Received: 28 May 2022 | Revised: 10 August 2022 | Accepted: 10 September 2022

DOI: 10.1002/jsp2.1225

REVIEW

JOR Spine OPEN ACCESS

Biomedical applications of silk and its role for intervertebral disc repair

Andreas S. Croft[1] | Eugenia Spessot[2,3] | Promita Bhattacharjee[4] | Yuejiao Yang[2,3,5] | Antonella Motta[2,3,5] | Michael Wöltje[6] | Benjamin Gantenbein[1,7]

[1]Tissue Engineering for Orthopaedic & Mechanobiology, Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland

[2]Department of Industrial Engineering and BIOtech Research Center, University of Trento, Trento, Italy

[3]European Institute of Excellence on Tissue Engineering and Regenerative Medicine Unit, Trento, Italy

[4]Department of Chemical Sciences, SSPC the Science Foundation Ireland Research Centre for Pharmaceuticals, Bernal Institute, University of Limerick, Limerick, Ireland

[5]INSTM, Trento Research Unit, Interuniversity Consortium for Science and Technology of Materials, Trento, Italy

[6]Institute of Textile Machinery and High Performance Material Technology, Dresden, Germany

[7]Department of Orthopaedic Surgery & Traumatology, Inselspital, Bern University Hospital, Medical Faculty, University of Bern, Bern, Switzerland

Correspondence
Benjamin Gantenbein, Tissue Engineering for Orthopaedics & Mechanobiology (TOM), Bone & Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland.
Email: benjamin.gantenbein@dbmr.unibe.ch

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: #437213841; Fondazione Caritro; H2020 Marie Skłodowska-Curie Actions, Grant/Award Numbers: 101008041, 778078; Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 310030E_192674/1

Abstract

Intervertebral disc (IVD) degeneration (IDD) is the main contributor to chronic low back pain. To date, the present therapies mainly focus on treating the symptoms caused by IDD rather than addressing the problem itself. For this reason, researchers have searched for a suitable biomaterial to repair and/or regenerate the IVD. A promising candidate to fill this gap is silk, which has already been used as a biomaterial for many years. Therefore, this review aims first to elaborate on the different origins from which silk is harvested, the individual composition, and the characteristics of each silk type. Another goal is to enlighten why silk is so suitable as a biomaterial, discuss its functionalization, and how it could be used for tissue engineering purposes. The second part of this review aims to provide an overview of preclinical studies using silk-based biomaterials to repair the inner region of the IVD, the nucleus pulposus (NP), and the IVD's outer area, the annulus fibrosus (AF). Since the NP and the AF differ fundamentally in their structure, different therapeutic approaches are required. Consequently, silk-containing hydrogels have been used mainly to repair the NP, and silk-based scaffolds have been used for the AF. Although most preclinical studies

Abbreviations: ACAN, aggrecan; AF, annulus fibrosus; ASmiR-214, anti-sense miR-214; BMP, bone morphogenetic protein; *Bombyx mori*, *B. mori*; CAD, computer-aided design; COL1, collagen type I; COL2, collagen type II; CS, chondroitin sulfate; ECM, extracellular matrix; GAG, glycosaminoglycan; GDF-6, growth and differentiation factor 6; GF, growth factor; GlcNAc, N-acetyl-glucosamine; GMO, genetically modified organism; HA, hyaluronic acid; IDD, intervertebral disc degeneration; IVD, intervertebral disc; LBP, low back pain; MaSp, major ampullate spidroin; miRNA, microRNA; MSC, mesenchymal stromal cell; NP, nucleus pulposus; PLGA, poly(lactic acid-co-glycolic acid); PTH, parathyroid hormone; RGD, arginine-glycine-aspartic acid; SELP, silk-elastin-like protein; SF, silk fibroin; SOX9, SRY-box transcription factor 9; SS, silk sericin; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor.

have shown promising results in IVD-related repair and regeneration, their clinical transition is yet to come.

KEYWORDS
Bombyx mori, degeneration, functionalization, intervertebral disc, low back pain, regeneration, repair, silk, stem cells

1 | INTRODUCTION

1.1 | The burden of low back pain and intervertebral disc degeneration

Every year, 266 million people worldwide report suffering from low back pain (LBP), with the highest incidence in Europe (5.7%).[1] The reasons for LBP are versatile. However, the main contributor to chronic LBP is intervertebral disc (IVD) degeneration (IDD).[2] Compared to other organs of the musculoskeletal apparatus, the onset of IDD often starts in early adolescence and then progressively aggravates with age.[3–5] The beginning of IDD is thought to be multifactorial, and many risk factors such as excessive mechanical stress,[6] genetics,[7] trauma,[8] and nutritional disorders within the IVD[9] can set the ball rolling responsible for the progression of IDD. And once the vicious circle of IDD is entered, it is of great challenge to escape it and reverse the process.[10] IDD usually starts in the IVD's inner region, known as the nucleus pulposus (NP).[11] The NP acts as the IVD's central pressure and weight absorber, which it is capable of due to its highly hydrated nature and abundance of collagen type II (COL2), elastin fibers and proteoglycans like aggrecan (ACAN).[12,13] During IDD, biochemical and cellular changes occur that promote catabolic turnover.[14] Hence, the NP's osmotic balance gets disturbed and consequently dehydrates.[11] Decreased hydration of the NP causes a shift of the compressive load from the NP to its surrounding tissue, the annulus fibrosus (AF), which is comprised of multiple concentric ring-like layers (lamellae) that are rich in collagen type I (COL1).[15,16] However, as the structure of the AF is preferentially made to resist tensile forces and less compressional forces, it becomes stiffer and weaker and aggravates the IVD's degeneration process overall.[15] As a result, the IVD can bulge and cause disc herniation with associated discogenic pain.[17]

To date, the clinical management of IDD has proven to be suboptimal in many cases since the current therapy methods primarily target the symptoms of IDD, mainly pain, and not the pathophysiology itself.[18] This issue can be largely traced back to a lack of available treatment options that would encourage the repair or regeneration of the IVD.[19] However, as an IVD is characterized by a low cell density, low turnover, avascularity, and poor nutritional supply, it poses a considerable challenge to researchers attempting to repair it.[20] Nevertheless, novel biomaterial-based therapies for the treatment of IDD have attracted considerable attention in recent years.[21] Biomaterial-based therapies have the great advantage of preserving the IVD's structure while either already containing cells that drive the regeneration and repair or stimulating the regenerative potential of the remaining cells in the tissue.[21]

One biomaterial that has historically been used time and time again for biomedical applications is silk.[22] Moreover, in orthopedics and especially in IVD-related research, silk was often used to support the IVD's repair or regeneration.[23] Therefore, this review aims to elaborate on the sources, types and properties of silk, how it has been used as a biomaterial and mainly, how the introduction of silk into IVD-related research has been implemented to repair the damage caused by IDD and to counteract further degeneration of the IVD.

1.2 | Silk—properties and the various species from which it can be harvested

Many insects and arachnids produce silk biopolymers as a protective shield during their life, such as the silkworms (Insecta: Lepidoptera: Bombycidae),[24] spiders (Chelicerata: Arachnida),[25] mites (Chelicerata: Arachnida: Acari: Tetranychidae),[26] and wasps (Insecta: Hymenoptera).[27] The most popular type of silk, mulberry silk, accounts for most silks produced globally (about 95%) (Figure 1).[28] Other commercially essential types of silk are classified as non-mulberry silk since they do not feed on mulberry plant leaves. The prominent representatives of this group are Eri silk, Tasar (Tussar) silk and Muga silk (all Insecta: Lepidoptera: Saturniidae) (Figure 1).[29] On the other hand, there is spider silk with its outstanding mechanical properties[30]; however, its commercialization is limited by the high-cost production and the difficulty in obtaining more significant amounts with the exception of some novel gene technology approaches.[22,31] Silks from silkworms and spiders have been widely studied for their use in tissue engineering and regenerative medicine.[32–34] Depending on the source, the biological and physicochemical properties change due to the different structural compositions.[35–37]

1.2.1 | Mulberry silk

Commercially available mulberry silk is produced from a single species, i.e., *Bombyx mori* Linnaeus, 1758 (Figure 1). Mulberry silkworms are entirely domesticated, and they do not occur naturally.[38] The silk protein is secreted from the silk glands of the mature fifth instar larva.[39] Their cocoon is formed by a structural protein core, that is, silk fibroin (SF), surrounded by a water-soluble coating named silk sericin (SS).[40] SF constitutes the significant portion of the cocoon and is the core silk protein. It is the same protein reeled from the cocoons into threads to be woven into cloth that forms a significant source of income for the sericulture industry.[41]

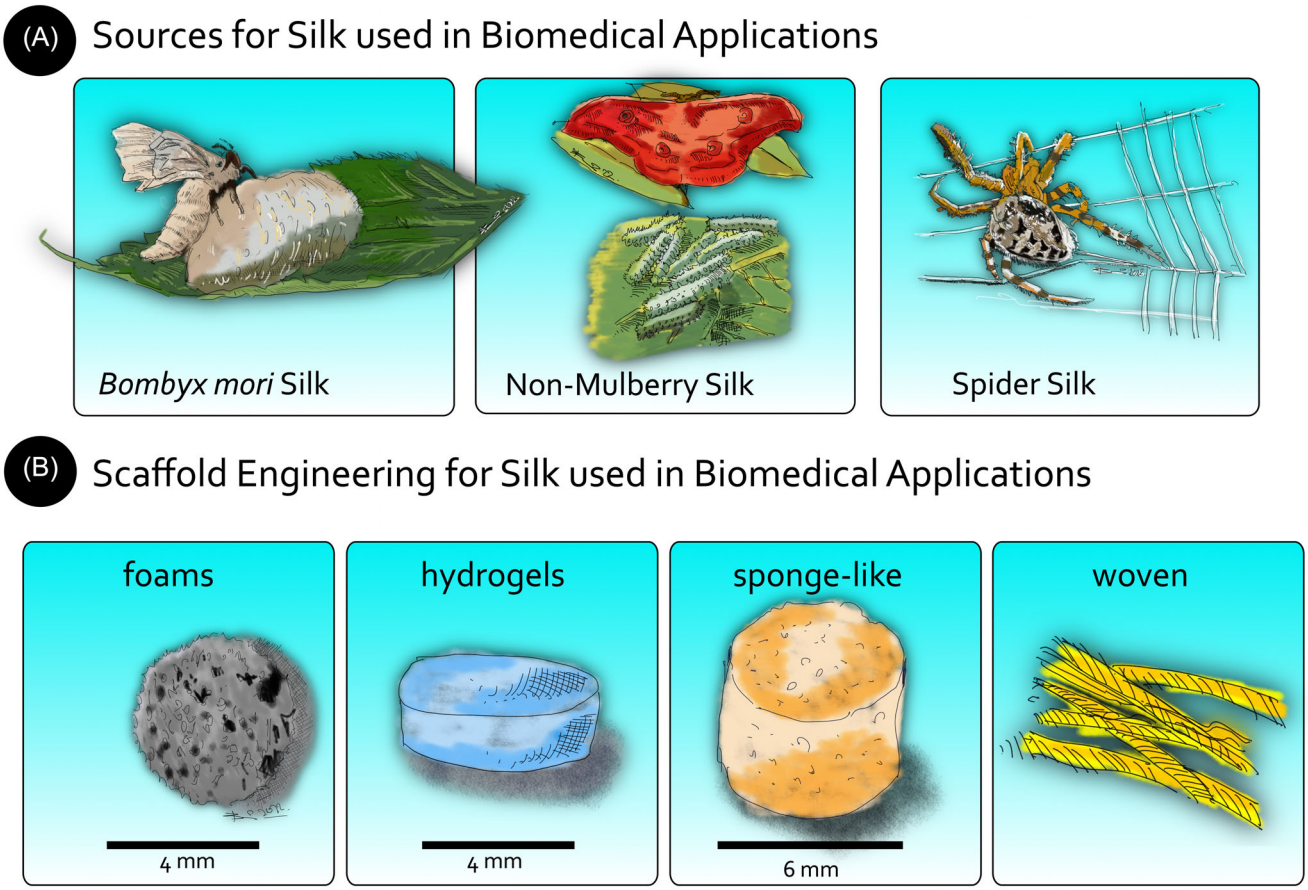


FIGURE 1 A schematic of silk origins and how it is applied for biomedical applications. (A) Sources of arthropod-derived silk. (B) Various forms of silk scaffolds for various biomedical applications: silk foams (redrawn, based on Hardy et al.[135]), silk hydrogels (redrawn, based on Singh et al.[131]), sponge-like silk (redrawn, based on Yu et al.[132]) and woven silk (redrawn, based on Hofmann et al.[250])

SF from *B. mori* is composed of a heavy chain (H-chain, 360–390 kDa) and a light chain (L-chain, 27 kDa), which are held together by a disulfide bond and a glycoprotein called P25, which is linked to both chains by noncovalent interactions in the molar ratio of 6:6:1, respectively (Figure 2).[42–44] The primary structure of the H-chain is a polypeptide that is mainly composed of glycine (43%–46%, G), alanine (30%, A), serine (12%, S), tyrosine (5.3%, Y) and other amino acids.[45–47] A SF H-chain is designed as a natural block-copolymer with a repetitive core formed by 12 domains forming the crystalline region of SF interspersed with 11 less organized domains composed of a nonrepetitive primary sequence.[35,43] This block-copolymer arrangement of the H-chain guarantees the characteristic mechanical properties of SF.[48]

The other protein constituting the silk cocoon is SS, a globular protein. It is an amorphous glycoprotein that acts as a cement to keep the SF filaments together during the spinning process and comprises about 20%–30% of the cocoon's mass. Its primary structure is mainly composed of serine (28%–34%), glycine (10%–19%), aspartic acid (14%–19%), and in minor parts, other amino acids such as histidine, tyrosine, glutamic acid, threonine, and others.[49,50] The high serine content and the polar side domains of other amino acids (hydroxyl, carboxyl, or amino groups) make it highly water-soluble. Moreover, they enable crosslinking, copolymerization, and blending.

SS obtained from *B. mori* exists mainly as random coil conformation with a molecular weight ranging between 20 and 400 kDa.[49] A small percentage of beta-sheets, along with beta-turns, contributes to small crystalline domains.[51] However, due to the principal presence of random coils, it behaves like an amorphous material, brittle in the dry state. Its properties can be improved by triggering beta-sheet formation upon drying, mechanical stretching, moisture absorption, or chemical modifications.

