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REVIEW PAPER

Biocompatible Scaffold Based on Silk Fibroin for Tissue Engineering Applications

Ali Imran Ansari[1] · **Nazir Ahmad Sheikh**[1]

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Abstract Silk fibroin (SF) is a protein with unique properties that make it an ideal scaffold for tissue engineering. Research in this area has improved its advantages by incorporating many other materials during synthetic stages and helping in use of (SF) in broader biomedical application. Silk is gaining popularity as an encouraging bio-sourced raw material due to superior mechanical qualities, flexibility, as well as bioactivity. This review focus on the current advances in silk-founded biologically inspired active and efficient scaffold in medical fields as well as the most recent applications and advancements in the use of SF as a biocompatible material. The authors begun with a short overview of silk, including its origins, characteristics, source, in addition procedures. The findings of this analysis are crucial in aiding designers in making proper design structure as well as the percentage of porosity required in scaffold for the cell's growth prior to fabrication of the scaffold. Furthermore, SF can be formed into numerous scaffold kinds such as sponges, mats, hydrogels, and films using both traditional and innovative bio-fabrication methodologies.

Keywords Silk fibroin · Biomaterial · Bone tissue engineering · Bone regeneration · Tissue engineering · Scaffold

Introduction

Osseous matter, seed, wood, spider silk, wild bear hair, as well as epidermis, for example, have exceptional mechanical qualities and perhaps some distinctive capabilities, which are largely due to their parametric configurations [1, 2]. Vast numbers of people across the globe suffering from serious pain caused by bony vascular trauma or disease. The reanimation of a defected bone poses significant diagnostic and therapeutic obstacles. During the last two decades, bone tissue engineering (TE) has evolved tremendously, with many breakthroughs in biomaterial scaffolds [3]. It is indeed important to note that significant biological materials are usually made up of degradable and sustainable constituents like amino acids, polysaccharides, or micronutrients [4]. It is becoming more essential as Petro polymeric materials are resistant to biodegradation, resulting in constantly rising pollution concerns and challenges worldwide [5]. As a result, leveraging biodegradable polymers as base materials to build biologically inspired substances, in portion or in whole, emerges as an appealing alternative. Fig. 1 shows the various stages of production of silk fiber from Silkworm (Bombyx Mori) cocoons [6, 30]. Numerous biomaterials have indeed been studied to create composite materials with biocompatible configurations, together with collagen, silk, fibers, chitosan, hydrogels, as well as alginate. Silk remains out among them for its exceptional mechanical quality, adaptability, as well as bioactivity [7, 8]. It is indeed an encouraging natural resource for constructing a variety of bioinspired structures. Silk generated by arthropods is a proteinic polymer which has been used in the textile manufacturing and also for clinical surgery for generations [9]. Its basic state consists of a central protein fiber made up of the protein silk fibroin as well as a sticky wrap made up of the protein sericin. Silk protein molecules have a highly

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✉ Ali Imran Ansari
aliimran_06phd19@nitsri.net

[1] Mechanical Engineering Department, National Institute of Technology, Srinagar, Jammu & Kashmir, India

repetitive series that allows them to have a lot of uniformity in their secondary structures, which reflects their significance as structural strength [10, 11]. *Bombyx mori* fibroin is a silk fibroin derived from *Bombyx mori*. Silk caterpillar is a collagen fibrils nutrient comprised of three subtypes: dense bonds SF (391 kDa), less dense SF (27 kDa), and P25 (25 kDa), all have a molar relationship of 6:6:1. This structure at 2.3 MDa is complex when taken as a whole [12]. Silk fibroin can take on a variety of shapes, all of which are created by physiochemical cross-linking [13, 14]. Silk fibroin's dense chain is made up of antiparallel ß-sheets, whose fibers run in parallel to the fiber orientation. Extended sections of SF are made up of crystalline structure that ranges of (-Gly-Ser-Gly-Ala-Gly-Ala-)n, which are obstructed by immense contaminants like Val, Leu, I Val, Leu, Ile, Asn, Pro, and Tyr [15]. It is hydrophobic in nature due to the crystals repeating patterns. The dense chain's N- and C-terminal effluents are non-repetitive and also have dissimilar charge statistics. The N-terminus incorporates numerous Asp contaminants that might aid in protein biosynthesis in reaction to pH fluctuations, whereas the C-terminus is high in Arg. The less dense chain of silk fibroin constitutes a disulfide bridge with the dense chain, restricting it from being retained in the endoplasmic reticulum. The less dense chain has a non-continuous amino acid composition and a non-repetitive sequential. It is much more hydrophilic, mildly stretchy, as well as has little or no crystalline phase than the heavy chain [16]. The configuration of silk fibroin produced by non-mulberry silkworms (Saturniidae) varies. Only the dense chain forms silk fibroin in approximately few species, which include *Antheraea mylitta* and A. assama. A polyalanine-based ß-sheet core is varied with patterns rich in Arg or Gly, resulting in fewer arranged antiparallel -sheets, -turns, and partial -helices in this heavy chain [17]. A few arthropods, such as spider silk fibroin (spidroin), have quite a configuration that is similar to silk fibroin. Spidroin is primarily made up of a dominant repetitive section that is high in Gly, Ala, and Ser [18]. Complications associated with farming spiders, natural spidroin is in short supply, so research has focused on recombinant spidroin [19]. Silk fibroin can be used to make successful quantifiable tissue-engineered scaffolds, such as foams, films, mats, textiles, porous, hydrogels, as well as particles. Silk fibroin, like every substance, could enhance the effects of many other compounds to enhance or acquire qualities such as cell adhesiveness, biocompatibility, immune regulation, antimicrobial properties, as well as super-paramagnetic behaviors [20, 21]. Synthetic oxides as well as salts, incorporated as nanoparticles, interpenetrating networks, surface coating, or even other methods, have become widely attractive for silk fibroin scaffolds with tissue engineering applications. A few of these relations are referred to as nanoparticles, which are multi stage structures composed of organic substances that are closely embedded with inorganic compounds that have at least in one dimension of sequence 100 nm or even less [22, 23]. Nanomaterials have the benefit of ensuring features that make them more beneficial as scaffolds for tissue engineering, such as toughness as well as persistence; in certain instances, they

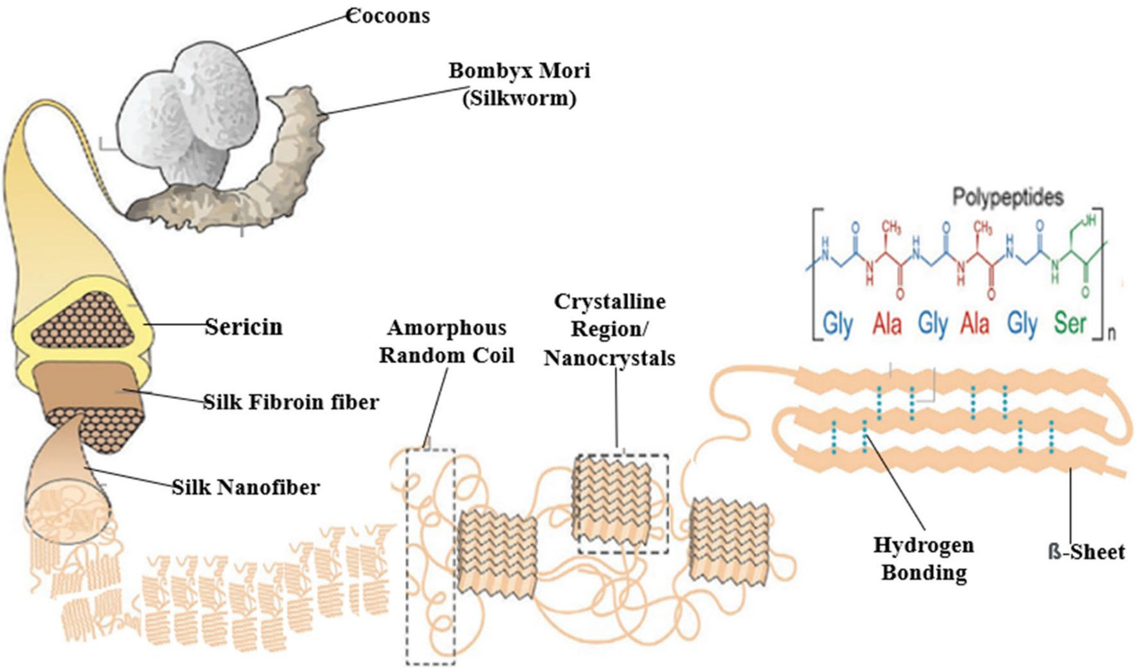

Fig. 1 Shows the hierarchical levels of silk fiber derived from B. Mori's (Silkworm) [6, 30]

might even become reinforced biomaterials. Mechanical behavior as well as bioactivity such as biocompatibility, innate immunity activation, as well as angiogenesis are all dependent on how the organic and inorganic aspects engage. Van der Waals forces, electrostatic interaction, as well as hydrogen bonds may be used to weakly interact the various stages. Heavy relations, such as covalent as well as ionic-covalent bonds, may indeed exist [24]. The objective of this study is to examine the different synthetic materials used to create silk fibroin bio composite material, as well as the different kinds of scaffolds as well as focused molecules and tissues used to examine them. This overview furthermore seeks to examine the impact of synthetic material incorporation on the structural as well as chemical behaviors of SF scaffolds, as well as the resulting bioactivity.

Silk Source

Spiders, silkworms, as well as other arthropods could perhaps yield silk, which is made up primarily of amino acids. Spider silk is less widely preferred on large scale owing to the challenges in cultivating spiders as well as gathering raw silk. Cocoon silk obtained from silkworms, particularly the *Bombyx mori* (*B. mori*) species, on the other hand, is being used for centuries and it is the most excellent source of silk protein. *Antheraea pernyi* (*A. pernyi*) and *Antheraea mylitta* (*A. mylitta*) are two wild silkworm species that can produce high-quality silk [25–27]. Furthermore, recent advances in transgenic technologies have allowed the production of recombinant spider silk protein in a variety of heterologous, including E.coli, yeast, plants, goats, and silkworms [28, 29]. As a result, silk's sources will be expanded even further, as well as fusion silk proteins will be customized. Silk is renowned for its enhanced mechanical features that also stem from hierarchical structures ranging from the molecular to the macroscopic scale. With nanoscale b-crystalline stage ingraining in an amorphous matrix, proteins patterns accumulate into particular areas, in which the crystal area specifies toughness as well as the amorphous site defines strength and durability [30]. The clustering frameworks then arrange into functionalized nanofibers, microfibers, as well as microfibers, creating massive intermolecular and intramolecular hydrogen bonds at multiple scales, endowing silk fiber with greater mechanical properties.

