

The use of silk fibroin as a biomaterial biology essay

[Science](#), [Biology](#)



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Introduction

The use of silk fibroin as a biomaterial has been developed recently for biomedical engineering approaches in several ways as drug carriers. The Water-oil emulsion solvent diffusion method, this method is a simple and fast method for preparing both noncrosslinked and genipin crosslinked SF microspheres. The crosslinked and non crosslinked SF microspheres show great potential to act as drug carriers. The SF microspheres are completely spherical in shape and smooth in surface which can act as drug carriers particularly for hydrophilic drug delivery. [1]Reverse Microemulsion method for the preparation of Nanoscale sized Silk fibroin particles which have the capacity to encapsulate fluorescent dyes, can slowly degrade and is very biocompatible which is used for Molecular imaging and bioassaying. [2] Silk

Fibroin microparticles prepared by spray dryer method would be used for the biomaterials with skin affinity and it is superior to other matrix materials and it might be applied for immobilization of drugs. [3] Capillary-microdot technique SF-derived curcumin nanoparticles show higher efficacy against breast cancer cells and have the potential to treat in vivo breast's tumors by local, sustained and long-term therapeutic delivery as a biodegradable system. Drug delivery to breast cancer cells (delivery of curcumin) is their main application. [4] Laminar jet break-up of an aqueous SF solution spheres, These spheres have great encapsulation efficiencies and sustained release kinetics it helps to preserve the bioactivity of the embedded growth factor, with a great sustained release profile. The applications of those spheres may range from the controlled delivery of labile (likely to change) drugs and protein therapeutics to their use as a platform for the delivery of growth factors for tissue repair. [5] As for the Desolvation technique the nanoparticles were non-toxic to the cells and showed normal cell cycle distribution without any visible signs of cell arrest. The In-vitro release of loaded VEGF in the nanoparticles showed a sustained release of over 3 weeks without an initial burst, SF nanoparticles have great biocompatibility and degradability, so they are used as Carriers to deliver drugs to the target cells for diagnostics and therapeutics. [6] Silk fibroin was conjugated with methoxypoly (ethylene glycol) derivatives to prepare silk nanoparticles. The sizes and shapes of SF nanoparticles observed were ranged about 150-400 nm in diameter and spherical morphology. UV/VIS spectrometry showed SF nanoparticles might be outer PEG and inner SF structure. [7] It is also emerging in the field of tissue engineering as a material for tissue

engineering anterior cruciate ligaments (ACL) as the matrix was built to match the natural human ACL Properties they found that SF can provide suitable biomaterial matrices to support adult stem cells differentiation toward ligament lineages along with its excellent mechanical properties and biocompatibility [8], using silk fibroin in conjugation with an inorganic compound as a material for bone tissue engineering The blend of gelatin/SF was used as a based protein scaffold which was used to initiate osteogenesis and showed great properties as porosity, good water absorption ability. However the biodegradability occurred in the protein part and the scaffold as a whole showed a low cytotoxicity level [9]. A novel bone-like biomaterial of hydroxyapatite (HAP) and silk fibroin (SF) composite was developed by biomimetic synthesis. The HAP/SF composite then demonstrated that it could promote osteoblast proliferation in vitro and new bone formation in vivo. The novel biomaterial is a promising material for bone replacement and regeneration [10]. Silk fibroin scaffolds were prepared from aqueous silk fibroin solutions by combining salt-leaching and freeze drying Methodologies. However the mechanical properties of the scaffold exhibited concentration dependence. The scaffold showed great stability and maintained its properties after in vitro degradation for 30 days. It also shows that it is suitable for meniscus and cartilage tissue engineering [11]. SF in conjugation with poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) has been used to fabricate the tissue engineered cardiovascular scaffolds due to its controllable and suitable mechanical properties. However it was found that SF-modified PHBHHx scaffolds are highly biocompatible with cardiovascular-related cells, showing that it can be utilized for the extensive applications of

PHBHHx in the cardiovascular regeneration. [12]. In another study it is found that silk fibroin has good biocompatibility with rat Dorsal Root Ganglia (DRG) and is also beneficial to the survival of Schwann cells without exerting any significant cytotoxic effects on their phenotype or functions, which can open a gateway for silk fibroin as a material for nerve tissue engineering [13].

