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A Review on Silk Fibroin as a Biomaterial in Tissue Engineering

Tkhu Chang Le[1,2,3], Qian Zhang[1,2], Qingdi Qu[1,2], Wentong Ding[1,2], Sergej Anatolyevich Lazarev[3], Shuang Pan[1,2*]

[1]The First Affiliated Hospital of Harbin Medical University, Harbin, China
[2]Department of Endodontics, School of Stomatology, Harbin Medical University, Harbin, China
[3]Department of Prosthodontics, Bashkir State Medical University, Ufa, Russia
Email: *panshuang@hrbmu.edu.cn

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Abstract

Regenerative medicine progress is based on the development of cell and tissue bioengineering. One of the aims of tissue engineering is the development of scaffolds, which should substitute the functions of the replaced organ after their implantation into the body. The tissue engineering material must meet a range of requirements, including biocompatibility, mechanical strength, and elasticity. Furthermore, the materials have to be attractive for cell growth: stimulate cell adhesion, migration, proliferation and differentiation. One of the natural biomaterials is silk and its component (silk fibroin). An increasing number of scientists in the world are studying silk and silk fibroin. The purpose of this review article is to provide information about the properties of natural silk (silk fibroin), as well as its manufacture and clinical application of each configuration of silk fibroin in medicine. Materials and research methods. Actual publications of foreign authors on resources PubMed, Medline, E-library have been analyzed. The selection criteria were materials containing information about the structure and components of silk, methods of its production in nature. This article placed strong emphasis on silk fibroin, the ways of artificial modification of it for use in various sphere of medicine.

Keywords

Tissue Engineering, Biomaterial, Scaffold, Silk, Fibroin

1. Backgrounds

Regenerative medicine progress is based on the development of cell and tissue bioengineering [1]. The bioartificial analogue creation of different tissue type

extracellular matrices is a challenge for tissue engineering. Bioartificial matrices are a substrate for adhesion, migration and proliferation of tissue and progenitor cells from surrounding tissues, providing regeneration at the damage site. The physical, chemical and biological properties of the scaffold material are key to its ability to support tissue regeneration. The tissue engineering material must meet a range of requirements, including biocompatibility, mechanical strength, and elasticity. Furthermore, the materials have to be attractive for cell growth: stimulate cell adhesion, migration, proliferation and differentiation. One of the natural biomaterials is silk. An increasing number of scientists in the world are studying it. According to Pubmed, for 20 years (2002-2022), the number of articles using the term "silk fibroin" as a key word has increased from 36 to 590, and since 2023 408 articles have been published (**Figure 1**). In this work, we reviewed literature related to silk fibroin (SF), its properties, fabrication, and application in tissue engineering.

The growth of the study of silk in bioengineering is understandable, as it aligns with most requirements for artificial bioengineering materials. Therefore, one of the versatile materials used as a frame component is silk fibroin. And in this literature review we would like to give a clearer understanding of the nature and properties of silk fibroin and potential applications in medicine.

Silk is a biomaterial produced mainly by *Bombyx mori* silkworms and is a fibrous protein in nature [2]. Silk is also produced by members of the class *Arachnida* (over 30,000 species of spiders) and several worms of the order *Lepidoptera* (ticks, butterflies and moths). Silk is produced by epithelial cells of these animals' glands [3] [4]. For the first time, silk was widely applied to surgical practice by a Nobel Prize winner in physiology and medicine, a Swiss surgeon Emil Theodor Kocher. And more recently, silk has attracted attention as a biomaterial because of a number of properties: ease of chemical modification, biocompatibility, low degradation rate in a living organism, variability of formats obtained on an aqueous solution or from an organic solvent [3] [5].

