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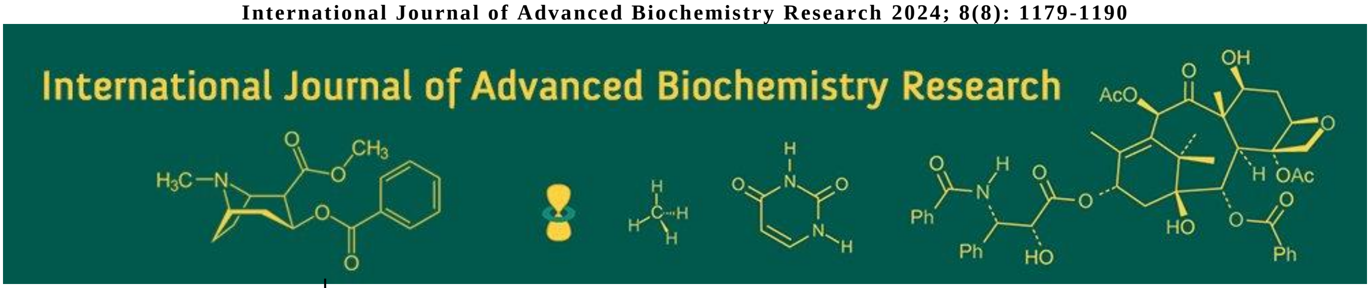

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Jasmeena Qadir
Division of Sericulture,
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Jammu,
Jammu and Kashmir, India

Shalini Aryan
Department of Agriculture,
Mata Gujri College, Fatehgarh
Sahib, Punjab, India

Somagaini Pavankumar
State Department of
Sericulture, Govt. of Andhra
Pradesh, Lakkireddypalli
Mandal, Andhra Pradesh,
India.

Sumiya Afreen
Division of Sericulture,
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Jammu,
Jammu and Kashmir, India

Corresponding Author:
Jasmeena Qadir
Division of Sericulture,
Sher-e-Kashmir University of
Agricultural Sciences and
Technology of Jammu,
Jammu and Kashmir, India

Potential of silk fibroin in biomedical science

Jasmeena Qadir, Shalini Aryan, Somagaini Pavankumar and Sumiya Afreen

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Abstract

Silk fibroin is a natural biopolymer produced by many sericigenous insects *viz.*, silkworms and spiders. Owing to its remarkable biological and mechanical attributes employs its wider utilization in biomedical science in addition to textiles. The efficient biocompatibility, biodegradation, unique mechanical features makes it a facile stuff effective for tissue regeneration, tissue engineering's and small molecular and drug deliveries. Silk fibroin have been designed in various models *viz.*, silk films, silk hydro-gels, silk 3-dimensional porous scaffolds, non-woven scaffolds, particles, silk fibers or silk composites for exploring their functionalization in different biomedical approaches. The fabrication of ideals designs of Silk fibroin biomaterials have been devised to improve the functional integrity and physiological relevance in tissue engineering techniques. These innovative SF tools represent absolute solutions for different clinical problems of tissues, bones, cartilage, heart, vascular system, nerves and skin regeneration. SF has been developed as an efficient drug and small molecular carrier/ vehicle for treatment of dreadful and challenging clinical problems like cancer. The current review discusses the topics pertaining to characteristics of silk fibroin and SF based biomaterials developed and evaluated for wider utilization in biomedical treatment of cardiac, neuron, skin, bone, liver and tooth related problems. The emphasis has been laid on perpetual use of effective silk fibroin based materials in future biomedical clinical care.

Keywords: Bone, dental, drug delivery, fibroin, scaffolds, tissue engineering

1. Introduction

The global jumps in tissue substitute demand have pressurized the medical management to adopt artificial regeneration and repairing strategies which pose a major problem (Sun *et al*., 2021) [98]. The most critical tissue imperfections are repaired by autografts and allografts but the scarcity of donors and higher risk of infections indicate the necessity of utilizing synthetic materials (Pihlman *et al*., 2018) [82]. However, it is essential for the synthetic structures to perform as primordial tissue without altering its microenvironment (Henning *et al*., 2021) [35]. Many biological polymers *viz*., *cellulose*, chitosan and collagen have been found as appropriate alternatives (Good *et al*., 2009; Muñoz-Bonilla *et al*., 2019) [28, 72]. In context to biomedical approach, biocompatibility and biodegradability with improved mechanical attributes of biomaterial is of utmost importance (Chakraborty *et al*., 2022; Armengol *et al*., 2024) [11, 2]. Silk was used as luxury garment, ornaments and surgical suture in medical since 150 AD (Muffly *et al*., 2011; Thilagavathi and Viju, 2015) [71, 103]. The potential properties and applications of silk fibroin are depicted in Figure 1 and 2.Silk is a fibrous protein crafted through the spinning characteristic of many sericigenous insects' *viz*., domestic silkworm (*Bombyxmori*), wild silkworms (*Antheraea species*) and Spider (*Nephilaclavipes*) (Pereira *et al*., 2015; Mahanta *et al*., 2023) [80, 60]. The production of spider silk is unachievable task (Tokareva *et al*., 2013; Yip and Rayor, 2014) [104, 129]. The mulberry silkworm dominates the status of producing silk in bulk (Fang *et al*., 2016) [23]. It involves different developmental stages, egg, larva, pupa and adult (Pereira *et al*., 2015; Babu, 2017) [80, 3]. It feeds on mulberry leaves to construct protective shelter, silken cocoons as defence against microbes, predators and harsh environment (Muzamil *et al*., 2023) [73]. The silk fibres are woven in an intricate model naturally by spinning (Huang *et al*., 2018) [41]. The noble silkworm is literally sacrificed by killing inside the pupa for unravelling the cocoon into silk fibres (Young, 2019) [127].

