealed good potential of the silk-based biomaterial in aiding the formation of cardiac tissues with enhanced features [87].

7.6. Neuron

Damage on peripheral nerves, spinal cord, brain injuries, and neurological diseases are some of the leading challenges in the medical field. Various approaches were tried and tested before neural tissue engineering finally came into existence [11]. While investigating the feasibility of SF for use in the central nervous system, substrates of SF fibres demonstrated good biocompatibility and supported the survival and growth of hippocampal neurons [88]. A study using SF with peripheral nerve cells and tissues was performed to explore the possibility of constructing artificial nerve grafts. Dorsal root ganglia (DRG) taken from a rat and Schwann cells taken from rat sciatic nerve were cultured on SF fibres substrate and SF fluid. Insignificant changes in the morphology of those tested cell lines with usual media was observed. This revealed the reliability of the SF with DRG and Schwann cells accompanied with no cytotoxic effect on their function [89]. Silk–tropoelastin protein films were developed and studied to evaluate their ability to act as a platform for nerve regeneration. The protein film greatly enhanced neurite extension while providing a tough template allowing its use in nerve cell control with further consideration for nerve repairs and neurite guidance [90]. Mesenchymal stem cells obtained from a dog's bone marrow were cultured with the composite scaffold and an extracellular matrix was observed on the surface of the scaffold. This neural scaffold served as a nerve graft for connecting the 60 mm nerve gap in the dog's sciatic nerve [91]. In another work, a novel heterostructure composite scaffold made up of reduced graphene oxide and silk nanofibers was fabricated for the induction of an oriented cell growth with improved differentiation of SH-SY5Y cells. The experimental results revealed significant oriental growth with enhanced SH-SY5Y neuronal differentiation [92].

7.7. Hepatic

Liver is the largest organ in the body responsible for a variety of metabolic, immune, and regulatory roles. A major portion of the cytoplasmic mass in the liver consists of hepatocytes that are engaged in release, storage, synthesis, and metabolism. The loss of any one of its functionalities can lead to liver failure and loss of life. Hence the lack of proper treatment necessitates the need for hepatic tissue engineering to save lives [93]. Different silk-based biomaterials are being explored for their potential roles in hepatic tissue engineering. In one of such work, a 3D porous sponge containing conjugates of lactose and SF (Lac-CY-SF), hepatocyte specific ligands and β-galactose residues was fabricated. These Lac-CY-SF sponges had a heterogeneous structure and were seeded with FLC-4 cells derived from human hepatocellular carcinoma. In a span of 5 days the FLC-4 cells forged multicellular spheroids with diameters ranging from 30 to 100 μm. After 3 weeks, the FLC-4 cells cultured in the sponge showed great promotion of albumin secretion with a constant albumin level. Genes related to specific liver functions