Extraction

Silk fibers can be spun from silkworm cocoons on a massive scale using industrial machinery, which is primarily used in the textile manufacturing. In the meantime, revived silk fibroin (RSF) dominates the construction of silk-based materials in a range of shapes. A specialized protocol is followed to extract RSF from *B. mori* cocoons [12], Degumming, rinse with water, solubilization, dialysis, as well as flocculation are common procedures. artificial salts, which have large portion of LiBr salt mixture, NaSCN salt mixture, ZnCl2 salt mixture, as well as a triple mixture of CaCl2/ ethanol/ H2O, are being utilized to disintegrate degummed silk fibers by splitting H^{2+} bonds and splitting the b-crystalline frameworks, after that a liquid mixture of natural RSF is acquired upon effectively eliminating the ions as well as contaminants. The RSF solution can be solubilized for extended storage, as well as the partially purified substance can then be dispersed in an organic solvent such as HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) to produce different substance. A few natural salts, such as AminCl and BminCl, can dissipate silk fibers to produce an RSF ionic liquid solution with a suitable concentration, and that they are biodegradable. Furthermore, because silk contains a significant proportion of side-chain proteins like serine, threonine, aspartic, glutamic, as well as tyrosine, the RSF molecules reveal a large number of reactive groups for further synthetic as well as operational reconfiguration [31]. However, due to the damage of silk fibroin protein chains as well as large molecules degeneration, the degumming procedure as well as solubilization highly predictable weaken the mechanical characteristics of ready to use RSF substances [32, 33]. On the one hand, degumming silk fibers with a non-alkali solution (such as urea) instead of an alkali solution (such as sodium carbonate) causes less harm. Because solubilized RSF particles exist as arbitrary coils, however it is liable to control the physical properties of RSF substance by adjusting their configuration transformation to b-crystalline framework that will fulfill the requirements in diverse utilization situations [34]. SNF molecules, in addition to RSF molecules, perform an essential role in the development of biologically inspired substances. Up-down depilation, bottom-up gathering, as well as electrospinning have all been found to be useful methodologies for preparing SNF, each with its own set of advantages and pitfalls. There are normally three main steps to exfoliation: To acquire silk fiber/SMF slurries, silk fibers are submerged in HFIP as well as nurtured at 60 °C for 24 h; the dehydrated silk fiber slurries are instead relocated to H2O solution as well as precipitates are eliminated; as well as eventually, SMF scattering is handled with ultrasound to extract SNF [35]. Despite the fact that this procedure necessitates numerous stages, the exfoliated SNF preserve their natural structure as well as physicochemical parameters. A small quantity of soluble RSF mixture is heated at 60 ^{0}C for one week to consolidate silk amino acids into SNF in the bottom-up technique [36]. The entire procedure is frequently motivated by thermodynamical process and takes a long time to complete; however, once initiated, SNF will grow on its own. Electrospinning is a method for extruding a charged silk solution through a syringe as well as sprinkling nanofibers that are then collected on an oppositely charged collector [37]. SNF's morphological characteristics as well as structures can be fine-tuned thanks to the high precision control of operating parameters. Nevertheless, to enhance the mechanical property as well as moisture consistency of the as-prepared SNF, post-treatments are generally required.

Silk Fibroin as a Biomaterial

Biological compounds, scaffolding substances, or just tissue were used to cure Musculoskeletal deficiencies [38]. Silk, a biocompatible polymer, has recently attracted considerable attention for its potential role in the regeneration of orthopedic tissue architecture. Silk is a protein biomaterial produced from silkworms, spiders, mites, as well as flies that is deduced from native sources. Silk has a range of characteristics; however, the major ingredient is proteins, with minuscule quantities of lipids and polysaccharides [39]. Cell growth, propagation, genetic mutation, as well as segmentation are all influenced by the biomaterial as well as surface topography of scaffolds in which cells adhere [40, 41]. The opportunity of SF as a biocompatible material on mesenchymal stem cells (MSCs) to distinguish with ECM secretion as well as mineralization has been demonstrated using SF scaffolds [42, 43]. The mechanical and physical properties of bone TE are also essential. Across several research, SF has showed outstanding mechanical characteristics, strength, durability, as well as elastic modulus. Bioactivity, good biocompatibility, as well as low cytotoxicity, which are all key aspects to consider when designing TE scaffolds, have produced remarkable results [44]. The aspects of SF as a biocompatible material will be discussed in the following sections.

Properties of Silk Fibroin (SF)

Silk is made up of two major proteins: SF, which is the fibrous portion of the filament, and sericin, which is a hydrophilic adhesive-like protein. Silk is derived from a variety of insects, including spiders and *Bombyx mori*. Beetles, on the other hand, use a variety of techniques. Various silks have unique mechanical and functional qualities [45]. Owing to its simplicity of processing, excellent mechanical properties, as well as biodegradability, SF from *Bombyx mori* is the most well-known silk. Due to its commercial manufacturing, SF is being used as a textile for centuries. Furthermore, there have been various research on the use of SF in biomedical applications. One of the very first steps in the manufacturing of an SF scaffold is to remove the sericin component. For diagnostic and therapeutic use, sericin, the outermost layer of silk, was known to cause an allergic reaction. Even so, it was discovered that a composite of sericin and fibroin induced the allergic response, but instead sericin has recently been discovered to be a biocompatible material [46, 47]. The degumming procedure requires heating cocoons to eliminate the glue-like protein sericin (Fig. 2). The primary component of degummed SF is the ß-crystalline sheet. Besides enhancing the glycine substance in the SF proteins series, potassium phosphate or methanol treatment ß can stimulate pretty reliable sheet nanocrystals. Because of the high glycine content, SF can pack tightly [48, 49]. Hydrogen bonds are the primary means of interaction between the crystalline sheets. Despite the fact that hydrogen bonds are said to have weak interactions, SF's hydrogen bonds assist it to arrange as well as heal itself when it reforms. Before being used in laboratories, the sericin, or outer layer of SF, is removed. SF is exposed to a harsh condition during the degumming procedure. This mostly alters the silk's architecture, as well as its mechanical characteristics. While the Young's modulus of degummed SF was similar, the tensile strength and elongation rate were not [50, 51]. Numerous investigators may be drawn to handle SF in various forms because of its sufficient mechanical capabilities.

Silk-based Bone-Inspired Materials

Bone appears to be a standard biological composite material with complex hierarchical structures (Fig. 3) [52]. The organic–inorganic phases were indeed closely coupled at the nanometer scale and then further assembled into macroscopic scales configurations with hydroxyapatite (HAP) nanocrystals disseminating all along collagen fibers produced from type I collagen molecules. Bone, as an important structure for the human body, seamlessly blends high toughness and strength, which are often thought to be mutually exclusive. Bone can also self-grow as well as self-heal, ensuring that it maintains its ordinary structure and wellbeing. Numerous bone-like composite materials have been invented, the majority of which are used for bone regeneration, and thus are influenced by the chemical structure, multiscale design, as well as versatility of bone. Due to its many advantages of silk, such as its similar fibrous protein configuration to type I collagen, favourable biotransformation capacity, ideal mechanical properties, and reduced immunogenicity, silk is an intriguing material in this context [53–55]. The impressive results of silk/HAP composites in bone regeneration have been well-covered, and this review provides brief introductions to several research of significant relevance. Collins et al. addressed the first illustration of a load-bearing bioinspired bone-like silk/HAP composite scaffold with mechanical characteristics nearly equivalent to cancellous bone in 2009 [56].

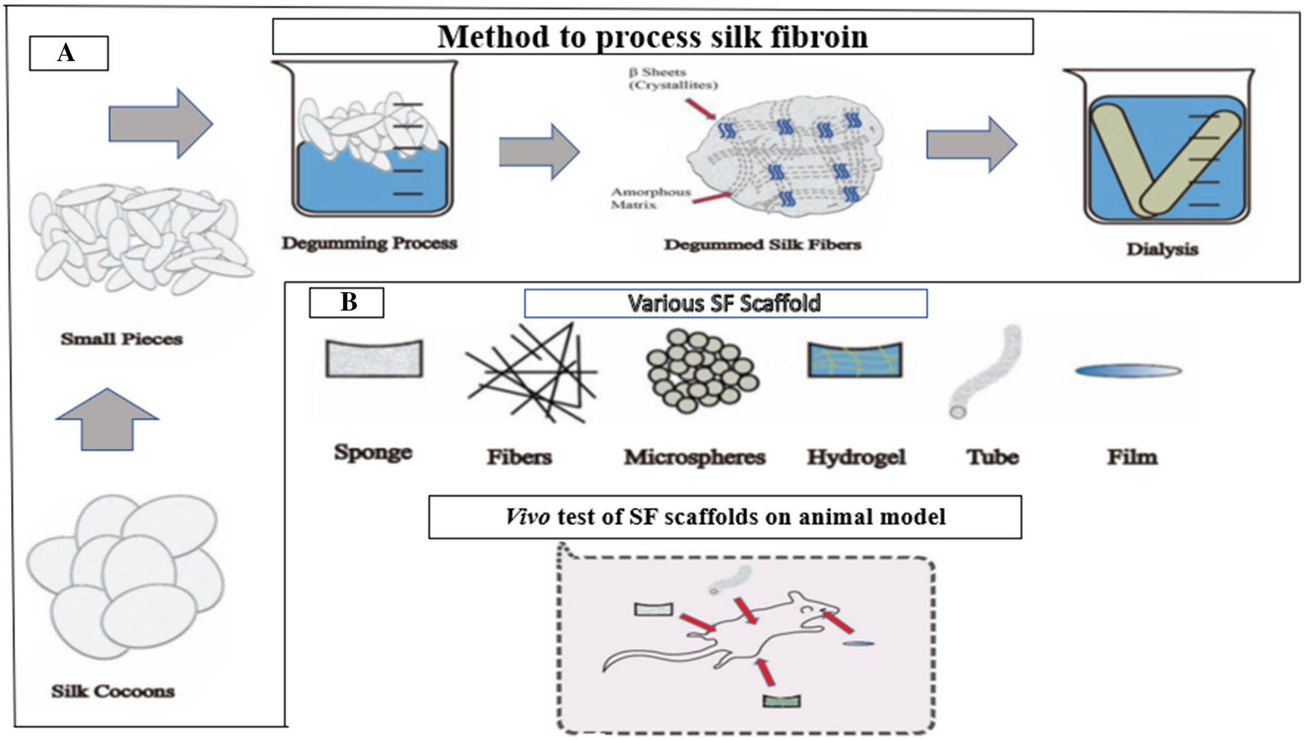

Fig. 2 Shows the method to process the silk fibroin as well as indicate the various types of SF based scaffold and vivo test of SF scaffolds on animal model [48, 49]

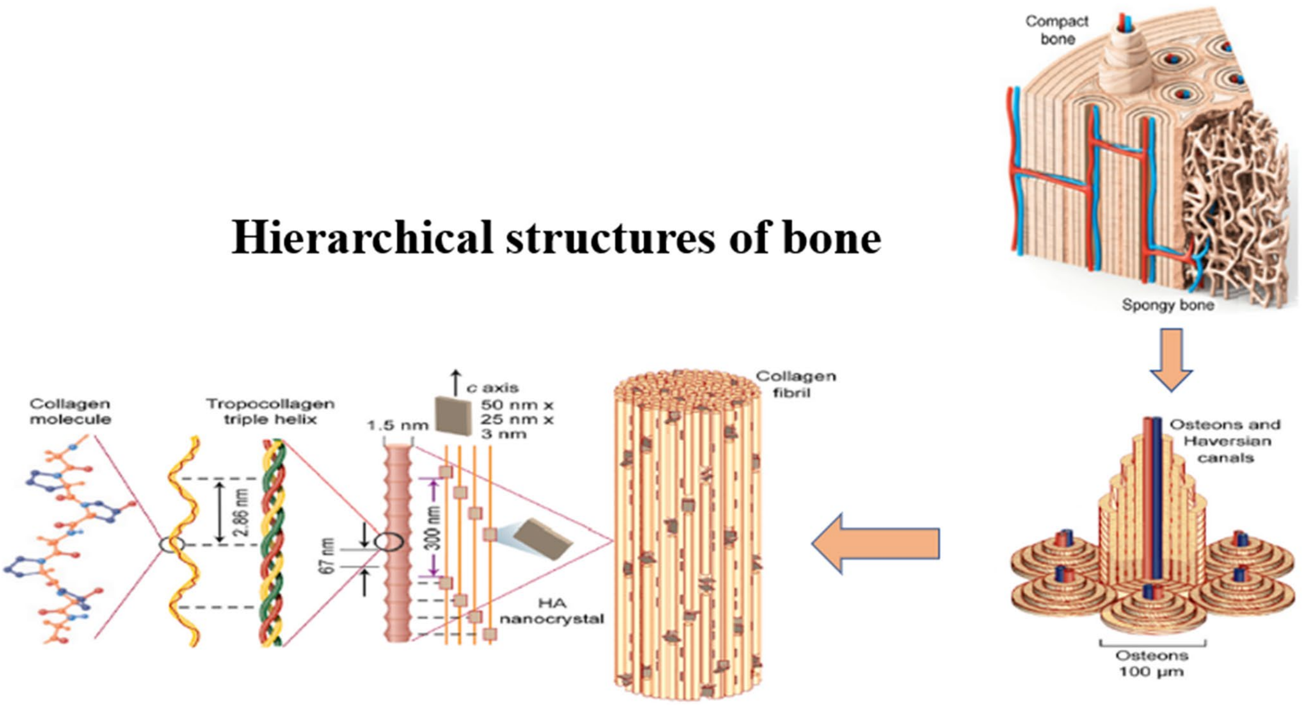

Fig. 3 Depicts a hierarchies of bone structure in which osteones have a lamellar morphology, with specific lamellas comprising of fibers organized in geometrical patterns, as well as the fibers consist of several mineralized [52]