The process of removing sericin from the silk fiber is named degumming. To extract and use sericin from the cocoon, different methods can be chosen: high temperature (with or without high pressure) by autoclaving, acidic solution (citric, tartaric, succinic acid), soap, and alkali solutions (sodium carbonate), highly concentrated urea or by enzymatic processes.[52–54] With different extraction protocols, the chemical structure of sericin and its amino acid composition changes, which could impact the potential biomedical application.[54–56] The degumming process is also fundamental for the extraction and regeneration of silk fibroin into an aqueous solution and for the structural integrity of the three subunits of the silk fibroin protein complex.[57]

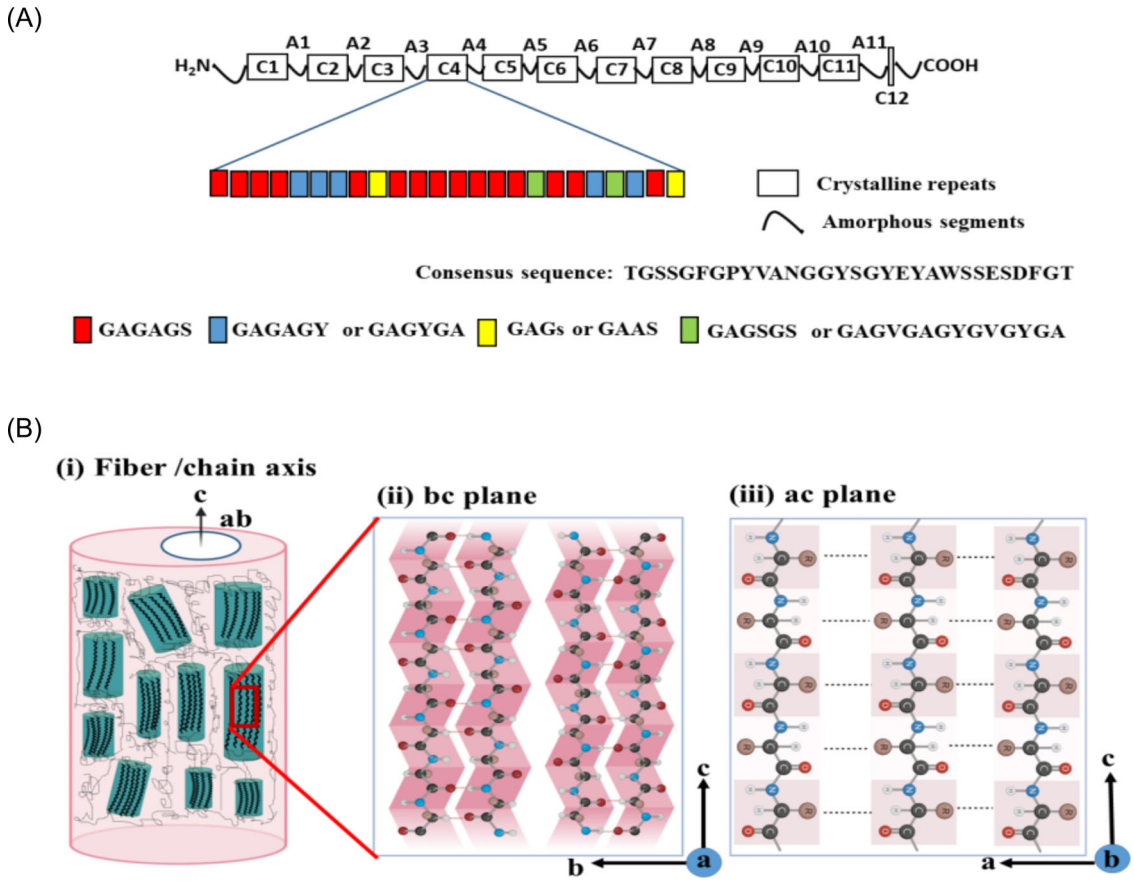


FIGURE 2 Chain configuration of silk fibroin: (A) Schematic representation of the primary structure of the silk fibroin heavy chain. Each of the 12 crystalline domains varies slightly in length and sequence, while the 11 amorphous domains are nearly identical. The amino acid sequence for one of the crystalline domains is given to highlight the repetitive nature of the protein. (B) Hierarchical structural organization in *Bombyx mori* silk fibroin. (i) Orientation of aligned beta-sheet crystallites and amorphous regions within a native fiber. (ii) Inter-sheet stacking within a beta crystallite, held together by van der Waals interactions between the glycine or alanine populated faces. (iii) Hydrogen bonding in the peptide chain organizes the crystalline blocks of the protein into anti-parallel beta-sheets

Thus, it is often a crucial first step for developing silk-based biomaterials.

In addition to SF and SS, the major silk proteins, several components of low molecular weight peptides have been identified. These silk protein components are called seroin.[58] Seroin is distinguished from other silk proteins by high proline content, lack of cysteines, and the presence of two kinds of short amino acid repeats.[58] It is assumed that seroin is involved in cocoon protection against predators and microbes.[59]

1.2.2 | Non-mulberry silk

Representatives of non-mulberry or "wild" silks are called tasar, eri, muga, fragaria, cricula (collectively called "Vanya silks," coming from Sanskrit language and standing for untamed, wild, or forest-based, Lepidoptera: Saturniidae), and shashe (Lepidoptera: Lasiocamidae) (Figure 1).[60] The process of non-mulberry silk production in the silkworm gland is the same as the mulberry silk. However, the spun silk displays significant characteristic differences.[61] A prominent feature is that non-mulberry silk has a higher cross-section since it has reduced packing capacity due to the higher content of bulky side-groups in the H-chain (dibasic acids and arginine). Its stability results from higher H-bonds in the H-chain, which limits the dissolution. Moreover, non-mulberry silks are more stable at high temperatures than mulberry silks and present attractive compressive strength, toughness, and elasticity.[29,62,63] The amorphous domains are formed by bulky and polar side chains, responsible for maintaining the silk properties under different external treatments. Finally, non-mulberry silk does not possess an L-chain and the P25 glycoprotein.[64,65] The big challenge with non-mulberry silk is the isolation and purification of SF from the cocoon. This difficulty arises from the high hydrophobic structural stability of non-mulberry cocoons and the high amount of H-bonds. They cannot be dissolved in lithium bromide and the other solutions used for mulberry silks. Therefore, other ionic liquids such as calcium nitrate, sodium thiocyanate, lithium thiocyanate and harsh organic solvents, such as trifluoroacetic acid, have been considered.[66,67] Due to this issue, non-mulberry SF is generally isolated from the silk gland of the fifth instar larvae by squeezing it in a distilled water solution with anionic surfactants.[68]

TABLE 1 Properties of various non-mulberry fibroin and sericin

Silk type	Silkworm species	Background	Silk proteins	Molecular weight (kDa)	Breaking strain (%)	Reference
Tasar silk	*Antheraea mylitta* *Antheraea perny* *Antheraea yamamai* *Antheraea roylei*	Can be divided into two types: tropical tasar and temperate tasar. These silkworms can be bivoltine or trivoltine depending upon variety and habitat. Among non-mulberry silkworms, *A. mylitta* has the highest silk production capacity and its cocoon is the largest.	Fibroin Sericin	Two fractions of 395 and 197 Five fractions ranging from 30 to more than 200	26–39	68,223,224
Muga silk	*Antheraea assama*	Muga is golden yellow colored silk and is mostly distributed in the north-eastern region of India. The silkworms are semi-domesticated and multivoltine.	Fibroin Sericin	Two fractions of 20 and 220 Single fraction of 66	26–41	223,225,226
Eri silk	*Philosamia ricini* (*Samia ricini*/*Cynthia ricini*)	Eri silk can regain greater amounts of moisture than mulberry silk. Its fibroin contains a many hydrophilic and positively charges amino acids. The silkworms can be domesticated, and they eat the leaves of several trees, not just mulberry leaves.	Fibroin Sericin	Two fractions of 45 and 97 Single fraction of 66	24–27	223,225,227,228
Fagaria silk	*Attacus atlas*	*Attacus atlas* can be found in southeast Asia. Its silk has a bivoltine nature and the tensile strength of the silk yarn is greater than that of tasar and muga.	Fibroin Sericin	N/A N/A	N/A	60,229,230
Shashe silk	*Gonometa postica*	*Gonometa postica* is a polyphagous African insect. Its high-quality silk has mainly been used for textiles and has only recently been implemented as a biomaterial.	Fibroin Sericin	N/A N/A	23–32	231–233
Cricula silk	*Cricula trifenestrata*	*Cricula trifenestrata* is a silkworm from South Asian countries. The cocoons of this species are small and perforated. The silk has good biocompatible properties.	Fibroin Sericin	Single fraction of 400 Single fraction of 350	12	60,234–236

Source: Modified from Kundu et al.[60]

A selection of non-mulberry silks and their properties is presented in Table 1.

1.2.3 | Spider silk

Spiders produce spin silks to perform many functions, such as mating,[69] flying,[70] and building their webs for hunting (Figure 1).[71] Among these, spider cobwebs are the most well-known ones. They are composed of at least five types of silk (i.e., minor and major ampullate silks, piriform silk, flagelliform silk and aggregate silk), produced by different glands with various functional properties.[72–75] The most extensively characterized spider silks are from *Nephila clavipes* and *Araneus diadematus*.[22]

Spider silk provides a greater diversity of physical and mechanical properties than silkworm-derived fibers due to multiple complex silk glands.[72] Silk derived from the spiders' major ampullate glands is a natural hierarchically ordered material that displays a unique combination of the tensile strength (1.3 GPa), extensibility (30% elongation to fracture) and toughness (158–180 J/cm^3). In contrast to *B. mori* silk, spider silk does not comprise any sericin.[76–80] Moreover, it is biocompatible and has a very high strength-to-density ratio, exceeding the one of high-performance steels and many commercial fibers.[81] For these reasons spider major ampullate silk has always inspired

researchers in the design of biomedical devices and tools with outstanding mechanical and biological properties.[32,82,83]

Unlike silkworms, which rely on two essential proteins—sericin and fibroin—spiders manufacture proteins (spidroins) whose composition and properties vary significantly between species.[84] However, they consist of two nonrepetitive hydrophilic terminal domains (amino- and carboxy-terminal) with a large internal repetitive hydrophobic region.[85] Major ampullate silk is mainly composed of major ampullate spidroin (MaSp) 1 and 2 that comprise poly-alanine and poly-glycine-rich domains in the repetitive region.[86] It contains crystalline beta-sheets formed by poly-alanine interconnected in an amorphous matrix composed of glycine.[87]

Despite the remarkable properties, native spider silk has limited uses as it is very challenging to achieve more extensive mass production. One reason for these limitations might be the cannibalistic nature of spiders, which makes it hard to harvest silk on a large scale.[88] A possible solution to overcome this hurdle is the production of artificial spider silk through their expression in heterologous hosts, such as bacteria.[89] Recently, it has been proposed to use plants such as potatoes and tobacco to amplify spider silk proteins for the industry.[31] Aside from increased scalability, protein engineering techniques allow scientists to design artificial silks with specific features that could outperform native spider silk for their use in the biomedical field, in textiles and others.[90]

2 | SILK AS A BIOMATERIAL

Biomaterial design is a fundamental ingredient of tissue engineering. An ideal biomaterial should: (i) be biocompatible and elicit little to no host immune response, (ii) integrate physical, chemical, and biological cues to guide cells into functional tissues via cell attachment, migration, proper cell–cell interactions, cell proliferation, and differentiation, (iii) degrade at a rate favorable with new tissue formation, (iv) offer mechanical support appropriate to the level of functional tissue development, and (v) possess versatile processing options and should be easily chemically modified to suit a wide range of targeted biomedical applications.[91]

Silks represent a unique family of proteins that fulfill all these criteria of a functional biomaterial.[80] The most studied silk type for biomaterial design is mulberry (*B. mori*) silk because it can be domesticated and regenerated in an aqueous solution. Non-mulberry silk is not widely used in the field of biomaterials, despite the presence of the tripeptide sequence arginine-glycine-aspartic acid (RGD) in the primary structure, which would enhance the interaction of integrin present on the cells' surface, giving non-mulberry silk an advantage over mulberry silk to enhanced cell adhesion and proliferation.[92–94] As previously mentioned, the use of non-mulberry silk, is hindered by cocoon dissolution issues, so harvesting is almost limited to direct extraction from the silk glands. Nevertheless, there are studies for its use as a potential biomaterial for different target tissues (bone,[95] cartilage,[96] skin,[97] tendon,[98] cornea[99]), for drug delivery[100] and as tissue models.[101,102] Finally, since the spread of spider silk as a biomaterial is limited because of the difficulties in obtaining large quantities of material, there is considerable interest in the production of recombinant spider silk proteins using heterologous hosts.[88,103] To date, silk has been processed in the form of fibers,[104] non-woven meshes,[105] films and coatings,[106] porous forms,[107] hydrogels,[108] and bioinks[109,110] for tissue engineering purposes (Figure 1B).

2.1 | Silk fibroin

SF from *B. mori* is an attractive biomaterial that has been used as suture material since ancient times. Meanwhile, some SF-based products have been approved by the FDA for their use in clinics. Degummed SF yarns are used for surgical sutures and for manufacturing the knitted surgical mesh (SERI surgical scaffold™).[32] Furthermore, a powder from freeze-dried regenerated silk fibroin solution is used as an injectable filler (Silk Voice™) for vocal fold medialization and vocal fold insufficiency.[37,111] One reason for the excellent biocompatibility of SF may depend on the crystallinity content and the method of material processing.[112] As an example, the thrombogenic response of regenerated SF films can be tuned by varying their beta-sheet content and decreasing their hydrophobicity so that they adsorb more serum proteins compared to the native fibers.[113]

The regeneration of SF is fundamental for obtaining an aqueous solution that can be processed in diverse ways by controlling and triggering the self-assembly of beta-sheets "on-demand" for the crystallization structure. The key features that make SF an exciting choice for tissue engineering applications are its tuneable biocompatibility,[114] low immunogenicity,[115] tuneable biodegradation,[116] versatile processability,[117] controllable and tailorable mechanical properties,[118] and its sustainability (easy accessibility, cost-effective and green processing).[119]

A soluble helical structure dominates the regenerated aqueous solution (Silk I-like).[45] When this structure is exposed to mechanical/physical and chemical treatments, the transition to Silk II occurs.[120] Silk II is a beta-sheet crystal-dominated structure and is the main contributor to the SF's strength, biodegradation kinetics, biological response and insolubility in water and other solvents, such as mild acids and alkaline environments. Depending on the beta-sheet content and the self-assembling method, the physicochemical and biological properties of the scaffold vary.[121]

When dealing with scaffolds, it is essential that their degradation kinetics are consistent with the rate of novel tissue formation. The relevant advantage of SF is that its degradation can be controlled by tuning the crystallinity, concentration, molecular weight, the scaffold morphology, such as scaffold pore size, porosity and processing technique.[122–124] Being a protein, SF is subjected to proteolytic digestion in vitro and in vivo by chymotrypsin, proteases, collagenases and matrix metalloproteinases.[116] Each enzyme has a specific cleavage site, and protease XIV has been considered the most efficient for degrading silk in any material construct.[123] Due to their long-term functional stability, porous silk scaffolds have been used in sustainable cultures for up to 6 months.[125]

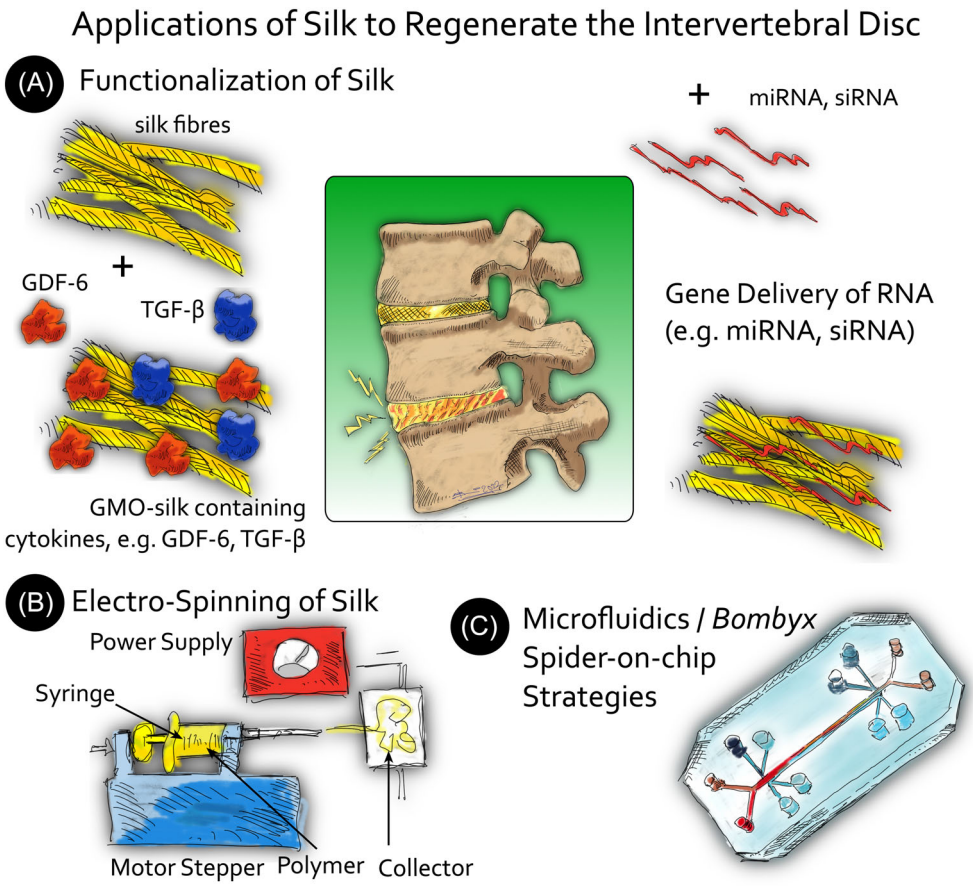


FIGURE 3 Illustration of recent advances of silk engineering for regeneration of spine applications. (A) Functionalization of silk, for example, with GMO modified *Bombyx mori* larvae overexpressing TGF-β or GDF-6 in silk glands based on ref or addition of RNA molecules such as miRNA, siRNA based on Frauchiger et al.[215] (B) Process of electrospinning of silk. (C) Microfluidics of *B. mori*/ spider-on-chip strategies

SF-based scaffolds also display high thermal stability, depending mainly on the primary and secondary structure.[35] Silk I and Silk II crystals melt at different temperatures, that is, 260–292°C and 286–350°C as a mean value, respectively.[126,127] Also, the processing technique and post-treatments influence the thermal stability of SF scaffolds.[128] Due to their thermal strength, SF-based scaffolds can withstand different sterilization techniques, such as autoclaving, ethylene oxide, ethanol, and UV- and gamma irradiation, without damaging the structure.[129] This feature is a crucial difference from many other natural polymers.

