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Review Article

Biomedical Applications of Silk fibroin: A Comprehensive Review

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Organ and tissue failure due to injury or disease presents a significant challenge in modern healthcare. Traditional repair strategies including organ transplantation, synthetic implants, and hybrid approaches are often limited by issues such as immune rejection, infection risk, and mechanical failure. Tissue Engineering and Regenerative Medicine (TERM), an emerging interdisciplinary field, aims to overcome these limitations by integrating cellular biology, materials science, and engineering to regenerate functional tissues. In dentistry, regenerative approaches are rapidly evolving, especially through the use of biomimetic scaffolds. Among various natural and synthetic scaffold materials, silk fibroin (SF), a protein derived from *Bombyx mori* silkworm cocoons has garnered increasing attention due to its excellent biocompatibility, tunable degradation rates, minimal immunogenicity, and robust mechanical properties. This narrative review explores the current state and future potential of SF-based biomaterials in biomedical and dental applications. SF is highly versatile, forming films, sponges, nanofibers, and 3D scaffolds, and can be combined with other polymers or bioactive molecules to enhance its functionality. It supports cellular adhesion, proliferation, and differentiation, making it suitable for hard and soft tissue regeneration. Despite its promise, clinical translation faces challenges such as ethical concerns, scalability, and complexity of fabrication processes. Recent advances in nanotechnology, such as 3D bioprinting and micropatterning, further highlight SF's potential to create structurally defined, biomimetic constructs at the micro- and nanoscale. In conclusion, silk fibroin stands out as a cost-effective, sustainable, and FDA-approved biomaterial with transformative potential in regenerative medicine and dentistry. Continued research and technological refinement are essential to fully harness its capabilities and overcome current limitations.

Introduction

The organ or tissue failure following an injury or disease remains one of the most challenging operations in healthcare worldwide. Traditional approaches to tissue and organ repair can be grouped into three categories: the biological approach involving organ or tissue transplantation; classical engineering approaches that utilize synthetic implantable materials or devices; and combination methods that integrate biological as well as the engineering techniques [1]. However, these approaches may carry the risk of transplant rejection, lifelong immunosuppressive therapy, infections, and mechanical failures, compromising their long-term effectiveness [2].

A novel interdisciplinary field namely the Tissue Engineering and Regenerative Medicine (TERM) combines expertise from cellular and molecular biology, materials science, and stem cell engineering. This discipline aims to regenerate and restore the function of damaged tissues for various conditions, ranging from minor injuries to severe organ damage [3]. Traditional treatment procedures like root canal therapy and vital pulp therapy have a negative influence over the success rate in addition to complicating the existing condition [4]. In dentistry, TERM is revolutionising the regeneration related treatment approaches [5].

Regenerative dentistry, a subset of TERM, focuses on the regeneration of the damaged structure. By involves three main components i.e, stem cells, growth factors and scaffolds presenting an environment for cell growth and differentiation [5]. Literature reports a variety of scaffold materials, ranging from naturally derived substances like collagen and chitosan to synthetic polymers such as polylactic acid (PLA) and polycaprolactone (PCL) [6].

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Though these materials have shown promising results in preclinical studies, each has its own set of limitations. Natural polymers can be expensive, exhibit poor mechanical properties, and be subjected to variability [7]. While synthetic polymers may degrade into acidic by-products that can cause adverse immune responses affecting the treatment outcomes [8].

In recent times silk fibroin (SF), a natural protein derived from silkworm silk, has been identified as a promising alternative biomaterial for various dental applications [9,10]11,12]. SF possesses several advantages like excellent biocompatibility, controllable biodegradability, minimal immunogenicity, and high mechanical strength [11]13]. It is adaptable to various formulations, including three-dimensional scaffolds, thin films, and nanofibers, and can be easily combined with other polymers to enhance its properties [12]14]. Additionally, SF is cost-effective, environmentally friendly, and readily available [13]15]. Studies have shown that silk fibroin scaffolds can effectively support cell adhesion, proliferation, and differentiation, and are well-tolerated in vivo with minimal immune response [8-10,14,15]-12].

This article will explore the potential of silk fibroin as a biomaterial in regenerative medicine and dentistry, highlighting its ability to overcome the limitations of existing materials and its promise in advancing tissue engineering strategies for dental care.