In correlation to collagen scaffolds (0.034 MPa) as well as viable calcium and phosphate scaffolds (< 5.0 MPa), such highly porous scaffolds had mean compressive strengths of 14 MPa as well as modulus of 175 MPa, which had been in the mid-range for cancellous tissue mechanical systems (7–10 MPa and 5–500 MPa). The scaffold was made by gradually intruding $Ca2^{+}$ ions into a refrigerated phosphate-containing silk fibroin gel and afterward treating this with ethanol as well as chemical cross-linking, resulting in uniform throughout vivo mineralization and tight protein/mineral incorporation. In addition, the silk/HAP scaffold promoted the angiogenesis of bone marrow stromal cells (HBMSCs) in vitro, as well as fresh osteoid was witnessed on the scaffold's surface once subcutaneously implanted in rats, implying that it could be used as a surgically implanted substitute to allograft, autograft, and xenograft. Instead of effectively combining the two aspects, which eventually results in phase transition as well as poor mechanical design, this research introduced an innovative way to construct silk/HAP composite scaffolds. In a latest systematic review, Farokhi et al. systematically represented silk/HAP composite materials for bone tissue regeneration [57]. They can be used alone rather than in conjunction with biologically active compounds as well as stem cells to enhance repair outcomes even more. For instance, a silk-nanohydroxyapatite (nHAP) scaffold loaded with bone morphogenetic protein-2 (BMP-2) as well as vascular endothelial growth factor (VEGF) has been formed for bone tissue regeneration, as BMP-2 has been shown to induce osteogenic differentiation and VEGF has been shown to perform a vital phase in blood vessel configuration [58]. In vitro and in vivo, the governed a double discharge of BMP-2 and VEGF encouraged osteogenesis; as per recently created bone content 12 weeks after incorporation in rat calvarial deficiencies, the composite scaffold reached a significant bone formation impact compared to incorporating BMP-2 or VEGF alone. Furthermore, VEGF may aid vasculature as well as expedite the degeneration of the silk scaffold, resulting in improved bone tissue regeneration. A good replacement between both the prosthesis as well as the patient's anatomical structures is necessary, in addition to equivalent structure and composition to native tissue. Due to its customized design as well as efficient control in designing and building bone regeneration scaffolds, 3D printing is surfacing as a much more viable approach in this respect. Fitzpatrick et al. published a 3D silk/HAP scaffold with governed microporosity as well as integrated porous structure throughout in 2021 [59]. This structure had good cytotoxic effects as well as osteoconductive, as well as mechanical characteristics appropriate for bone. Undoubtedly, in the upcoming years, 3D printing could provide a route to genuinely replicating one's fine configurations at multiple scales. Collagen fibrils are consisted of collagen protein complexes (tropocollagen) constituted from triple chains of protein molecules as well as hydroxyapatite nanomaterials connected by an organic phase to produce fibril configurations.

Silk-Based Skin-Inspired Materials

Skin serves as a protective barrier against the outside world for vertebrates, as well as a comprehensive sensor ability to respond to numerous environmental stimulation at the same time [60, 61]. Skin is also gentle, tough, stretchy, as well as self-healing, attracting a lot of interest in wearable electronics, advanced materials, as well as wound repair to mimic its form and composition. The explosive growth of skin-inspired optoelectronic devices has been especially noticeable in recent times [62]. Silk is an encouraging basic raw material in this flourishing field because of its structural reliability, stretchability, demic affinity, as well as ecological sustainability. Silk-based substances have been used in a variety of versatile electronics applications, which include conductors, sensors, actuators, as well as optoelectronic devices, as characterized across several recently reported publications [63, 64]. The bioinspired structure design influenced by skin and bone will be the main focus of this paper. The epidermis, dermis, as well as endodermis are the three main levels of skin, with the dermis, as the thickest layer, playing the most important role in bearing stress distribution and avoiding damage [65]. Wang et al. mentioned SCP gel, a bioinspired gel sensor based on silk fibroin/cellulose nanocrystals (CNC) as a "hard" element as well as polyacrylamide (PAM) as a "elastomeric" component, motivated by the 3D architectures generated by hard as well as stretchable fibers in the dermis CNC motivated the promising transition of SF from a-helixes to b-crystallites in combination with silk fibrous networks as well as multiple H^{2+} bonding in SCP gel, nearly increasing the mechanical integrity of the embedded design. Even through a 40% deformation, the SCP gel was capable of maintaining 88.8% of its peak performance after 1000 compression cycles. The SCP gel also showed excellent adherence as well as self-healing characteristics, making it perfect for use as sensors attached directly to human skin [66]. This characteristic is also critical for flexible electronics, as deficiencies are almost always unavoidable during processing and then use, particularly for those with pure polymer praising surfaces. Whenever a notch is present, the tensile strength and fracture strain of the most frequently utilized polymer, PDMS, are both reduced by 90%. Humans replicated skin's tension allocation approach in a fiber dispersed composite film based on flat silk cocoon to fulfil this need, as well as demonstrated remarkable mechanical performance and excellent tear resistance [67]. When evaluated by comparing to pure polymer, incorporating just 1% silk fiber increased tensile strength and modulus by 300% to 612% percent, respectively, whereas the composite film remained flexible and transparent. The composite film also outperformed the pure film in terms of tear as well as fatigue resistance, with nearly 30,000 loading–unloading tensile cycles, nearly 7.5 times those of the pure film. The synergistic impact of fiat silk cocoon representing as both a reinforcement phase as well as a crack inhibitor in the polymermatrix resulted in superior mechanical effectiveness. More intriguingly, this paper reviews that by simply changing the spinning behaviors of silkworms, an innovative form of silk fiber with a unique purpose could be produced, spurring the advancement of silk fiber-reinforced synthetic structures.

Types of Organic and Inorganic Materials in Silk Fibroin Composite Scaffold

The use of SF as well as other biomimetic material in bone regeneration has been shown to benefits. In vitro and in vivo, the addition of calcium phosphate, collagen, as well as various nature-derived biomaterials resulted in bone tissue regeneration. Titanium alloys are among the inorganic compounds that were used to create scaffolds [68], calcium phosphate [69–72], calcium carbonate [73], molybdenum disulfide [74], nanoparticles based on silver [75], titanium oxide [76], magnetite [77], zirconia [78], cerium oxide [79], gold [80], alumina [81], β-tricalcium phosphate (β-TCP) and ZnSr-doped β-TCP [82]. Bioactive ceramics, such as hydroxyapatite [83], diopside [84], Laponite [85], hardystonite [86], and halloysite [87], have also been used as inorganic phases. Numerous different inorganic materials, like strontium and silver, have been mixed with hydroxyapatite in some cases [88, 89]. The synthetic phase, which is usually made up of nanomaterials, was added to the silk fibroin network to increase its mechanical characteristics. This could be explained by a decrease in pore diameter as well as an enhance in scaffold wall thickness. Even so, because micropores sizes can impact nutritional dispersion, cell–cell interrelations, cell migration, multiplication, adhesion, and segmentation, a such transformation must be properly balanced [90]. In comparison to non-modified scaffolds, composite SF scaffolds have recent times shown to have higher osteoconductive, osteogenic, and new bone remodeling rates. The electrospinning process has used the integration of HAp with SF due to the high osteoconductive and mechanical characteristics. Incorporating BMP-2, HAp, and SF into hMSCs has also been shown to endorse propagation, osteogenic differentiation, as well as calcium concentration [91]. BK has been shown to improve osteogenesis as well as excellent biocompatibility when combined with natural components such as aloe vera as well as chitosan [92]. The demineralized bone matrix (DBM), which contains a large concentration of collagen and BMP-2, is a commonly used material. DBM powder or DBM particulate mixed with SF not only encouraged osteogenic differentiation in rat ASCs but it also made it easier to effectively manage DBM. Surface morphology as well as mechanical characteristics, that are essential for cell attachment and mobility, were enhanced by combining DBM and SF [93]. SF has recent times been coupled with ionic-doped calcium phosphates, which have the ability to modify stem cellular proliferation and osteogenic possibilities [94]. Besides introducing new biomaterial or reinforcing the SF itself, the appropriate compressive strength of the scaffold for bone regeneration can be accomplished. SF microfibers and microspheres were used to improve mechanical characteristics in an earlier study. The addition of SF particles increases the solidity, harshness, as well as osteoblastic cell differentiation in the scaffolds.

3D Printing

Inner configuration as well as permeability, which affect cellular functions, are controlled by 3D printing. Ability to control the properties of a material with existing scaffold processing technologies is ineffective. Freeze drying, porogen leaching, electrospinning, as well as gas foaming are some of the traditional methods for creating arbitrary porous scaffolds. Biomaterials, cell lines, as well as supporting components are combined with pre-defined inner architectural style scaffolds in 3D printing, eliminating the shortcomings of the existing methods [95]. Cells can also be encapsulated in biomimetic hydrogels that provide a structural as well as biologically active positive environment using 3D printing. There are about just few investigations that have used SF for 3D printing. The toughness as well as degeneration of SF scaffolds in 3D printing are controlled by the -sheet product reconfiguration [96]. Costa et al. recently published a paper on quick setting SF bioinks, which expands the bioproduction of memory-shape prosthetics for personalized TE [97]. Further research is required, but 3D printing is seen as a probable new methodology in bone TE.

Types of Scaffolds Based on Silk Fibroin

Porous scaffold, Hydrogel, Particles, mats and textiles, films, as well as mixed conformations were used to distinguish various scaffolds based on silk fibroin. Such configurations are depicted in (Fig. 4) and explained further down.