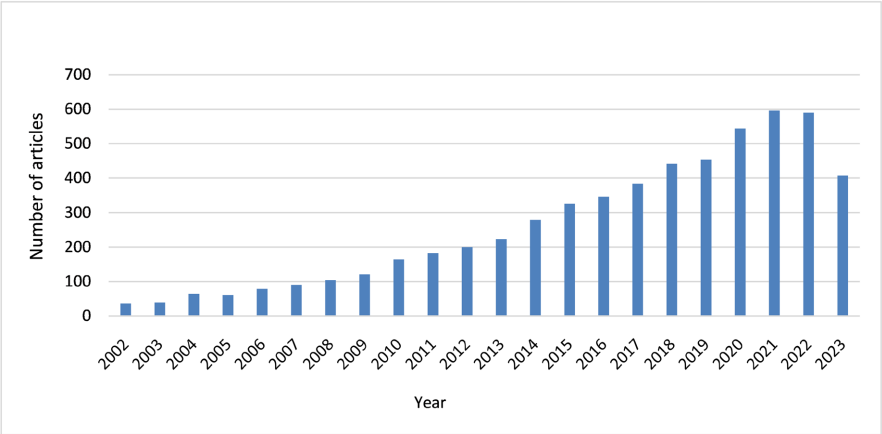

Figure 1. Dynamics of publications using the term "silk fibroin" as keywords, 2002-2023.

The primary structure of silk consists of repeating blocks of heavy and light polypeptide chains, and each of these chains consists of different amino acids. These hydrophobic blocks are predominant and lead to extensive hydrogen and hydrophobic interactions throughout all protein chains (anti-parallel β-hydrogen bonded layers). Such structure determines the homogeneity of the secondary SF structure [6]. Crystallization of the protein is caused by these interactions, which in turn increases the environmental SF stability [7]. These high crystalline regions associate with less organized amino acid regions, which alternate between crystalline regions and result in noticeable strength and elasticity of SF [7]. The second structure of this protein can be divided into three crystalline forms:

1) Silk I is liquid, and mainly has a structure of α-spirals and random turns.

2) Silk II has a crystal structure of β-antiparallel folding.

3) Silk III has a triple spiral chain that accumulates on the water-air surface [8] [9].

Silkworms produce two kinds of silk proteins: hydrophilic sericin and hydrophobic fibroin [2]. Fiber protein is coated with sericin proteins. Sericin are glutinous proteins that account for 25% - 30% of total cocoon weight. SF consists of a light chain (approximately 26 kDa) and a heavy chain (approximately 390 kDa) connected by disulfide bridges. SF is a hydrophobic β-fold domain connected by small hydrophilic linkers. Crystalline regions mainly consist of glycine-X repeats, where X is alanine, serine, threonine, valine. These domains contain subdomains rich in glycine, alanine, serine and tyrosine. These structures allow dense packing of stacked sheets of hydrogen bonds of antiparallel protein chains [3]. This protein organization ensures the hydrophobicity and strength of the fibers [3] [5]. Silk tensile strength is 635 ± 108 MPa, Young's modulus of silk—11.7 ± 2.2 GPa, breaking strain of silk—21.9% ± 4.9% [10]. In terms of strength, silkworm silk exceeds widely used polymer degradable biomaterials such as collagen and polylactic acid (**Table 1**).

Polylactic acid and collagen lack the mechanical properties of silk. Silk has more mechanical strength due to β-antiparallel folds. Thus, silk can be given any shapes: non-woven fibroin scaffolds, fibroin spongy porous matrices, fibroin (silk) films, microparticles, tubes etc. [11]. In addition, the extraction of sericine during the degumming process increases the strength of SF by 50%, making it more durable during pharmaceutical production [12]. Therefore, due to its properties silk is a suitable material for drug delivery and tissue engineering [13].

Table 1. Comparison of biomaterial mechanical properties.