Silk Fibroin

Silks are protein polymers spun into fibres by certain species under normal conditions. Among the various silk producing organisms-spiders, bees, mites, etc., silkworms produce the majority of natural silk [14]. Discovered in China around 2700 BC and it is now cultured in Asian and European countries, primarily in China, India, and Japan. Silk has a long history of biomaterial applications, having been utilised as a surgical suture material for decades. It has lately been employed in numerous biomedical applications, including soft and hard tissue engineering scaffolds and medication delivery [13-15]. They can support the adhesion, proliferation, and differentiation of a wide range of cell types while remaining biocompatible. Silk fibroin (SF) is enzymatically biodegradable and can be transformed into porous sponges, injectable hydrogels, and water-insoluble implants. The ability of SF to improve vascularization while also providing robust anticoagulant action and platelet reactivity is appealing for tissue engineering in dentistry. It has great mechanical strength, elasticity, biodegradability, morphologic flexibility, oxygen and water permeability, and a moderate breakdown rate, which allows fibroin to be gradually replaced by freshly formed tissue. SF is less immunogenic and inflammatory, compared to either polylactic co glycolic acid (PLGA) or collagens [16-19].

Bombyx mori silkworm cocoon

Each cocoon comprises a continuous silk filament called bave, which ranges between 700 to 1500 meters long. The bave is typically split into two main proteins: fibroin, an internal structural protein, and sericin, an exterior glue-like protein [20]. The cocoon comprises fibroin (75%-83%), sericin (17%-25%) and other impurities such as carbohydrates, phytochemicals, pigments, etc (1%-4%) [21]. The amino acid residues contained in silk proteins could be functionally summarised into three classes: charged (aspartic acid), polar (serine), and hydrophobic (glycine) amino acids [22]. Silk fibroin comprises two chains, one light (Mw ~26 kDa) and one heavy (Mw ~390 kDa), connected by a disulphide bond. Silk fibroin is a block copolymer having hydrophobic β-sheet-forming blocks connected by tiny hydrophilic linker segments or spacers. The crystalline regions are primarily composed of glycine-X repeats (X= alanine, serine, threonine or valine). Within these domains there are the subdomains that are rich in glycine, alanine, serine and tyrosine [23]. This results in a hydrophobic protein that self assembles to form strong and resilient material. The fibroin structure's dominant β-sheet-forming regimes provide excellent mechanical strength and toughness to the material. As mentioned earlier, silkworm silk is superior to degradable materials like collagen, PLA. The ultimate tensile strength of B. mori silk fibers is 740 MPa whereas collagen has an ultimate tensile strength of 0.9–7.4 MPa and PLA 28–50 MPa [24]. Therefore, silk fibroin is an ideal polymer for biomedical purposes. Research indicates that sericin is responsible for allergic reactions in certain patients. Thus, sericin must be eliminated from the fibroin to ensure biocompatibility [25]. The inflammatory response to silk films was assessed in vitro using hMSCs or by seeding rat MSCs onto the films and implanting them in vivo. Silk fibroin elicited equivalent in vitro responses as collagen and tissue culture plastic controls. In vivo, silk had a reduced inflammatory response compared to collagen and PLA. In vitro testing with macrophages revealed that silk, like tissue culture plastic, had high quantities of tumour necrosis factor-α, a cytokine associated with inflammation [26].

Processing of Silk Fibroin

Natural silk requires minimal modification (e.g., dying or spinning) for textile applications; but for biomedical applications they require a specific morphology at micro or nano scale. Natural silk threads cannot be modified directly; they must first be dissolved. Once dissolved, it can be used to fabricate films, gels, nanofibers, spheres, etc [20]. The process of extraction of silk fibroin is best described by *Sofi et al.*

There are three main stages in the processing of silkworm silk –(i) Degumming of silkworm cocoons – removal of sericin, (ii) Dissolution in aqueous ionic solution – removal of inorganic ions and (iii) Fabrication of biomaterial with regenerated silk.

The silk cocoons are cut into small pieces and then treated with a boiling aqueous solution of 0.02M Na_2CO_3 for 30 minutes twice to remove sericin protein, a gumming substance, before being washed with deionised water to remove contaminants and allowed to dry in the air at room temperature. The degummed silk is dissolved in a 9.3M LiBr aqueous salt solution at 60°C for 3-4 hours. The solution is then dialysed using a dialysis cassette in deionised water for 3 days at room temperature to get rid of the ions. Finally, the obtained clear solution is centrifuged at 4000 rpm/ 20 minutes to further remove any silk aggregates and is stored at 4°C [21].