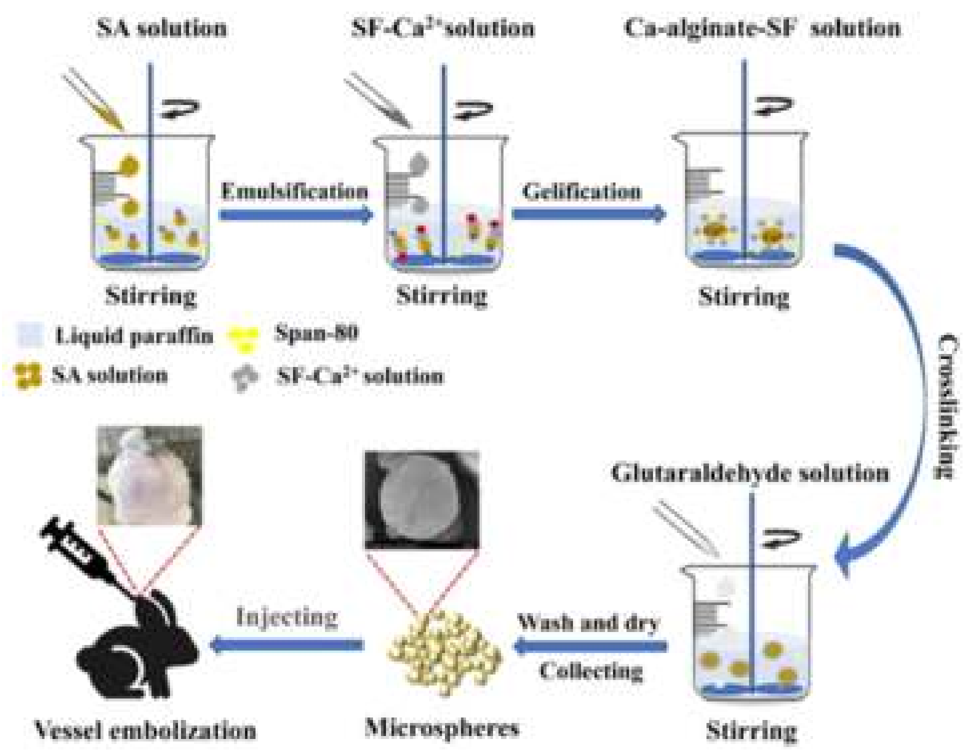

Fig. 6. Schematic presentation of the preparation method of silk fibroin microspheres modified with Sodium alginate for use in arterial embolization. Reproduced from Chen et al., (2020).

like HNF-4 and transferrin were found to be expressed in the FLC-4 cells cultured in the Lac-CY-SF sponges. The results from this study revealed the promising features of the Lac-CY-SF sponges for a prolonged FLC-4 cell culture in studying hepatocyte metabolism, drug toxicity and also in the development of a model bio artificial liver [94]. Another study reported the investigation of composites of SF and gelatin for their *in vivo* biocompatibility and *in vitro* cytotoxicity for applications in liver tissue engineering. Human hepatic QZC cells were cultured on various blends of the 2D SF and gelatin scaffolds. The relative and proliferative growth rates were assessed using MTT assay and the results revealed significant cell proliferation and attachment on the composite. For further analysis, subcutaneous implantation of the composite was performed and only slight signs of inflammation were observed. On the 30th day, the scaffold was fully infiltrated by fibroblasts and inflammatory cells and owing to their fast degradation with an increase in the gelatin concentration, they were considered as promising candidates for implantable bio-artificial livers [89]. Modified sodium alginate (mSA) and SF microspheres were developed as novel embolic agents and tested for their embolization ability. The overall illustration of the preparation process is shown in Fig. 6. The results obtained from the study revealed that the microspheres had sensitivity to temperatures, pH, and swelling ratio necessary for an arterial embolic agent. Excellent blood compatibility was seen in anticoagulant and haemolytic tests and further cytotoxicity tests indicated the possible promotion of HUVEC and fibroblast proliferation. *In vivo* embolization studies showed that the arteries could undergo embolization using these mSA modified microspheres and all the findings confirmed its potential use in liver therapy as an arterial embolic agent [95].

8. Applications in biotechnology

8.1. Sensors

With its outstanding processibility and exceptional properties, silk-based biosensors have become a growing area of interest for many researchers [96]. Silk-based food sensors developed in the form of passive metamaterial antennas have provided an in-depth monitoring of biological analytes [97] as well as food quality [98]. Silk protein-based substrates adhered on an apple surface have been fabricated to allow intimate contact with the nano and micro-structures that could probe and monitor the surrounding environment for changes. The sensor consisted of all biodegradable components and is edible, signifying a possible use in health care and food market [99]. In the field of on-skin sensors, a highly thermal-wet, comfortable, and conformational silk-based electrode was fabricated by Li and colleagues as illustrated in Fig. 7. This on-skin sensor displayed sweat tolerance and could be employed for electrophysiological measurement of skin under sweaty conditions. High stretchability, low resistance to evaporation, low thermal insulation, and high transmission of water vapour were observed allowing further engagement for developmental progress with regards to on-skin sensors [100]. Oxygen sensors fabricated from silk films were evaluated for both *in vivo* and *in vitro* experiments. These silk films contained a water insoluble Pd (II) tetramethacrylated benzoporphyrin (PdBMAP) and an oxygen sensing chromophore. The self-assembly of the cross-linked protein network stabilizes the silk film and the de-aerated phosphorescence lifetime is decreased to 50 % of its initial value indicating good functionality in sensing oxygen within the normal physiological range. *In vivo* and *in vitro* studies revealed that the chromophore composite films were cyto- and biocompatible with excellent mechanical properties suitable for subcutaneous implantation. The composite film also maintained oxygen sensing functions during *in vivo* test and demonstrated sensing abilities in real life for several physiological states like hyperoxia, normoxia, and hypoxia [101]. A reliable glucose sensor was developed from a composite of reduced graphene oxide (RGO)/glucose oxidase (GOx) and silk nanofibril. This sensor displayed detection even at extremely low limits (300 nM) and had high sensitivity at 18.0 μA/Mm which is within the sweat glucose range, thereby providing coverage for both diabetic patients (0–100 μM) and healthy people. Upon testing on sweat samples, the results indicated good reliability and correlation with the data from a normal commercial glucometer (Chen *et al.*, 2022). In another work, spider dragline silk (SDS) was used to create a novel humidity sensor that relies on the mechanism of refractive change of the dragline silk with respect to humidity changes in the environment by Liu and team. The SDS was wrapped on a tapered single mode fibre allowing the formation of a multimode interference structure to obtain an optical spectrum. As the refractive index of SDS changed with varia-