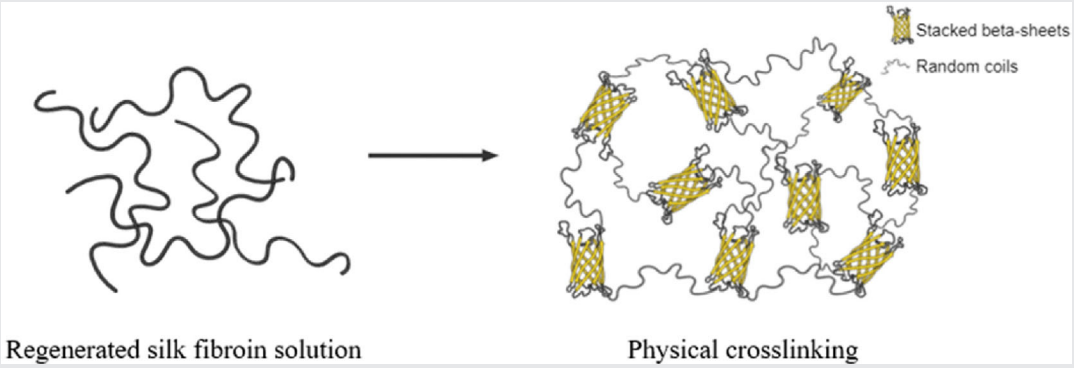
The regenerated SF solution can be processed differently to produce different scaffold morphologies such as hydrogels,[130–132] foams[133] and sponges,[134,135] 3D printed constructs,[136] micro- and nano-particles,[137] electrospun membranes,[138] and 2D films (Figures 1B and 3).[139] This processing versatility allows it to adapt to the needs and requirements of different target tissues with diverse physicochemical and biological responses.[36,113] For example, hydrogels are highly hydrated polymer networks that can be crosslinked by other methods and permit cell seeding and encapsulation due to their capacity to retain large amounts of water. Hydrogels have been widely explored in tissue engineering because of their unique biocompatibility and biodegradability.[140] Usually, traditional SF-hydrogels are based on physical and chemical crosslinking (summarized in Table 2). The properties of SF hydrogels depend on the number of beta-sheets in the material.[141] Physical stabilization can then be achieved using shear stresses,[142] an electric field[143] and ultrasound[144] or varying the temperature[145] or pH[146] to allow the conformational change from Silk I to Silk II. Besides physical crosslinking, however, chemical crosslinking can also be involved to improve the stability and the mechanical properties thanks to the abundance of functional groups on SF chains (i.e., tyrosine, lysine).[130]

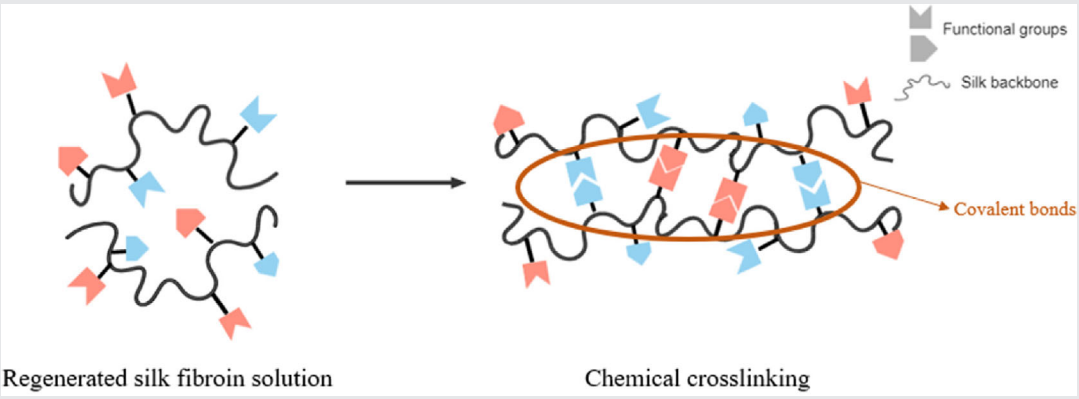
Sponges and foams are interconnected porous structures whose properties can be controlled by the processing method. SF sponges can be produced by salt leaching,[147] freeze drying,[134] or gas foaming.[133] They have been widely used for orthopedic applications and soft tissue engineering due to their macroporous structure, which can be adjusted for tissue regeneration and vascularization.[148] Sponges and foams can also be used in combination with 3D printed synthetic polymer structures to promote the bioactivity of the construct. For example, in the past, SF was combined with a 3D printed polycaprolactone structure to fabricate an "entrapped in cage" scaffold for meniscus tissue engineering.[149] The presence of silk enhanced the mechanical properties in the wet state thanks to its swelling properties and favored cell adhesion, proliferation, and metabolic activity in vitro and neovascularization in vivo.

Due to its different chemical structure, the biocompatibility of SF can be enhanced by chemical modifications of the amino acid side chains to graft bioactive molecules (peptides, growth factors [GFs]). This process includes coupling reactions (i.e., carbodiimide chemistry,[150,151] diazonium coupling[152] or cyanuric chloride

TABLE 2 Types of crosslinking used for silk fibroin hydrogels

Crosslinking type	Methods	Main interactions
Physical crosslinking	• Self-assembly • Ultrasonication • Shear stresses • Electric field application • Temperature changes • pH variations • Organic solvents (methanol, ethanol) • Surfactants (sodium lautoyl sarcosinate, sodium lauryl sulfate, poloxamer)	Noncovalent bonds (hydrogen bonding, hydrophobic interaction, electrostatic interaction, ionic interaction).[128,237–242]
Chemical crosslinking	• Photopolymerization (UV or visible light) • Irradiation (gamma-rays) • Chemical crosslinking agent (carbodiimide, genipin, glutaraldehyde) • Enzyme crosslinking (horseradish peroxidase, glutamine transferase, carbonic anhydrase, alcohol oxidase, tyrosinase, laccase)	Formation of covalent bonds via enzymes, chemical agents or others.[150,152,243–247]





Note: Sketches created in the Mind the Graph platform (www.mindthegraph.com).

activated[153]), which can facilitate the addition of another polymer chain,[154] oligosaccharides[155] or specific peptide chains.[156] A former study showed that biocompatibility was increased by the covalent addition of RGD and parathyroid hormone (PTH).[157] The scaffold biocompatibility can also be improved by blending SF with other materials, i.e. with calcium phosphates or specific inorganic components to enhance osteogenic properties.[158] Furthermore, silk fibroin scaffolds have also been shown to promote differentiation of mesenchymal stromal cells (MSC) with extracellular matrix (ECM) secretion and mineralization, making them an optimal candidate for orthopaedical tissue regeneration.[159] Moreover, the incorporation of specific GFs (i.e., bone morphogenetic protein 2 [BMP-2],[160] BMP-7,[161] vascular endothelial growth factor [VEGF])[162] further increased the osteogenic and angiogenic potential of SF scaffolds.

2.2 | Silk sericin

SS is a natural polymer protein material produced by the silkworm *B. mori* that covers and holds the silk fibroin filaments together. However, SS is usually considered a side-product of the cocoon during the degumming process, becoming an unutilized waste product. Researchers started to extract it as biomaterial due to its natural origin, availability, and interesting biological properties. SS has been

recognized as the immunogenic element of the silk filament for years, which has fuelled research into the purification of SF and its regeneration. However, researchers demonstrated that the immunogenicity of the silk fiber is mainly due to the combination of SS with SF, although the mechanism responsible for initiating the immune response is not yet fully understood.[163]

SS has been used in cosmetics for years due to its properties such as antioxidant,[164] moisturizing,[165] UV-protective potential,[166] and oxygen permeability.[167] For tissue engineering approaches, SS-biomaterials have been synthesized in various forms, such as hydrogels,[168] sponges,[169] films,[170] and inks.[171] Furthermore, with the development of SS-based 3D scaffolds and films, its biological effects could be investigated. SS showed an increase in the migration, proliferation and production of COL1 in skin cells.[172] Additionally, it favors the growth of keratinocytes and fibroblasts, which makes it a potential candidate for epithelial tissue repair and wound dressings.[173] Moreover, SS can favor the nucleation of bone-like hydroxyapatite, raising interest in its use in bone tissue engineering and the coating of titanium surfaces.[174,175] Finally, due to its chemical reactivity, pH sensitivity and amphiphilic structure, SS has been employed for the design of drug delivery systems and for targeting purposes.[174]

Despite the interesting biological properties, the widespread use of SS-based scaffolding materials is limited because it is characterized by fast degradation rates and weak mechanical properties. However, thanks to the presence of hydroxyl, carboxyl and amino groups present in the polar side chains, it can be crosslinked,[176] co-polymerized[177] and blended[178] with other polymers to improve biomechanical features or to conjugate bioactive molecules. For example, in a previous study, SS was combined with gelatin methacrylate, and it was then used as ink for 3D printing purposes favoring the proliferation and stratification of keratinocytes.[171]

2.3 | Functionalized silk

As mentioned above, silk as a biomaterial already has a lot of advantages in tissue engineering applications. However, when silk is fabricated into a scaffold with designed functions and in contact with the tissues, the microenvironment with which the scaffold is in contact is complicated, so the requirements of the material are more crucial. For this reason, the silk or silk-based scaffold should be functionalized to have a controllable performance according to the final purpose. The general principle of functionalization is the use of physical or chemical methods to make silk as a delivery and sustainable controlled-release system of some targeted molecules, in order to improve the tissue-specific biological properties of the scaffold.

2.3.1 | Growth factor and cytokine functionalization on silk

GFs and cytokines are two major biological signal molecules, which regulate cellular function, and have an essential contribution to ECM synthesis.[179] Since silk is a well-studied material in this field, functionalized silk with GFs and cytokines has presented excellent performances in both delivering and releasing. In the past, the biological properties of the dual GFs BMP-2 and transforming growth factor β1 (TGF-β1) functionalized silk-based (non-mulberry silk fibroin, from *Antheraea mylitta*) scaffold have been studied for bone regeneration using different functional methods. Bhattacharjee et al.[180] loaded the two GFs using the carbodiimide-coupling reaction, while Naskar et al.[181] loaded the same GFs by simple physical blending. Even though the architectures of these two studies were completely different, both results showed that the functionalized silk scaffolds had a sustained GF release profile, good cell adhesion, proliferation, and migration, as well as an earlier stage differentiation. In the study by Wang et al., osteochondral GFs were either encapsulated in poly(lactic acid-co-glycolic acid) (PLGA)-based or mulberry SF-based microspheres and then further incorporated in alginate or silk scaffolds to create concentration gradients.[182] The results showed that both microsphere types were able to form concentration gradients and induced human MSCs to differentiate along the concentration gradient into an osteochondral phenotype.

2.3.2 | Functionalizing silk with miRNA

MicroRNAs (miRNAs) are short, noncoding RNA molecules that regulate gene expression. Importantly, due to the "small size" of these RNAs, the therapeutic miRNAs will not integrate into DNA, thus eliminating the scruples about genetic alterations (Figure 3A).[183] Although the regulation of miRNA on skeletal tissues is widely studied, the application of miRNA to functionalize silk or silk-based scaffolds for tissue engineering is still an emerging field. One such sparse study where miRNA-functionalized-silk was used for orthopedic research was conducted by James et al.[184] Here, they developed an all-aqueous, silk-based device to enhance the osteoinduction of MSCs. Just by simply doping the silk fibroin solution blended with anti-sense miR-214 (ASmiR-214) on the surface of the silk-based screw, the continuous release of miRNA that inhibits the expression of osteoinductive antagonists could be detected up to 7 days. The in vitro evaluations demonstrated that the osteoblastic commitment and osseous integration were enhanced.

2.3.3 | Microfluidics using silk

Recently, lab-on-chip approaches were followed for the production of nano-particles of silk and nano-films (Figure 3C).[185,186] Also, bioinks for the usage in 3D printing engineering have been developed.[187,188] Jeon et al. for instance, investigated on silk-elastin-like protein (SELP) polymers that can be used for predicted drug release depots.[189] Peng et al. mimicked the complex interplay of different silk glands of spiders as a "spider-on-chip" approach.[190] Silk in general seems to be very advantageous for the usage of lab-on-chip devices compared to other materials such as ceramics and polymers.[191,192] This is specifically true

if advantage is taken of hydrophilic or hydrophobic yarns with high elasticity.[191,193,194]

3 | SILK USED FOR INTERVERTEBRAL DISC REPAIR

Regarding the silk's biomechanical properties and its versatile biomedical applications, this biomaterial has also been considered and used for IVD research with the aim to repair damaged and/or degenerated IVDs.[23] Anatomically spoken, there are two approaches how to repair the IVD using silk; either by targeting the NP or the AF.