Silk fibre constitutes two structural proteins, fibroin (72-81%) and sericin (19-28%) (Koh *et al*., 2015) [50]. Fibroin being the inner core provides mechanical strength and sericin acts as outer gluey coating fibroin (Silva *et al*., 2022) [90]. Fibroin is composed of smaller units known as nano-fibrils which form the building blocks of silk. Many nano-fibrils assemble as a strong interlocking unit to form larger fibril units known as micro fibrils (Ling *et al*., 2018; Wang and Schniepp, 2019) [55, 108]. The nano-fibrils and micro fibrils get arranged in parallel to form twisted bundle of silk microfiber with excellent mechanical property (Niu *et al*., 2018) [75]. Structurally, silk constitutes heavy chain, a light chain and a glycoprotein, p25. The heavy chain (390 kda) specifies fibroinwhich is linked with light chain (26 kda) by a single disulfide bond in a ratio of 6:6:1 (Inoue *et al*., 2000; Reizabal *et al*., 2023) [42, 86]. These proteins are non-covalently linked to disulphide linkage of cys-c20 (heavy chain), cys-172 (light chain) and p25 (glycoprotein) (Inoue *et al*., 2000; Zafar *et al*., 2015) [42, 132]. Heavy chain is fibrous protein responsible for silk fiber properties while as light chain essentially secretes silk from silk gland (Kundu *et al*., 2014) [51]. Silk proteins are fascinating due to biocompatibility, cbiodegradability, self-assembly, mechanical stability with controllable structure and morphology (Liu *et al*., 2005; Nguyen *et al*., 2019) [56, 76]. This intricate natural structure proved as excellent material for tailoring technological materials with defined functions in biosensors, electronics, tissue engineering and drug delivery systems (Melke *et al*., 2016; Gou *et al*., 2019 [66, 30]. The full potential of this ancient material fibroin in modern technology have been approved in the fields of nano science and nanotechnology (Nguyen *et al*., 2019) [76]. Throughout this article, we intended to throw light on significance of silk fibroin and its potential applications in biomedical science.

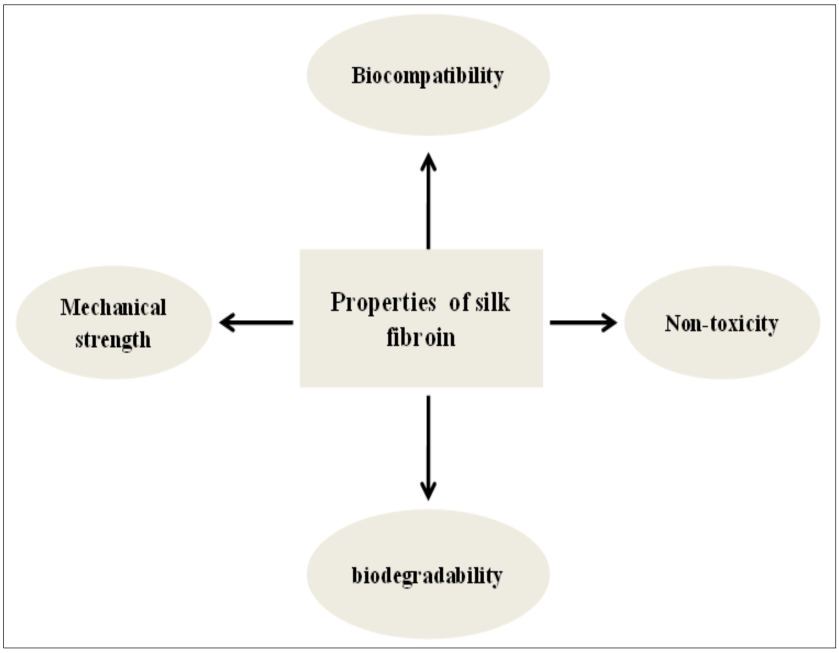

Fig 1: Illustration depicting different properties of silk fibroin.