Biomaterial	Tensile strength (MPa)	Young's modulus (GPa)	Breaking strain (%)	Reference
B. mori silk	635 ± 108	11.7 ± 2.2	21.9 ± 4.9	[10]
Collagen	0.0018 - 0.046	0.9 - 7.4	24 - 68	[3]
Polylactic acid	1.2 - 3.0	28 - 50	2 - 6	[3]

2. Fibroin

Fibroin is a promising material for tissue engineering, due to good mechanical properties, biocompatibility and biodegradability, as well as good permeability [14]. However, the regenerated SF matrices are water soluble. The use of chemical binding to prevent the SF solubility may not be effective due to the low primary amino group content in its structure. Because the SF solubility depends on the conformational state: water-soluble α-helices and insoluble β-folding structures, the creation of conditions for the conformational transformation $\alpha \rightarrow \beta$ in the fibroin hydrogel production allows influence on its solubility. The degradation rate of silk biomaterials directly affects the mesenchymal stem cells (MSC) metabolism, which in turn changes the rate of osteogenesis [15].

2.1. Biodegradation of SF

SF, as a natural biomaterial, is biodegradable. The speed of crystalline SF degradation is dependent on the implant site used to make the implant [16]. Natural polymers such as collagen and silk are degraded by proteases. The SF degradation has been studied in the SF incubation in protease XIV for 12 weeks, and it has been shown that the enzyme cleaves silk protein at several locations along the chain [17]. In addition, the degradation rate of SF can be corrected by changing the way of SF fabrication, affecting the degree of β-folding. The following correlation is observed: the rate of degradation diminishes as the total content of the β-layers increases. It has also been shown that the silk degradation rate directly affects the metabolism of MSC, which in turn changes the rate of osteogenesis. In addition to the site of implantation, the way SF is fabricated and SF concentration during manufacturing, the degradation rate is affected by changes in crystallinity, porosity, pore size, molecular weight distribution (MWD). MWD is modified by SF treating in alkaline conditions and heat [3] [18]. The SF degradation in the body is accompanied by the formation of non-toxic and, in some cases, even beneficial to regeneration products, and can be controlled [15].

2.2. Biocompatibility and Immunological Responses

The immune system responds to an antigen in the body through a complex sequence of processes called immunological responses. Therefore, biocompatibility is an important requirement for the scaffold. Recent research has shown that the combination of sericin-fibroin, which is composed of silk, leads to an active immune response. However, patients have no allergic reaction when sericin and fibroin are used in isolation [16] [19]. *In vitro*, de-gummed SF showed a lower adhesion of immunocompetent cells compared to polystyrene and poly (2-hydroxyethyl methacrylate) in the inflammatory response, *in vivo*—lower than collagen and polylactic acid [17] [20].

Immune response affects the rate of biodegradation of SF scaffolds. In a rat experiment, hexafluoroisopropanol-based SF scaffolds and water-based SF scaffolds were injected subcutaneously. The results of the study showed that im-

mune-induced scaffold biodegradation from aqueous SF solution was more active than from hexafluoroisopropanol-based scaffolds [21] [22].

In long-term applications SFalso showed promising results. Panilaitis with co-authors during SF potential immunogenicity study determined that SF fibers are mmunologically inert in short- and long-term culture with RAW 264.7 murine macrophage cells [23]. A.Yu. Kolesnikov and colleagues made SF vascular patches and implanted in the wall of the sheep carotid arteries for 2 and 6 months. The functioning and endotheliation of vascular patches in the carotid artery were investigated using optical coherence tomography. Histological studies indicate that SF patches in the vascular wall greater degrade after 6 months than after 2 months. The patch was represented by the divergent SF layers, between which there was a connective tissue filled with cells; new blood capillaries were found in the patch thickness (vasa vasorum) [24].

2.3. Sterilizability

Compared to collagen, SF scaffold can be sterilized in various ways, such as autoclaving, γ-radiation, with the use of ethylene oxide and 70% ethanol [17]. However, the sterilization method influences the MWD and degree of beta-sheet formation, which affects the SF scaffolds characteristics (degradation and stiffness) [5] [25].