3.1 | Nucleus pulposus repair

In the past, the NP has been a popular target to repair a degenerated IVD. This is likely related to the fact that IDD has its origins in the NP, where a reduced ECM turnover accompanied by a loss of internal proteoglycans leads to the disc's dehydration.[11] Most NP replacement biomaterials, made at least partially of silk, are hydrogels. This seems like the most obvious choice since hydrogels and the NP both share a lot of common ground. Hydrogels can absorb significant amounts of liquid, and they can be designed to have similar mechanobiological properties as the highly hydrated NP.[195–197]

One of the first approaches to use a hydrogel with incorporated silk to regenerate NP tissue was carried out by Park et al.[198] Their approach was to encapsulate chondrocytes with a hydrogel that consisted of fibrin/hyaluronic acid (HA) only, 2% silk or a combination of both biomaterials either with 1%, 1.5%, or 2% silk. They supplemented silk to the fibrin/HA hydrogel to achieve superior mechanical strength compared to plain fibrin/HA gels. After 1 week of culture, all five groups showed a defined chondrogenic area stained with alcian blue. Furthermore, all silk groups expressed a significantly higher GAG content than the fibrin/HA only group after 1 week. However, based on the gene expression of *COL2*, *SOX9*, and *ACAN*, the 2% silk gel and the 2% hybrid were inferior to the other groups. Interesting results were also found regarding the mechanical properties of the different gels. As hypothesized, all samples treated with silk presented a significantly higher compressive modulus and yield strength than those without silk, making them a better substitute for NP tissue.

Recently, a similar approach has been carried out that also explored the influence of silk/HA-composite hydrogel concentrations on the samples' biomechanical behavior.[199] This study confirmed the correlation between higher silk to hyaluronic acid ratio and a higher viscoelastic modulus. Furthermore, in combination with TGF-β3 enriched chondrogenic inductive medium, the hydrogels promoted considerable NP-like differentiation of bone marrow-derived MSCs after only 7 days. This differentiation was most clearly noticeable through a significantly enhanced expression of GAG and COL2 in the ECM and a significantly higher gene expression of *ACAN* and *COL2*.

Another study on silk-based hydrogels was conducted by Hu et al.[200] Here, they were working on an injectable hydrogel with the ultimate goal of replacing a degenerated NP. They proposed a cross-linked hydrogel composed of silk fibroin and polyurethane, which could be prepared in a liquid or semi-liquid state at room temperature. The hydrogel showed great cytocompatible properties during a one-week culture period using bone marrow-derived MSCs, good radiographical visibility and a Young's modulus comparable to that of a natural NP. In a follow-up study, the group further focused on the mechanical features of the same hydrogel as well as its in vivo biocompatibility.[201] Confined compression and fatigue tests revealed adequate physical-mechanical characteristics and the ability to withstand a million cycles at an axial strain of 15% and a frequency of 5 Hz. The hydrogel's biocompatibility was shown with an in vivo rabbit model, where they transplanted the dried implants into the paravertebral muscle. After 3 months, no inflammatory response was observed in the surrounding tissue, nor did the hydrogel display any apparent signs of deformation or degradation. In conclusion, the investigators state that due to the good biomechanical properties, which resemble those of a healthy NP, further animal trials and, ultimately, its clinical transition seems realistic.

The wide range of possible applications of silk fibroin for the regeneration of the NP was nicely shown by Murab et al.[202] Not only was the silk used as a hydrogel for improved mechanical support, but within the hydrogel, silk fibroin was shaped into hollow microspheres and used as carriers for N-acetyl-D-glucosamine (GlcNAc). As GlcNAc has been known to regulate the expression of TGF-β1[203] and for the formation of large proteoglycan aggregates,[204] it was hypothesized that its spatiotemporal release would enhance the differentiation of human adipose-derived stem cells towards a NP-like phenotype. Indeed, cells cultured in hydrogels containing GlcNAc-loaded microspheres expressed significantly more *COL2* and *ACAN* than controls lacking GlcNAc. Furthermore, the hydrogel's rheological characterization demonstrated its injectability, and cyclic compressive testing using degenerated IVDs with subsequent hydrogel injection revealed a compressive strength similar to that of a healthy IVD.

Almost all trials that aimed to regenerate/repair the NP with silk used such from the silkworm *B. mori*. In this context, only a single study has assessed how non-mulberry silk could be used for NP regeneration.[196] Here, composite hydrogels were formed using different ratios of silk fibroin proteins derived from *Antheraea assamensis* and *B. mori*. The aim was then to find a suitable mixture of these two silk fibroins for in situ replacement of the NP. After testing different ratios of silk fibroin blends, the investigators observed that the higher the concentration of *Antheraea assamensis* derived silk, the faster the hydrogel's gelation, but also degradation time, the higher the proliferation rate of NP cells and the greater its ability to swell as well as to withstand cyclic compression. In conclusion, they state that the hydrogel's properties can be adjusted by varying the silk fibroin proportions, making it a potential candidate for clinical translation.

Nevertheless, despite the promising results obtained with hydrogels for NP repair, they also have their limitations. Due to their viscous nature, cell migration and the exchange of nutrients and waste products into and out of the hydrogel can be hindered, and the synthesis of newly formed ECM can be impaired.[205,206] To tackle these

TABLE 3 Overview of published studies where silk was used to repair/regenerate the nucleus pulposus

Silk origin	Silk structure	Study	Conclusions	References
Bombyx mori	Hydrogel	Assess whether a composite hydrogel made of silk-fibrin and hyaluronic acid causes greater mechanical strength and more chondrogenesis than silk-fibrin/hyaluronic acid alone.	Silk-fibrin/hyaluronic acid hydrogels had improved mechanical strength and a decreased degradation rate while maintaining the chondrogenic phenotype of NP cells.	198
Bombyx mori	Hydrogel	Comparing the mechanical properties and chondrogenic inductive potential of hydrogels made of hyaluronic acid and different silk fibroin strains with varying weight ratios.	The higher the weight ratio of silk fibroin to hyaluronic acid, the greater the viscoelastic modulus. Moreover, the hydrogels promoted NP-like differentiation of MSCs.	199
N/A	Hydrogel	Testing the biomechanical properties of a NP replacement hydrogel consisting of silk fibroin and polyurethane.	The hydrogel possessed adequate physical-mechanical properties to replace the NP as a prosthetic biomaterial.	200
N/A	Hydrogel	Determine the compressive mechanic characteristics, stability and biocompatibility of a composite hydrogel made of silk fibroin and polyurethane in vivo.	The hydrogel showed great biocompatibility in vivo and no obvious signs of degradation were found after 3 months of implantation.	201
Bombyx mori	Cryogel	Assess the effect of a silk fibroin enriched poly (vinyl) alcohol cryogel on its hydrophilicity and how it influences the cellular attachment and proliferation of adipose-derived MSCs.	The enrichment of silk improved the cryogels' rehydration ratio, water content, hoop stress, and compressive modulus. Moreover, cell-hosting abilities were improved.	248
Bombyx mori	Hydrogel	Creating a hydrogel that resembles the NP's ECM. The hydrogel was chitosan and COL2 based and to increase the hydrophilicity and stability, gelatin and silk fibroin were added.	The hydrogel was injectable at 4°C and started gelation after 30 min at 37°C. The addition of silk increased the hydrogel's stability and durability.	249
Bombyx mori	Silk microspheres embedded in a silk hydrogel	Study the effect of GlcNAc loaded hollow spheres on the NP-like differentiation of adipose-derived MSCs, which are embedded in silk fibroin together with the spheres.	Spatiotemporally controlled release of GlcNAc enhanced the expression of COL2, ACAN and GAG. Furthermore, the hydrogel brought adequate structural support during cyclic compression.	202
Bombyx mori and *Antheraea assamensis*	Hydrogel	Blending two different silk variations to design a suitable hydrogel for in situ NP replacement applications.	The gelation time and mechanical properties of the hydrogel could be tuned depending on the ratio of the silk variants. NP cells proliferated on all variants tested.	196
Bombyx mori	Scaffold	Determine the feasibility of porous silk fibroin scaffolds seeded with NP cells for NP regeneration.	NP cells proliferated in the scaffold and produced significant amounts of COL2 and proteoglycans. A higher cell number resulted in a greater compressive elastic modulus of the scaffold.	206

Abbreviations: ACAN, aggrecan; COL2, collagen type II; ECM, extracellular matrix; GAG, glycosaminoglycan; GlcNAc, N-acetyl-glucasamine; MSC, mesenchymal stromal cell; NP, nucleus pulposus; N/A, non-available.

issues, Zeng et al. created a highly porous silk fibroin scaffold with interconnected macropores, leaving enough space for NP cells to infiltrate, increase, and to deposit newly synthesized ECM.[206] As hypothesized, NP cells infiltrated the scaffolds and proliferated well therein, as a significant increase in DNA content was found over a culture period of 3 weeks. Moreover, quantitative analysis showed that

TABLE 4 Overview of published studies where silk was used to repair/regenerate the annulus fibrosus

Silk origin	Silk structure	Study	Conclusions	References
Bombyx mori	Scaffold	Assess whether either lamellar or porous spongy silk scaffolds are better suited for AF tissue formation and function. Both scaffolds were seeded with porcine AF cells.	Both scaffolds showed similar mechanical properties after 2 weeks of culture. However, lamellar scaffolds enabled a significantly higher GAG and collagen production than porous scaffolds.	207
Bombyx mori	Scaffold	Creating a biphasic structure that mimics the IVD. The AF-like structure was made of silk and was cultured with porcine AF cells and the NP scaffold consisted of fibrin and hyaluronic acid and was cultured with porcine chondrocytes.	The amount of GAG significantly increased with the lamellar AF scaffolds during a culture period of 4 weeks. Lamellar NP scaffolds showed significantly more collagen after 2 weeks and more GAG after 4 weeks.	208
Bombyx mori	Scaffold	Assess whether porous silk scaffolds with or without RGD allow AF cells to attach and promote ECM production.	AF cells attached and proliferated on the scaffold regardless of whether the silk was functionalized with RGD or not. However, RGD silk improved *ACAN* and *COL2* expression.	214
N/A	Scaffold	Integrating MSC-sheets onto silk scaffolds that were wrapped around a disc made of silicon. The artificial IVD was cultured for 4 weeks in static conditions.	MSCs-sheets adhered well to the silk scaffolds. During a culture period of 4 weeks, MSCs remained metabolically active and the COL2 to COL1 ratio increased over time.	209
N/A	Scaffold	Using alternating layers of silk scaffolds that were wrapped around a silicon disc to simulate and IVD-like assembly. MSC-sheets were put onto the scaffolds and the construct was mechanically stimulated.	Cells remained viable over a culture period of 4 weeks, however, the viability gradually decreased. Mechanical stimulation guided the MSCs to differentiate towards a phenotype that resembles the inner AF.	210
Bombyx mori	Scaffold	A multilayered, angle-ply scaffold was created to mimic the anatomical structure of the AF. Porcine AF cells or human MSCs were seeded onto the scaffold.	AF cells and MSCs proliferated during 14 days of culture and produced significant amounts of ECM. The scaffolds also displayed good mechanical properties.	211
N/A	Scaffold	Creating a biomimetic multilamellar angle-ply AF-like scaffold made of polycaprolactone and silk fibroin fibers with ±30° alternating orientation. Leporine AF cells were seeded onto the scaffold.	The AF-like scaffolds possessed mechanical properties similar to those of a natural AF. AF cells managed to adhere, proliferate, infiltrate into the scaffold and deposited ECM.	212
Antheraea mylitta	Scaffold	Creating a silk fibroin scaffold using crisscross-oriented fibers to mimic the structure of a native AF tissue. Fibers were made with or without crosslinked CS.	Nasal chondrocytes aligned along the silk fibers and produced GAG and COL2 after 4 weeks of culture, regardless of the presence of CS.	216
Antheraea mylitta	Scaffold	Study the cellular response of CS functionalized silk scaffolds using articular chondrocytes.	Chondrocytes showed enhanced chondrogenic redifferentiation potential and a higher metabolic activity in the presence of CS functionalized silk.	217
Antheraea mylitta	Scaffold	Mimicking the inner and outer AF using crisscross-orientated silk fibers and articular chondrocytes. The fibers of the inner AF were functionalized with CS. The scaffolds were loaded statically and dynamically.	A tissue gradient was formed that mimicked the characteristics of the inner and outer AF. Cells in the inner AF produced more GAG and expressed more *COL2* and *ACAN*, whereas more *COL1* was found in the outer AF.	218
Bombyx mori	Scaffold	3D printing of an anatomically-shaped AF using a composite bioink made of silk fibroin and elastin. Adipose derived stem cells were used to test the scaffold's cytocompatibility.	The bioprinted scaffolds morphologically resembled an anatomically-shaped AF and displayed mechanical characteristics similar to a native AF. Moreover, cells were metabolically active for 21 days.	221
Bombyx mori	Scaffold	Assess how well genetically engineered silk containing TGF-β3 or GDF-6 promotes IVD-like differentiation of MSCs and how well it maintains the phenotype of AF cells.	MSCs expressed about 10 times more *ACAN* than *COL2*, indicating a trend towards NP-like differentiation. AF cells were not negatively affected by the silk.	215

TABLE 4 (Continued)

Silk origin	Silk structure	Study	Conclusions	References
Bombyx mori	Scaffold	An AF injury was induced in bovine IVDs. To repair the IVD, the created cavity was filled with a genipin-enhanced fibrin hydrogel and sealed with a silk scaffold. Then, the IVDs were mechanically tested.	The repair was considered as a success because no herniation occurred regardless of the loading condition. However, the IVDs' height could not be recovered.	222

Abbreviations: ACAN, aggrecan; AF, annulus fibrosus; CS, chondroitin sulfate; COL1, collagen type I; COL2, collagen type II; ECM, extracellular matrix; GAG, glycosaminoglycan; GDF-6, growth and differentiation factor 6; IVD, intervertebral disc; MSC, mesenchymal stromal cell; NP, nucleus pulposus; RGD, arginine-glycine-aspartic acid; TGF-β3, transforming growth factor β3; N/A, non-available.

significantly more COL2 and proteoglycans were deposited on the scaffold after 3 weeks of culture, which then further improved the compressive elastic modulus of the scaffold itself.

A summary of published articles concerning the application of silk-based biomaterials for the repair and regeneration of the NP can be found in Table 3.

3.2 | Annulus fibrosus repair

Just as hydrogels have been used almost exclusively for the repair/regeneration of the NP, only firm scaffolds can be found for the application of the AF. Early investigations on how to implement silk specifically for the repair or regeneration of the AF were done by Park et al.[207,208] In two related studies, silk scaffolds with a lamellar morphology were compared to ones with a porous, spongy structure. The aim was to find out whether an AF-like lamellar structure would positively influence the tissue formation of porcine AF cells. Both studies concluded that the lamellar orientation of the scaffolds improved the construction of AF-like tissue during a culture period of 2 weeks. Furthermore, compared to the porous scaffolds, the amount of GAG gradually and significantly increased in the lamellar samples, and significantly more collagen was detected towards the end of each experiment. Surprisingly, however, the porous scaffold displayed a superior elastic modulus and tensile strength after 1 day of culture but then showed comparable values after 2 weeks.