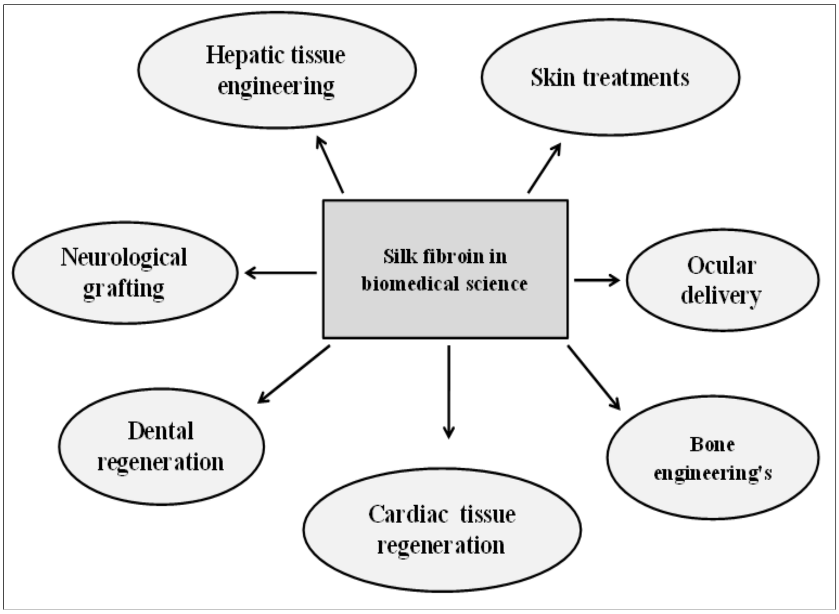

Fig 2: Biomedical applications of silk fibroin.

2. Biomedical Approaches Employing Fibroin

Drug delivery systems are conspicuously leading strategies for their efficacy and safety (Yu *et al*., 2023) [130]. In context of drug delivery, silk fibroin possess remarkable features, *viz*., extension of half-life of drugs, reduction of dosage, toxicity and frequency of drug administration with improved drug accumulation and curability in addition to feasibility of wide range of drug administration routes (Chen *et al*., 2017; Hu *et al*., 2020; Zhao *et al*., 2021) [137, 38, 13]. Thus, silk fibroin is becoming a viable biomaterial to be utilized in drug delivery. The silk fibroin dissolution technique utilizes different solvents such as, lithium bromide, zinc chloride, calcium nitrate and many others. Many limiting factors *viz*., solubility strength, reaction time and temperature necessitate the need of effective and sustainable technique which generates better yield (DeGiorgio *et al*., 2024) [19]. The purification of silk fibroin by dialysis method considerably yields pure aqueous silk fibroin solution as illustrated in figure 4. The silk fibroin can be fabricated into a wide range of frameworks with defined characteristics such as hydrogels, porous scaffolds, rods, fibers, and nanoparticles (Zhao *et al*., 2015) [138]. Different types of fibroin frameworks are depicted in Figure 3.It has been extensively studied as drug carrier for antitumour activity against breast cancer cell lines(Al saqr *et al*., 2021) [1], HIV (Crakes *et al*., 2020) [18], vaccine (Stinson *et al*., 2020) [94] and treating cancer (Xu *et al*., 2019; Zuluaga-Velez *et al*., 2021) [121, 141]. Silk fibroin has been utilized as electro-spinning-fibres in drug delivery as well (Farokhi *et al*., 2020) [24]. Tissue engineering is an outstanding way to revitalize bone, tissue, ligament and cartilage (Melke *et al*., 2016). It is the most viable option in present clinical procedures for repairing tissue and bone damages. SF-based devices are fabricated to aid in cartilage tissue regeneration, artificial vascular grafts for treating cardiovascular ailments, devising of artificial skin grafts, electro-spun silk fibers as insulation material for treating nervous system ailments, SF sponges to treat pancreatic diseases (DeGiorgio *et al*., 2024) [19]. In addition to other biomedical applications such as biomedical implants to treat breast cancer, hernia, abdominal wall defects, suture material in surgical procedures (Pasquier *et al*., 2023; Mehrabi *et al*., 2023) [78, 64]. Different types of silk fibroin based biomaterials have been devised to perform different functionality in clinics and biomedical sciences (Table 1).