Another study about a developed novel method for cross-linking a silk fibroin solution by gamma irradiation to form a hydrogel that might serve as a dermal scaffold. The silk fibroin hydrogel is not cytotoxic to human fibroblasts or human keratinocytes. The mechanical properties of the silk fibroin hydrogel formed by gamma irradiation method were superior to that of silk fibroin hydrogel created using a freeze-drying method. The silk fibroin hydrogel created using this novel method is suitable as a dermal scaffold in Skin Tissue Engineering [14]. Recently silk fibroin and elastin scaffolds were produced for the treatment of burn wounds SF & Elastin scaffolds were produced for the treatment of burn wounds. The excellent properties of SF were combined with elastin protein and resulted in a scaffold which mimics the extracellular matrix (ECM). By using genipin as a cross-linker they obtained scaffolds with smaller pore size and reduced swelling ratio degradation and release rates. The composition had a great effect on the scaffold's physical properties; composition can be easily controlled to make the scaffold suitable for biological applications. The cytocompatibility with human skin fibroblasts along with the healing improvements make these scaffolds suitable for wound dressing applications [15]. Silk fibroin has a unique and useful combination of properties, including good biocompatibility and excellent mechanical performance. These features provided early clues

to the utility of regenerated silk fibroin as a scaffold/matrix for tissue engineering. The silk fibroin scaffolds used for tissue engineering should degrade at a rate that matches the tissue growth rate. The results show that a high content of β -sheet structure leads to a low degradation rate. The results demonstrate that it is possible to control the degradation rate of a silk fibroin scaffold by controlling the content of β -sheet structure [16]. The preparation of SF for applications in tissue engineering was studied, Pure SF was extracted from silk worm cocoons by degumming and solubilizing and then further purification was carried out. However the results indicate that the regenerated silk fibroin can be used for fabrication of porous silk fibroin scaffolds for various tissue engineering applications [17]. For tissue engineering, it is very important to design and control the pore architecture of three-dimensional (3D) polymeric scaffolds, which plays an important role in directing tissue formation and function. Different pore structures were formed according to the pH of silk fibroin because silk fibroin exhibits water-like behavior under basic conditions and gel-like behavior under acidic conditions [18]. The pore architecture of scaffolds plays a critical role in tissue engineering as it provides the vital framework for seeded cells to organize into a functioning tissue. By manipulating the concentration the pore sizes of the scaffolds decreased as the concentration of fibroin protein increased. Human bone marrow mesenchymal stromal cells (BMSC) transfected with the BMP7 gene were cultured in different pore sized scaffolds. The results showed that BMSC expressing BMP7 preferred a pore size between 100 and 300 microns in silk fibroin protein fabricated scaffolds, with better cell proliferation and ECM production. Furthermore, in vivo

transplantation of the silk fibroin scaffolds combined with BMSC expressing BMP7 induced new bone formation. This means that optimized pore architecture of silk fibroin scaffolds can modulate the bioactivity of BMP7-transfected BMSC in bone formation [19].

Chemical Properties of silk Fibroin

Fibroin is an insoluble protein created by spiders, the larvae of *Bombyx mori*, and numerous other insects. Silk in its raw state consists of two main proteins, sericin and fibroin, fibroin being the structural center of the silk, and sericin being the sticky material surrounding it. [20]http://upload.wikimedia.org/wikipedia/commons/thumb/3/3f/Silk_fibroin_primary_structure.svg/300px-Silk_fibroin_primary_structure.svg.png