Fig. 4 Scaffolds focused on silk fibroin nanomaterials that are used for bioengineering

Porous Scaffold

The most widely accepted design between many silk fibroin scaffolds is sponge-like frameworks, also recognized as porous scaffolds in publications [98]. These are 3D polymers with a high percentage of pore sizes that cells could occupy. Because of their appealing properties, they are mainly used in the manufacture of orthopedic implants [99]. In comparison to certain other scaffold categories, sponge-like configurations have a lot of structural diversifications. Despite this, only *B. mori* silk fibroin was being used to fabricate it. The synthetic stage was available in all of the configurations in the shape of nanometric-sized particles. Even though bone was the target tissue in all instances, the accumulation of silk fibroin differed from 2 to 16%, indicating a wide range of scaffold fabrication. 3D-printing or a combination of the two methods were used to create the network [36], directional temperature field freezing technology [68, 100], the use of cross-linkers [101], sonication [102], salt leaching [77] or "silk-on-silk" layering followed by freeze drying [36]. A few researchers have used silk fibroin hydrogels to create hierarchies sponge-like frameworks, under which the synthetic component was combined with silk fibroin prior to actually hydrogelation [103]. Methanol was being used to crystallize silk fibroin as well as boost its -sheet content in certain situations [104]. The immunogenicity of such scaffolds varies, and it is largely defined by the nature of synthetic phase used. Yan et al. [70] found that the inclusion of calcium phosphate as the synthetic part will not produce epitopes that might induce powerful immune function. Such finding was also recognized by Ribeiro et al. [103] who found no inflammatory reaction upon implant placement of silk fibroin bi-layer scaffolds comprising -TCP (BTCP) or -TCP doped with zinc and strontium (BdTCP) upon eight weeks**.** However, Gholipourmalekabadi et al. [102] witnessed the Macrophage recruitment all around hydroxyapatite nanocomposite. There have been no assessments of usual macrophage M2 specific population indicators, including such CD206 or arginase-1 appearance [105]; the recruitment of macrophages may be linked to a pro-inflammatory reaction. Recently, Afjeh-Dana et al. [106] studied the silk fibroin nanocomposites' application in tissue regeneration. They created a sponge-like configuration aimed at marginal neural tissue, demonstrating that gold nanorod encouragement of silk fibroin scaffolds permitted neural-specific peptides nestin as well as neuron-specific enolase to be expressed. The feature, multiplication, self-renewal, as well as biochemical functions of nerve cells are all influenced by these amino acids. Gold nanoparticles also improved electrical properties and boosted cell adhesion.

Hydrogel

Hydrogels are cross-linked synthetic polymer media, also recognized as hydrophilic blocks, that are dissolved in a large water matrix. They can soak up large quantities of various elements, including such as water, stimulants, or cell lines, bloating as well as rising their volume in the methodology [107]. Hydrogels have been infused with a variety of inorganic compounds, including hydroxyapatite, halloysite, strontium, silver, as well as magnetite. The synthetic phase was usually organized as particulate engrained in the gel matrix. Furthermore, silk fibroin hydrogels have been created utilizing natural constituent compositions such as chitosan [108], collagen [109], carboxymethyl cellulose [75], and polyvinyl alcohol [110]. The natural cross-linking increased the biomaterial's elastic properties significantly; however, the fracture stress did sometimes not alter. Hydrogels are important today because, in addition to their bioactivity as well as sufficient physiochemical features, their unique properties enable for the inclusion of biologically active compounds that can be controlled-released in the lesion as well as stimulate particular genetic reactions, like osteoconductivity [87] and neovascularization [111]. Mechanical cross-linking stimulated by ultrasonic pulses or changes in temperature, for example, are simple as well as cost-effective methods for fabricating hydrogels based on silk fibroin. They can also be made with horseradish peroxidase as well as hydrogen peroxide (HRP cross-linking) that also permits for more precise control of the sol–gel transformation as well as higher -sheet content, probably due to di-tyrosine bridges facilitated by the enzyme [112]. Moses et al. [112] used this approach recently to create bioinks for 3D-bioprinting an osteochondral framework. The spatial progression as well as segmentation of adipose-derived stem cells was aided by the Microextrusion methodology.

Particles

Particles have diameters ranging from 43 to 700 nm, so they have high surface area due to their small size, allowing them to interact with their surroundings as well as soak up huge quantities of bioactive components with ease. Owing to lack of particular shape and mechanical characteristics, they are not appropriate for the repair of comprehensive size deficiencies [113]. Silk fibroin nanoparticles were used to mimic the structure of eggs in one investigation [73]. The "yolk" was represented by nanomaterials made of silk fibroin that have been coated with polyethyleneimine as well as embedded by "shells" made of calcium carbonate that were connected by electrostatic interactions. BMP-2 (Bone morphogenetic protein-2) was also present in these nanocomposites, which aided osteogenesis of mesenchymal stem cells by increasing calcium accumulation as well as alkaline phosphatase activity. The in vivo modification of the scaffold design was a focus of this article. The shells disintegrated after phosphate stimulation, forming hydroxyl-carbonate apatite as well as hydroxyapatite. The scaffold's compressive strength was enhanced six times, allowing for easier cell infiltration as well as neo-tissue colonization. Nanomaterials have the opportunity over the other scaffolds in that they can be effortlessly traced when fluorescently tagged, allowing researchers to determine their intracellular position as well as investigate cell-scaffold interrelations [114]. Silk fibroin can be covalently bound to the fluorophore. Similar strategies in the future could make use of the native fluorescence found in some silk fibroin variants [115].

Silk-Based Mats and Textiles Scaffold

Mats and textiles are fiber-based structures that are highly permeable. They are distinct from films in that they have a flat structure formed by fibers. They can be woven or stitched (mats). Apart from one created by spray-drying/pressing, almost all of the fibers investigated in the identified articles were created by electrospinning [116], and one where a knitting machine was used to create a micro-porous silk mesh with hydroxyapatite [83]. The composition of silk fibroin in nanofibers ranged from 2 to 17%, indicating that there is little agreement on the particular silk fibroin composition required for tissue regeneration. This scaffold type is used for a wide range of different tissues. On the mats studied, osteoblasts, mesenchymal stem cells, PC-12 cells, and cardiomyocytes were all grown. In the case of bone tissue regeneration, incorporating synthetic nanoparticles into silk fibroin mats can stimulate both natural and inorganic aspects [117]. Increased alkaline phosphatase behaviors as well as subsequent activation of osteoblastic gene regulation have indeed been witnessed as favorable findings of the incorporation of bioceramics such as hydroxyapatite, hardystonite, as well as Laponite—a silicate-based disk-shaped nanoparticulated ceramic [118]. When Laponite was added to silk fibroin at significant amounts, it enhanced its mechanical properties, percentage of elongation, strength, as well as cell growth [85]. This occurred since the addition of bioceramics enhanced the percentage of -sheet crystalline regions as well as engrossed the fiber diameter, enhancing mineral accumulation prerequisites and, as a result, cell attachment. Biologically active ceramic materials can discharge ions that promote tissue formation by enhancing differentiation and proliferation. For example, hardystonite releases Zn and Si, endorsing neovascularization as well as bone tissue formation [86]. The addition of gold and molybdenum to mats has proven to be beneficial. If gold nanoparticles were mixed with EMC and SF, Dong et al. [116] found that cardiomyocyte proliferation and expansion improved. Gold's conductive qualities appeared to expedite inter-cellular electrical interactions in this particular instance, as well as the nanomaterials could thus be used to heal myocardial infarctions. While TBX18–hiPSCs were cultured in silk fibroin mats containing molybdenum disulfide nanosheets, an equivalent phase occurred, with enhanced confirmation of mature as well as cardiogenic differentiation markers (cardiac troponin T and -Myosin heavy chain) [74]. According to another study, adding gold nanoparticles to mats enhanced the Elastic modulus of silk nanostructures by 70% when compared to non-composite nanofibers. Due to improved cell attachment, gold permitted cells to grow larger as well as inhabited more space [80].

Silk-Based Films

Films are flat, 2D structures made composed of thin layers stacked on top of one another. The synthetic component covers the surface of the SF layer in specific. The method for creating them is well-known, and it is based on rinsing as well as deposition techniques equivalent to those used in semiconductor film fabrication. This is demonstrated by the

Matrix-Assisted Pulsed Laser Evaporation (MAPLE) technique [119], and the procedure for plasma splashing [89], and also in vivo to co-precipitation methodology [120]. The composition of SF in films ranged from 2 to 12%, and the synthetic phases included hydroxyapatite and silver. Films are conveniently used in microbial growth as well as cell attachment immunoassay because of their bi-dimensional environment, which can be identified utilizing microscopy. Films with low cytotoxic effects and the ability to advance cellular proliferation have been developed. Rabbit knee joint anterior cruciate ligament restoration was achieved using a SF nanostructure focusing on silver and hydroxyapatite [89]. In this particular instance, the films limited bone passage remineralization as well as enhanced the bone–joint complex's biomechanical performance.

Mixed Conformations

All nanostructures are made up of a variety of conformations, most commonly cross-linked natural connections containing synthetic nanomaterials. Mixed conformations are classified as natural or synthetic polymer network ingrained inside another polymer system with a varying configuration or Integrating multiple configurations, such as a porous structure containing a hydrogel, results in mixed conformations [121], or alternatively, an electrospun mat can be used. [71] or a polymeric matrix [122]. They were used in cartilage and bone engineering, especially to heal osteochondral deformities, because each layered tissue has unique mechanical characteristics as well as cellular structures as well as operations. Such hybrid rearrangements are aimed at producing a layered scaffold that closely mimics the structure of native tissue, resulting in bioinspired scaffolds. They could be improved further by developing a framework to stimulate the development of various tissue molecules in each layer. This method will mimic the complex nature of the biological structure. Some other benefit consist of slow release as well as embedding of tissue, cell growth factor, as demonstrated by Dong et al. [122] who created a three-layered nanostructure scaffold that continuously released human recombinant BMP-2 and TGF-3 (Transforming growth factor beta 3), effectively targeting osteochondral regeneration. Another study looked at a porous configuration that provided as a medium for an electrospun silk fibroin layer [71]. A heating assessment using differential scanning calorimetry (DSC) revealed an increment in crystalline phase when silk fibroin was integrated with calcium phosphate. When especially in comparison to a porous structure made of naked silk fibroin, the nanocomposite enhanced cell adhesion, survivability, and alkaline enzymatic activities in osteoblasts. All of these positive attributes, however, were enhanced when an additional electrospun mat layer was incorporated [123]. Felfel et al. [121] used two-photon stereolithography to incorporate SELRs combined with hydroxyapatite into a porous structure made of poly(D, L-lactide-co-caprolactone). The goal of this hybrid was to enhance the scaffold's bioactivity as well as its physical and chemical properties. The porous structure of poly(D,L-lactide-co-caprolactone) provided the majority of the structural and mechanical qualities. The bioactivities of the scaffold improved slightly, as evidenced by an increased DNA content. As a bone-filling material candidate for tissue engineering, a cotton wool-like scaffold was created by Colpankan Gunes et al. [124]. This scaffold was made of hydroxyapatite and a biobased polyester called poly(3-hydroxybutyrate-co-3-hydroxyvalerate). The addition of silk fibroin improved fiber shape and size, as well as thermal properties, but had no effect on nutrient deposition or alkaline phosphatase activity. Nonetheless, it allowed for the absorption of type I collagen, which is needed for bone graft substitutes, as well as improved cell regeneration of an osteosarcoma-derived cell line.