Biocompatibility and Biodegradation

The immunogenicity and antigenicity of silk scaffolds have been thoroughly investigated. Silk fibroin bio-conjugates have demonstrated a well-tolerated response in the therapy of musculoskeletal diseases [22]. For up to 8 weeks after subcutaneous implantation of electrospun fibre mats in rats, there is no evidence of infection, however there is some normal accumulation of phagocytes and lymphocytes. Furthermore, examination of haematoxylin and eosin-stained tissues revealed only mild inflammation [23]. When subcutaneously implanted in Lewis rats, silk 3-D scaffolds produce a minimal immune response after one year. Expression levels for immune response genes such as TNF-α, IFN-δ, IL-4, IL-6, and IL-13 are undetectable for most types of silk sponges [24]. A pig model used in ligament tissue engineering showed no evidence of malfunction after 24 weeks of *in vivo* culture [25]. Degradation is examined in animal models by measuring mechanical properties of silk after implantation for a certain period

of time and examining structural integrity using histological investigations, fluorescence staining, and different biochemical assays. As an implant, regenerated silk fibroin biomaterial degrades significantly faster than fibres. The degradation rate is determined by the secondary structure of silk resulting from the manufacture of regenerated silk materials [26]. Wang et al reported that upon implantation in Lewis rats, Water-based 3-D scaffolds deteriorated in a matter of weeks and disappeared entirely after a year. The host immune system has a major impact on the breakdown of 3-D silk fibroin porous scaffolds, and the degradation of silk sponge is demonstrated to be mediated by macrophages, indicating that silk is not only biodegradable but also bio-resorbable [27].

Applications of Silk Fibroin in Various Fields of Tissue Engineering

The versatility silk fibroin has enabled its application across a wide range of medical disciplines. In particular, silk fibroin has been explored for use in bone and cartilage tissue engineering, ligament and tendon repair, tympanic membrane regeneration, skin wound healing, vascular grafts, ocular surface reconstruction, regenerative dentistry, and even cancer therapy. These diverse applications underscore its potential as a promising biomaterial in both regenerative medicine and therapeutic delivery systems. Table 1 presents the biomedical applications of silk fibroin across various clinical fields, highlighting specific use cases and key benefits. The general characteristics of the studies included in this review, encompassing the various biomedical applications of silk fibroin, are summarized in table 2.

Bone and cartilage tissue engineering

Bone and cartilage tissues are classified as connective tissue. Bone is a hard structure that has the vital function of sustaining muscle contraction, acting as a mineral deposit, and protecting specific internal organs structurally [28]. On the other hand, the cartilage tissue is not vascularised nor innervated and has limited regeneration potential. With continuous friction and pressure, it can lead to wear and damage. SF can be employed for such damages [29]. For bone tissue engineering, scaffold materials must be designed to provide matrix toughness and permit ECM deposition. SF has been extensively researched in bone TE and has demonstrated great toughness, mechanical strength, and biocompatibility [30]. For instance, it has been demonstrated that RSF scaffolds promote osteogenic differentiation of human mesenchymal stem cells (HMSC) in vitro. They have been shown to heal femoral defects in vivo in nude rat models [31]. Recently, Wu et al. produced PLLA/SF composite nanofiber mesh by electrospinning, and coated osteoblast-derived extracellular matrix (O-ECM) on the nanofiber scaffold. In vitro tests have demonstrated that the new nanofiber scaffold (O-ECM/PLLA/SF) significantly enhanced the osteogenic differentiation of cultured stem cells [32]. A number of materials, such hydroxyapatite (HA) and tricalcium α-phosphate (α-TCP), have been utilised to create bioplastics for cell scaffolds; nevertheless, it's interesting to note that throughout time, it has been demonstrated that incorporating silk into these substances enhanced the characteristics of the final product [33]. Compared to pure fibroin scaffolds, the SF-hydroxyapatite (HAp) nanocomposite has demonstrated higher mechanical strength and cytocompatibility. Bi et al. modified a silk-collagen scaffold with HAp at both ends. Results showed that the modified group outperformed the silk-collagen group in terms of osteoarthritis prevention,extensive formation of more mature bone at the tendon-bone interface, increased collagen I and osteocalcin deposition, and bone mineral formation [34]. In another study, bone clips with good biocompatibility and satisfactory mechanical qualities were made from a three-component bioplastic made of polylactic acid, hydroxyapatite, and silk [35].