Around the same time, See et al. worked on silk scaffolds for AF regeneration. In a first attempt, cell sheets consisting of bone-marrow-derived MSCs were transferred onto a porous silk scaffold and wrapped around an artificial NP made of silicone.[209] The IVD-like assembly was then cultured for 4 weeks under static conditions. During this culture period, the cell activity remained unchanged, and the cell sheets' initial COL1-rich ECM shifted towards a COL2-dominant environment, resembling the ECM found in the inner AF. The same construct was then used in a follow-up study, but this time they cultured it in a bioreactor that enabled dynamic compressional loading.[210] Results revealed that, on the one hand, the MSCs' metabolic activity decreased significantly after 4 weeks of culture compared to the static load. On the other hand, dynamic loading improved the gene expression profile of the MSCs that were seeded onto the scaffold, as essential IVD-related genes such as *SOX9*, *COL1*, *COL2*, *ACAN*, and *biglycan* were significantly higher expressed than in the static condition. Since then, multiple studies have attempted to mimic the AF's anatomical structure using a silk scaffold, and they all share the same conclusion: The more precisely the scaffold can replicate the human AF, the better the phenotype of the cultured cells and the closer the mechanical properties of the scaffold compared to native AF tissue.[211,212]

Although the morphology of the scaffold has a crucial impact on the synthesis of AF-like tissue, the composition and the properties of the biomaterial itself are also known to be just as ground-breaking for successful tissue formation. The surface of silk fibroin can be functionalized by covalent conjugation of biomolecules, thereby making its features tuneable and consequently allowing a more efficient and better-defined differentiation or retention of an AF-like phenotype.[213] For example, RGD functionalization of silk fibroin scaffolds has shown to enhance the expression of *ACAN* and *COL2* in AF cells compared to the nonmodified silk.[214] Another example would be the application of genetically engineered silk that was functionalized either with TGF-β3 or growth and differentiation factor 6 (GDF-6) and was able to preserve the phenotype of human AF cells (Figure 3).[215] Ideally, however, surface functionalization is combined with a suitable scaffold that tries to imitate the structure of the AF as closely as possible. The functionalization was nicely illustrated in a series of studies by Bhattacharjee et al.[216–218] Here, silk fibroin fibers were functionalized with chondroitin sulfate (CS) and were aligned in a crisscross orientated manner to resemble the AF. Although first attempts could not reveal any notable differences between the functionalized and the plain scaffold,[216] a refined setup using articular chondrocytes instead of nasal chondrocytes led to an upregulation of *SOX9*, *ACAN*, and *biglycan*, an improved production of GAG and collagens, an eminent increase of the metabolic activity and a significantly higher compressive strength with the CS-treated scaffolds compared to controls.[217] However, the full potential of their functionalized scaffold was achieved when the scaffolds were cultured in a hydrodynamic environment and a distinction was made between inner and outer AF.[218] Previous studies had shown that the presence of CS can cause MSCs to adopt a phenotype comparable to that of inner AF cells.[219,220] Consequently, only the inner part of the scaffold was functionalized with CS. With this study design, they demonstrated that the dynamic culture conditions enhanced the metabolic activity and the production of ECM. Furthermore, due to the CS in the inner AF, a chondrogenic tissue gradient

was formed, with the inner part expressing significantly more *COL2*, *ACAN*, *biglycan* and significantly less *COL1* and *elastin* than the outer part of the scaffold, thereby mimicking the properties of native AF tissue.

A relatively recent and very ambitious study on the use of anatomically-shaped silk scaffolds for the regeneration of the AF was conducted by Costa et al.[221] Therefore, a reverse engineering approach was followed in which a human patient was subjected to an MRI scan and then, based on the segmented morphologic scan of the L1-L2 IVD, a 3D model of the AF's ultrastructure was printed with a bioink made of SF and elastin. Remarkably, the mechanical characteristics, including the compressive modulus and stress–strain-curve, turned out to be very similar to those of fibrocartilage cartilage tissue found in the AF.[221] Moreover, human adipose-derived stem cells adhered well onto the scaffold and remained metabolically active for 21 days.

Finally, it is important to point out that silk scaffolds or scaffolds in general do not always have to be the basis for novel tissue formation and as a result aim to replace the defective or degenerative tissue itself. Still, they can be also used specifically to support the reparative process of a defective site. A corresponding example of this is provided by Frauchiger et al. using a bovine IVD damage model.[222] Here, an AF defect was induced with a biopsy punch and the created cavity was subsequently filled with a genipin-enhanced fibrin hydrogel, sealed with a silk fibroin scaffold and then the entire IVD was tested under different culture/loading regimes. Even though the IVDs' height could not be recovered, the silk scaffold reliably sealed the repaired site, as no herniation of the hydrogel could be detected during extensive dynamic loading.

A summary of published articles concerning the application of silk-based biomaterials for the regeneration and repair of the AF can be found in Table 4.

4 | CONCLUSION

Humans have bred silkworms for centuries, and their silk has found many uses thanks to its excellent biomechanical properties. There are many different species from which silk can be harvested, and each has its characteristics, advantages, and disadvantages. However, the clear dominator on the market is SF, derived from the silkworm *B. mori*.

In the clinic nowadays, silk is mainly used for surgical sutures. However, many preclinical studies show its great potential and versatility as a tissue repair and regenerative biomaterial. Especially in the past decade, the application of silk has also found its way into the field of IVD-related research, where mainly silk-based hydrogels have been used for the regeneration of the NP and only firm silk-based scaffolds have been investigated for the repair of the AF. Some of these scaffolds and hydrogels show auspicious outcomes. They thus indicate a transition into clinics in the foreseeable future, where they will hopefully present themselves as another missing puzzle piece to treat and ultimately cure patients suffering from IDD.

AUTHOR CONTRIBUTIONS

The study conception and design were proposed by Andreas S. Croft and Benjamin Gantenbein. The literature search and data analysis were done by Andreas S. Croft, Eugenia Spessot, Promita Bhattacharjee, and Yuejiao Yang. Benjamin Gantenbein, Promita Bhattacharjee and Eugenia Spessot designed the figure arts. The initial draft of the manuscript was written by Andreas S. Croft, Eugenia Spessot, Promita Bhattacharjee, and Yuejiao Yang and was then reviewed and edited by Antonella Motta, Michael Wöltje, and Benjamin Gantenbein. Funding was provided by Benjamin Gantenbein, Antonella Motta and Michael Wöltje.

ACKNOWLEDGMENTS

This study was supported by the Swiss National Science Foundation # 310030E_192674/1, by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation #437213841) and by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778078, the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101008041, and Fondazione Caritro (Italy) with the project "One-step 3D bioprinting and in vitro validation of a full thickness corneal tissue."

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Andreas S. Croft https://orcid.org/0000-0003-1472-1979
Eugenia Spessot https://orcid.org/0000-0002-8367-7847
Promita Bhattacharjee https://orcid.org/0000-0002-8105-8516
Yuejiao Yang https://orcid.org/0000-0001-9566-3314
Antonella Motta https://orcid.org/0000-0003-4893-6863
Michael Wöltje https://orcid.org/0000-0002-5405-0981
Benjamin Gantenbein https://orcid.org/0000-0002-9005-0655

REFERENCES

1. Ravindra VM, Senglaub SS, Rattani A, et al. Degenerative lumbar spine disease: estimating global incidence and worldwide volume. *Global Spine J*. 2018;*8*(8):784-794. doi:10.1177/2192568218770769
2. Cheung KM, Karppinen J, Chan D, et al. Prevalence and pattern of lumbar magnetic resonance imaging changes in a population study of one thousand forty-three individuals. *Spine (Phila Pa 1976)*. 2009;*34*(9):934-940. doi:10.1097/BRS.0b013e3181a01b3f
3. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine (Phila Pa 1976)*. 2002;*27*(23):2631-2644. doi:10.1097/00007632-200212010-00002
4. Kos N, Gradisnik L, Velnar T. A brief review of the degenerative intervertebral disc disease. *Med Arch*. 2019;*73*(6):421-424. doi:10.5455/medarh.2019.73.421-424
5. Miller JA, Schmatz C, Schultz AB. Lumbar disc degeneration: correlation with age, sex, and spine level in 600 autopsy specimens. *Spine (Phila Pa 1976)*. 1988;*13*(2):173-178.
6. Battie MC, Videman T, Gibbons LE, Fisher LD, Manninen H, Gill K. Volvo Award in clinical sciences. Determinants of lumbar disc degeneration. A study relating lifetime exposures and magnetic resonance

imaging findings in identical twins. *Spine (Phila Pa 1976)*. 1995; *20*(24):2601-2612.
7. Kalichman L, Hunter DJ. The genetics of intervertebral disc degeneration. Familial predisposition and heritability estimation. *Joint Bone Spine*. 2008;*75*(4):383-387. doi:10.1016/j.jbspin.2007.11.003
8. Carragee EJ, Don AS, Hurwitz EL, Cuellar JM, Carrino JA, Herzog R. ISSLS Prize Winner: does discography cause accelerated progression of degeneration changes in the lumbar disc: a ten-year matched cohort study. *Spine (Phila Pa 1976)*. 2009;*34*(21):2338-2345. doi:10.1097/BRS.0b013e3181ab5432
9. Grunhagen T, Wilde G, Soukane DM, Shirazi-Adl SA, Urban JP. Nutrient supply and intervertebral disc metabolism. *J Bone Joint Surg Am*. 2006;*88*(Suppl 2):30-35. doi:10.2106/JBJS.E.01290
10. Vergroesen PP, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. *Osteoarthr Cartil*. 2015;*23*(7):1057-1070. doi:10.1016/j.joca.2015.03.028
11. Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? *Spine (Phila Pa 1976)*. 2006;*31*(18):2151-2161. doi:10.1097/01.brs.0000231761.73859.2c
12. Iatridis JC, MacLean JJ, O'Brien M, Stokes IA. Measurements of proteoglycan and water content distribution in human lumbar intervertebral discs. *Spine (Phila Pa 1976)*. 2007;*32*(14):1493-1497. doi:10.1097/BRS.0b013e318067dd3f
13. Adams P, Eyre DR, Muir H. Biochemical aspects of development and ageing of human lumbar intervertebral discs. *Rheumatol Rehabil*. 1977;*16*(1):22-29. doi:10.1093/rheumatology/16.1.22
14. Galbusera F, van Rijsbergen M, Ito K, Huyghe JM, Brayda-Bruno M, Wilke HJ. Ageing and degenerative changes of the intervertebral disc and their impact on spinal flexibility. *Eur Spine J*. 2014;*23*(Suppl 3):S324-S332. doi:10.1007/s00586-014-3203-4
15. Dowdell J, Erwin M, Choma T, Vaccaro A, Iatridis J, Cho SK. Intervertebral disk degeneration and repair. *Neurosurgery*. 2017;*80*(3S): S46-S54. doi:10.1093/neuros/nyw078
16. Ambard D, Cherblanc F. Mechanical behavior of annulus fibrosus: a microstructural model of fibers reorientation. *Ann Biomed Eng*. 2009; *37*(11):2256-2265. doi:10.1007/s10439-009-9761-7
17. Martin MD, Boxell CM, Malone DG. Pathophysiology of lumbar disc degeneration: a review of the literature. *Neurosurg Focus*. 2002; *13*(2):E1-E6. doi:10.3171/foc.2002.13.2.2
18. Sampara P, Banala RR, Vemuri SK, Av GR, Gpv S. Understanding the molecular biology of intervertebral disc degeneration and potential gene therapy strategies for regeneration: a review. *Gene Ther*. 2018; *25*(2):67-82. doi:10.1038/s41434-018-0004-0
19. Bowles RD, Setton LA. Biomaterials for intervertebral disc regeneration and repair. *Biomaterials*. 2017;*129*:54-67. doi:10.1016/j.biomaterials.2017.03.013
20. Vernengo A, Li Z, Grad S. Editorial – disc biology special issue. *Eur Cell Mater*. 2022;*43*:1-3. doi:10.22203/eCM.v043a01
21. Yamada K, Iwasaki N, Sudo H. Biomaterials and cell-based regenerative therapies for intervertebral disc degeneration with a focus on biological and biomechanical functional repair: targeting treatments for disc herniation. *Cell*. 2022;*11*(4):602. doi:10.3390/cells11040602
22. Altman GH, Diaz F, Jakuba C, et al. Silk-based biomaterials. *Biomaterials*. 2003;*24*(3):401-416. doi:10.1016/s0142-9612(02)00353-8
23. Frauchiger DA, Tekari A, Woltje M, Fortunato G, Benneker LM, Gantenbein B. A review of the application of reinforced hydrogels and silk as biomaterials for intervertebral disc repair. *Eur Cell Mater*. 2017;*34*:271-290. doi:10.22203/eCM.v034a17
24. Collin MA, Mita K, Sehnal F, Hayashi CY. Molecular evolution of lepidopteran silk proteins: insights from the ghost moth, *Hepialus californicus*. *J Mol Evol*. 2010;*70*(5):519-529. doi:10.1007/s00239-010-9349-8
25. Mariano-Martins P, Monfardini RD, Lo-Man-Hung N, Torres TT. Evidence of positive selection on six spider developmental genes. *J Exp Zool B Mol Dev Evol*. 2022;*338*:314-322. doi:10.1002/jez.b.23119
26. Arakawa K, Mori M, Kono N, Suzuki T, Gotoh T, Shimano S. Proteomic evidence for the silk fibroin genes of spider mites (order Trombidiformes: family Tetranychidae). *J Proteomics*. 2021;*239*:104195. doi:10.1016/j.jprot.2021.104195
27. Fraser RDB, Parry DAD. The molecular structure of the silk fibers from *Hymenoptera aculeata* (bees, wasps, ants). *J Struct Biol*. 2015; *192*(3):528-538. doi:10.1016/j.jsb.2015.10.017
28. Melesse G, Atalie D, Koyrita A. Structural and thermal properties of Ethiopian Eri and Mulberry silk fibres. *Adv Mater Sci Eng*. 2020;*2020*: 9750393. doi:10.1155/2020/9750393
29. Mazzi S, Zulker E, Buchicchio J, Anderson B, Hu X. Comparative thermal analysis of Eri, Mori, Muga, and Tussar silk cocoons and fibroin fibers. *J Therm Anal Calorim*. 2014;*116*(3):1337-1343. doi:10.1007/s10973-013-3631-0
30. Rising A, Nimmervoll H, Grip S, et al. Spider silk proteins—mechanical property and gene sequence. *Zoolog Sci*. 2005;*22*(3): 273-281. doi:10.2108/zsj.22.273
31. Scheller J, Guhrs KH, Grosse F, Conrad U. Production of spider silk proteins in tobacco and potato. *Nat Biotechnol*. 2001;*19*(6):573-577. doi:10.1038/89335
32. Holland C, Numata K, Rnjak-Kovacina J, Seib FP. The biomedical use of silk: past, present, future. *Adv Healthc Mater*. 2019;*8*(1): e1800465. doi:10.1002/adhm.201800465
33. Neubauer VJ, Dobl A, Scheibel T. Silk-based materials for hard tissue engineering. *Materials (Basel)*. 2021;*14*(3):674. doi:10.3390/ma14030674
34. Jao D, Mou X, Hu X. Tissue regeneration: a silk road. *J Funct Biomater*. 2016;*7*(3):22. doi:10.3390/jfb7030022
35. Aksakal B, Akdere Ü, Günay SD, Çağın T, Taşseven Ç. Influence of repeating sequence on structural and thermal stability of crystalline domain of bombyx mori silk fibroin. *Mater Res Express*. 2020;*6*(12): 125356. doi:10.1088/2053-1591/ab6548
36. Bhattacharjee M, Schultz-Thater E, Trella E, et al. The role of 3D structure and protein conformation on the innate and adaptive immune responses to silk-based biomaterials. *Biomaterials*. 2013; *34*(33):8161-8171. doi:10.1016/j.biomaterials.2013.07.018
37. Janani G, Kumar M, Chouhan D, et al. Insight into silk-based biomaterials: from physicochemical attributes to recent biomedical applications. *ACS Appl Bio Mater*. 2019;*2*(12):5460-5491. doi:10.1021/acsabm.9b00576
38. Rossiter CH. *Silk and the Silk Worm. A Complete Book of Instruction on Silk Culture*. CH Rossiter; 1881.
39. Wei-cheng WU, Qi-kang GAO, Jin-e C, et al. Analysis of two-dimensional gel electrophoresis images of protein from posterior silk gland of silkworm (*Bombyx mori*) on day 1 and day 4 in the 5th instar stage. *Agric Sci China*. 2007;*6*(2):249-254. doi:10.1016/S1671-2927(07)60042-X
40. Kundu B, Kurland NE, Bano S, et al. Silk proteins for biomedical applications: bioengineering perspectives. *Prog Polym Sci*. 2014; *39*(2):251-267. doi:10.1016/j.progpolymsci.2013.09.002
41. Sasaki T, Noda H. Studies on silk fibroin of *Bombyx mori* directly extracted from the silk gland. II. Effect of reduction of disulfide bonds and subunit structure. *Biochim Biophys Acta*. 1973;*310*(1):91-103. doi:10.1016/0005-2795(73)90011-1
42. Inoue S, Tanaka K, Arisaka F, Kimura S, Ohtomo K, Mizuno S. Silk fibroin of *Bombyx mori* is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain, and P25, with a 6:6:1 molar ratio. *J Biol Chem*. 2000;*275*(51):40517-40528. doi:10.1074/jbc.M006897200
43. Zafar MS, Belton DJ, Hanby B, Kaplan DL, Perry CC. Functional material features of Bombyx mori silk light versus heavy chain proteins. *Biomacromolecules*. 2015;*16*(2):606-614. doi:10.1021/bm501667j
44. Asakura T, Yao J, Yamane T, Umemura K, Ulrich AS. Heterogeneous structure of silk fibers from *Bombyx mori* resolved by 13C solid-state