E.S. Gil and coauthors studied the influence of two types of sterilization (autoclaving and immersion in 70% ethanol solution) on SF characteristics. Autoclaving significantly reduces degradation and increases SF mechanical properties because this type of sterilization increases the b-sheet content and size of the crystals. SF mechanical properties do not change after sterilization with the usage of 70% ethanol solution due to a small impact [26]. Y. Zhao and colleagues confirmed the results of the Gil and coauthors study, showing a change in the SF secondary structure and SF mechanical properties after autoclaving [27]. K.A. George and coauthors have also determined that autoclaving not only alters the structure of silk but also reduces SF film transparency. Silk properties remain unchanged during the gamma-irradiation sterilization process. Steam-sterilization, like autoclaving, makes the scaffold stiffer [28].

3. Manufacturing of SF

Because SF is soluble in high-ion or acid solutions and remains in solution when exchanged with less harsh solutions, it is feasible to create SF scaffolds of various shapes and purposes: non-woven fibroin scaffolds, fibroin spongy porous matrices, fibroin (silk) films, microparticles, tubes, electrospinning fibers of fibroin, aqueous solutions of fibroin, fibroin hydrogels [3] [5] [16]. Further, the formation of crystalline beta-sheets is induced, usually by the influence of either heat or solvents, resulting in silk material becoming insoluble in water. The mechanical and degradation properties can be modified by silk fabrication changing, method of β-sheet (crystal) induce formation and silk concentration. Accordingly,

the final properties of silk will also change [16].

Many SF products have been tested *in vivo* and have been shown to be effective for skin regeneration [29], bone tissue, cartilage [30], heart [31], vascular, liver, and as carriers of medicinal compounds with regulated release [5].

3.1. SF Hydrogel and Clinical Application of SF Hydrogel

Hydrogels are gels constructed from cells of hydrophilic polymer chains. It is an analogue of the natural extracellular matrix produced tissue—three-dimensional structure of proteins and proteoglycans. Due to its architecture and mechanical properties, the presence of cellular adhesion molecules and growth factors, this design supports the attachment, settlement and differentiation of cells, the transmission of signals of cell interaction between itself and the matrix [32]. Their nature allows them to absorb large amounts of water into a three-dimensional network that is ideal for supporting cell growth. Gel-like biomaterials are permeable for cytokines and metabolic products, which is important for cell culture. SF hydrogels made from silk aqueous solutions form β-layered structures. T.P. Nguyen *et al*. in the study found that SF can be converted into hydrogel and various environmental factors such as pH can increase the rate of gelatinization [9]. Pore size in hydrogel controlled by SF concentration and temperature [33]. The mechanical compressive strength and hydrogel module increase as the protein concentration and gelation temperature increase. Apart from temperature, pH and SF concentration, the rate of gelatinization is influenced by Ca^{2+} and poly (ethylene oxide) (PEO). Increasing protein concentration, decreasing pH, rising temperature, adding Ca^{2+} and PEOs led to a decrease in gelatinization time [26].

SF hydrogels are used in skin wound healing, bone and cartilage tissue engineering [17].

S. He *et al*. investigate the wound healing in Sprague-Dawley rat animal model. The authors divide rats in four groups and treat the wound with different wound dressing: heparinized SF hydrogel, heparinized SF hydrogel with acidic fibroblast growth factor 1 (FGF1), commercial chitosan dressing (positive control), and the untreated blank group. Results shows significant healing process in wound of first three groups, but heparinized SF hydrogel with FGF1 showed the smallest size of wound, because FGF1 promote cell migration and proliferation [35].

Osteoblastic cells are well attached to 2% SF hydrogels, thus confirming the biocompatibility of the material. Adding 30% glycerin to the hydrogel increases their proliferation [34]. Implanted into rabbit femur defects f SF hydrogels stimulated the bone regeneration and accelerated mineralization [36].

SF hydrogel was prepared by using 1,4-Butanediol diglycidyl ether (BDDE). SF/BDDE hydrogels was biocompatible and had good mechanical properties: high elasticity (compression module of 166 ± 15.0 kPa), stable structure and mechanical strength in aqueous solution. SF/BDDE hydrogels reduced pain and cartilage damage in rats, indicating the utility for pain relief and delayed release medications in osteoarthritis treatments [37].