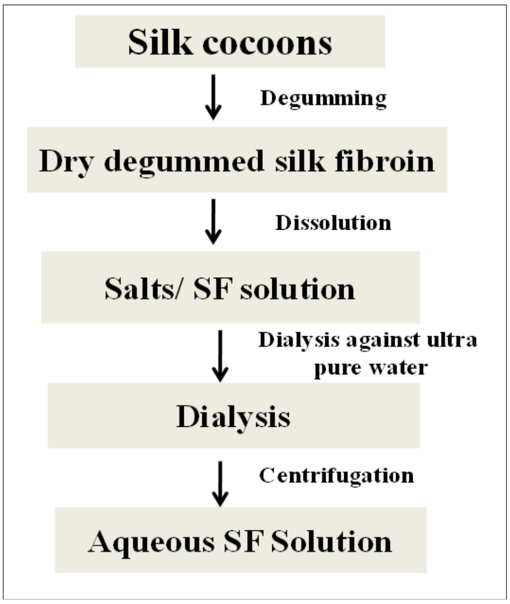

Fig 3: Schematic representation of silk fibroin purification

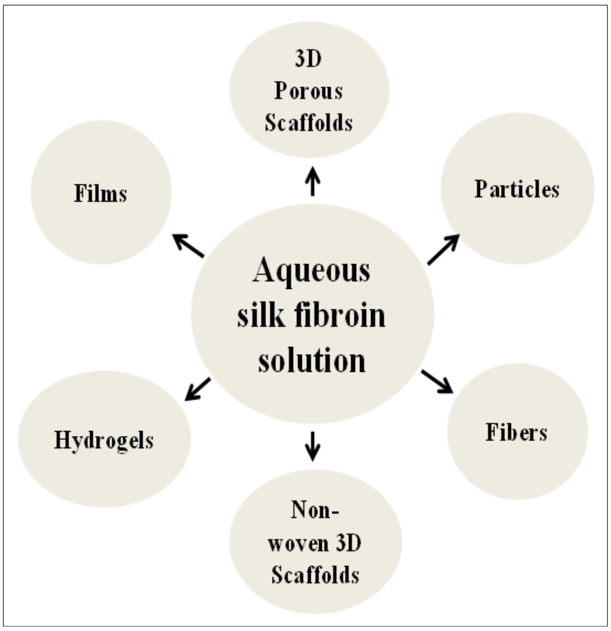

Fig 4: Illustration of silk fibroin based biomaterials prepared from aqueous SF solution

3. Properties of Silk fibroin

The innate capability of silk fibroin signifies its remarkability in mechanical strength, vigorous solubility and biodegradability. The secondary structure of silk fibroin can be modified to embark its external features for the applicability in biomedical science (Nguyen *et al*., 2019) [76].

3.1 Biocompatibility

Biocompatibility is one of the most challenging standards to be attained by materials for medical use. It is very limited characteristic utilized according to prior requirements of its use (Williams, 2008) [114]. Silk has been proved as clinically approved biomaterial for human consumption in many pre-clinical studies. The potential of silk fibroin in biocompatibility was first investigated in 1995. The silk fibroin of *Bombyx mori* manifested remarkable attachment to access the growth and nourishment of fibroblast cells (Melke *et al*., 2016) [66]. Earlier, silk was extensively used as suture material due to its superior biocompatibility and was further used for wound dressing and other clinical uses (Padol *et al*., 2011) [77]. Food and Drug Administration (FDA) approved fabrication of many silk based materials *viz*., surgical mesh Seri scaffold and Seri ACLTM due to their prolonged utilization as bioresorbable material and ligament graft respectively (Wharram *et al*., 2010) [112]. The fabrication of silk based scaffolds revealed extremely low or negligible immune response upon implantation (Meinel *et al*., 2005; Gou *et al*., 2019) [65, 30].

3.2 Biodegradation

Biodegradation is the important aspect of tissue engineering. It may be defined as the ability of a biological material to disintegrate and isolate the implanted materials when new tissues are generated (Kundu *et al*., 2013) [52]. It is the important event in tissue engineering for the easy removal of implanted device after use. Silk possesses excellent biodegradability regulated by proteolytic inhibitors and depends on many factors *viz*., cystallinity, degradation time extrinsic pressure, material composition and texture, treatment determinants and features of implanted site and other processing parameters (Nguyen *et al*., 2019; Holland *et al*., 2019) [76, 37]. Silk scaffolds degradation is controlled mechanism which supports new tissue, bone, ligament formation, depending upon its use. Silk fibroin based materials posses the remarkable features, the resultant amino acid formed can be absorbed according to the micro-environment i.e., in-vitro/ in-vivo in biomedical applications. Water-insoluble silk fibroin films with minimum beta-sheet content controlled drying process and revealed faster degradation (Jin *et al*., 2005: Lu *et al*., 2010) [44, 58]. Hence the beta-sheets of fibroin controls degradation rate by regulating porosity,pore-dimensions and molecular weight distribution (Tomeh *et al*., 2019) [105].