Scaffold Physical and Chemical Characterization

Table 1 illustrates the methodologies that were most commonly used to characterize the scaffolds. It is also used to investigate its morphology, chemical bonding, as well as mechanical characteristics, indicating the biomaterial's physical and chemical consistency, as well as the interrelations in between organic and inorganic processes. Most silk fibroin nanostructures have indeed been studied utilizing mechanical tests depending on the Elastic modulus of the classic stress–strain curve in the preliminary linear range, with compression examinations being one of the most common. Contradictory to DMA (Dynamic Mechanical Analysis), the above experiment does not quite permit for the examination of the material's chance to restore as well as lose strength, as depicted by the storage as well as loss modulus, which is a good estimate for rheological properties [125]. DMA is a more accurate depiction of the oscillatory modifications that the scaffold undergoes in vivo [126]. Dynamic tests are consistently lacking, as well as certain tensile properties are limited to nanofibers, limiting potential comparability throughout distinct scaffold morphologies. Even though it is tough to distinguish diagnostic measurements, nanocomposites demonstrated an improvement in mechanical characteristics. The elevated level of control achieved in the synthesizing of the inorganic phase enabled the biomaterial characteristics to be fine-tuned. This was ascertained when Wang et al. [100] were able to design an oriented porous structure with sufficient mechanical qualities, which aided in vascularization as well as bone regeneration. Even though FTIR is used in a few examinations to characterize nanocomposites, it is mostly used to analyze the synthetic phase and also to demonstrate its integration

Table 1 The following are the major physicochemical, mechanical, as well as cytocompatibility methodologies used to characterize silk fibroin nanostructures scaffold

Technique	Objective	References
Physicochemical analysis		
Fourier transform infra-red spectroscopy (FTIR)	Identification of a synthetic phase within an organic phase	[77]
Thermal gravimetric analysis (TGA)	Thermal stability of the scaffold	[134]
Scanning electron microscopy (SEM) and similar techniques (FE- SEM, SEM–EDX)	The surface microstructure of a nanostructured scaffold, its elemental analysis, as well as subcellular localization and adhesion	[129]
Transmission electron microscopy (TEM) similar techniques (FE- TEM)	The size and shape of nanoparticles	[116]
X-ray diffraction (XRD)	Nanoparticle as well as nanostructure scaffold crystalline structure assessment	[112]
In vitro compounds release testing	In cultured cells pharmacokinetic and pharmacodynamic as well as leaching	[68]
In vitro degradation assays	Stability as well as biocompatibility of nanostructure scaffolds	[135]
Mechanical analysis		
Static compression tests	Nanocomposite scaffolds' elastic mechanical characteristics (compression modulus and compressive strength)	[73]
Dynamic-mechanic analysis	Nanocomposite scaffolds' viscoelastic properties (storage modulus, loss modulus, and complex modulus)	[70]
Static tensile tests	Nanocomposite scaffolds' elastic mechanical characteristics (tensile modulus and tensile strength)	[74]
Loaded-unloaded hysteresis	Nanocomposite scaffolds' mechanical hysteresis behavior	[121]
Atomic force microscopy nano-indentation tests	Surface mechanical reaction as well as uniformity assessment of nanostructured scaffolds	[80]
Cell viability and cytocompatibility assays		
Metabolic reduction of resazurin	Cell mitochondrial behavior in nanostructured scaffolds	[121]
Metabolic reduction of tetrazolium salts	Cell mitochondrial behavior in nanostructured scaffolds	[75]
Fluorescence-based membrane integrity monitoring	Cell growth in nanostructured scaffolds is determined by membrane integrity as well as active metabolic activity	[83]
LDH cytotoxicity assays	Cell growth in nanostructured scaffolds is determined by membrane stability	[129]
DNA quantification	Proliferation of cells in nanocomposite scaffolds	[82]

into the organic solution. Numerous authors suggest amide I, II, and III bands as modifications in -sheet content when evaluating the organic phase, but they do not assess trends in secondary structure by deconvolution as anticipated [127]. Current findings has shown that the succeeding bands can be used to assess conformation reliability: 1712 cm^{-1} (side chains), 1693 cm^{-1} (intermolecular -sheets), 1680 cm^{-1} (turn), 1656 cm^{-1} (random coil), 1644 cm^{-1} (-helix), 1628 cm^{-1} (intramolecular -sheets), and 1615 cm^{-1} (intermolecular -sheet) [128]. Benefits to be derived from identifying the appropriate conditions for maximizing -sheet content, as well as recognizing how synthetic sequence integration impacts the conformational changes of silk fibroin. XRD has also been used mainly to assess the synthetic phase, though it has been limited in some cases to registering the synthetic phase's integration. Tanasa et al. [129] and Wang et al. [113] recently indicated an improvement in peak intensity as well as crystalline nature when a synthetic phase was incorporated to scaffolds. Ghorbanian et al. [84] and Ran et al. [108] were indeed able to identify conformational alternation in silk fibroin. Silk fibroin scaffolds' reduced crystallization essence is among the XRD inherent limitations. The tightly packed synthetic phases alter slight shifts changes in its secondary structure. XRD was used by Zafar et al. [81] to assess the progress of mineralization within a week of osteogenesis of rabbit adipose-derived stem cells. Circular dichroism spectroscopy can be used as a supplement or substitute to FTIR and XRD [130] or perhaps even solid-phase nuclear magnetic resonance (NMR), which might, in theory, expose more information about the structural complexities of silk fibroin [131]. UV–Vis spectroscopy can be used to track the incorporation of the synthetic phase under certain situations. The existence of characteristic absorbance peak at 554 and 774 nm, for instance, has been used to prove the existence of gold nanoparticle [106]. Thermal gravimetric analysis (TGA) is being used to demonstrate the inclusion of inorganic materials through an increment in ash content [101]. Even though the physical interaction processes among silk fibroin and nanostructured materials are sluggish, the force of attraction have been intense enough in those situations to enhance the substance thermal decomposition characteristics. Scanning electron microscopy (SEM) and various techniques (FE-SEM, SEM–EDX) were used to study scaffold structure as well as morphological cell assessment. Even so, in certain cases, SEM revealed no visible reticular configuration or pore volume, a classic issue associated with soft porous structures disintegrating during dehydration [132]. The presence of large volume fraction of nanoparticles appeared to be associated with reduced porosities but improved mechanical characteristics [133]. Furthermore, when combined with some other constituents, such as graphene oxide [100], the synthetic phase has enabled the creation of micro-architectural configurations with oriented porous networks, which enhance mechanical characteristics and aid biochemical mechanisms such as neovascularization as well as bone tissue regeneration.

Biomedical Applications of Silk Fibroin

Silk fibroin is being used to make scaffolds for a variety of tissue engineering applications. Silk fibroin-containing compositions have been used as remedy for skin [136], abdominal wall [137], liver [138], vascular tissue [139], nerves [140] and nucleus pulposus [141]. Silk fibroin nanostructures, on the other hand, have often been used in cells with high physiological prerequisites, like cartilage, bone, and osteochondral cells. Silk fibroin nanostructures in recent times are being used as implants for many other tissues supporting structure in biomedical projects that involve quick regeneration, such as the cardiac muscle, neuromuscular junctions, cornea, solid tumors, respiratory epithelial layer, anterior cruciate ligament, as well as periodontium. This demonstrates the diverse potential application of silk fibroin, which is boosted by the incorporation of synthetic molecules in nanocomposites. There are clinically relevant complex tissues that have yet to be targeted by SF nanocomposites, such as the liver, pancreas, urinary tissue, vagina, trachea, etc.

Conclusion and Future-Scopes

Despite recent advancements made in silk-based biomimetic material properties over the last few decades, architectural integrity as well as bulk production remains obstacle. At environmental temperature, it is difficult to fully imitate the widescale hierarchy configurations of organic bioactive molecules. To that end, the authors have attempted to present a perspective that combines reductionism as well as integratism. Reductionism, in particular, necessitates a perfect analysis of the composition and properties of biomolecules from the macroscopic level to the nanometer scale. Reznikov et al. 3D's observation and characterization, for example, helped expand the hierarchy of bone structure previously identified [52], with a special focus on revealing the organisation and connection among bone's primary components—mineral and collagen—which will undoubtedly help in understanding the sensitive architecture of bone in every detail. Integratism, in overall, serves to remind us to construct bioinspired substances with essentially identical constituents, with an emphasis on the combination of various aspects or multiscale structures. Mao et al., for instance, created a millimeter-thick artificial nacre that accurately reflects the chemical properties as well as hierarchical system of organic nacre [142]. This was achieved using a "assembly and mineralization" strategy inspired by the natural process in mollusks, where silk fibroin was crucial for attachment and created the organic layers between aragonite layers. With this in mind, using catechol-group-modified silk materials would be a better decision [143]. The solution is unidentified. In the case of bone-like composite materials, bioengineered silk protein merged with the HAP-binding domain VTKHLNQISQSY (VTK) has been shown to enhance biomineralization and therefore increase osteoinductivity [144]. Furthermore, the underlying arginine–glycine–aspartate (RGD) pattern found in the some wild silk could stimulate cell adhesion and proliferation in tissue engineering applications [145]. As a result, maximizing silk's potential through directional selection or optimization of its properties could provide a robust material foundation for future silk-based biomimetic usable composite materials. Scientists and researchers require a proper mechanism for interacting with the structural complexity of a truly biologically inspired configuration whose dimensions span from the nanometer scale to the macroscopic level, whereas combining these two or even more design and manufacturing innovations may collaborate out as a solution. This journey will surely be aided by the incorporation of biologically inspired structural or functional components based on silk. Hopefully, a significant development will occur in the near future.

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Declarations

Conflict of interest Both the authors have no conflict of interest in any part of the work and with any institution.