NMR spectroscopy. *J Am Chem Soc*. 2002;*124*(30):8794-8795. doi:10.1021/ja020244e
45. Callone E, Dire S, Hu X, Motta A. Processing influence on molecular assembling and structural conformations in silk fibroin: elucidation by solid-state NMR. *ACS Biomater Sci Eng*. 2016;*2*(5):758-767. doi:10.1021/acsbiomaterials.5b00507
46. Asakura T, Tanaka T, Tanaka R. Advanced silk fibroin biomaterials and application to small-diameter silk vascular grafts. *ACS Biomater Sci Eng*. 2019;*5*(11):5561-5577. doi:10.1021/acsbiomaterials.8b01482
47. Asakura T, Aoki A, Komatsu K, et al. Lamellar structure in alanine-glycine copolypeptides studied by solid-state NMR spectroscopy: a model for the crystalline domain of *Bombyx mori* silk fibroin in silk II form. *Biomacromolecules*. 2020;*21*(8):3102-3111. doi:10.1021/acs.biomac.0c00486
48. Cheng Y, Koh LD, Li D, Ji B, Han MY, Zhang YW. On the strength of beta-sheet crystallites of *Bombyx mori* silk fibroin. *J R Soc Interface*. 2014;*11*(96):20140305. doi:10.1098/rsif.2014.0305
49. Kunz RI, Brancalhao RM, Ribeiro LF, Natali MR. Silkworm Sericin: properties and biomedical applications. *Biomed Res Int*. 2016;*2016*:8175701. doi:10.1155/2016/8175701
50. Omar A, Gao Y, Wubulikasimu A, Arken A, Aisa HA, Yili A. Effects of trypsin-induced limited hydrolysis on the structural, functional, and bioactive properties of sericin. *RSC Adv*. 2021;*11*(41):25431-25440. doi:10.1039/D1RA03772B
51. Das G, Shin HS, Campos EVR, et al. Sericin based nanoformulations: a comprehensive review on molecular mechanisms of interaction with organisms to biological applications. *J Nanobiotechnol*. 2021;*19*(1):30. doi:10.1186/s12951-021-00774-y
52. Cherdchom S, Sereemaspun A, Aramwit P. Urea-extracted sericin is potentially better than kojic acid in the inhibition of melanogenesis through increased reactive oxygen species generation. *J Tradit Complement Med*. 2021;*11*(6):570-580. doi:10.1016/j.jtcme.2021.06.005
53. Silva VR, Ribani M, Gimenes ML, Scheer AP. High molecular weight Sericin obtained by high temperature and ultrafiltration process. *Proc Eng*. 2012;*42*:833-841. doi:10.1016/j.proeng.2012.07.476
54. Aramwit P, Siritientong T, Srichana T. Potential applications of silk sericin, a natural protein from textile industry by-products. *Waste Manag Res*. 2012;*30*(3):217-224. doi:10.1177/0734242X11404733
55. Chirila TV, Suzuki S, McKirdy NC. Further development of silk sericin as a biomaterial: comparative investigation of the procedures for its isolation from *Bombyx mori* silk cocoons. *Prog Biomater*. 2016;*5*:135-145. doi:10.1007/s40204-016-0052-8
56. Jaramillo-Quiceno N, Callone E, Dirè S, Álvarez-López C, Motta A. Boosting sericin extraction through alternative silk sources. *Polym J*. 2021;*53*(12):1425-1437. doi:10.1038/s41428-021-00539-2
57. Woltje M, Kolbel A, Aibibu D, Cherif C. A fast and reliable process to fabricate regenerated silk fibroin solution from degummed silk in 4 hours. *Int J Mol Sci*. 2021;*22*(19):10565. doi:10.3390/ijms221910565
58. Zurovec M, Yang C, Kodrik D, Sehnal F. Identification of a novel type of silk protein and regulation of its expression. *J Biol Chem*. 1998;*273*(25):15423-15428. doi:10.1074/jbc.273.25.15423
59. Nirmala X, Mita K, Vanisree V, Zurovec M, Sehnal F. Identification of four small molecular mass proteins in the silk of *Bombyx mori*. *Insect Mol Biol*. 2001;*10*(5):437-445. doi:10.1046/j.0962-1075.2001.00282.x
60. Kundu SC, Kundu B, Talukdar S, et al. Invited review nonmulberry silk biopolymers. *Biopolymers*. 2012;*97*(6):455-467. doi:10.1002/bip.22024
61. Fang G, Sapru S, Behera S, et al. Exploration of the tight structural–mechanical relationship in mulberry and non-mulberry silkworm silks. *J Mater Chem B*. 2016;*4*(24):4337-4347. doi:10.1039/c6tb01049k
62. Naskar D, Sapru S, Ghosh AK, Reis RL, Dey T, Kundu SC. Nonmulberry silk proteins: multipurpose ingredient in bio-functional assembly. *Biomed Mater*. 2021;*16*(6):062002. doi:10.1088/1748-605X/ac20a0
63. Guo C, Zhang J, Jordan JS, Wang X, Henning RW, Yarger JL. Structural comparison of various silkworm silks: an insight into the structure-property relationship. *Biomacromolecules*. 2018;*19*(3):906-917. doi:10.1021/acs.biomac.7b01687
64. Tsubota T, Yamamoto K, Mita K, Sezutsu H. Gene expression analysis in the larval silk gland of the eri silkworm *Samia ricini*. *Insect Sci*. 2016;*23*(6):791-804. doi:10.1111/1744-7917.12251
65. Guo Y, Li X, Zhang Q, Yan S, You R. Dissolution and regeneration of non-mulberry Eriogyna Pyretorum silk fibroin. *Mater Res Express*. 2017;*4*(10):105404. doi:10.1088/2053-1591/aa8e07
66. Silva SS, Gomes JM, Vale AC, Lu S, Reis RL, Kundu SC. Green pathway for processing non-mulberry *Antheraea pernyi* silk fibroin/chitin-based sponges: biophysical and biochemical characterization. original research. *Front Mater*. 2020;*7*:135. doi:10.3389/fmats.2020.00135
67. Balan KK, Sundaramoorthy S. Hydroentangled nonwoven eri silk fibroin scaffold for tissue engineering applications. *J Ind Text*. 2018;*48*(8):1291-1309. doi:10.1177/1528083718763779
68. Mandal BB, Kundu SC. A novel method for dissolution and stabilization of non-mulberry silk gland protein fibroin using anionic surfactant sodium dodecyl sulfate. *Biotechnol Bioeng*. 2008;*99*(6):1482-1489. doi:10.1002/bit.21699
69. Catherine ES, Alissa GA, Maydianne CBA. A review of the mechanisms and functional roles of male silk use in spider courtship and mating. *J Arachnol*. 2018;*46*(2):173-206. doi:10.1636/JoA-S-17-093.1
70. Morley EL, Robert D. Electric fields elicit ballooning in spiders. *Curr Biol*. 2018;*28*(14):2324-2330 e2. doi:10.1016/j.cub.2018.05.057
71. Greco G, Pugno NM. How spiders hunt heavy prey: the tangle web as a pulley and spider's lifting mechanics observed and quantified in the laboratory. *J R Soc Interface*. 2021;*18*(175):20200907. doi:10.1098/rsif.2020.0907
72. Romer L, Scheibel T. The elaborate structure of spider silk: structure and function of a natural high performance fiber. *Prion*. 2008;*2*(4):154-161. doi:10.4161/pri.2.4.7490
73. Greco G, Pantano MF, Mazzolai B, Pugno NM. Imaging and mechanical characterization of different junctions in spider orb webs. *Sci Rep*. 2019;*9*(1):5776. doi:10.1038/s41598-019-42070-8
74. Eberhard W. *Spider Webs: Behavior, Function, and Evolution*. University of Chicago Press; 2020.
75. Eisoldt L, Smith A, Scheibel T. Decoding the secrets of spider silk. *Mater Today*. 2011;*14*(3):80-86. doi:10.1016/S1369-7021(11)70057-8
76. Brown CP, Rosei F, Traversa E, Licoccia S. Spider silk as a load bearing biomaterial: tailoring mechanical properties via structural modifications. *Nanoscale*. 2011;*3*(3):870-876. doi:10.1039/c0nr00752h
77. Kiseleva AP, Krivoshapkin PV, Krivoshapkina EF. Recent advances in development of functional spider silk-based hybrid materials. *Front Chem*. 2020;*8*:554. doi:10.3389/fchem.2020.00554
78. Blackledge TA. Spider silk: a brief review and prospectus on research linking biomechanics and ecology in draglines and orb webs. *J Arachnol*. 2012;*40*(1):1-12.
79. Greco G, Francis J, Arndt T, et al. Properties of biomimetic artificial spider silk fibers tuned by PostSpin bath incubation. *Molecules*. 2020;*25*(14):3248. doi:10.3390/molecules25143248
80. Vepari C, Kaplan DL. Silk as a biomaterial. *Prog Polym Sci*. 2007;*32*(8–9):991-1007. doi:10.1016/j.progpolymsci.2007.05.013
81. Yarger JL, Cherry BR, van der Vaart A. Uncovering the structure–function relationship in spider silk. *Nat Rev Mater*. 2018;*3*(3):18008. doi:10.1038/natrevmats.2018.8

82. Lewis RV. Spider silk: ancient ideas for new biomaterials. *Chem Rev*. 2006;*106*(9):3762-3774. doi:10.1021/cr010194g
83. Greco G, Mastellari V, Holland C, Pugno NM. Comparing modern and classical perspectives on spider silks and webs. *Perspect Sci*. 2021;*29*(2):133-156. doi:10.1162/posc_a_00363
84. Scheibel T. Spider silks: recombinant synthesis, assembly, spinning, and engineering of synthetic proteins. *Microb Cell Fact*. 2004;*3*(1): 14. doi:10.1186/1475-2859-3-14
85. Malay AD, Arakawa K, Numata K. Analysis of repetitive amino acid motifs reveals the essential features of spider dragline silk proteins. *PLoS One*. 2017;*12*(8):e0183397. doi:10.1371/journal.pone.0183397
86. An B, Jenkins JE, Sampath S, et al. Reproducing natural spider silks' copolymer behavior in synthetic silk mimics. *Biomacromolecules*. 2012;*13*(12):3938-3948. doi:10.1021/bm301110s
87. Chan NJ, Gu D, Tan S, et al. Spider-silk inspired polymeric networks by harnessing the mechanical potential of beta-sheets through network guided assembly. *Nat Commun*. 2020;*11*(1):1630. doi:10.1038/s41467-020-15312-x
88. Foelix R. *Biology of Spiders*. 3rd ed. Oxford University Press; 2011.
89. Andersson M, Johansson J, Rising A. Silk spinning in silkworms and spiders. *Int J Mol Sci*. 2016;*17*(8):1290. doi:10.3390/ijms17081290
90. Johansson J, Rising A. Doing what spiders cannot-a road map to supreme artificial silk fibers. *ACS Nano*. 2021;*15*(2):1952-1959. doi:10.1021/acsnano.0c08933
91. Burdick JA, Mauck RL. Introduction. *Biomater Tissue Eng Appl: Rev Past Future Trends*. 2011;1-564.
92. Sehnal F, Zurovec M. Construction of silk fiber core in lepidoptera. *Biomacromolecules*. 2004;*5*(3):666-674. doi:10.1021/bm0344046
93. Chen J, Altman GH, Karageorgiou V, et al. Human bone marrow stromal cell and ligament fibroblast responses on RGD-modified silk fibers. *J Biomed Mater Res A*. 2003;*67*(2):559-570. doi:10.1002/jbm.a.10120
94. Kardestuncer T, McCarthy MB, Karageorgiou V, Kaplan D, Gronowicz G. RGD-tethered silk substrate stimulates the differentiation of human tendon cells. *Clin Orthop Relat Res*. 2006;*448*:234-239. doi:10.1097/01.blo.0000205879.50834.fe
95. Bhattacharjee P, Naskar D, Kim H-W, Maiti TK, Bhattacharya D, Kundu SC. Non-mulberry silk fibroin grafted PCL nanofibrous scaffold: promising ECM for bone tissue engineering. *Eur Polym J*. 2015;*71*:490-509. doi:10.1016/j.eurpolymj.2015.08.025
96. Singh BN, Pramanik K. Fabrication and evaluation of non-mulberry silk fibroin fiber reinforced chitosan based porous composite scaffold for cartilage tissue engineering. *Tissue Cell*. 2018;*55*:83-90. doi:10.1016/j.tice.2018.10.003
97. Chouhan D, Chakraborty B, Nandi SK, Mandal BB. Role of non-mulberry silk fibroin in deposition and regulation of extracellular matrix towards accelerated wound healing. *Acta Biomater*. 2017;*48*: 157-174. doi:10.1016/j.actbio.2016.10.019
98. Musson DS, Naot D, Chhana A, et al. In vitro evaluation of a novel non-mulberry silk scaffold for use in tendon regeneration. *Tissue Eng Part A*. 2015;*21*(9–10):1539-1551. doi:10.1089/ten.TEA.2014.0128
99. Hazra S, Nandi S, Naskar D, et al. Non-mulberry silk fibroin biomaterial for corneal regeneration. *Sci Rep*. 2016;*6*:21840. doi:10.1038/srep21840
100. Wang J, Yin Z, Xue X, Kundu SC, Mo X, Lu S. Natural non-mulberry silk nanoparticles for potential-controlled drug release. *Int J Mol Sci*. 2016;*17*(12):2012. doi:10.3390/ijms17122012
101. Bissoyi A, Kumar Singh A, Kumar Pattanayak S, et al. Understanding the molecular mechanism of improved proliferation and osteogenic potential of human mesenchymal stem cells grown on a polyelectrolyte complex derived from non-mulberry silk fibroin and chitosan. *Biomed Mater*. 2017;*13*(1):015011. doi:10.1088/1748-605X/aa890c
102. Talukdar S, Kundu SC. A non-mulberry silk fibroin protein based 3D in vitro tumor model for evaluation of anticancer drug activity. *Adv Funct Mater*. 2012;*22*(22):4778-4788. doi:10.1002/adfm.201200375
103. Humenik M, Smith AM, Scheibel T. Recombinant spider silks—biopolymers with potential for future applications. *Polymers*. 2011; *3*(1):640-661. doi:10.3390/polym3010640
104. Salehi S, Koeck K, Scheibel T. Spider silk for tissue engineering applications. *Molecules*. 2020;*25*(3):737. doi:10.3390/molecules25030737
105. Muller F, Zainuddin S, Scheibel T. Roll-to-roll production of spider silk nanofiber nonwoven meshes using centrifugal electrospinning for filtration applications. *Molecules*. 2020;*25*(23):5540. doi:10.3390/molecules25235540
106. Borkner CB, Wohlrab S, Moller E, Lang G, Scheibel T. Surface modification of polymeric biomaterials using recombinant spider silk proteins. *ACS Biomater Sci Eng*. 2017;*3*(5):767-775. doi:10.1021/acsbiomaterials.6b00306
107. Gellynck K, Verdonk PC, Van Nimmen E, et al. Silkworm and spider silk scaffolds for chondrocyte support. *J Mater Sci Mater Med*. 2008; *19*(11):3399-3409. doi:10.1007/s10856-008-3474-6
108. Withanage S, Savin A, Nikolaeva V, et al. Native spider silk-based antimicrobial hydrogels for biomedical applications. *Polymers (Basel)*. 2021;*13*(11):1796. doi:10.3390/polym13111796
109. DeSimone E, Schacht K, Pellert A, Scheibel T. Recombinant spider silk-based bioinks. *Biofabrication*. 2017;*9*(4):044104. doi:10.1088/1758-5090/aa90db
110. Schacht K, Jungst T, Schweinlin M, Ewald A, Groll J, Scheibel T. Biofabrication of cell-loaded 3D spider silk constructs. *Angew Chem Int Ed Engl*. 2015;*54*(9):2816-2820. doi:10.1002/anie.201409846
111. Rameau A, Hong RS, Djalilian H, et al. New medical device and therapeutic approvals in otolaryngology: state of the art review of 2019. *OTO Open*. 2020;*4*(2):2473974X20932506. doi:10.1177/2473974X20932506
112. Motta A, Migliaresi C, Lloyd AW, Denyer SP, Santin M. Serum protein absorption on silk fibroin fibers and films: surface opsonization and binding strength. *J Bioactive Compat Polym*. 2002;*17*(1):23-35. doi:10.1177/0883911502017001195
113. Motta A, Maniglio D, Migliaresi C, et al. Silk fibroin processing and thrombogenic responses. *J Biomater Sci Polym Ed*. 2009;*20*(13): 1875-1897. doi:10.1163/156856208X399936
114. Guo X, Lin N, Lu S, Zhang F, Zuo B. Preparation and biocompatibility characterization of silk fibroin 3D scaffolds. *ACS Appl Bio Mater*. 2021;*4*(2):1369-1380. doi:10.1021/acsabm.0c01239
115. Zhang YQ, Zhou WL, Shen WD, et al. Synthesis, characterization and immunogenicity of silk fibroin-L-asparaginase bioconjugates. *J Biotechnol*. 2005;*120*(3):315-326. doi:10.1016/j.jbiotec.2005.06.027
116. Cao Y, Wang B. Biodegradation of silk biomaterials. *Int J Mol Sci*. 2009;*10*(4):1514-1524. doi:10.3390/ijms10041514
117. Zhao Y, Zhu ZS, Guan J, Wu SJ. Processing, mechanical properties and bio-applications of silk fibroin-based high-strength hydrogels. *Acta Biomater*. 2021;*125*:57-71. doi:10.1016/j.actbio.2021.02.018
118. Johari N, Moroni L, Samadikuchaksaraei A. Tuning the conformation and mechanical properties of silk fibroin hydrogels. *Eur Polym J*. 2020;*134*:109842. doi:10.1016/j.eurpolymj.2020.109842
119. DeBari MK, King CI 3rd, Altgold TA, Abbott RD. Silk fibroin as a green material. *ACS Biomater Sci Eng*. 2021;*7*(8):3530-3544. doi:10.1021/acsbiomaterials.1c00493
120. Jiang T, Zhou P. *Environment-Induced Silk Fibroin Conformation Based on the Magnetic Resonance Spectroscopy*. IntechOpen; 2011.
121. Han H, Ning H, Liu S, et al. Silk biomaterials with vascularization capacity. *Adv Funct Mater*. 2016;*26*(3):421-436. doi:10.1002/adfm.201504160
122. Brown J, Lu CL, Coburn J, Kaplan DL. Impact of silk biomaterial structure on proteolysis. *Acta Biomater*. 2015;*11*:212-221. doi:10.1016/j.actbio.2014.09.013