In SF-based scaffolds for cartilage and osteochondral tissue regeneration, pore size and porosity had a major impact on cell adhesion and penetration. Pore sizes smaller than 300 μm promoted osteogenesis while those larger than 300 μm aided in endochondral ossification [36]. Scaffolds made of SF may function as a releasing system to promote cartilage development. Wu et al., developed an Rb1/TGF-β1 loaded SFgelatin porous scaffold (GSTR). It enhanced hyaline cartilage regeneration *in vitro*, suppressed inflammatory levels in vivo, and promoted chondrogenesis by creating an environment for cartilage regeneration [37]. In comparison to collagen-only dense mats, electrospun RSF–collagen dense mats seeded with MSCs demonstrated enhanced chondrogenic differentiation of MSCs and promoted expression of cartilaginous matrix. Wang et al. fabricated porous RSF–collagen scaffolds combined with poly-lactic-co-glycolic acid (PLGA) microspheres which demonstrated strong cell affinity and promoted the growth of articular cartilage in rabbits [38]. Sharafat-Vaziri et al. conducted a preliminary clinical study on two patients with osteochondral lesions in the knee using engineered tissue composed of autologous chondrocytes and collagen/SF scaffold. Clinical data has shown that the SF-based scaffold is safe and useful for repairing large chondral lesions [39].

Ligament and tendon regeneration

Ligament and tendon tissues are composed of collagen and fibrocytes which are made up of a dense fibrous connective tissue that can be rapidly damaged and has a very limited capacity for natural regeneration [40]. The anterior cruciate ligament is a crucial part of the knee joint (ACL). An ACL injury can result from improper sports practice or an excessive amount of external force. This injury causes instability and progressive deterioration to the knee joint. It has been established that SF can promote the

Table 1: Biomedical applications of silk fibroin across various clinical fields, highlighting specific use cases and key benefits

Application Field	Specific Use Cases	Key Benefits
Bone and Cartilage	Scaffolds for osteogenesis and chondrogenesis	Enhanced mechanical support, promotes mineralization
Ligaments and Tendons	ACL repair, scaffold-assisted regeneration	Promotes cellular migration and long-term joint stability
Tympanic Membrane	Repair of TM perforations	Promotes keratinocyte growth, biodegradable, acoustic match
Skin	Wound healing, burn treatment, skin substitutes	Mimics ECM, promotes re-epithelialization
Vascular	Small-diameter vascular grafts	Supports endothelialization and remodeling
Ocular	Corneal epithelium, limbal stem cell carrier, PSR	Biocompatible, promotes regeneration
Regenerative Dentistry	Dentine-pulp complex, bone grafts, barrier membranes	Supports stem cell growth, mineralization
Cancer Therapy	Nanoparticles for drug delivery, tumor models	Targeted delivery, reduced systemic toxicity

Table 2: General characteristics of the studies included for the biomedical applications of silk fibroin