Figure 1: Primary structure of fibroin, (Gly-Ser-Gly-Ala-Gly-Ala)_nThe fibroin protein consists of layers of antiparallel beta sheets. Its primary structure mainly consists of the recurrent amino acid sequence (Gly-Ser-Gly-Ala-Gly-Ala)_n. The high glycine and alanine contents allows for tight packing of the sheets, which helps to give the silk's rigid structure that can't be stretched (tensile strength). A combination of stiffness and toughness make it a material with applications in several areas, including biomedicine and textile manufacture. Figure 2: The schematic of structure of *Bombyx mori* silk fibroin protein. The primary structure consists of 12 repetitive regions interspaced by 11 nonrepetitive regions. The repetitive region is responsible for the formation of β -sheets crystals of size 10x15x200 Å³. The nonrepetitive region forms the amorphous part of the protein

3: a, Hydrophobicity pattern in *B. mori* silk fibroin heavy chain primary sequence with possible chain folding intra- and inter-molecular schemes. b, Micelle assembly of silk fibroin in water (8%), based on hydrophilic-hydrophobic multiblock co-polymer structure leaving internal smaller hydrophilic domains to promote solubility in water, with larger-chain terminal hydrophilic blocks in contact with the surrounding aqueous solution. c, 'Globule' formation driven by increased fibroin concentration and lower water content, further hydrophobic interactions, and at the final stages by the presence of sericin or PEO. d, Elongation and alignment of globules and interactions among globules promoted by physical shear, leading to fibrillar structure [46]. Fibroin is known to arrange itself in three structures, called silk I, II, and III. Silk I is the natural form of fibroin, as emitted from the *Bombyx mori* silk glands. Silk II refers to the arrangement of fibroin molecules in spun silk, which has greater strength and is often used in various commercial applications. Silk III is a newly discovered structure of fibroin. Silk III is formed principally in solutions of fibroin at an interface (i. e. air-water interface, water-oil interface, etc.)[21].

Physical Properties

Mechanical Properties

Silk is a very versatile biomaterial with its significant crystallinity, high elasticity, strength and toughness, and resistance to failure in compression. The combination of the β -sheet crystals, the interphase between the crystals, the semi-crystalline regions and the shear alignment of the molecular chains are the basis for silk's unique mechanical properties. While

the highly organized β -sheet regions of the protein provide the tensile integrity, the semi-crystalline regions are the basis for the protein's elasticity [22]. The β -sheet structure affects the tensile properties, degradation rate and elasticity of the scaffold, so the tailoring of these properties can be done in part with the cross-linking process. The transition depends on the length of time exposed to the solvent as well as the solvent concentration. Methanol treatment is a widely used process to induce β -sheet formation although it does not transform all molecular regions. Ethanol, 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide [23] hydrochloride (EDC), glutaraldehyde or genipin also cause the transition from random coils to β -sheet configurations [24, 25, 26, and 27]. Studies show that regenerated silk fibers can hold their initial tensile integrity for 21 days under immune deficient in vitro culture conditions [8]. Moreover, the solvent used for electrospinning can affect the β -sheet formation of the scaffold's secondary structure, which in turn can alter the mechanical properties. Formic acid, HFIP and water have been used to electrospin silk scaffolds, and of those, water and formic acid seem to enhance the mechanical properties of the scaffolds [28, 29].

Degradation

According to the US Pharmacopia, an absorbable biomaterial loses most of its tensile strength 60 days after implantation. Even though silk is considered non-degradable by this definition, it does in fact degrade but over a longer period of time. Silk will lose most of its tensile strength within a year in vivo, and will be unrecognizable at the implantation site within 2 years. However, the rate of degradation depends on the animal model and the tissue