References

1. M. Eder, S. Amini, P. Fratzl, Biological composites—complex structures for functional diversity. Science (80-.) **362**(6414), 543–547 (2018). https://doi.org/10.1126/science.aat8297
2. U.G.K. Wegst, H. Bai, E. Saiz, A.P. Tomsia, R.O. Ritchie, Bioinspired structural materials. Nat. Mater. **14**(1), 23–36 (2015). https://doi.org/10.1038/nmat4089
3. L. Fuentes-Mera, Scaffolds based on silk fibroin for osteochondral tissue engineering. Res. Dev. Mater. Sci. (2019). https://doi.org/10.31031/rdms.2019.10.000740
4. A.K. Mohanty, S. Vivekanandhan, J.M. Pin, M. Misra, Composites from renewable and sustainable resources: challenges and innovations. Science (80-.) **362**(6414), 536–542 (2018). https://doi.org/10.1126/science.aat9072
5. R. Geyer, J.R. Jambeck, K.L. Law, Production, use, and fate of all plastics ever made. Sci. Adv. (2017). https://doi.org/10.1126/sciadv.1700782
6. C. Li et al., Fiber-Based Biopolymer Processing as a Route toward Sustainability. Adv. Mater. **34**(1), 1–27 (2022). https://doi.org/10.1002/adma.202105196
7. C. Holland, K. Numata, J. Rnjak-Kovacina, F.P. Seib, The biomedical use of silk: past, present, future. Adv. Healthc. Mater. (2019). https://doi.org/10.1002/adhm.201800465
8. F.G. Omenetto, D.L. Kaplan, New opportunities for an ancient material. Science (80-.) **329**(5991), 528–531 (2010). https://doi.org/10.1126/science.1188936
9. X. Hu, D.L. Kaplan, Silk biomaterials. Compr. Biomater. **2**, 207–219 (2011). https://doi.org/10.1016/b978-0-08-055294-1.00070-2
10. T. Lefèvre, M.E. Rousseau, M. Pézolet, Protein secondary structure and orientation in silk as revealed by Raman spectromicroscopy. Biophys. J. **92**(8), 2885–2895 (2007). https://doi.org/10.1529/biophysj.106.100339
11. Y. Qi et al., A review of structure construction of silk fibroin biomaterials from single structures to multi-level structures. Int. J. Mol. Sci. (2017). https://doi.org/10.3390/ijms18030237
12. D.N. Rockwood, R.C. Preda, T. Yücel, X. Wang, M.L. Lovett, D.L. Kaplan, Materials fabrication from *Bombyx mori* silk fibroin. Nat. Protoc. **6**(10), 1612–1631 (2011). https://doi.org/10.1038/nprot.2011.379
13. L.D. Koh, J. Yeo, Y.Y. Lee, Q. Ong, M. Han, B.C.K. Tee, Advancing the frontiers of silk fibroin protein-based materials for futuristic electronics and clinical wound-healing (Invited review). Mater. Sci. Eng. C **86**(January), 151–172 (2018). https://doi.org/10.1016/j.msec.2018.01.007
14. P.M. Gore, M. Naebe, X. Wang, B. Kandasubramanian, Progress in silk materials for integrated water treatments: Fabrication, modification and applications. Chem. Eng. J. **374**(May), 437–470 (2019). https://doi.org/10.1016/j.cej.2019.05.163
15. R. Fedič, M. Žurovec, F. Sehnal, Correlation between fibroin amino acid sequence and physical silk properties. J. Biol. Chem. **278**(37), 35255–35264 (2003). https://doi.org/10.1074/jbc.M305304200
16. M.S. Zafar, D.J. Belton, B. Hanby, D.L. Kaplan, C.C. Perry, Functional material features of *Bombyx mori* silk light versus heavy chain proteins. Biomacromol **16**(2), 606–614 (2015). https://doi.org/10.1021/bm501667j
17. K. Adarsh Gupta, K. Mita, K.P. Arunkumar, J. Nagaraju, Molecular architecture of silk fibroin of Indian golden silkmoth, Antheraea assama. Sci. Rep. **5**, 1–17 (2015). https://doi.org/10.1038/srep12706
18. S.M. Correa-Garhwal et al., Spidroins and Silk Fibers of Aquatic Spiders. Sci. Rep. **9**(1), 1–12 (2019). https://doi.org/10.1038/s41598-019-49587-y
19. S. Salehi, K. Koeck, T. Scheibel, Spider silk for tissue engineering applications. Molecules (2020). https://doi.org/10.3390/molecules25030737
20. Y.J. Yang et al., Multifunctional adhesive silk fibroin with blending of RGD-bioconjugated mussel adhesive protein. Biomacromol **15**(4), 1390–1398 (2014). https://doi.org/10.1021/bm500001n
21. S. Ullah, X. Chen, Fabrication, applications and challenges of natural biomaterials in tissue engineering. Appl. Mater. Today (2020). https://doi.org/10.1016/j.apmt.2020.100656
22. M. Jiang, Review of 'Glossary of Biotechnology and Nanobiotechnology Terms' by Kimball Nill. Biomed. Eng. Online **6**, 5 (2007). https://doi.org/10.1186/1475-925X-6-5
23. R. C. Cammarata, "Robert C. Cammarata."
24. W. Park, H. Shin, B. Choi, W.K. Rhim, K. Na, D. Keun Han, Advanced hybrid nanomaterials for biomedical applications. Prog. Mater. Sci. **114**(March), 100686 (2020). https://doi.org/10.1016/j.pmatsci.2020.100686
25. F. Chen, D. Porter, F. Vollrath, Morphology and structure of silkworm cocoons. Mater. Sci. Eng. C **32**(4), 772–778 (2012). https://doi.org/10.1016/j.msec.2012.01.023
26. C. Fu, D. Porter, X. Chen, F. Vollrath, Z. Shao, Understanding the mechanical properties of *Antheraea pernyi* Silka-From primary structure to condensed structure of the protein. Adv. Funct. Mater. **21**(4), 729–737 (2011). https://doi.org/10.1002/adfm.201001046
27. C. Fu, Y. Wang, J. Guan, X. Chen, F. Vollrath, Z. Shao, Cryogenic toughness of natural silk and a proposed structure-function relationship. Mater. Chem. Front. **3**(11), 2507–2513 (2019). https://doi.org/10.1039/c9qm00282k
28. D.R. Whittall, K.V. Baker, R. Breitling, E. Takano, Host systems for the production of recombinant spider silk. Trends Biotechnol. **39**(6), 560–573 (2021). https://doi.org/10.1016/j.tibtech.2020.09.007
29. T.B. Aigner, E. DeSimone, T. Scheibel, Biomedical applications of recombinant silk-based materials. Adv. Mater. **30**(19), 1–28 (2018). https://doi.org/10.1002/adma.201704636
30. S. Ling, D.L. Kaplan, M.J. Buehler, Nanofibrils in nature and materials engineering. Nat. Rev. Mater. **3**, 1–15 (2018). https://doi.org/10.1038/natrevmats.2018.16
31. A.R. Murphy, D.L. Kaplan, Biomedical applications of chemically-modified silk fibroin. J. Mater. Chem. **19**(36), 6443–6450 (2009). https://doi.org/10.1039/b905802h
32. M.P. Ho, H. Wang, K.T. Lau, Effect of degumming time on silkworm silk fibre for biodegradable polymer composites. Appl. Surf. Sci. **258**(8), 3948–3955 (2012). https://doi.org/10.1016/j.apsusc.2011.12.068
33. H. Yang, Z. Wang, M. Wang, C. Li, Structure and properties of silk fibroin aerogels prepared by non-alkali degumming process. Polymer (Guildf.) **192**(February), 122298 (2020). https://doi.org/10.1016/j.polymer.2020.122298
34. C. Guo et al., Thermoplastic moulding of regenerated silk. Nat. Mater. **19**(1), 102–108 (2020). https://doi.org/10.1038/s41563-019-0560-8
35. S. Ling, C. Li, K. Jin, D.L. Kaplan, M.J. Buehler, Liquid exfoliated natural silk nanofibrils: applications in optical and electrical devices. Adv. Mater. **28**(35), 7783–7790 (2016). https://doi.org/10.1002/adma.201601783
36. S. Ling et al., Combining in silico design and biomimetic assembly: a new approach for developing high-performance dynamic responsive bio-nanomaterials. Adv. Mater. **30**(43), 1–11 (2018). https://doi.org/10.1002/adma.201802306
37. M. Humenik, G. Lang, T. Scheibel, Silk nanofibril self-assembly versus electrospinning. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. **10**(4), 1–24 (2018). https://doi.org/10.1002/wnan.1509

38. L.P. Corrales, M.L. Esteves, J.E. Ramirez-Vick, Scaffold design for bone regeneration. Journal of nanoscience and nanotechnology. J. Nanosci. Nanotechnol. **14**(1), 15–56 (2014)
39. J.G. Hardy, T.R. Scheibel, Composite materials based on silk proteins. Prog. Polym. Sci. **35**(9), 1093–1115 (2010). https://doi.org/10.1016/j.progpolymsci.2010.04.005
40. H.V. Unadkat, et al., "Erratum: An algorithm-based topographical biomaterials library to instruct cell fate (Proceedings of the National Academy of Sciences of the United States of America (2011) 108, 40 (16565–16570). https://doi.org/10.1073/pnas.1109861108. Proc. Natl. Acad. Sci. USA 109(15), 5905 (2012). https://doi.org/10.1073/pnas.1204360109.
41. Y.W. Won, A.N. Patel, D.A. Bull, Cell surface engineering to enhance mesenchymal stem cell migration toward an SDF-1 gradient. Biomaterials **35**(21), 5627–5635 (2014). https://doi.org/10.1016/j.biomaterials.2014.03.070
42. D.K. Kim, J.I. Kim, T.I. Hwang, B.R. Sim, G. Khang, Bioengineered osteoinductive broussonetia kazinoki/silk fibroin composite scaffolds for bone tissue regeneration. ACS Appl. Mater. Interfaces **9**(2), 1384–1394 (2017). https://doi.org/10.1021/acsami.6b14351
43. J.A. Apablaza, M.F. Lezcano, A. Lopez Marquez, K. Godoy Sánchez, G.H. Oporto, F.J. Dias, Main morphological characteristics of tubular polymeric scaffolds to promote peripheral nerve regeneration—a scoping review. Polymers (Basel) (2021). https://doi.org/10.3390/polym13152563
44. C. Correia et al., Development of silk-based scaffolds for tissue engineering of bone from human adipose-derived stem cells. Acta Biomater. **8**(7), 2483–2492 (2012). https://doi.org/10.1016/j.actbio.2012.03.019
45. T.D. Sutherland, J.H. Young, S. Weisman, C.Y. Hayashi, D.J. Merritt, Insect silk: one name, many materials. Annu. Rev. Entomol. **55**, 171–188 (2010). https://doi.org/10.1146/annurev-ento-112408-085401
46. K. Felix, R. Ferrándiz, R. Einarsson, S. Dreborg, Allergens of horse dander: comparison among breeds and individual animals by immunoblotting. J. Allergy Clin. Immunol. **98**(1), 169–171 (1996)
47. H.K. Soong, K.R. Kenyon, Adverse reactions to Virgin Silk sutures in cataract surgery. Ophthalmology **91**(5), 479–483 (1984). https://doi.org/10.1016/S0161-6420(84)34273-7
48. J.H. Choi, H. Jeon, J.E. Song, J.M. Oliveira, R.L. Reis, G. Khang, Biofunctionalized lysophosphatidic acid/silk fibroin film for cornea endothelial cell regeneration. Nanomaterials **8**(5), 1–14 (2018). https://doi.org/10.3390/nano8050290
49. K. Na et al., Osteogenic differentiation of rabbit mesenchymal stem cells in thermo-reversible hydrogel constructs containing hydroxyapatite and bone morphogenic protein-2 (BMP-2). Biomaterials **28**(16), 2631–2637 (2007). https://doi.org/10.1016/j.biomaterials.2007.02.008
50. D. Yao et al., Salt-leached silk scaffolds with tunable mechanical properties. Biomacromol **13**(11), 3723–3729 (2012). https://doi.org/10.1021/bm301197h
51. Y. Wang, H.J. Kim, G. Vunjak-Novakovic, D.L. Kaplan, Stem cell-based tissue engineering with silk biomaterials. Biomaterials **27**(36), 6064–6082 (2006). https://doi.org/10.1016/j.biomaterials.2006.07.008
52. N. Reznikov, M. Bilton, L. Lari, M.M. Stevens, R. Kröger, Fractal-like hierarchical organization of bone begins at the nanoscale. Science (2018). https://doi.org/10.1126/science.aao2189
53. P. Bhattacharjee et al., Silk scaffolds in bone tissue engineering: an overview. Acta Biomater. **63**, 1–17 (2017). https://doi.org/10.1016/j.actbio.2017.09.027
54. J. Melke, S. Midha, S. Ghosh, K. Ito, S. Hofmann, Silk fibroin as biomaterial for bone tissue engineering. Acta Biomater. **31**, 1–16 (2016). https://doi.org/10.1016/j.actbio.2015.09.005
55. M. Saleem, S. Rasheed, C. Yougen, Silk fibroin/hydroxyapatite scaffold: a highly compatible material for bone regeneration. Sci. Technol. Adv. Mater. **21**(1), 242–266 (2020). https://doi.org/10.1080/14686996.2020.1748520
56. A.M. Collins et al., Bone-like resorbable silk-based scaffolds for load-bearing osteoregenerative applications. Adv. Mater. **21**(1), 75–78 (2009). https://doi.org/10.1002/adma.200802239
57. M. Farokhi et al., Silk fibroin/hydroxyapatite composites for bone tissue engineering. Biotechnol. Adv. **36**(1), 68–91 (2018). https://doi.org/10.1016/j.biotechadv.2017.10.001
58. Q. Wang, Y. Zhang, B. Li, L. Chen, Controlled dual delivery of low doses of BMP-2 and VEGF in a silk fibroin-nanohydroxyapatite scaffold for vascularized bone regeneration. J. Mater. Chem. B **5**(33), 6963–6972 (2017). https://doi.org/10.1039/c7tb00949f
59. V. Fitzpatrick et al., Functionalized 3D-printed silk-hydroxyapatite scaffolds for enhanced bone regeneration with innervation and vascularization. Biomaterials **276**(July), 120995 (2021). https://doi.org/10.1016/j.biomaterials.2021.120995
60. A. Ní Annaidh, K. Bruyère, M. Destrade, M.D. Gilchrist, M. Otténio, Characterization of the anisotropic mechanical properties of excised human skin. J. Mech. Behav. Biomed. Mater. **5**(1), 139–148 (2012). https://doi.org/10.1016/j.jmbbm.2011.08.016
61. K. Liu, Y. Jiang, Z. Bao, X. Yan, Skin-inspired electronics enabled by supramolecular polymeric materials. CCS Chem. **1**(4), 431–447 (2019). https://doi.org/10.31635/ccschem.019.20190048
62. A. Chortos, Z. Bao, Skin-inspired electronic devices. Mater. Today **17**(7), 321–331 (2014). https://doi.org/10.1016/j.mattod.2014.05.006
63. C. Shi et al., New silk road: from mesoscopic reconstruction/functionalization to flexible meso-electronics/photonics based on cocoon silk materials. Adv. Mater. **33**(50), 1–32 (2021). https://doi.org/10.1002/adma.202005910
64. B. Zhu et al., Silk fibroin for flexible electronic devices. Adv. Mater. **28**(22), 4250–4265 (2016). https://doi.org/10.1002/adma.201504276
65. W. Yang et al., On the tear resistance of skin. Nat. Commun. (2015). https://doi.org/10.1038/ncomms7649
66. C. Wang et al., Multifunctional biosensors made with self-healable silk fibroin imitating skin. ACS Appl. Mater. Interfaces **13**(28), 33371–33382 (2021). https://doi.org/10.1021/acsami.1c08568
67. Z. Xu, M. Wu, W. Gao, H. Bai, A transparent, skin-inspired composite film with outstanding tear resistance based on flat silk cocoon. Adv. Mater. **32**(34), 1–8 (2020). https://doi.org/10.1002/adma.202002695
68. Z. Jia et al., Constructing multilayer silk protein/nanosilver biofunctionalized hierarchically structured 3D printed Ti6Al4 v scaffold for repair of infective bone defects. ACS Biomater. Sci. Eng. **5**(1), 244–261 (2019). https://doi.org/10.1021/acsbiomaterials.8b00857
69. W.I. Abdel-Fattah, A.S.M. Sallam, A.M. Diab, G.W. Ali, Tailoring the properties and functions of phosphate/silk/Ag/chitosan scaffolds. Mater. Sci. Eng. C **54**, 158–168 (2015). https://doi.org/10.1016/j.msec.2015.05.015
70. L.P. Yan et al., Bilayered silk/silk-nanoCaP scaffolds for osteochondral tissue engineering: in vitro and in vivo assessment of biological performance. Acta Biomater. **12**(1), 227–241 (2015). https://doi.org/10.1016/j.actbio.2014.10.021
71. M. Farokhi, F. Mottaghitalab, M.A. Shokrgozar, J. Ai, J. Hadjati, M. Azami, Bio-hybrid silk fibroin/calcium phosphate/PLGA nanocomposite scaffold to control the delivery of vascular endothelial growth factor. Mater. Sci. Eng. C **35**(1), 401–410 (2014). https://doi.org/10.1016/j.msec.2013.11.023