Author/Year	Study design	Scaffold composition	Application	Study outcome
G.H. Altman et al/ 2002	In vitro	Silk fibres	Anterior cruciate ligaments TE	Silk fibre matrices provide better mechanical properties, biocompatibility, slow degradability, and support differentiation
Fan et al./ 2009	Animal study (pig model)	Pure silk fibroin	Anterior cruciate ligaments TE	Remarkable scaffold degradation, the maximum tensile load of regenerated ligament could be maintained after 24 weeks of implantation
Etienne et al./ 2009	In vitro and In vivo Study	Pure silk fibroin	Soft tissue augmentation	Silk-gel material created three dimensional soft tissue augmentation and is promising for periodontal and maxillofacial therapies.
R. Ghassemifar et al/ 2010	In vitro	SF membrane seeded with human tympanic membrane cells	Tissue engineered replacements for the tympanic membrane	Cell proliferation analysis revealed that SF is a suitable substratum for growing tympanic cells when compared to a commercial tissue-culture plastic
J. Liu et al/ 2012	In vitro	SF film	Substrate for corneal wound repair and tissue-engineering	Could support corneal epithelial cells to proliferate, differentiate, and stratify, retaining the normal characteristic epithelium phenotype
Lozano et al./ 2014	In vitro	Silk fibroin and graphene oxide	Tissue engineered replacements for the tympanic membrane	Combination of human dental stem cells/fibroin/GO based-bioengineered constructs have strong potential in regenerative dentistry.
Yang et al/ 2015	In vitro and In vivo Study	SF scaffold alone; bFGF-incorporated SF scaffold	Dental pulp tissue engineering	Significantly promoted the cell viability of DPSCs and the generated dentin-like tissue
Xie et al/ 2016	In vitro and In vivo study	Electrospun SF/ gelatin nanofibres	Skin anterior cruciate ligaments TE - skin re-epithelialization	Promising scaffold for the stem cells transplantation in skin TE
J.Wang et al/ 2016	In vitro and In vivo study	SF/Collagen scaffold incorporated with PLGA microsphere	Cartilage tissue engineering	Collagen/SF composite scaffold with PLGA microspheres could enhance articular cartilage regeneration and integration
Mottaghitalab/ 2017	Animal study (mice model)	Gemcitabine loaded silk fobroin	Drug delivery for cancer therapy	Higher potential of targeted Gem-loaded SFNPs in treating induced lung tumor
Rider et al./ 2018	In vitro	Silk fibres	Dental implantology	Excellent biocompatibility, mechanical properties, degradation rate, and non-toxic degradation by-products make it ideal for dental barrier membrane.
Jizhao Li et al/ 2018	In vitro	SF + Chitosan scaffold	3D tumor models for evaluation of anti-cancer drugs	3D scaffolds showed in vivo tumor-like morphological and biological characteristics. Accurate predictions in therapeutic efficiency
Zhang et al/ 2019	In vitro and In vivo Study	HAp/SF composite scaffolds labeled USPIO	Dental pulp tissue engineering	Composite scaffolds performed good biocompatibility, biomechanical properties with ability of promoting regeneration of dental pulp tissue
Sun et al./ 2019	In vitro Study	Doxorubicin & folic acid loaded silk fibroin	Drug delivery for cancer therapy	The specific binding of fibroin particles to folate receptors provides the particles to with target functions to the tumor cells
Zhi et al/ 2019	In vivo	Silk/PET hybrid [polyethylene terephthalate (PET)]	Ligamentization of the PET artificial ligament in a canine ACL reconstruction model.	Silk/PET hybrid ligament kept greater ability to induce the ingrowth of the autologous tissue, indicating that the silk hybrid had enhanced the ligamentization of the PET artificial ligament.
Sharafat-Vaziri, Arash et al/ 2020	pilot clinical trial study	SF/Collagen with autologous chondrocytes	Cartilage anterior cruciate ligaments TE - repair of osteochondral defects.	MRI showed great coverage and integration of the graft in patients, with no effusion, decreased edema and cartilage formation signals.
Shimada et al/ 2020	In vivo	Silk fibroin/ polyurethane/ SVVYGLR peptide	Angiogenesis promoting	This study suggested that SVVYGLR peptide could give the angiogenic-promoting activity to silk fibroin-based vascular repairing sheet
A. Moin et al/ 2021	In vitro	SF nanoparticles loaded with selective estrogen receptor modulator; tamoxifen citrate	Delivery of anti-cancer drugs (Breast cancer)	Initial burst effect followed by a sustained release for 48 h. showed cytotoxic efficacy against breast cancer cell lines. TC-SF-NPs are expected to efficiently restrain tumor progression at a lower dose than required for conventional oral tamoxifen treatment
Wu et al/ 2021	In vitro study	PLLA/SF electrospun nanofiber scaffold coated with osteoblast derived ECM	Bone tissue engineering	Enhanced the osteogenic differentiation potential of BMSCs
Jiang et al/ 2021	In vitro	Type I collagen/ silk fibroin	3D tumor models for evaluation of anti-cancer drugs	Biocompatible and stimulates the adhesion and proliferation of HDPCs. The scaffolds with the aperture sizes of CSF1 and CSF-2 enhanced the adhesion and ALP activity of HDPCs
Man et al./ 2022	In vitro	MI192 pre-treated hDPSCs with porous Bombyx Mori-lyophilised silk scaffolds	Bone tissue engineering	Scaffold constructs enhanced the vascularisation and extracellular matrix mineralisation. demonstrate the potential to promote hDPSCs bone formation efficacy
Yule Xu et al/ 2023	Animal study (rabbits)	Regenerated silk fibroin (RSF) hydrogels	Posterior scleral reinforcement	RSF hydrogel exhibits excellent biocompatibility and is safe for all layers of the retina and optic nerve. Possess superior biomechanical properties.