implantation site. Silk is considered biodegradable because it is vulnerable to bacterial and enzymatic degradation. Studies show that proteases will cleave the protein at the less-crystalline regions after which the resulting peptides can be phagocytized by the cell [22, 24]. The solvent used to electrospin the silk scaffold may affect the degradation of the scaffold in vitro and in vivo. It was studied that electrospinning from an aqueous solution instead of an organic solvent like HFIP can increase the degradation rate while promoting cell proliferation and penetration. The silk scaffold electrospun from an aqueous solution degraded between 2 and 6 months while those electrospun out of HFIP lasted over a year. Also methanol treatment can significantly decrease the degradation rate [24, 30]. There is a wide variety of biocompatible polymers used for tissue applications aside from silk fibroin. However, the degradation rates of other polymers cannot be tailored within such a high range as that of silk. Collagen, which is a widely used biomaterial, degrades between 1 to 4 weeks and sometimes longer depending on the cross-linking process [31]. Polycaprolactone (PCL) can last within the body for more than 2 years [32]. Another synthetic polymer, poly (lactic-co-glycolic acid) (PLGA) (85: 15) usually degrades within 26 weeks, while PLGA (50: 50) degrades between 6 and 8 weeks in vitro [33, 34, 35]. Silk scaffolds, however, can be modified to have similar degradation rates by changing the solvent for electrospinning [30].

Swelling properties

The degree of swelling depends on the ionization of the network, its degree of crosslinking and its hydrophilic/ hydrophobic balance [36] Changes in

polymer compositions can influence the degree of swelling [37]. This can potentially increase the cumulative amount and rate of drug release. The swelling ratio of SF scaffolds has also decreased with an increase in Silk Fibroin concentration. Blending of Silk Fibroin with other materials such as chitosan [38, 39] and hyaluronic acid [40] led to increase the swelling when compared with pure Silk Fibroin.

Solubility

Crystalline SF is insoluble in most solvents as well as in Water, Commonly applied to dissolve SF are highly concentrated salt solutions of lithium bromide, lithium thiocyanate calcium thiocyanate or calcium chloride [41]. These electrolyte solutions are able to disrupt the hydrogen bonds that stabilize β -sheets [42]. The possibility to control the solubility of SF not only allows for longer storage times for SF solutions but also for an increase in SF concentration without aggregation.

Biocompatibility

The foreign body response after the implantation of Silk Fibroin in vivo has been shown to be comparable to or even less than the other biomaterials in use today [22, 43, 44]. All-aqueous- and Hexafluoroisopropanol (HFIP)-derived scaffolds have been tested in a one-year implantation study in rats. Those scaffolds were accepted by the host animals and the host immune response to the implanted scaffolds was low and local [45]. This matches another study with SF films [41].

Scaffolds and Cell Culturing

The design of new scaffolds mimicking the natural environment during tissue formation is an important issue in biomaterials research. Silk fibroin and hyaluronan scaffolds were prepared with porous microstructures by freeze-drying aqueous solutions of silk fibroin and hyaluronan also incubation in methanol to induce water insolubility of silk fibroin. Mesenchymal stem cells were seeded on silk fibroin/hyaluronan scaffolds and cultured for three weeks. Histology of the constructs after cell culture showed enhanced cellular ingrowth into silk fibroin/hyaluronan scaffolds as compared to plain silk fibroin scaffolds. However Silk fibroin/hyaluronan scaffolds are good as a biomimetic platform for mesenchymal stem cells in tissue engineering.

[47] Scaffold composition, configuration and resulting properties critically affects tissue development. The influence of silk fibroin concentration and correspondent processing method (aqueous or HFIP-derived) and three-dimensional scaffold structure (lamellar or porous, with distinct pore size) on bone tissue formation by osteogenic differentiation of human adipose tissue derived stem cells (hASC) were studied. We observed that very similar bone tissue was formed in all silk fibroin scaffold groups, evaluated by alkaline phosphatase activity, calcium production, collagen type I deposition and scaffold bone volume fraction. [48] Fibers with nanoscale diameters provide benefits due to high surface area for biomaterial scaffolds. Adhesion, spreading and proliferation of human bone marrow stromal cells (BMSCs) on these silk matrices was studied. The ability of electrospun silk matrices to support BMSC attachment, spreading and growth in vitro, combined with a biocompatibility and biodegradable properties of the silk protein matrix,