3.2. SF Sponge and Clinical Application of SF Sponge

Porous SF sponge can be obtained by water-based or solvent-based SF (e.g., hexafluoro propanol) using gas foaming, porogens and lyophilization [17]. Lyophilization is a soft low-temperature drying process using vacuum. Before molding and freezing, a small amount of solvent (ethanol, methanol, DMSO) can be added to the aqueous SF fiber solution for the preparation of porous solvent-based sponges [38].

Pores can also be created with porogens. Porogens are specified shape and size particles, which are used to make pores. Salt (e.g., 490 to 940 μm sodium chloride) or sugar are used as a porogen. Pore size is controlled by the porogen size, porogen stacking and SF concentration. SEM results showed that water-based sponges have a rougher surface morphology than solvent-based sponges due to the limited solubilization of the crystals surface during supersaturation of the SF solution prior to solidification. This rough surface of this sponge was more effective in attaching to cells than solvent-based sponges. In addition, water-based sponges degrade faster than solvent-based sponges [39].

SF sponges are used in skin wound dressings, bone and cartilage tissue engineering [17].

M.M. Moisenovich *et al*. examined two types of scaffolds: SF sponge and containing SF and 30% gelatin composite-based scaffold. Starting from the 1st day, cell line 3T3 actively proliferated on SF scaffolds, and the presence of gelatin of composite-based scaffold reduced the proliferation of such cells. SF sponge scaffold is suitable for effective keratinocyte cultivation *in vitro*. SF scaffolds exhibited the ability to regulate the proliferation of fibroblast, which is important in the cell delivery to the injured site, because intensive fibroblast proliferation can lead to the fibrosis development and the scar tissue formation. Balanced fibroblast growth is significant to create optimal growth conditions for keratinocyte in tissue engineering [40].

Deshpande R. *et al*. during the study of MSC in bone tissue regeneration with the use of SF sponges found that SF increases osteogenesis biomarkers such as bone morphogenetic proteins, osteopontin, osteocalcin, osterix, runt-related transcription factor 2 compared to ceramic-based scaffolds [41].

Chondrocyte distribution and cartilage formation were measured in three types of SF sponges of various pore sizes (40 - 80, 80 - 120 and 100 - 140 μm). Cartilage tissue was well formed in each group after 21 days. These results suggest that the initial condensation of cells within 24 hours after seeding plays an important role in the formation of cartilage [42].

3.3. SF Mats and Clinical Application of SF Mats

The structure of SF mats increases the surface area for cell attachment, which make SF mats appealing in tissue engineering. SF mats can be fabricated by reprocessed native SF fibers or electrospinning. The main elements of the electrospinning apparatus are a needle through which the polymer solution is supplied,

and a collector designed to collect the incoming polymer. These elements are combined into one electrical circuit. As the electrical voltage increases, at the end of the needle, the forces of the surface tension of the polymer solution are overcome, resulting in the formation of a Taylor cone—a conical droplet of the polymer [43] [44]. As soon as the voltage is sufficient, from the top of the cone in the collector direction polymer jet is directed, the diameter of which depends on a variety of conditions. In the air, a part of the solvent is evaporated, and the jet is split, resulting in the collection of pure polymers in the form of randomized or directionally laid fibers with nano or micrometer dimensions. The resulting material is in the form of a thin, fibrous, porous, soft fabric or a thin elastic coating [14] [45]. SF has excellent biocompatibility, high strength and minimal inflammatory response [3]. However, the material produced by electrospinning has a small pore size, which prevents proper cell infiltration. The team of researchers obtained fibers from a mixture of SF and polyethylene oxide with simultaneous application of NaCl crystals during electrospinning [46]. There was good adhesion and infiltration of 3T3 fibroblasts on the matrix. In wound treatment experiments in rats, the scaffold closed wounds faster and degraded more efficiently than the commercially available MatriDerm regenerative material [47].

SF mats are used in skin wound healing, nerve, bone, connective tissue engineering [17].