differentiation of adult stem cells into ligament lineages [41]. In order to facilitate the regeneration of tendons and ligaments, researchers began creating knitted scaffolds based on SF. Liu et al., for instance, created web-like SF sponges on knitted scaffolds that were seeded with HMSCs; these scaffolds exhibit higher cellular activity in comparison to RSF hydrogel knitted scaffolds. The findings showed that while web-like microporous RSF sponges can increase cellular activity, SF-based knitted scaffolds proved to have structural strength [42]. Shen et al. utilised a rabbit model of anterior cruciate ligament injury to study the long-term repair impact of silk-collagen scaffolds. Two months following surgery, spindle-shaped cells were seen migrating and adhering to the scaffolds. A more improved microstructural morphology was seen after six months. Furthermore, for eighteen months following surgery, the knitted silk-collagen sponge scaffold successfully shielded the articular surface cartilage and maintained the joint space [43]. A three-phase scaffold composed of collagen and SF that mimics the natural tendon bone structure of a was recently developed by Geng et al. It promoted tendon development and showed good biocompatibility in cellular studies [44]. Incorporating cells into the SF-based scaffold prior to implantation to guide ligament bone insertion was another technique used for ligament regeneration. The tensile strength of scaffolds and MSCs may meet the mechanical requirements of daily operations [45]. Jiang et al. employed SF to modify the surface of polyethylene terephthalate (PET) [used in the ligament advanced reinforcement system (LARS) for posterior cruciate ligament replacement] to change its hydrophilicity and biocompatibility. In vitro experiments confirmed that SF coating improved cell adhesion and proliferation, as well as the material's biocompatibility and "ligamentization" process [46]. In another study, a silk hybrid on the ligamentization was developed for a canine ACL reconstruction. In comparison to the PET artificial ligament, the regenerated ligament in the silk/PET hybrid group had a compact structure and more regenerated autologous tissue and collagen [47].

Tympanic membrane regeneration

Tympanic membrane (TM) is a transparent structure situated between the outer and middle ear, whose functions include receiving sound vibrations and protecting the middle ear. It is composed of three layers - epidermal outer layer, fibrous middle layer and mucosal inner layer, which mainly consist of keratinocytes, fibroblasts, and collagen (type II and type III). Typically, traumatic ruptures caused by pressure bursts and mechanical trauma induce TM perforations, as well as middle ear infections. If the rupture doesn't heal itself within 3 months, it will develop a chronic perforation, which can cause hearing loss and recurrent infections [48,49]. SF is an ideal material for tympanic membrane tissue regeneration as it supports the growth and spread of keratinocytes derived from human TM cells. Shen et al. observed that SF films implanted in rat and guinea pig models repaired TM perforations and accelerated TM regeneration, indicating faster hearing recovery. Furthermore, the films showed no significant macrophage response in host tissue, less inflammation, and was degradable in vivo [50-52]. According to Allardyce et al. SF membranes have good tensile strengths to cartilage and good acoustic energy transfer capabilities and thereby serve as excellent materials for regeneration of chronic TM perforations in vivo [53].

Skin regeneration

The skin's primary layers, the epidermis and dermis, are made mostly of keratinocytes and extracellular matrix (ECM), which especially consists of collagen and elastin. Severe burns, for example, might result in a significant loss of skin integrity, which can cause disability or even death [54]. It has been demonstrated that RSF biomaterials impact keratinocyte and fibroblast attachment [55]. Two forms of three-dimensional fibroin structures - nanofibrous mats and microporous scaffolds - are used to produce medical materials based on silk that are therapeutically effective and, consequently, widely accessible. These structures are then coated or mixed with particular chemicals to provide them bioactive properties [56]. Researchers have created an SF based fibrous scaffold to deliver stem cells to rats with burn wounds as part of their scientific investigation into wound healing. In this study, SF containing stem cells closely mimicked the physicochemical and biochemical properties of the natural extracellular matrix (ECM) while providing a vast surface area, cellular behaviour guidance, and scar removal. The findings suggest that SF's architectural characteristics offered an early biomimetic system for stem cell development [57]. Another study also showed that the use of an SF nanofibrous scaffold loaded with mesenchymal stem cells (MSCs) and epidermal stem cells can significantly accelerate the synthesis of collagen and skin re-epithelization; additionally, the histological characteristics and skin appendages of the reconstructed skins resembled those of normal rat skin [58]. The development of SF scaffolds with antibacterial qualities for use as wound dressings has attracted an increasing amount of research attention in recent years [59,60]. Sen et al. immobilised SF onto polyurethane (PU) scaffold surfaces. At 8 mg/mL, SF was able to limit the growth of K. pneumonia, a bacterium that is present in wound infections [61]. However some of the antimicrobial substances used in SF dressings might have negative side effects. Achieving the right balance between the antibacterial characteristics and biocompatibility remains a difficulty. Genetic engineering was utilised to create more effective based SF wound healing products and it was observed that minimal scarring could be achieved by producing inexpensive artificial skin with additional properties using genetically modified SF-based scaffolds [62].