72. L.P. Yan, J.M. Oliveira, A.L. Oliveira, R.L. Reis, In vitro evaluation of the biological performance of macro/micro-porous silk fibroin and silk-nano calcium phosphate scaffolds. J. Biomed. Mater. Res. Part B. Appl. Biomater. **103**(4), 888–898 (2015). https://doi.org/10.1002/jbm.b.33267
73. P. Shi et al., Yolk shell nanocomposite particles as bioactive bone fillers and growth factor carriers. Nanoscale **9**(38), 14520–14532 (2017). https://doi.org/10.1039/c7nr03093b
74. H. Nazari et al., Incorporation of two-dimensional nanomaterials into silk fibroin nanofibers for cardiac tissue engineering. Polym. Adv. Technol. **31**(2), 248–259 (2020). https://doi.org/10.1002/pat.4765
75. R. Raho et al., Photo-assisted green synthesis of silver doped silk fibroin/carboxymethyl cellulose nanocomposite hydrogels for biomedical applications. Mater. Sci. Eng. C **107**(July), 110219 (2019). https://doi.org/10.1016/j.msec.2019.110219
76. N. Johari, H.R. Madaah Hosseini, N. Taromi, S. Arasteh, S. Kazemnejad, A. Samadikuchaksaraei, Evaluation of bioactivity and biocompatibility of silk fibroin/TiO_2 nanocomposite. J. Med. Biol. Eng. **38**(1), 99–105 (2018). https://doi.org/10.1007/s40846-017-0295-4
77. S.K. Samal et al., Biomimetic magnetic silk scaffolds. ACS Appl. Mater. Interfaces **7**(11), 6282–6292 (2015). https://doi.org/10.1021/acsami.5b00529
78. A. Teimouri, L. Ghorbanian, H. Salavati, A.N. Chermahini, Fabrication and characterization of POM/ZrO2/silk fibroin composite scaffolds. Mater. Lett. **157**, 85–88 (2015). https://doi.org/10.1016/j.matlet.2015.05.064
79. M. Passi, V. Kumar, G. Packirisamy, Theranostic nanozyme: Silk fibroin based multifunctional nanocomposites to combat oxidative stress. Mater. Sci. Eng. C **107**(May), 2020 (2019). https://doi.org/10.1016/j.msec.2019.110255
80. J. Pyun et al., Nanocomposite Gold-Silk Nano fi bers. Nano Lett. **102**, 10–13 (2012)
81. B. Zafar, F. Mottaghitalab, Z. Shahosseini, B. Negahdari, M. Farokhi, Silk fibroin/alumina nanoparticle scaffold using for osteogenic differentiation of rabbit adipose-derived stem cells. Materialia **9**(July), 100518 (2020). https://doi.org/10.1016/j.mtla.2019.100518
82. V.P. Ribeiro et al., Enzymatically cross-linked silk fibroin-based hierarchical scaffolds for osteochondral regeneration. ACS Appl. Mater. Interfaces **11**(4), 3781–3799 (2019). https://doi.org/10.1021/acsami.8b21259
83. P. Shi, T.K.H. Teh, S.L. Toh, J.C.H. Goh, Variation of the effect of calcium phosphate enhancement of implanted silk fibroin ligament bone integration. Biomaterials **34**(24), 5947–5957 (2013). https://doi.org/10.1016/j.biomaterials.2013.04.046
84. L. Ghorbanian, R. Emadi, S.M. Razavi, H. Shin, A. Teimouri, Fabrication and characterization of novel diopside/silk fibroin nanocomposite scaffolds for potential application in maxillofacial bone regeneration. Int. J. Biol. Macromol. **58**, 275–280 (2013). https://doi.org/10.1016/j.ijbiomac.2013.04.004
85. M. Atrian, M. Kharaziha, R. Emadi, F. Alihosseini, Silk-Laponite® fibrous membranes for bone tissue engineering. Appl. Clay Sci. **174**(March), 90–99 (2019). https://doi.org/10.1016/j.clay.2019.03.038
86. Z. Hadisi et al., In vitro and in vivo evaluation of silk fibroin-hardystonite-gentamicin nanofibrous scaffold for tissue engineering applications. Polym. Test. **91**(June), 106698 (2020). https://doi.org/10.1016/j.polymertesting.2020.106698
87. F. Avani, S. Damoogh, F. Mottaghitalab, A. Karkhaneh, M. Farokhi, Vancomycin loaded halloysite nanotubes embedded in silk fibroin hydrogel applicable for bone tissue engineering. Int. J. Polym. Mater. Polym. Biomater. **69**(1), 32–43 (2020). https://doi.org/10.1080/00914037.2019.1616201
88. X.-Y. Zhang et al., Biocompatiable silk fibroin/carboxymethyl chitosan/strontium substituted hydroxyapatite/cellulose nanocrystal composite scaffolds for bone tissue engineering. Int. J. Biol. Macromol. **136**, 1247–1257 (2019). https://doi.org/10.1016/j.ijbiomac.2019.06.172
89. S. Jiang, X. Liu, Y. Liu, J. Liu, W. He, Y. Dong, Synthesis of silver @hydroxyapatite nanoparticles based biocomposite and their assessment for viability of Osseointegration for rabbit knee joint anterior cruciate ligament rehabilitation. J. Photochem. Photobiol. B Biol. **202**(6), 111677 (2020). https://doi.org/10.1016/j.jphotobiol.2019.111677
90. I. Bružauskaitė, D. Bironaitė, E. Bagdonas, E. Bernotienė, Scaffolds and cells for tissue regeneration: different scaffold pore sizes—different cell effects. Cytotechnology **68**(3), 355–369 (2016). https://doi.org/10.1007/s10616-015-9895-4
91. N. Panda, A. Bissoyi, K. Pramanik, A. Biswas, Directing osteogenesis of stem cells with hydroxyapatite precipitated electrospun eri-tasar silk fibroin nanofibrous scaffold. J. Biomater. Sci. Polym. Ed. **25**(13), 1440–1457 (2014). https://doi.org/10.1080/09205063.2014.943548
92. J.A. Apablaza, M.F. Lezcano, K.G. Sánchez, G.H. Oporto, F.J. Dias, Optimal morphometric characteristics of a tubular polymeric scaffold to promote peripheral nerve regeneration: a scoping review. Polymers (2022). https://doi.org/10.3390/polym14030397
93. M. Tian, Z. Yang, K. Kuwahara, M.E. Nimni, C. Wan, B. Han, Delivery of demineralized bone matrix powder using a thermogelling chitosan carrier. Acta Biomater. **8**(2), 753–762 (2012). https://doi.org/10.1016/j.actbio.2011.10.030
94. S. Pina et al., Biofunctional ionic-doped calcium phosphates: silk fibroin composites for bone tissue engineering scaffolding. Cells Tissues Organs **204**(3–4), 150–163 (2017). https://doi.org/10.1159/000469703
95. Y. Tan et al., 3D printing facilitated scaffold-free tissue unit fabrication. Biofabrication (2014). https://doi.org/10.1088/1758-5082/6/2/024111
96. K. Schacht, T. Jüngst, M. Schweinlin, A. Ewald, J. Groll, T. Scheibel, Biofabrication of cell-loaded 3D spider silk constructs. Angew. Chemie - Int. Ed. **54**(9), 2816–2820 (2015). https://doi.org/10.1002/anie.201409846
97. J.B. Costa, J. Silva-Correia, J.M. Oliveira, R.L. Reis, Fast setting silk fibroin bioink for bioprinting of patient-specific memory-shape implants. Adv. Healthc. Mater. **6**(22), 1–8 (2017). https://doi.org/10.1002/adhm.201701021
98. S.J. Hollister, Porous scaffold design for tissue engineering. Nat. Mater. **5**(7), 590 (2006)
99. X. Wang et al., Topological design and additive manufacturing of porous metals for bone scaffolds and orthopaedic implants: a review. Biomaterials **83**, 127–141 (2016). https://doi.org/10.1016/j.biomaterials.2016.01.012
100. L. Wang et al., Preparation and biological properties of silk fibroin/nano-hydroxyapatite/graphene oxide scaffolds with an oriented channel-like structure. RSC Adv. **10**(17), 10118–10128 (2020). https://doi.org/10.1039/c9ra09710d
101. A. Teimouri, R. Ebrahimi, R. Emadi, B.H. Beni, A.N. Chermahini, Nano-composite of silk fibroin-chitosan/Nano ZrO2 for tissue engineering applications: Fabrication and morphology. Int. J. Biol. Macromol. **76**, 292–302 (2015)
102. M. Gholipourmalekabadi et al., In vitro and in vivo evaluations of three-dimensional hydroxyapatite/silk fibroin nanocomposites scaffolds. Biotechnol. Appl. Biochem. **62**(4), 441–450 (2015). https://doi.org/10.1002/bab.1285
103. V.P. Ribeiro, S. Pina, R.F. Canadas, S. Morais, C. Vilela, S. Vieira, In vivo performance of hierarchical HRP-crosslinked silk fibroin/β-TCP scaffolds for osteochondral tissue regeneration.