suggest potential use of these biomaterial matrices as scaffolds for tissue engineering. [49]The morphology of freeze-dried silk fibroin 3D-scaffolds was modified by varying both the NaCl concentration and the freezing temperature of the silk fibroin solution prior to lyophilization. Freezing at -22 C generated sponge-like interconnected porous networks, whereas fast freezing at -73 C formed stacked leaflet structures. The presence of millimolar NaCl (50-250mM) increased the porosity of the scaffolds. The seeding of P19 embryonic carcinoma cells showed that the presence of salt and freezing conditions influenced the cell distribution into the scaffolds, with salt addition increasing the access of cells to deeper regions. [50]Pore architecture of scaffolds is known to play a critical role in tissue engineering as it provides the vital framework for the seeded cells to organize into a functioning tissue, in this study different concentrations and pore sizes were investigated, The pore size of the scaffold decreases as the concentration of fibroin protein increases, Human bone marrow mesenchymal stromal cells (BMSCs) transfected with BMP7 gene were cultured in these scaffolds, The results showed that BMP7 expressing BMSCs preferred a pore size between 100 and 300 μm of silk fibroin protein fabricated scaffolds, with better cell proliferation and ECM production , in vivo transplantation of the silk fibroin scaffolds combined with BMP7-expressing BMSCs induced new bone formation. This study identified that optimized pore architecture of silk fibroin scaffolds could modulate the bioactivities of BMP7 transfected BMSCs in bone formation. [51]Cartilage tissue can be engineered by starting from a variety of cell sources, including stem cell based and primary cell-based platforms, cellular responses of isolated human chondrocytes, human

embryonic stem cells and mesenchymal stem cells (MSCs) derived from three sources, human embryonic stem cells, bone marrow and adipose tissue, were used with two biomaterials silk and chitosan scaffolds, in the presence and absence of bone morphogenetic protein 6 (BMP6), Embryonic stem cells-derived MSCs showed unique characteristics, with preserved chondrogenic phenotype in both scaffolds with regard to chondrogenesis, embryonic stem cells-derived MSCs were promising for chondrogenesis, particularly in the silk scaffolds with BMP6. The results show that cell source differences are important for chondrogenic outcomes, and the human embryonic stem cells-derived MSCs were the preferred cell source.

[52]Porous biodegradable silk scaffolds and human bone marrow derived mesenchymal stem cells (hMSCs) were used to engineer bone-like tissue in vitro. It is shown that RGD-silk scaffolds are particularly suitable for cells from a patient's own body bone tissue engineering, because of their stable macroporous structure, mechanical properties matching those of native bone, and slow degradation. [53]Natural bone consists of cortical and rod-shaped cell morphologies, the latter having variable pore sizes. SF scaffolds with different pore diameters were prepared and seeded with human mesenchymal stem cells (hMSC). As compared to static seeding, dynamic cell seeding in spinner flasks resulted in equal cell viability and proliferation, and better cell distribution throughout the scaffold. Differentiation of hMSC in osteogenic cell culture medium in spinner flasks for 3 and 5 weeks resulted in increased alkaline phosphatase activity and calcium deposition when compared to control medium. The pore structures of the newly formed tissue and that the structure of tissue-engineered bone was controlled by the

underlying scaffold geometry. [54] Human bone marrow-derived mesenchymal stem cells (MSCs) were seeded on silk, collagen, and crosslinked collagen scaffolds. Cells proliferated more rapidly on the silk fibroin scaffolds than on the collagen matrices. The total content of glycosaminoglycan deposition was three times higher on silk as compared to collagen scaffolds. Cartilage-like tissue was homogeneously distributed throughout the entire silk scaffolds; Round or angular-shaped cells resided in deep gaps in the silk systems. These results suggest that silk fibroin scaffolds are suitable for cells from patients own body cartilage tissue engineering in serum-free medium and enable mechanical improvements along with compositional features suitable for long-lasting implants to regenerate or generate cartilage. [55]