3.3 Mechanical strength

Polymeric scaffolds of higher mechanical strength are of utmost importance in drug delivery, tissue engineering and other biotechnological approaches (Bhrany *et al*., 2008) [7]. Silk fibroin scaffolds bear higher young’s modulus and altered tensile strength of 450-700 MPa at an elongation of 12-24% reveals considerably higher mechanical strength and load-bearing capacity than collagen and poly-lactic-co-glycolic acid (PGLA) scaffolds (Gou *et al*., 2019; Melke *et al*., 2016) [30, 66]. Although, the mechanical property of processed scaffolds may vary according to processing techniques, composition, matrix stiffness, b-sheet content as well as scaffold morphology and topology. Comparatively, silk fibroin proved superior mechanical property with higher resistance effective for fabrication of load bearing composites. Crystalline silk fibroin bears excellent mechanical properties due to beta-sheet conformation and can be effectively utilized in bone tissue engineering. Silk scaffolds can be improved for utilization by improvingthe compressive strength and modulus while enhancing fibroin concentration and uniformity in pore number along with decrease in pore size (Kim *et al*., 2005) [49].

4. Practical Applications of Silk fibroin In Biomedical Science

4.1 Applicability of fibroin in treatment of Cardiac issues

The non functionality of cardiac tissues is a major issue and often leads to severe disease complications and death. The cardiac tissue regeneration can be facilitated by fabrication of scaffolds which can be useful in providing the suitable environment to enhance cell growth and functionality (Tandon *et al*., 2020) [99]. The ischemic heart disease was successfully treated with injectable silk sericin hydrogel in in-vivo conditions. Silk sericin hydrogel provided aided in improved functionality with reduced inflammation and enhancement in micro vessel density and attenuated apoptosis (Song *et al*., 2016) [92]. Silk fibroin was used to improve cardiomyocyte functionality in an electrospun scaffold. The scaffolds manifested a better guiding ability in re-organization of cardiac cells and tissues. Hence fibroin based biomaterial possess a good potential in treating the cardiac issues with improved features (Zhao *et al*., 2020; Babu and Saumte, 2024) [136, 4]. Many studies have been conducted to explore the applicability of silk fibroin in biomedical science (Table 2).

4.2 Applicability of silk fibroin in treatment of Skin issues

Different silk based biomaterials were used to reflect compatibility with human skin so far. Silk possess the intricate capability to help in growth and proliferation of fibroblasts and keratinocytes (Zhang *et al*., 2009) [133]. The silk fibroin capsulated in silver oxide nanoparticles revealed superior antibacterial activity against pathogenic and non-pathogenic bacteria as well (Babu *et al*., 2018) [5]. Silk fibroin blended films revealed good biocompatibility with no toxicity effects when co-cultured with fibroblast cells. Hence SF films can be effectively used for skin tissue engineering and wound healing applications (Luangbudnark *et al*., 2012) [59]. The keratin epidermal layer when coated with silk fibroin film and was co-cultured with human dermal fibroblasts, it revealed wound healing effects (Chouhan *et al*., 2018) [16]. Hence fabrication of fibroin based material for skin possesses promising attributes to be utilized in future.

4.3 Applicability of silk fibroin in treatment Dental issues

Tooth regenerations techniques and effective inputs utilized for enhancing the development in the dentistry has been studied by researchers from a decade. Silk based biomaterials were found to be the promising candidates in pulp (soft tissue), cementum, dentin and enamel (hard tissue) engineerings in dentistry (Jindal *et al*., 2014) [45]. SF scaffolds were developed for mineralized dental tissue engineerings. It employs four kinds of compounds, *viz*., hexafluoro isopropanol (HFIP) to facilitate the formation of sound bio-engineered mineralized tissue. The study revealed the importance of silk scaffolds in developing mineralized osteodentin (Xu *et al*., 2008) [120]. The porous silk fibroin scaffold was used to generate a pulp like tissue formation with improved blood supply, formation of a hard tissue like dentin, and a new matrix deposition in host-originated and transplanted cells. It was supported by fibroblast growth factor inserted silk-based scaffold which are observed as facile candidates for treating root canal (regenerative endodontic) (Yang *et al*., 2015) [123].

4.4 Applicability of Silk fibroin in Ocular issues

The cornea of eye is the portion of visualization which is transparent from front side. Any injury to cornea in terms of physical damage, illness or other ailments disrupts the vision of eye (Harkin *et al*., 2011) [31]. The unique and critical environment in the eye makes ocular drug delivery the most challenging physical task. The application of ibuprofen-liposomal formulation encapsulated with silk fibroin for topical ocular delivery revealed sustained drug release with no cell toxicity was investigated (Dong *et al*., 2015) [22]. The grafting of corneal cells on a SF membrane in rabbit stroma revealed better vascularization in the limbus and growth of news vessels (Yang and Zhang, 2008) [124]. Silk fibroin films were used as carrier for epithelial cells to rejuvenate corneal epithelium and showed clear medium in comparison to other membranes (Higa and Shimazaki, 2008) [36]. The maintenance of surface integrity, electro-retinography and intraocular pressure in grafted eyes exhibited the silk fibroin film as efficient biomaterial for utilization as corneal scaffolds (Hazra *et al*., 2016) [33].