Regen. Med. Front. (2019). https://doi.org/10.20900/rmf20190007

104. L. Nie et al., Nano-hydroxyapatite mineralized silk fibroin porous scaffold for tooth extraction site preservation. Dent. Mater. **35**(10), 1397–1407 (2019). https://doi.org/10.1016/j.dental.2019.07.024
105. N. Hirosawa et al., Vein wrapping promotes M2 macrophage polarization in a rat chronic constriction injury model. J. Orthop. Res. **36**(8), 2210–2217 (2018). https://doi.org/10.1002/jor.23875
106. E. Afjeh-Dana et al., Gold nanorods reinforced silk fibroin nanocomposite for peripheral nerve tissue engineering applications. Int. J. Biol. Macromol. **129**, 1034–1039 (2019). https://doi.org/10.1016/j.ijbiomac.2019.02.050
107. I.M. El-Sherbiny, M.H. Yacoub, Hydrogel scaffolds for tissue engineering: progress and challenges. Glob. Cardiol. Sci. Pract. **2013**(3), 38 (2013). https://doi.org/10.5339/gcsp.2013.38
108. J. Ran et al., A novel chitosan-tussah silk fibroin/nano-hydroxyapatite composite bone scaffold platform with tunable mechanical strength in a wide range. Int. J. Biol. Macromol. **93**, 87–97 (2016). https://doi.org/10.1016/j.ijbiomac.2016.08.062
109. L. Chen, J. Hu, J. Ran, X. Shen, H. Tong, Preparation and evaluation of collagen-silk fibroin/hydroxyapatite nanocomposites for bone tissue engineering. Int. J. Biol. Macromol. **65**, 1–7 (2014). https://doi.org/10.1016/j.ijbiomac.2014.01.003
110. H. Zhou et al., Genipin-crosslinked polyvinyl alcohol/silk fibroin/nano-hydroxyapatite hydrogel for fabrication of artificial cornea scaffolds—a novel approach to corneal tissue engineering. J. Biomater. Sci. Polym. Ed. **30**(17), 1604–1619 (2019). https://doi.org/10.1080/09205063.2019.1652418
111. K. Wang et al., Injectable silk/hydroxyapatite nanocomposite hydrogels with vascularization capacity for bone regeneration. J. Mater. Sci. Technol. **63**, 172–181 (2021). https://doi.org/10.1016/j.jmst.2020.02.030
112. J.C. Moses, T. Saha, B.B. Mandal, Chondroprotective and osteogenic effects of silk-based bioinks in developing 3D bioprinted osteochondral interface. Bioprinting **17**, e00067 (2020)
113. L. Wang et al., Fabrication and characterization of strontium-hydroxyapatite/silk fibroin biocomposite nanospheres for bone-tissue engineering applications. Int. J. Biol. Macromol. **142**, 366–375 (2020). https://doi.org/10.1016/j.ijbiomac.2019.09.107
114. M. Passi, V. Kumar, G. Packirisamy, Theranostic nanozyme : silk fi broin based multifunctional nanocomposites to combat oxidative stress. Mater. Sci. Eng. C (2020). https://doi.org/10.1016/j.msec.2019.110255
115. M. Amirikia, S.M.A. Shariatzadeh, S.G.A. Jorsaraei, M.S. Mehranjani, Auto-fluorescence of a silk fibroin-based scaffold and its interference with fluorophores in labeled cells. Eur. Biophys. J. **47**(5), 573–581 (2018). https://doi.org/10.1007/s00249-018-1279-1
116. Y. Dong, M. Hong, R. Dai, H. Wu, P. Zhu, Engineered bioactive nanoparticles incorporated biofunctionalized ECM/silk proteins based cardiac patches combined with MSCs for the repair of myocardial infarction: In vitro and in vivo evaluations. Sci. Total Environ. **707**, 135976 (2020). https://doi.org/10.1016/j.scitotenv.2019.135976
117. E.I. Paşcu, J. Stokes, G.B. McGuinness, Electrospun composites of PHBV, silk fibroin and nano-hydroxyapatite for bone tissue engineering. Mater. Sci. Eng. C **33**(8), 4905–4916 (2013). https://doi.org/10.1016/j.msec.2013.08.012
118. T. Liu et al., Biomimetic fabrication of new bioceramics-introduced fibrous scaffolds: from physicochemical characteristics to in vitro biological properties. Mater. Sci. Eng. C **94**(October), 547–557 (2019). https://doi.org/10.1016/j.msec.2018.09.063
119. F.M. Miroiu et al., Composite biocompatible hydroxyapatite-silk fibroin coatings for medical implants obtained by Matrix Assisted Pulsed Laser Evaporation. Mater. Sci. Eng. B Solid-State Mater. Adv. Technol. **169**(1), 151–158 (2010). https://doi.org/10.1016/j.mseb.2009.10.004
120. J. Mobika, M. Rajkumar, S.P. Linto Sibi, V. Nithya Priya, Fabrication of bioactive hydroxyapatite/silk fibroin/gelatin cross-linked nanocomposite for biomedical application. Mater. Chem. Phys. **250**(12), 123187 (2020). https://doi.org/10.1016/j.matchemphys.2020.123187
121. R.M. Felfel et al., Performance of multiphase scaffolds for bone repair based on two-photon polymerized poly(D, L-lactide-co-ε-caprolactone), recombinamers hydrogel and nano-HA. Mater. Des. **160**, 455–467 (2018). https://doi.org/10.1016/j.matdes.2018.09.035
122. Y. Dong et al., Regional and sustained dual-release of growth factors from biomimetic tri-layered scaffolds for the repair of large-scale osteochondral defects. Appl. Mater. Today **19**, 100548 (2020). https://doi.org/10.1016/j.apmt.2019.100548
123. K. Hu, B.R. Olsen, The roles of vascular endothelial growth factor in bone repair and regeneration. Bone **91**, 30–38 (2016). https://doi.org/10.1016/j.bone.2016.06.013
124. O. Colpankan Gunes et al., Three-dimensional silk impregnated HAp/PHBV nanofibrous scaffolds for bone regeneration. Int. J. Polym. Mater. Polym. Biomater. **68**(5), 217–228 (2019). https://doi.org/10.1080/00914037.2018.1443928
125. K.P. Menard, N.R. Menard, *Dynamic Mechanical Analysis in the Analysis of Polymers and Rubbers*, no. 9 (2015). https://doi.org/10.1002/0471440264.pst102.pub2
126. D. Warnecke et al., Biomechanical, structural and biological characterisation of a new silk fibroin scaffold for meniscal repair. J. Mech. Behav. Biomed. Mater. **86**(June), 314–324 (2018). https://doi.org/10.1016/j.jmbbm.2018.06.041
127. K.A. Oberg, J.M. Ruysschaert, E. Goormaghtigh, The optimization of protein secondary structure determination with infrared and circular dichroism spectra. Eur. J. Biochem. **271**(14), 2937–2948 (2004). https://doi.org/10.1111/j.1432-1033.2004.04220.x
128. D.J. Belton, R. Plowright, D.L. Kaplan, C.C. Perry, A robust spectroscopic method for the determination of protein conformational composition—application to the annealing of silk. Acta Biomater. **73**, 355–364 (2018). https://doi.org/10.1016/j.actbio.2018.03.058
129. E. Tanasa, C. Zaharia, A. Hudita, I.C. Radu, M. Costache, B. Galateanu, Impact of the magnetic field on 3T3-E1 preosteoblasts inside SMART silk fibroin-based scaffolds decorated with magnetic nanoparticles. Mater. Sci. Eng. C **110**(June), 1–13 (2020). https://doi.org/10.1016/j.msec.2020.110714
130. H.Y. Hu, Q. Li, H.C. Cheng, H.N. Du, B-sheet structure formation of proteins in solid state as revealed by circular dichroism spectroscopy. Biopolym. Biospectroscopy Sect. **62**(1), 15–21 (2001). https://doi.org/10.1002/1097-0282(2001)62:1%3c15::AID-BIP30%3e3.0.CO;2-J
131. J. Cheng, T.J. Deming, Synthesis of polypeptides by ROP of NCAs. Pept. Mater. **310**(June), 1–26 (2011). https://doi.org/10.1007/128
132. M.S. Rahman, et al., *Morphological Characterization of Hydrogels* (2019). https://doi.org/10.1007/978-3-319-77830-3_28
133. N. Johari, H.R. Madaah Hosseini, A. Samadikuchaksaraei, Optimized composition of nanocomposite scaffolds formed from silk fibroin and nano-TiO2 for bone tissue engineering. Mater. Sci. Eng. C **79**, 783–792 (2017). https://doi.org/10.1016/j.msec.2017.05.105
134. P. Ravi, P.S. Shiakolas, A.D. Thorat, Analyzing the effects of temperature, nozzle-bed distance, and their interactions on the width of fused deposition modeled struts using statistical techniques toward precision scaffold fabrication. J. Manuf. Sci. Eng. Trans. ASME **139**(7), 1–9 (2017). https://doi.org/10.1115/1.4035963

135. N. Johari, H.R. Madaah-Hosseini, A. Samadikuchaksaraei, Novel fluoridated silk fibroin/ TiO2 nanocomposite scaffolds for bone tissue engineering. Mater. Sci. Eng. C **82**(September), 265–276 (2018). https://doi.org/10.1016/j.msec.2017.09.001
136. X. Zhang, C. Jia, X. Qiao, T. Liu, K. Sun, Silk fibroin microfibers and chitosan modified poly (glycerol sebacate) composite scaffolds for skin tissue engineering. Polym. Test. **62**, 88–95 (2017). https://doi.org/10.1016/j.polymertesting.2017.06.012
137. A.S. Gobin, C.E. Butler, A.B. Mathur, Repair and regeneration of the abdominal wall musculofascial defect using silk fibroin-chitosan blend. Tissue Eng. **12**(12), 3383–3394 (2006). https://doi.org/10.1089/ten.2006.12.3383
138. G. Janani, S.K. Nandi, B.B. Mandal, Functional hepatocyte clusters on bioactive blend silk matrices towards generating bioartificial liver constructs. Acta Biomater. **67**(2018), 167–182 (2018). https://doi.org/10.1016/j.actbio.2017.11.053
139. P. Chantawong et al., Silk fibroin-Pellethane® cardiovascular patches: Effect of silk fibroin concentration on vascular remodeling in rat model. J. Mater. Sci. Mater. Med. (2017). https://doi.org/10.1007/s10856-017-5999-z
140. C. Wang, Y. Jia, W. Yang, C. Zhang, K. Zhang, Y. Chai, Silk fibroin enhances peripheral nerve regeneration by improving vascularization within nerve conduits. J. Biomed. Mater. Res. Part A **106**(7), 2070–2077 (2018). https://doi.org/10.1002/jbm.a.36390
141. J. Hu et al., Injectable silk fibroin/polyurethane composite hydrogel for nucleus pulposus replacement. J. Mater. Sci. Mater. Med. **23**(3), 711–722 (2012). https://doi.org/10.1007/s10856-011-4533-y
142. L.B. Mao et al., Synthetic nacre by predesigned matrix-directed mineralization. Science (80-.) **354**(6308), 107–110 (2016). https://doi.org/10.1126/science.aaf8991
143. A.D. Graham et al., High-Resolution Patterned Cellular Constructs by Droplet-Based 3D Printing. Sci. Rep. **7**(1), 1–11 (2017). https://doi.org/10.1038/s41598-017-06358-x
144. N. Dinjaski, R. Plowright, S. Zhou, D.J. Belton, C.C. Perry, D.L. Kaplan, Osteoinductive recombinant silk fusion proteins for bone regeneration. Acta Biomater. **49**, 127–139 (2017). https://doi.org/10.1016/j.actbio.2016.12.002
145. S. Behera et al., Hydroxyapatite reinforced inherent RGD containing silk fibroin composite scaffolds: promising platform for bone tissue engineering. Nanomed. Nanotechnol. Biol. Med. **13**(5), 1745–1759 (2017). https://doi.org/10.1016/j.nano.2017.02.016