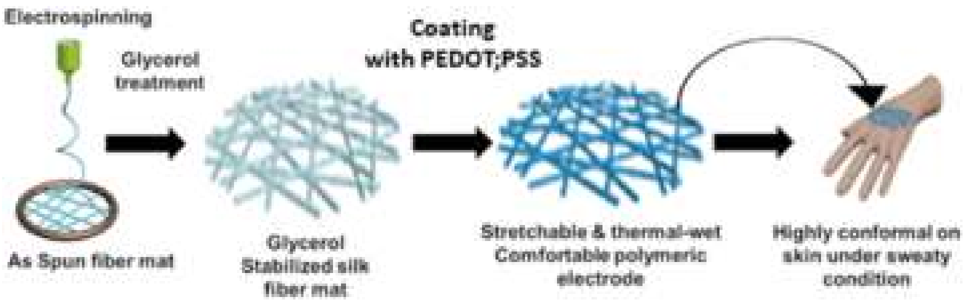

Fig. 7. Preparation of gas permeable and stretchable silk-based electrodes. Reproduced from Li et al., 2021.

tions in the humidity of the environment, this led to a shift of the multimode interference spectrum. The experimental results displayed the average sensitivity at 0.532 nm/%RH at a range of 70 %RH-89 %RH for the proposed SDS-based sensor. The maximum sensitivity was observed at 0.789 nm/%RH at a range of 70 %RH/- 89 %RH with the humidity sensing curve approximately fitted using the linear method [100].

8.2. Textiles

Silk-based biomaterials have already been utilized as clinical sutures for a long time and an increased recognition for their use in biomedical textile has grown in the recent years [102]. Even though multifunctional textiles have a promising use as wearable electronics, there is still a major issue associated with the maintenance of their porous features and innate flexibility. Using a layer-by-layer assembly technique assisted by a vacuum, Liu and team embedded textiles with electrically conductive substances for fabrication of a multifunctional silk textile having good flexibility, hydrophobicity, and excellent electromagnetic interference (EMI) shielding with a highly sensitive response to humidity. A biomimetic leaf-like nanostructure containing nanosheets of transition metal carbide/carbonitride (MXene) as the lamina and highly conductive skeleton of silver nanowires (AgNWs) were used for electrical conductance. The fabricated textile displayed low sheet resistance and outstanding EMI shielding efficiency with good response to humidity all while preserving its porosity and permeability. The self-derived hydrophobicity was achieved through aging of the Mxene-coated silk which was hydrophilic in nature. The production of these wearable textiles having multifunctionality are highly promising for further applications in smart garments, EMI shielding, and humidity sensors [103]. With the known capability of functionalized textiles, a skin comfortable Janus Silk E-Textile was prepared from natural silk materials for the analysis of biofluid (Fig. 8). Due to its biocompatibility, silk was chosen and modified into a sensing electrode and a textile substrate. The unidirectional biofluid behaviour of the Janus silk substrate provided a comfortable microenvironment for the skin and avoided any excess cold or heat allowing the formation of a new type of smart textile for health monitoring and wet-thermal management [104]. High performance and ultra-stable silk textiles have exhibited broad application potential in integration of intelligent clothing, human interactive interfaces, and harvesting energy [105]. Silk textiles have also been used as reinforcements for natural rubber (NR) to obtain a green elastomeric composite having good flexibility and strength. This composite exhibited a highly dynamic and static mechanical feature on comparison with other NR and nylon composites which could be attributed to the strong matrix adhesion and enhanced wettability [106]. There are reports of high-quality colour reproduction from digital images achieved using silk textile. This reproduction was performed with a precise colour matching technique done between the original digital image and woven fabrics [107]