Chouhan D. *et al*. used electrospinned SF mats modified by epidermal growth factor (EGF) and ciprofloxacin HCl as a wound dressing in skin regeneration studying. Modified SF mat scaffold increased the proliferation of dermal fibroblasts and keratinocytes *in vitro* compared to non-modified scaffold. Modified SF mat scaffold exhibited more rapid wound healing and re-epithelialization controlling the deposition of elastin, reticulin and collagen in rabbit animal model. Results indicate the effectiveness of EGF delivery in wound healing, as well as good biocompatibility, acceptable good moisture retention capacity, water vapour transmission rate, suitable elasticity, antibacterial effects and controlled drug release [48].

SF mat scaffolds can be used in neural engineering. Li G. *et al*. examined aligned and random eletrospun fabricated SF mat scaffolds. The authors determined that SF mat scaffold increased neural progenitor cell proliferation (143.8% ± 13.3% and 156.3% ± 14.7% respectively) and cell differentiation (93.2% ± 6.4% and 3167.1% ± 4.8% respectively). SF mat provides a favorable functional microenvironment for neurogenesis, making it a promising material in neural engineering [49].

SF scaffolds containing bone morphogenetic protein (BMP-2) and (or) hydroxyapatite nanoparticles obtained by electrospinning, used to form bone tissue in *vitro* with the use of human bone marrow MSC [50]. BMP-2 was electrospunned in an aquatic environment and remained bioactive. The cells were cultivated on the scaffolds for 31 days in an osteogenic environment. Scaffold supported cell growth and osteogenic differentiation. The application of BMP-2 and

hydroxyapatite together resulted in the highest calcium deposition and transcription levels of BMP-2 compared to other systems [51]. According to the study, SF is involved in the formation and maturation of bone tissue.

3T3 fibroblasts have a higher proliferative activity on scaffolds made of gelatin and electrospunned SF than on SF films obtained by casting.SF to gelatin mass ratio – 1:3. Cell proliferation is increased by adding gelatin to the scaffold. The number of cells in these samples was 2.5 times greater than in pure gelatin coatings [51].

3.4. SF Fiber and Clinical Application of SF Fiber

SF fiber can be produced by silk cocoons reeling. SF fiber are prepared by partial solubilization of native SF fibers in formic acid and calcium chloride solution. SF fibers also can be pre-mechanically homogenized and then partially solubilized. An alternative method of SF fiber fabrication is electrospinning [21].

SF fiber are used in skin wound healing, ligament tissue, tendon tissue engineering [17].

Zhang *et al*. examined two kinds of electrospunned SF scaffolds (ESF), one of them was modified by poly-dopamine (PESF). Results show that PESF enhance the hydrophilicity and protein adsorption ability, attachment, spreading and proliferation of fibroblasts which affect the acceleration of the healing process comparing to others. Therefore, PESF membrane scaffold could be promising wound dressing for skin regeneration [53].

SF fiber are used as an artificial ligament treatment, for instance anterior cruciate ligament (ACL) reconstruction. The artificial ligament woven from regenerated SF and bioactive clay Laponite (RSF/LAP) hybrid fibers showed better cytocompatibility and osteogenic differentiation. In addition, RSF/LAP hybrid fibers significantly enhanced the process of transplant osteointegration and improved the corresponding biomechanical properties in the ACL rat model reconstruction *in vivo* [54].

SF fibers was combined with gelatin methacryloyl (GelMA) to create and bioactive nanofibrous scaffold with strong mechanical properties. By changing the ratio of SF to GelMA, the mechanical properties of scaffold nanofibers can be flexibly altered. This scaffold promotes MSC proliferation, the production of vascular endothelial growth factor, and the expression of tenogenic genes. These results in the promotion of tenogenesis: migration and proliferation of tenocytes. In the experiment *in vivo*, these scaffolds demonstrate enhanced tendon regeneration, allowing them to be applied in tendon tissue engineering [55].