4.5 Applicability of silk fibroin in treatment of Nervous system related issues

The greatest challenges involve nerve damages, brain injuries, spinal cord damages and all neurological diseases in medical field. Neurological tissue engineering is one the modern approach for impairment of neurological disorders (Tandon *et al*., 2020) [99]. Silk fibroin fibres proved better biocompatibility and enhanced growth of hippocampal neurons (Tang *et al*., 2009) [100]. Artificial nerve grafts were fabricated by using silk fibroin with schwan nerve cells and tissues of rat, dorsal root ganglia (DRG), cultured on silk fibroin fibres and fluid as media. The study revealed least toxicity with improvement in cellular physiology and functioning of dorsal root ganglia and schwan cells (Yang *et al*., 2007) [125]. Silk fibroin possesses the remarkable ability to facilitate nerve regenerations in combination with tropoelastin protein. The combo ofsilk and tropoelastin protein fabricated films revealed enhanced neurite extension, nerve repairs and neurite guidance (White *et al*., 2015) [113]. Silk nano-fibres were induced in novel heterostucture composite scaffold to improvecell growth and cell differentiation of SH-SY5Y nerve (Qing *et al*., 2018) [85].

4.6 Applicability of silk fibroin in treatment of Hepatic issues

Liver is the largest organ responsible for regulating body metabolism and immune functions. The main function of hepatic cells is the regulation of carbohydrate, lipid and protein metabolism in addition to detoxification or removal of waste products and maintenance of homeostasis. Therefore, deprivation of any function can cause liver damage and death. The hepatic tissue engineering is necessary to save the life of such patients (Kasoju and Bora, 2012) [48]. Silk fibroin based biomaterials have been explored to play an effective role in repairing the hepatic tissues. For instance, three-dimensional porous sponge constituting conjugates of lactose and silk fibroin were fabricated to encapsulate FLC-4 cells derived from human hepatic cancer cells. The results revealed the constant albumin secretion in shorter span of time. Hence, the compound sponge was found to be effective for use in prolonged cell culture with promising attributes for development of biological artificial liver (Gotoh *et al*., 2011) [29]. Composites of silk fibroin and gelatin revealed higher biocompatibility and cytotoxicity in in-vivo and in-vitro conditions respectively in liver tissue engineering. The composites/scaffolds were found to be facile candidates for grafting bio-artificial livers (Yang *et al*., 2007) [125]. The embolization of arteries re-structured sodium alginate (MSA) and silk fibroin microparticles under in-vivo conditions proved the potential of silk fibroin to be utilized as arterial embolic vehicle in liver therapy (Chen *et al*., 2020) [12].

4.7 Applicability of silk fibroin in treatment of Bone related issues

The active connective tissue of human body known as bone suffers many defects due to injuries, infections and tumours. The scaffolding materials are found to be the most viable and cost effective solutions to these defects (Buck and Dumanian, 2012) [9]. Tissue engineering represents natural reconstruction of bone which employstechniques. The rejuvenation of a bone-ligament-bone graft can be acquired by scaffolds with preferably improved graft retention along with host bone.The scaffold had the ability to generate a bone-ligament-bone graft which improves graft osteo integration with the host bone. The composites of hydroxyapatite (HA) had proved better features in terms of toughness and mechanical strength when compared with mineralized macroporous SF scaffold (Collins *et al*., 2009) [17]. Silk fibroin based materials are actively utilized in orthodontics due to their promising characters of combination with calcium phosphate bioceramics such as hydroxyapatite, calcium sulphate which are readily available materials used for bone grafting. The capabilities of silk based biomaterials have been extensively studied for cell adhesion, cell proliferation and cell differentiation in microenvironments in both in-vivo and in-vitro conditions (Deshpande *et al*., 2021; Jing *et al*., 2022) [20, 46]. A porous scaffold developed by freeze-drying approach constituting silk fibroin, cellulose nanowhisker chitosan revealed improved biocompatibility, decreased porosity and mechanical properties. The porous scaffold upon culturing with Human MG-63 osteosarcoma cells enhanced the osteocalcin cell proliferation and expression (He *et al*., 2016) [34]. The scaffolds constituting silk fibroin and nano-Haphydrogels revealed higher biodegradability with improved osteogenic properties (Ribeiro *et al*., 2018) [87]. In the present times, SF based biomaterials are combined with metallic elements to enhance the anti-bacterial properties of scaffolds/ hydrogels used in orthodontics. Silver and gold nanoparticles are of prime importance to induce bacterial cell death. The combination of SF hydrogel composites with gold nanoparticles proved the reduced bacterial cell proliferation of *Staphylococcus aureus* and *Escherichia coli*. Therefore can be effectively used for healing and prevention of post-surgery infection by regulating osseointegration (Ribeiro *et al*., 2017; Ribeiro *et al*., 2018) [88, 87].