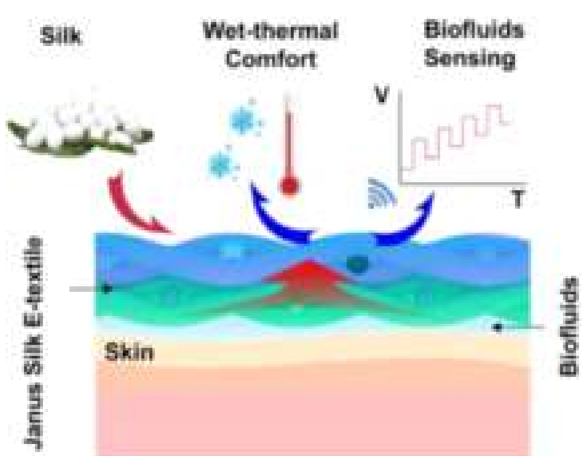

Fig. 8. Janus Silk E- textile for biofluid sensing. Reproduced from He et al., 2021.

8.3. Food industry

Proving the many potential applications of silk, several silk-based food packaging has been proposed in the food industry. Silk-based coatings of SF and dragline silk have established an application in the preservation of fruits and vegetables. These coatings reduce transpiration, respiration, microbial infections, and provide protection to their physiochemical and phytochemical properties [108–110]. A water-based protein suspension that gets self-assembled after dip coating on the surface of food was developed using SF. Since the post-processing control is based on water, it allowed modulation of diffused gases through the thin SF membrane, which is an important parameter for maintaining freshness of foods. The formation of a thin micrometer ranged SF covering the fruit contributed in maintaining the post-harvest physiology by reducing the water evaporation and cell respiration rates [111]. SF hybridized with poly vinyl alcohol (PVOH) at a ratio of 1:1 was used to form a coating solution. The hybridization regulated the gas barrier and mechanical properties of SF by increasing the β-sheet content. Using freshly chopped apple coated with the solution, the reliability for use as a food preservative was evaluated. While drying, a bilayer structure was observed wherein the SF came in direct contact with the food and PVOH formed another layer above the silk. The colour change and weight loss of the coated fresh apples were much lower than those of the uncoated controls kept for over 14 days and stored at 4°C [112]. Another study has demonstrated the fabrication of an edible coating for the preservation of apricots and extension of their shelf life by preventing contamination by fungus. In this work, a degummed sterilized silk was prepared for a coating solution. The solution was used for coating fresh apricots followed by water annealing at different intervals which lead to a rise in the β-sheet content. A 14 days shelf-life was observed in the water annealed and coated apricots while the coated but un-annealed apricots displayed no fungal contamination but high-water loss. Signs of spoilage were observed in 3 days on the uncoated apricots followed by a change in texture on the 4th day and complete growth of fungus on the 7th day [113]. Promising use of silk waste to prepare thin films are reported to have genuine capability for food coating. SF thin films prepared from silk waste were investigated for their use as edible strawberry coatings. Two set of thin films were prepared in which one had a water annealing treatment and the other was untreated. The strawberries coated with the water annealed thin films showed good performance of reduced weight loss while storing and preserving the visual appearance. Analysis for metal contaminants was also performed which revealed that the silk fibroin obtained from the waste had no metal toxicity [114]