3.5. SF Film and Clinical Application of SF Film

SF film is derived from aqueous and organic SF solution that has been treated with various solutions (e.g., methanol, polyethylene oxide). The ratio of SF to solutions and processing time will determine its mechanical properties and biodegradability. Another method of SF film manufacturing is the layer-by-layer

assembly [56] [57]. These ultrathin films are stabilized by hydrophobic interactions, the film thickness is regulated by the concentration of SF in solution. Films support MSC adhesion and proliferation [29].

SF films are used in ophthalmology, skin wound healing, bone tissue engineering [17].

S.Wang and his colleagues created artificial corneas using 3D bioprinting, which are made of collagen and SF due to the shortage of donor corneas. The SF stiffness is comparable to that of corneas (silk fibroin—67.7 kPa, cornea—40 - 60 kPa) [58]. Its modification variability allows to create an optically clear films that are suitable for use as a regenerative scaffold for cornea. By transforming SF into a sponge, the scaffold cansupport neuron growth nd the formation of neuronal connections [59].

Li S. *et al*. developed Strontium loaded SF/sodium alginate blend film. This film scaffold exhibits good biocompatibility, permeability, mechanical strength, high swelling ratio. Release Sr^{2+} ions stimulate endothelial cells proliferation and migration and enhance blood vessels formation [60]. Mentioned above factors are important for wound dressing materials [61].

Electrospunned SF films comprising a copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HHx) [P(3HB-co-3HHx)] and were examined by S.L. Ang in bone regeneration study. Histological analysis showed that this scaffold promotes osteogenic differentiation of human umbilical cord-derived MSC. In addition, this scaffold adjusts osteogenic marker genes expression alkaline phosphatase and osteocalcin [62].

4. Conclusion

An analysis of the articles was conducted, whose authors studied the properties of SF: their biocompatibility, structure and mechanical properties, biodegradation ability, ability to maintain adhesion, migration, proliferation of different types of cells. Notwithstanding the above, there are still some serious obstacles to the development of biomedical silk-based devices. For example, the problem of synthetic manufacturing of artificial silk or silk-like fibers, which characteristics will be comparable to natural silk or will be better. Alternative option of manufacturing silk is producing *in vitro*. Another relevant issue is the possibility of improving the characteristics of the natural silk. In this case, it is necessary to pay attention on selective breeding of animals (for example, silkworm spiders), which will produce silk with desired properties for regenerative application. There remain doubts about the long-term safety of silk biomaterials for medical use. Silk sutures are present in the body for a limited time due to the healing rate of the wound, while tissue engineering scaffold should be in contact with the tissues for a long period of time. The responses of the innate and adaptive immune system to scaffold require long-term research. The rate of biodegradation and immunogenicity is influenced by the type of scaffold and the site of application, which requires further research. In addition, there may be unwanted body

reactions to SF scaffolds due to their size and morphology. For example, microparticles resulting from the scaffold biodegradation may cause immune response. With the greater development and wider use of SF scaffolds, sterilization plays an important role in the study of these materials. Currently, there is no universal and superior method to sterilize different variations of SF scaffolds without altering their characteristics. There is also great interest in studying mechanical and biological characteristics, conducting experiments *in vitro* using materials from SF derivatives (for example, Methacrylated silk fibroin (SFMA)), as well as combining them with other materials, commonly used in regenerative medicine.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of abbreviations

SF—silk fibroin
SEM—Scanning electron microscope
MSC—mesenchymal stem cells
MWD—molecular weight distribution
FGF1—fibroblast growth factor 1
BDDE—1,4-Butanediol diglycidyl ether
EGF—epidermal growth factor
BMP-2—bone morphogenetic protein 2
ESF—electrospunned silk fibroin
PESF—poly-dopamine electrospunned silk fibroin
ACL—anterior cruciate ligament
RSF—regenerated silk fibroin
LAP—Laponite
GelMA—gelatin methacryloyl
3HB—3-hydroxybutyrate
3HHx—3-hydroxyhexanoate