123. Guo C, Li C, Kaplan DL. Enzymatic degradation of *Bombyx mori* silk materials: a review. *Biomacromolecules*. 2020;*21*(5):1678-1686. doi:10.1021/acs.biomac.0c00090
124. Hu Y, Zhang Q, You R, Wang L, Li M. The relationship between secondary structure and biodegradation behavior of silk fibroin scaffolds. *Adv Mater Sci Eng*. 2012;*2012*:185905. doi:10.1155/2012/185905
125. Abbott RD, Kimmerling EP, Cairns DM, Kaplan DL. Silk as a biomaterial to support long-term three-dimensional tissue cultures. *ACS Appl Mater Interfaces*. 2016;*8*(34):21861-21868. doi:10.1021/acsami.5b12114
126. Cebe P, Partlow BP, Kaplan DL, Wurm A, Zhuravlev E, Schick C. Silk I and silk II studied by fast scanning calorimetry. *Acta Biomater*. 2017;*55*:323-332. doi:10.1016/j.actbio.2017.04.001
127. Zhao M, Qi Z, Tao X, Newkirk C, Hu X, Lu S. Chemical, thermal, time, and enzymatic stability of silk materials with silk I structure. *Int J Mol Sci*. 2021;*22*(8):4136. doi:10.3390/ijms22084136
128. Puerta M, Peresin MS, Restrepo-Osorio A. Effects of chemical post-treatments on structural and physicochemical properties of silk fibroin films obtained from silk fibrous waste. *Front Bioeng Biotechnol*. 2020;*8*:523949. doi:10.3389/fbioe.2020.523949
129. Rnjak-Kovacina J, Tang F, Whitelock JM, Lord MS. Silk biomaterials functionalized with recombinant domain V of human perlecan modulate endothelial cell and platelet interactions for vascular applications. *Colloids Surf B Biointerfaces*. 2016;*148*:130-138. doi:10.1016/j.colsurfb.2016.08.039
130. Zheng H, Zuo B. Functional silk fibroin hydrogels: preparation, properties and applications. *J Mater Chem B*. 2021;*9*(5):1238-1258. doi:10.1039/d0tb02099k
131. Singh YP, Bhardwaj N, Mandal BB. Potential of agarose/silk fibroin blended hydrogel for in vitro cartilage tissue engineering. *ACS Appl Mater Interfaces*. 2016;*8*(33):21236-21249. doi:10.1021/acsami.6b08285
132. Yu LM, Liu T, Ma YL, Zhang F, Huang YC, Fan ZH. Fabrication of silk-hyaluronan composite as a potential scaffold for tissue repair. *Front Bioeng Biotechnol*. 2020;*8*:578988. doi:10.3389/fbioe.2020.578988
133. Maniglio D, Bonani W, Migliaresi C, Motta A. Silk fibroin porous scaffolds by N_2O foaming. *J Biomater Sci Polym Ed*. 2018;*29*(5):491-506. doi:10.1080/09205063.2018.1423811
134. Ferreira BMP, Andersson N, Atterling E, Engqvist J, Hall S, Dicko C. 3D structure and mechanics of silk sponge scaffolds is governed by larger pore sizes. Original research. *Front Mater*. 2020;*7*:211.
135. Hardy JG, Geissler SA, Aguilar D Jr, et al. Instructive conductive 3D silk foam-based bone tissue scaffolds enable electrical stimulation of stem cells for enhanced osteogenic differentiation. *Macromol Biosci*. 2015;*15*(11):1490-1496. doi:10.1002/mabi.201500171
136. Wang Q, Han G, Yan S, Zhang Q. 3D printing of silk fibroin for biomedical applications. *Materials (Basel)*. 2019;*12*(3):504. doi:10.3390/ma12030504
137. Mwangi TK, Bowles RD, Tainter DM, Bell RD, Kaplan DL, Setton LA. Synthesis and characterization of silk fibroin microparticles for intra-articular drug delivery. *Int J Pharm*. 2015;*485*(1–2):7-14. doi:10.1016/j.ijpharm.2015.02.059
138. Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials*. 2004;*25*(7–8):1289-1297. doi:10.1016/j.biomaterials.2003.08.045
139. Agostinacchio F, Maniglio D, Callone E, Migliaresi C, Dire S, Motta A. A novel and selective silk fibroin fragmentation method. *Soft Matter*. 2021;*17*(28):6863-6872. doi:10.1039/d1sm00566a
140. Floren M, Migliaresi C, Motta A. Processing techniques and applications of silk hydrogels in bioengineering. *J Funct Biomater*. 2016;*7*(3):26. doi:10.3390/jfb7030026
141. Su D, Yao M, Liu J, Zhong Y, Chen X, Shao Z. Enhancing mechanical properties of silk fibroin hydrogel through restricting the growth of beta-sheet domains. *ACS Appl Mater Interfaces*. 2017;*9*(20):17489-17498. doi:10.1021/acsami.7b04623
142. Yucel T, Cebe P, Kaplan DL. Vortex-induced injectable silk fibroin hydrogels. *Biophys J*. 2009;*97*(7):2044-2050. doi:10.1016/j.bpj.2009.07.028
143. Wang L, Song D, Zhang X, et al. Silk-graphene hybrid hydrogels with multiple cues to induce nerve cell behavior. *ACS Biomater Sci Eng*. 2019;*5*(2):613-622. doi:10.1021/acsbiomaterials.8b01481
144. Hu X, Lu Q, Sun L, et al. Biomaterials from ultrasonication-induced silk fibroin-hyaluronic acid hydrogels. *Biomacromolecules*. 2010;*11*(11):3178-3188. doi:10.1021/bm1010504
145. Kim UJ, Park J, Li C, Jin HJ, Valluzzi R, Kaplan DL. Structure and properties of silk hydrogels. *Biomacromolecules*. 2004;*5*(3):786-792. doi:10.1021/bm0345460
146. Nagarkar S, Patil A, Lele A, Bhat S, Bellare J, Mashelkar RA. Some mechanistic insights into the gelation of regenerated silk fibroin sol. *Ind Eng Chem Res*. 2009;*48*(17):8014-8023. doi:10.1021/ie801723f
147. Yao D, Dong S, Lu Q, et al. Salt-leached silk scaffolds with tunable mechanical properties. *Biomacromolecules*. 2012;*13*(11):3723-3729. doi:10.1021/bm301197h
148. Rnjak-Kovacina J, Wray LS, Burke KA, et al. Lyophilized silk sponges: a versatile biomaterial platform for soft tissue engineering. *ACS Biomater Sci Eng*. 2015;*1*(4):260-270. doi:10.1021/ab500149p
149. Cengiz IF, Maia FR, da Silva MA, et al. Entrapped in cage (EiC) scaffolds of 3D-printed polycaprolactone and porous silk fibroin for meniscus tissue engineering. *Biofabrication*. 2020;*12*(2):025028. doi:10.1088/1758-5090/ab779f
150. Cai J, Zhang L, Chen J, Chen S. Silk fibroin coating through EDC/-NHS crosslink is an effective method to promote graft remodeling of a polyethylene terephthalate artificial ligament. *J Biomater Appl*. 2019;*33*(10):1407-1414. doi:10.1177/0885328219836625
151. Santi S, Mancini I, Dire S, et al. A bio-inspired multifunctionalized silk fibroin. *ACS Biomater Sci Eng*. 2021;*7*(2):507-516. doi:10.1021/acsbiomaterials.0c01567
152. Murphy AR, St John P, Kaplan DL. Modification of silk fibroin using diazonium coupling chemistry and the effects on hMSC proliferation and differentiation. *Biomaterials*. 2008;*29*(19):2829-2838. doi:10.1016/j.biomaterials.2008.03.039
153. Acharya C, Hinz B, Kundu SC. The effect of lactose-conjugated silk biomaterials on the development of fibrogenic fibroblasts. *Biomaterials*. 2008;*29*(35):4665-4675. doi:10.1016/j.biomaterials.2008.08.033
154. Galeotti F, Andicsová A, Bertini F, Botta C. A versatile click-grafting approach to surface modification of silk fibroin films. *J Mater Sci*. 2013;*48*:7004-7010. doi:10.1007/s10853-013-7509-0
155. Gotoh Y, Tsukada M, Aiba S, Minoura N. Chemical modification of silk fibroin with N-acetyl-chito-oligosaccharides. *Int J Biol Macromol*. 1996;*18*(1–2):19-26. doi:10.1016/0141-8130(95)01039-4
156. McGill M, Grant JM, Kaplan DL. Enzyme-mediated conjugation of peptides to silk fibroin for facile hydrogel functionalization. *Ann Biomed Eng*. 2020;*48*(7):1905-1915. doi:10.1007/s10439-020-02503-2
157. Sofia S, McCarthy MB, Gronowicz G, Kaplan DL. Functionalized silk-based biomaterials for bone formation. *J Biomed Mater Res*. 2001;*54*(1):139-148. doi:10.1002/1097-4636(200101)54:13.0.co;2-7
158. Saleem M, Rasheed S, Yougen C. Silk fibroin/hydroxyapatite scaffold: a highly compatible material for bone regeneration. *Sci Technol Adv Mater*. 2020;*21*(1):242-266. doi:10.1080/14686996.2020.1748520
159. Kim DK, Kim JI, Hwang TI, Sim BR, Khang G. Bioengineered osteoinductive *Broussonetia kazinoki*/silk fibroin composite scaffolds for bone tissue regeneration. *ACS Appl Mater Interfaces*. 2017;*9*(2):1384-1394. doi:10.1021/acsami.6b14351