Table 1: Fabrication of silk-based biomaterials for biomedical use.

Bio-material	Fabrication methods employed	Function
Silk fibroin films	Casting (Motta *et al*., 2002) [69]	Dermal wound dressing (Srivastava *et al*., 2015) [93]
	Spin coating method, spin-aided layer-by-layer fabrication (Jiang *et al*., 2007) [43]	Optical applications (Malinowski *et al*., 2019; Dong *et al*., 2022) [62, 21]
	Three-dimensional printing (Mu *et al*., 2020) [70]	Controlled drug release (Mu *et al*., 2020) [70]
	Inkjet printing (Tao *et al*., 2015) [101]	Tissue engineering (Huang *et al*., 2017) [40]
Silk fibroin fibres	Wet spinning (Frydrych *et al*., 2019; Woltje *et al*., 2023) [26, 115]	Biomedical scaffolds (Biagiotti *et al*., 2022) [8]
	Dry spinning (Wei *et al*., 2012) [111]	Regenerative medicine (Frydrych *et al*., 2019; Woltje *et al*., 2023) [26, 115]
	Micro-fluidics method (Peng *et al*., 2016) [79]	Mechanical function (Peng *et al*., 2016) [79]
	Stable jet electro-spinning (Yi *et al*., 2018)	Load-bearing in connective tissues tissues (Yi *et al*., 2018) [128]
Silk fibroin hydrogels	Gelation technique (Wang *et al*., 2008; Mitropoulos *et al*., 2019) [110, 68]	Tissue engineering scaffolds, tissue adhesives, () (Zheng and Zuo, 2020) [140]
	Hydrogel fabrication using gamma-radiation (Wu *et al*., 2020) [117]	Electronic skin, bioelectronics and biosensors (Wang *et al*., 2015) [106]
Silk fibroin scaffolds	Foaming (Maniglio *et al*., 2018) [63]	Regenerative medicine (Mitropoulos *et al*., 2019) [68]
	freeze-drying (Li *et al*., 2023) [54]	Biological sensing, and energy storage (Maleki *et al*., 2018) [61,]
	particulate leaching (Zhang *et al*., 2012) [134]	Tissue engineering, microfluidics, and organic electronics (Yetiskin and Okay, 2017) [126]
Silk fibroin particles	Electro spraying (Gholami *et al*., 2011) [27], Salting out (Lammel *et al*., 2010) [53]	Drug encapsulation (Zhao *et al*., 2015) [138]
	Desolvation (Zhang *et al*., 2007) [135] Micro emulsion (Myung *et al*., 2008) [74]	delivery (Pham and Tiyaboonchai, 2020) [81]
	Powdering (Xiao *et al*., 2014) [118]	PH-dependent drug release (Sun *et al*., 2019) [97]
Silk fibroin Composites	Chitosan-fibroin blending method (Xing *et al*., 2023) [119] Silk fibroin incorporated ethylene dioxythiophene and styrene sulfonate composite (Kundu *et al*., 2013) [52]	Corneal epithelial regeneration (Bhattacharjee and Ahearne, 2022) [6]
	SF composites with polyurethane (PU) by wet spinning (Koto*et al*., 2022) [96]	Artificial vascular grafts (Koto*et al*., 2022) [96]

Table 2: Studies on bio-medical applications of silk-fibroin conducted so far.