Within the past years, several kinds of active and intelligent food packaging have been presented and produced [98]. The emergence of nanotechnology for food packaging have shown good potential with the use of nanomaterials that can enhance the properties of existing materials for preservation and maintenance of food [115,116]. In a recent work by Zhao and team, a thermoregulating hydrogel from silk was fabricated as an active packaging material for food products that are temperature sensitive. This hydrogel composite comprised of cellulose, SF, and a temperature buffer n-octadecane. It significantly slowed food spoilage by absorbing moisture from fruits that are temperature sensitive [117]. An anti-bacterial packaging material was developed from a SF/ poly vinyl alcohol hybrid with silver NPs (AgNPs) by Tao and team. The SF and PVOH interaction formed a matrix which exhibited amazing mechanical properties. Antimicrobial activity evaluated using food-borne pathogens of gram-negative and gram-positive bacteria revealed good inhibition against both pathogens offering more options for use in the field of active packaging [118]. Another study of SF films embedded with active compounds like Poly (ethylene oxide) (PEO) and thyme essential oil (TO) were reported to show an enhanced anti-bacterial activity. The cold plasma treated nanofibres of SF-PEO and TO could give protection to poultry meats from getting contaminated by *Salmonella typhimurium* for 7 days [119]. Valentini and colleagues developed a hybrid composite for smart food packaging using single cell fungi and regenerated silk nanofibrils obtained through yeast fermentation for monitoring heat sensitive foods. This hybrid composite was laminated onto a parafilm substrate and since parafilm has a great thermal expansion coefficient, it allowed the hybrid composite to switch to two states, wrinkled and wrinkle free states. On application to the food surface at a high temperature and cooling at an ambient temperature, wrinkling was observed as a result of compressive strength produced on the interface of the SF/parafilm/yeast composite. The process can be reversed and upon reheating it could return to a wrinkle-free state. As the films had low permeability of water, the shelf life was extended to more than 7 days using the activated metabolism of microbes present in the regenerated silk [120].

8.4. Cosmetic applications

The derivatives obtained from silk proteins of spiders and *B. mori* are used efficiently in many commercial skin care lotions, dyes, gels, bleaches, mousses, cleansing products, anti-aging creams, shampoos, and conditioners as an active ingredient [121]. The soft lustre of many expensive cosmetics can be credited to the presence of finely chopped silk [122]. The use of sericin has a long line of history with cosmetics. With the discovery of sericin having moistening ability, anti-wrinkle effects and reduction of other signs of skin ageing, however, more studies are being conducted to discover its true potential [123]. Nail cosmetics that contain 0.2 to20 % sericin have been reported to prevent nail brittleness, chapping and impart inherent gloss in nails [124]. For use as a bioactive mask in facial treatment, Aramwit and Bang developed a silk sericin releasing bacterial nanocellulose gel. This gel had a pure fibrous network and on comparison with commercially present paper mask, the gel had enhanced mechanical features, good moisture absorption, and less adhesiveness. Cytotoxicity test revealed no toxicity to HaCaT human keratinocyte and L929 mouse fibroblast cells. Subcutaneous implantation was evaluated based on ISO10993–6 standard and it showed that the gel was non-irritant to tissues [125]. Biosilk is among one of the most commonly used hair treatments on the market containing formulations of weightless treatments that protect all kinds of hair [126]. Investigations on hair cosmetics containing silk peptide were evaluated for their hair care effects using different tests like fluctuation in hair weight, moisture, thickness, and absorbance rates. Treatment using silk peptides revealed good penetration into hairs that were severely damaged by a chemical treatment. The hair weight and thickness were retained and increased recovery of the hair cuticle was observed, displaying its effective ability in recovering damaged hair to normal conditions [127]. In another study, a novel nanofibrous mat of SF was loaded with vitamin E and fabricated using a green process for use in skin care. Tocopherol polyethylene glycol 1000 succinate (VE TPGS), a derivative of vitamin E was embedded into the SF nanofibres. The nanofibrous mats displayed good water resistance and *in vitro* study revealed a sustained release of the VE TPGS. Excellent strengthening abilities were observed which protected the cells against oxidative stress. The results obtained gave an impressive and promising use of this vitamin E-loaded SF nanofibrous mats [128]. Silk NPs have also been used for the delivery and protection of guava leaf extracts containing phenolic compounds which are used in a variety of cosmetic products. These phenolics require heat protection and retainment of their anti-oxidant property which is attained by encapsulation within these silk NPs [129].