Vascular regeneration

Based on existing studies, a significant amount of circulatory system damage can be repaired through transplants employing polymer-based materials such as SF. But not every polymer is suitable for this kind of graft as the material needs to fulfill a number of requirements before being implanted into an artery or other channel, most important being thrombogenicity and patency index [63]. In one study, SF along with glycidyl methacrylate (Sil-MA) hydrogel was prepared to build small-diameter blood vessels. It showed good mechanical and rheological properties and was considered to be a potential treatment option of vascular injuries in the brain and ear [64]. Another combination of SF with polyurethane and the SVVYGLR peptide was implanted in the abdominal aorta of rats and tested for angiogenicity. Results confirmed the presence of endothelial cells, smooth muscles and fibroin [65]. SF with polyethylene terephthalate (PET) was studied as a material for blood vessel grafts with a diameter less than 6mm. It was tested on 24 rats (12- PET coated with SF glycine, 12- PET coated with gelatin). In the SF graft, fibroblasts, collagen fibres and blood vessels were observed, thereby confirming its ability to remodel into native tissue [66].

Ocular regeneration

Ocular disorders account for substantial rates of global vision loss. Silk has recently emerged as a biomimetic material of interest for tissue engineering approaches aimed at regenerating, repairing, or replacing various ocular structures [67]. The researchers Harkin et al. hypothesized that silk fibroin could be used as a biomimetic material for the bioengineering and potential regeneration, repair, or replacement of various complex ocular structures, including the

corneoscleral limbus, corneal stroma, corneal endothelium, and Ruysch's complex [68]. Liu et al. replaced the human amniotic membrane with silk fibroin because of the material's transparency, which may help prevent problems with mechanical stability, disease transmission, and transparency [69]. It has been demonstrated that the topographic features on the silk surface, particularly the groove pattern, may help to guide human corneal fibroblasts and stem cells as they form a corneal-like stromal construct. Furthermore, it has been found that the RGD features incorporated into the silk promote corneal stromal cell growth and multiplication [70]. Xu et al. developed RSF hydrogels posterior scleral reinforcement (PSR) to prevent the progression of high myopia. RSF hydrogels demonstrated strong biocompatibility and promoted the in vivo development of fibrous capsules at the posterior sclera. The biomechanical properties of the reinforced sclera were enhanced [71,72]. In another study, patients with inadequate limbal stem cells exhibited decreased corneal clarity and increased neovascularization. The development of silk fibroin with improved surface roughness and tensile strength, which could tolerate surgical manipulation, was achieved by blending silk film with polyethylene glycol (PEG) and then removing the PEG [73,74]. Research using a rabbit model lacking limbal stem cells showed that corneal epithelium was regenerated and new blood vessel growth was prevented by limbal epithelial stem cell/silk fibroin grafts. This shows that normal corneal epithelium may be produced from cultured limbal epithelial stem cells to replace damaged or diseased epithelium [75].

Regenerative dentistry

Silk fibroin-based biomaterial has been found suitable for periodontal, maxillofacial therapies and endodontic therapies, as a cell scaffold or on its own [76]. Owing to its high biocompatibility and promotion of cell growth, silk fibroin is evolving to be a promising biomaterial for tissue engineering in dentistry [77]. Currently, the commercially available barrier membranes for oral surgery are either non-resorbable and require a second surgery for their extraction or they are resorbable but have poor structural integrity and are yet to achieve a perfect design. Rider et al. had demonstrated reactive inkjet printing as an efficient method for modifying silk into regenerated silk fibroin form (RSF). It can be utilised to alter the structural properties of RSF and gradually induce crystallinity. The possibility to incorporate bioactive components, such as nHA, into printed films enhances inkjet-printed films' potential to aid in site repair and healing process [78]. An invitro study showed that hDPSCs have the potential to form mineralised matrix when grown on porous 3D silk fibroin scaffolds for applications in bone regeneration.