Research conducted	Findings	References
Enhancement of fibroin sutures by incorporating controlled release mechanisms to create infection-resistant sutures.	Feasibility of short-term infection-resistant sutures that release antibiotics.	Choi *et al*., 2004 [15]
Human skin Fibroblast adhesion and proliferation	N-terminal region peptides as the primary contributors to increased fibroblast growth enhancing fibroblast proliferation	Yamada *et al*., 2004 [122]
Wound healing capabilities of silk fibroin	promoted the adhesion and growth of human keratinocytes, crucial for wound re-epithelialization	Min *et al*., 2004 [67]
Wound healing capabilities of silk fibroin, alginate, and silk fibroin/alginate blend sponges.	Sponges significantly reduced wound healing time (by approximately 50%), increased collagen deposition, and enhanced cell proliferation.	Roh *et al*., 2006 [89]
Silk nano-layer for protein and drug release kinetics.	Silk nano-layer coatings exhibited outstanding mechanical attributes. However, the release kinetics could be adjusted by regulating β-sheet crystallinity and thickness of the coating.	Wang *et al*., 2007 [109]
Regeneration of silk fibroin films for controlled release employing matrix diffusion and membrane permeation techniques.	The implantation of anti-coagulant drug (heparin) blended with polyurethane and silk fibroin membrane proved sustained release of heparin with longer and greater membrane thickness, higher drug loading, and increased fibroin content.	Liu *et al*., 2009 [57]
Fibroin for drug release	can be modified into various drug release vehicles *viz*., fibers, films, nano-layers, hydro-gels, sponges, and microspheres	Pritchard and Kaplan, 2011 [83]
regenerated fibroin materials	mass transfer and release kinetics can be adjusted by altering the β-sheet crystalline content of regenerated fibroin	Karve *et al*., 2011 [47]
Drug release kinetics of regenerated fibroin	Drug release kinetics can be enhanced by co-releasing proteinase inhibitors such as ethylene-diamine tetra-acetic acid alongside the target drug to disrupt proteolytic activity.	Pritchard *et al*., 2011 [84]
Silk fibroin as ideal candidate for graft.	Silk fibroin exhibits exceptional mechanical, degradation, and biocompatibility properties, making it an ideal candidate for generating highly loaded grafts, particularly in the musculoskeletal field.	Yucel *et al*., 2014 [131]
Silk fibroin nano-particle for lung targeting	SF nanoparticle loaded with anti-cancer drug like doxorubicin serve as promising candidate for biomedical application in cancer research	Subia *et al*., 2014 [95]
Silk fibroin nano-particle for ocular drug delivery labeled with fluorescienisothiocyanate and bovine serum albumin (FITC- BSA-SFNs)	FITC- BSA-SFNs possessed sustained release, bio-adhesive, and co-permeation characteristics. The SFNs proved rapid and lasting adhesion on the outer sclera tissues.	Huang *et al*., 2014 [39]

Textile-engineered nerve guidance conduit utilizing silk fibers.	These conduits were designed to serve as guiding structures for the regeneration of peripheral nerves.	Teuschl *et al.*, 2015 [102]
Silk fibroin for delivery of bio-active molecules.	Owing to the robust mechanical properties of SF based materials, therapeutic agents can be sustained and transported to particular sites for controlled release.	Wu *et al.*, 2016 [116]
Delivery of natural antitumor compounds, curcumin encapsulated with fibroin.	The system revealed enhanced cellular uptake and effective inhibition of human breast cancer cells *i.e*, MDA-MB-231 cells.	Song *et al.*, 2017 [91]
Silk fibroin for bone regeneration	Silk fibroin scaffolds blended with hydroxyapatite (HA) were found efficient candidates for bone regeneration.	Farokhi *et al.*, 2018 [25]
Evaluation of silk fibroin meso-structures for electronic applications.	The top-down technique revealed higher mechanical strength with increased heterogeneity in comparison bottom-up approach with lower strength and provide homogeneity while maintaining fibril structure.	Zheng *et al.*, 2018 [139]
Chemical modification of silk fibroin chain	The modification in chemical structure of silk fibroin chains led to development of promising silk derivatives and conjugate products with photosensitivity and cell adhesion capability.	Chen *et al.*, 2018 [14]
Silk fibroin/ hydroxyapatite as efficient ceramic/ polymer combo for dental treatments.	Hydroxyapatite is a bioceramic material which showed osteogenic properties while in addition to fibroin, the flexibility and elasticity increased. The complex proved to mimic natural bone. Hence both the materials can be used as effective bone constituents.	Cacciotti, 2019 [10]
Assessment of sodium alginate-modified silk fibroin microspheres as potential hepatic arterial embolization agent	SA-modified SF microspheres proved smooth surfaces with good sphericity and blood compatibility. The microspheres can promote proliferation of fibroblasts and can be effectively used as potential biodegradable arterial embolic agent for liver cancer therapy.	Chen *et al.*, 2020 [12]
Encapsulation of ferulic acid-loaded silk fibroin nanoparticle carrying neutrophilmembranesagainst the oxidative stress and inflammatory reactions in treatment of acute pancreatists.	Neutrophil membrane-coated SF-NPs proved controlled and sustained release of FA into the inflammatory pancreas lesion.	Hassanzadeh *et al.*, 2021 [32]
Development of artificial silkwormsilkstronger than natural spider silks.	The development of artificial silkworm silk with regenerated proteins of commercial silkworm revealed superior tensile strength and strongest natural silk than spider silk.	Wang *et al.*, 2022 [107]

5. Conclusion

The remarkable mechanical strength, biocompatibility and controllable biodegradation make silk fibroin a superior biomaterial for use in clinical trials. However, revolution in the field of nanotechnology increased demand for more innovative designs of fibroin. Different designs with improved and durable clinical trials were fabricated. The complexity of cell and tissue organization is unlocking the challenge of implementing smart materials in near future. The silk fibroin as an ideal biomaterial can serve the need of innovative implants required for clinical microenvironments.

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