8.5. Drug delivery

Drug delivery refers to the use of a carrier molecule for delivering an active drug into a target site [130]. Silk-based biomaterials are found to have a wide utilization as drug delivery systems for different bioactive molecules such as drugs, genes, and other small molecules [131]. A sericin-alginate hydrogel having an interpenetrating polymer network (IPN) was developed for use in drug and cell delivery. These IPNs had high swelling ratios beneficial for use in drug delivery and were found to sustain a controlled release of the drug molecules loaded in the hydrogel [33]. Another hydrogel fabricated from sericin and dextran was used as a drug delivery system for treating malignant melanoma [132]. Tao and team designed a silk sericin/PVOH hydrogel having enhanced mechanical properties with the ability of being loaded with small drug molecules and NPs with sustained release. This hydrogel was loaded with gentamicin and no toxicity was observed towards the mammalian cell line. Anti-bacterial assay revealed effective inhibition of bacterial growth with a gradual release of the loaded drug from the hydrogel and these findings indicated a possible use of the hydrogel in promoting wound healing [133]. In another research on drug delivery, hybrid scaffolds of recombinant spider silk and silica were loaded with antimycotics and antibiotics to allow a sustained release of the antibiotics (Fig. 9). The antibiotics showed a sustained release within a course of 15 days and did not affect the cytocompatibility of the hybrid scaffold. Promotion of adhesion and proliferation of fibroblast cells were also observed, revealing an insight into the probable use of spider silk in further drug delivery systems [134]. Another study developed a SF hydrogel for protein drug delivery. This hydrogel network was biocompatible with good drug loading capacity and had a cumulative release rate of 80% within a span of 12 hrs after loading the protein [135]. Gharehnazifam and team also developed a SF hydrogel loaded with vincristine. The results obtained from the study revealed good drug release of 80% within the first 5 days after injection into an enzyme solution compared to another enzyme solution where only a 10% release was observed during 40 days [136].

8.6. Catalytic activity

Silk materials are investigated to serve as support system for immobilization of enzymes which can play a key role in a variety of therapeutic applications and allow recycling of costly enzymes for their reuse and expanded usage [137]. The characterization of enzymes that are immobilized using SF are done using microscopy and spectroscopy, evaluations obtained through biosensors also revealed high stability [138]. Immobilization of glucose oxidase was reported by Zhang and team in

Fig. 9. Schematic fabrication process for hybrid scaffolds composed of recombinant spider silk and silica loaded with antimycotics and antibiotics. Reproduced from Kumari et al., 2020.

methanol treated films of SF. High storage stability was observed along with maintenance of activity in broad temperature and pH levels. Based on this SF membrane immobilized with glucose, a glucose sensor with a wide range of linear response was fabricated and this sensor had the ability to detect 60 bio samples within an hour [139]. Flowerlike composites of gold NPs and reduced graphene oxide were synthesized using SF for use as an oxygen reduction electrocatalyst. The composite showed superior catalytic activity in oxygen reduction reactions and absorption through near-infrared and visible region in calorimetric detections [140]. A novel regenerated SF-based hydrogel was developed with a generated magnetic ferriferous oxide. The prepared hydrogel displayed enhanced power of magnetic stabilization and outstanding catalytic abilities. Detection of H_2O_2 at low concentrations of 1×10^{-6} mol L^{-1} was achieved and the catalytic activity was maintained for a long period under different conditions [141]. In another study by Li and team, the effective use of spider silk as a template was revealed through the synthesis of a microtube using electroless deposition in which spider silk was used as a template [142].

9. Conclusion

Silk-based biomaterials have emerged as a fascinating and promising area of research and development in the field of regenerative medicine. Silk is a unique polymer with high mechanical strength surpassing other biopolymers. The clinical success of silk as a biomaterial in the past has greatly influenced the expansion of silk-based biomaterials for various biomedical and biotechnological applications. Different kinds of silk, like mulberry silk, non-mulberry silk, and spider silk have been utilized in the fabrication of many biomaterials. These silk proteins can be prepared and regenerated to form films, sponges, hydrogels, and nanofibers. The versatility in biocompatibility and processibility into different kinds of biomaterial, ease in sterilization, controlled degradation, and surface chemistry for chemical modifications makes silk a promising biomaterial to be used in various fields such as skin regeneration, ophthalmic, dentistry, bone tissue engineering, hepatic, and neurological. Furthermore, the advancements of silk-based biomaterials in the different fields of biotechnology such as sensors, food coating and packaging, textiles, drug delivery, and even in cosmetics pave a path for new discoveries for the benefit of mankind. In conclusion, silk-based biomaterials offer a compelling platform for advancing medical technology and improving patient outcomes. Their unique combination of biocompatibility, mechanical strength, and controlled degradation make them valuable tools for a wide range of biomedical applications. As research in this field progresses, we can anticipate even more exciting developments and applications of silk-based biomaterials in the future.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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