Regenerative endodontics is becoming a highly preferred treatment with the development of bioactive materials and tissue engineering technologies, as dentine-pulp complex regeneration is identified to be a definitive procedure to repair and restore dental function [79,80]. Jiang et al. suggested that the type I collagen/silk fibroin scaffold material prepared by low-temperature deposition 3D printing is appropriate for dentine-pulp complex regeneration. Furthermore, this scaffold was biocompatible and enhanced the adhesion and proliferation of hDPCs. The scaffolds with the aperture sizes of 421.27 μm and 579.36 μm enhanced the adhesion and upregulate the ALP activity of hDPCs [81]. Another study for pulp regeneration with basic fibroblast growth factor (bFGF) and DPSCs in freeze-dried porous silk fibroin scaffolds generated pulp-like and vascularization, new matrix deposition and dentin-like tissue formation was observed histologically [82]. *Zhang et al* designed an ultrasmall superparamagnetic iron oxide (USPIO)(0.025mg/ml)-labeled hydroxyapatite (HA)(5mg/ml)/silk fibroin (SF) scaffold-loaded dental pulp stem cells (DPSCs) which showed stable physical properties, accurate MRI images, and low cytotoxicity in vitro. *In vivo* study revealed revascularization and mineralization, thereby providing another promising scaffold for pulp tissue regeneration [83]. Recently several invitro studies have shown that silk fibroin support the adhesion and proliferation of mesenchymal stem cell.

With regards to bone tissue engineering, current gold standard therapies to repair critical-sized bone defects, autografts face limited supply, and morbidity at the donor site [84]. Man et al., investigated the potential of a selective HDAC2 and 3 inhibitors, MI192, in promoting hDPSCs bone-like tissue formation in vitro and in vivo within the silk scaffolds. It promoted bone ECM deposition and mineralization as well as enhanced the vascularization [85]. Implant-related infections are among the most feared consequences of orthopaedic and dental surgery. When the medical device is implanted in the host, it creates an ideal environment for pathogens to attach and colonise its abiotic surface. When microorganisms adhere to the implant surface, they secrete a thick, extracellular polymeric matrix that protects against immune system attacks and antibacterial therapies [86,87]. Sideratou et al synthesised a novel, antibiotic-loaded, functionalized nanoparticles (AFN) -based on carboxylic acid functionalized hyperbranched aliphatic polyester (CHAP) that can be integrated into peptide-enriched silk fibroin (PSF) matrices with osteoconductive properties. The biocompatibility and microbiological tests confirm it to be a promising material against implant-related infections [88,89].

Cancer therapy

Currently, chemotherapy is the primary treatment of choice for a variety of cancers. But one of the biggest disadvantages is its high toxicity. As a result, there is a significant demand for the development of medications with lower toxicity. SF offers tremendous potential as a drug delivery system given its unique features. Mottaghitalab et al. encapsulated gemcitabine in SF nanoparticles to target lung cancer cells. Animal studies revealed that the SF nanoparticles could reduce the side effects of gemcitabine [90]. Similarly, Sun et al. encapsulated doxorubicin in SF-based nanoparticles and they covalently grafted the surface with folic acid. The nanoparticles did not inhibit the cell proliferation or viability and could target the tumour cells. Doxorubicin was also released in a controlled manner [91]. Moin et al. synthesised nano and micro-fibroin-based particles as drug delivery systems for encapsulating doxorubicin. They used two types of cancer cell lines (SAO-S2, MCF-7) and one normal human cell line (HFF). MTT cytotoxicity assessment revealed that cell growth was inhibited. The p53 gene expression study showed that the doxorubicin-loaded SF microparticles decreased p53 expression in the SAO-S2 cell line and significantly increased it in the MCF-7 and HFF cell lines. They also observed the ROS levels and found that it was lower in MCF-7 and HFF cell lines [92]. In another study, SF-based double-layer microneedles were developed successfully for the encapsulation and administration of triptorelin (a drug widely used in the treatment of prostate gland carcinomas) in a controlled release manner [93]. Moreover, SF is now employed as a tumour model to study cancer biology which can provide valuable insights [94,95].

Conclusion

Silk fibroin is proving to be a promising biomaterial for future regenerative techniques in medicine. Dentistry may benefit greatly from the various silk fibroin-derived materials that are now being tested, given the vast majority of studies focus on the regeneration of osteodental tissues. Nevertheless, there are limitations, including the procedures' complexity, ethics issues, potential treatment complications, etc. Silk Fibroin, derived from silkworm cocoons, is

an FDA-approved biomaterial that has gained widespread acceptance for use in TE applications due to its unique biomedical properties, mechanical performance, and tunability. Recent breakthroughs have placed a strong emphasis on bio nanotechnology tools such as micropatterning and 3D bioprinting to create SF multi-level structures with great structural definition down to the nanoscale. Many studies have demonstrated that this improves cell proliferation, differentiation, migration, and adhesion. Overall, silk is a versatile biomaterial with several intriguing applications in TE.

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