160. Li C, Vepari C, Jin HJ, Kim HJ, Kaplan DL. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials*. 2006;*27*(16):3115-3124. doi:10.1016/j.biomaterials.2006.01.022
161. Zhang Y, Wu C, Luo T, Li S, Cheng X, Miron RJ. Synthesis and inflammatory response of a novel silk fibroin scaffold containing BMP7 adenovirus for bone regeneration. *Bone*. 2012;*51*(4):704-713. doi:10.1016/j.bone.2012.06.029
162. Wang Q, Zhang Y, Li B, Chen L. 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*. 2017;*5*(33):6963-6972. doi:10.1039/c7tb00949f
163. Ode Boni BO, Bakadia BM, Osi AR, et al. Immune response to silk Sericin-fibroin composites: potential immunogenic elements and alternatives for immunomodulation. *Macromol Biosci*. 2022;*22*(1):e2100292. doi:10.1002/mabi.202100292
164. Fan J-B, Wu L-P, Chen L-S, Mao X-Y, Ren F-Z. Antioxidant activities of silk sericin from silkworm *Bombyx mori*. *J Food Biochem*. 2009;*33*(1):74-88. doi:10.1111/j.1745-4514.2008.00204.x
165. Padamwar MN, Pawar AP, Daithankar AV, Mahadik KR. Silk sericin as a moisturizer: an in vivo study. *J Cosmet Dermatol*. 2005;*4*(4):250-257. doi:10.1111/j.1473-2165.2005.00200.x
166. Kumar JP, Alam S, Jain AK, Ansari KM, Mandal BB. Protective activity of silk Sericin against UV radiation-induced skin damage by downregulating oxidative stress. *ACS Appl Bio Mater*. 2018;*1*(6):2120-2132. doi:10.1021/acsabm.8b00558
167. Arango MC, Montoya Y, Peresin MS, Bustamante J, Álvarez-López C. Silk sericin as a biomaterial for tissue engineering: a review. *Int J Polym Mater Polym Biomater*. 2021;*70*(16):1115-1129. doi:10.1080/00914037.2020.1785454
168. Baptista-Silva S, Borges S, Costa-Pinto AR, et al. In situ forming silk Sericin-based hydrogel: a novel wound healing biomaterial. *ACS Biomater Sci Eng*. 2021;*7*(4):1573-1586. doi:10.1021/acsbiomaterials.0c01745
169. Arango MC, Álvarez-López C. Effect of freezing temperature on the properties of lyophilized silk sericin scaffold. *Mater Res Express*. 2019;*6*(9):095414. doi:10.1088/2053-1591/ab3594
170. Yun H, Kim MK, Kwak HW, Lee JY, Kim MH, Lee KH. The role of glycerol and water in flexible silk sericin film. *Int J Biol Macromol*. 2016;*82*:945-951. doi:10.1016/j.ijbiomac.2015.11.016
171. Chen CS, Zeng F, Xiao X, et al. Three-dimensionally printed silk-Sericin-based hydrogel scaffold: a promising visualized dressing material for real-time monitoring of wounds. *ACS Appl Mater Interfaces*. 2018;*10*(40):33879-33890. doi:10.1021/acsami.8b10072
172. Aramwit P, Kanokpanont S, De-Eknamkul W, Kamei K, Srichana T. The effect of sericin with variable amino-acid content from different silk strains on the production of collagen and nitric oxide. *J Biomater Sci Polym Ed*. 2009;*20*(9):1295-1306. doi:10.1163/156856209X453006
173. Nayak S, Dey S, Kundu SC. Skin equivalent tissue-engineered construct: co-cultured fibroblasts/ keratinocytes on 3D matrices of sericin hope cocoons. *PLoS One*. 2013;*8*(9):e74779. doi:10.1371/journal.pone.0074779
174. Lamboni L, Gauthier M, Yang G, Wang Q. Silk sericin: a versatile material for tissue engineering and drug delivery. *Biotechnol Adv*. 2015;*33*(8):1855-1867. doi:10.1016/j.biotechadv.2015.10.014
175. Nayak S, Dey T, Naskar D, Kundu SC. The promotion of osseointegration of titanium surfaces by coating with silk protein sericin. *Biomaterials*. 2013;*34*(12):2855-2864. doi:10.1016/j.biomaterials.2013.01.019
176. Kumar JP, Bhardwaj N, Mandal BB. Cross-linked silk sericin–gelatin 2D and 3D matrices for prospective tissue engineering applications. *RSC Adv*. 2016;*6*(107):105125-105136. doi:10.1039/C6RA18654H
177. He M, Hu H, Wang P, et al. Preparation of a bio-composite of sericin-g-PMMA via HRP-mediated graft copolymerization. *Int J Biol Macromol*. 2018;*117*:323-330. doi:10.1016/j.ijbiomac.2018.05.190
178. Hang Y, Zhang Y, Jin Y, Shao H, Hu X. Preparation and characterization of electrospun silk fibroin/sericin blend fibers. *J Mater Res*. 2011;*26*(23):2931-2937. doi:10.1557/jmr.2011.356
179. Timin AS, Muslimov AR, Zyuzin MV, et al. Multifunctional scaffolds with improved antimicrobial properties and osteogenicity based on piezoelectric electrospun fibers decorated with bioactive composite microcapsules. *ACS Appl Mater Interfaces*. 2018;*10*(41):34849-34868. doi:10.1021/acsami.8b09810
180. Bhattacharjee P, Naskar D, Maiti TK, Bhattacharya D, Kundu SC. Investigating the potential of combined growth factors delivery, from non-mulberry silk fibroin grafted poly(ε-caprolactone)/hydroxyapatite nanofibrous scaffold, in bone tissue engineering. *Appl Mater Today*. 2016;*5*:52-67. doi:10.1016/j.apmt.2016.09.007
181. Naskar D, Ghosh AK, Mandal M, Das P, Nandi SK, Kundu SC. Dual growth factor loaded nonmulberry silk fibroin/carbon nanofiber composite 3D scaffolds for in vitro and in vivo bone regeneration. *Biomaterials*. 2017;*136*:67-85. doi:10.1016/j.biomaterials.2017.05.014
182. Wang X, Wenk E, Zhang X, Meinel L, Vunjak-Novakovic G, Kaplan DL. Growth factor gradients via microsphere delivery in biopolymer scaffolds for osteochondral tissue engineering. *J Control Release*. 2009;*134*(2):81-90. doi:10.1016/j.jconrel.2008.10.021
183. Kharaghani D, Kurniwan EB, Khan MQ, Yoshiko Y. MiRNA-nanofiber, the next generation of bioactive scaffolds for bone regeneration: a review. *Micromachines (Basel)*. 2021;*12*(12):1472. doi:10.3390/mi12121472
184. James EN, Van Doren E, Li C, Kaplan DL. Silk biomaterials-mediated miRNA functionalized orthopedic devices. *Tissue Eng Part A*. 2019;*25*(1–2):12-23. doi:10.1089/ten.TEA.2017.0455
185. Li J, Wu S, Kim E, et al. Electrobiofabrication: electrically based fabrication with biologically derived materials. *Biofabrication*. 2019;*11*(3):032002. doi:10.1088/1758-5090/ab06ea
186. Toprakcioglu Z, Levin A, Knowles TPJ. Hierarchical biomolecular emulsions using 3-D microfluidics with uniform surface chemistry. *Biomacromolecules*. 2017;*18*(11):3642-3651. doi:10.1021/acs.biomac.7b01159
187. Konwarh R, Gupta P, Mandal BB. Silk-microfluidics for advanced biotechnological applications: a progressive review. *Biotechnol Adv*. 2016;*34*(5):845-858. doi:10.1016/j.biotechadv.2016.05.001
188. Cheng J, Park D, Jun Y, Lee J, Hyun J, Lee SH. Biomimetic spinning of silk fibers and in situ cell encapsulation. *Lab Chip*. 2016;*16*(14):2654-2661. doi:10.1039/c6lc00488a
189. Jeon HY, Jung SH, Jung YM, Kim YM, Ghandehari H, Ha KS. Array-based high-throughput analysis of silk-elastinlike protein polymer degradation and C-peptide release by proteases. *Anal Chem*. 2016;*88*(10):5398-5405. doi:10.1021/acs.analchem.6b00739
190. Peng Q, Zhang Y, Lu L, et al. Recombinant spider silk from aqueous solutions via a bio-inspired microfluidic chip. *Sci Rep*. 2016;*6*:36473. doi:10.1038/srep36473
191. Choudhary T, Rajamanickam GP, Dendukuri D. Woven electrochemical fabric-based test sensors (WEFTS): a new class of multiplexed electrochemical sensors. *Lab Chip*. 2015;*15*(9):2064-2072. doi:10.1039/c5lc00041f
192. Brody H. Biomaterials. *Nature*. 2015;*519*(7544):S1. doi:10.1038/519S1a
193. Bhandari P, Narahari T, Dendukuri D. 'Fab-chips': a versatile, fabric-based platform for low-cost, rapid and multiplexed diagnostics. *Lab Chip*. 2011;*11*(15):2493-2499. doi:10.1039/c1lc20373h
194. Domachuk P, Tsioris K, Omenetto FG, Kaplan DL. Bio-microfluidics: biomaterials and biomimetic designs. *Adv Mater*. 2010;*22*(2):249-260. doi:10.1002/adma.200900821
195. Zhu J, Marchant RE. Design properties of hydrogel tissue-engineering scaffolds. *Expert Rev Med Devices*. 2011;*8*(5):607-626. doi:10.1586/erd.11.27

196. Bhunia BK, Mandal BB. Exploring gelation and physicochemical behavior of in situ bioresponsive silk hydrogels for disc degeneration therapy. *ACS Biomater Sci Eng*. 2019;*5*(2):870-886. doi:10.1021/acsbiomaterials.8b01099
197. Cassidy JJ, Hiltner A, Baer E. Hierarchical structure of the intervertebral disc. *Connect Tissue Res*. 1989;*23*(1):75-88. doi:10.3109/03008208909103905
198. Park SH, Cho H, Gil ES, Mandal BB, Min BH, Kaplan DL. Silk-fibrin/hyaluronic acid composite gels for nucleus pulposus tissue regeneration. *Tissue Eng Part A*. 2011;*17*(23–24):2999-3009. doi:10.1089/ten.TEA.2010.0747
199. Chung TW, Chen WP, Tai PW, Lo HY, Wu TY. Roles of silk fibroin on characteristics of hyaluronic acid/silk fibroin hydrogels for tissue engineering of nucleus pulposus. *Materials (Basel)*. 2020;*13*(12):2750. doi:10.3390/ma13122750
200. Hu J, Chen B, Guo F, et al. Injectable silk fibroin/polyurethane composite hydrogel for nucleus pulposus replacement. *J Mater Sci Mater Med*. 2012;*23*(3):711-722. doi:10.1007/s10856-011-4533-y
201. Hu J, Lu Y, Cai L, et al. Functional compressive mechanics and tissue biocompatibility of an injectable SF/PU hydrogel for nucleus pulposus replacement. *Sci Rep*. 2017;*7*(1):2347. doi:10.1038/s41598-017-02497-3
202. Murab S, Samal J, Shrivastava A, Ray AR, Pandit A, Ghosh S. Glucosamine loaded injectable silk-in-silk integrated system modulate mechanical properties in bovine ex-vivo degenerated intervertebral disc model. *Biomaterials*. 2015;*55*:64-83. doi:10.1016/j.biomaterials.2015.03.032
203. Lau KS, Partridge EA, Grigorian A, et al. Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. *Cell*. 2007;*129*(1):123-134. doi:10.1016/j.cell.2007.01.049
204. Prydz K, Dalen KT. Synthesis and sorting of proteoglycans. *J Cell Sci*. 2000;*113*(Pt 2):193-205.
205. Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials*. 2003;*24*(24):4337-4351. doi:10.1016/s0142-9612(03)00340-5
206. Zeng C, Yang Q, Zhu M, et al. Silk fibroin porous scaffolds for nucleus pulposus tissue engineering. *Mater Sci Eng C Mater Biol Appl*. 2014;*37*:232-240. doi:10.1016/j.msec.2014.01.012
207. Park SH, Gil ES, Mandal BB, et al. Annulus fibrosus tissue engineering using lamellar silk scaffolds. *J Tissue Eng Regen Med*. 2012;*6*-(Suppl 3):s24-s33. doi:10.1002/term.541
208. Park SH, Gil ES, Cho H, et al. Intervertebral disk tissue engineering using biphasic silk composite scaffolds. *Tissue Eng Part A*. 2012;*18*(5–6):447-458. doi:10.1089/ten.TEA.2011.0195
209. See EY, Toh SL, Goh JC. Simulated intervertebral disc-like assembly using bone marrow-derived mesenchymal stem cell sheets and silk scaffolds for annulus fibrosus regeneration. *J Tissue Eng Regen Med*. 2012;*6*(7):528-535. doi:10.1002/term.457
210. See EY, Toh SL, Goh JC. Effects of radial compression on a novel simulated intervertebral disc-like assembly using bone marrow-derived mesenchymal stem cell cell-sheets for annulus fibrosus regeneration. *Spine (Phila Pa 1976)*. 2011;*36*(21):1744-1751. doi:10.1097/brs.0b013e31821986b3
211. Bhunia BK, Kaplan DL, Mandal BB. Silk-based multilayered angle-ply annulus fibrosus construct to recapitulate form and function of the intervertebral disc. *Proc Natl Acad Sci U S A*. 2018;*115*(3):477-482. doi:10.1073/pnas.1715912115
212. Zhang T, Du L, Zhao J, et al. Biomimetic angle-ply multi-lamellar scaffold for annulus fibrosus tissue engineering. *J Mater Sci Mater Med*. 2020;*31*(8):67. doi:10.1007/s10856-020-06404-7
213. Saric M, Scheibel T. Engineering of silk proteins for materials applications. *Curr Opin Biotechnol*. 2019;*60*:213-220. doi:10.1016/j.copbio.2019.05.005
214. Chang G, Kim HJ, Kaplan D, Vunjak-Novakovic G, Kandel RA. Porous silk scaffolds can be used for tissue engineering annulus fibrosus. *Eur Spine J*. 2007;*16*(11):1848-1857. doi:10.1007/s00586-007-0364-4
215. Frauchiger DA, Heeb SR, May RD, Woltje M, Benneker LM, Gantenbein B. Differentiation of MSC and annulus fibrosus cells on genetically engineered silk fleece-membrane-composites enriched for GDF-6 or TGF-beta3. *J Orthop Res*. 2018;*36*(5):1324-1333. doi:10.1002/jor.23778
216. Bhattacharjee M, Miot S, Gorecka A, et al. Oriented lamellar silk fibrous scaffolds to drive cartilage matrix orientation: towards annulus fibrosus tissue engineering. *Acta Biomater*. 2012;*8*(9):3313-3325. doi:10.1016/j.actbio.2012.05.023
217. Bhattacharjee M, Chawla S, Chameettachal S, Murab S, Bhavesh NS, Ghosh S. Role of chondroitin sulphate tethered silk scaffold in cartilaginous disc tissue regeneration. *Biomed Mater*. 2016;*11*(2):025014. doi:10.1088/1748-6041/11/2/025014
218. Bhattacharjee M, Chameettachal S, Pahwa S, Ray AR, Ghosh S. Strategies for replicating anatomical cartilaginous tissue gradient in engineered intervertebral disc. *ACS Appl Mater Interfaces*. 2014;*6*(1):183-193. doi:10.1021/am403835t
219. Varghese S, Hwang NS, Canver AC, Theprungsirikul P, Lin DW, Elisseeff J. Chondroitin sulfate based niches for chondrogenic differentiation of mesenchymal stem cells. *Matrix Biol*. 2008;*27*(1):12-21. doi:10.1016/j.matbio.2007.07.002
220. Chou AI, Bansal A, Miller GJ, Nicoll SB. The effect of serial monolayer passaging on the collagen expression profile of outer and inner anulus fibrosus cells. *Spine (Phila Pa 1976)*. 2006;*31*(17):1875-1881. doi:10.1097/01.brs.0000229222.98051.9a
221. Costa JB, Silva-Correia J, Ribeiro VP, da Silva MA, Oliveira JM, Reis RL. Engineering patient-specific bioprinted constructs for treatment of degenerated intervertebral disc. *Mater Today Commun*. 2019;*19*:506-512. doi:10.1016/j.mtcomm.2018.01.011
222. Frauchiger DA, May RD, Bakirci E, et al. Genipin-enhanced fibrin hydrogel and novel silk for intervertebral disc repair in a loaded bovine organ culture model. *J Funct Biomater*. 2018;*9*(3):40. doi:10.3390/jfb9030040
223. Rajkhowa R, Gupta VB, Kothari VK. Tensile stress–strain and recovery behavior of Indian silk fibers and their structural dependence. *J Appl Polym Sci*. 2000;*77*(11):2418-2429. doi:10.1002/1097-4628(20000912)77:113.0.CO;2-Q
224. Dash R, Ghosh SK, Kaplan DL, Kundu SC. Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. *Comp Biochem Physiol B Biochem Mol Biol*. 2007;*147*(1):129-134. doi:10.1016/j.cbpb.2007.01.009
225. Ahmad R, Kamra A, Hasnain SE. Fibroin silk proteins from the non-mulberry silkworm *Philosamia ricini* are biochemically and immunochemically distinct from those of the mulberry silkworm *Bombyx mori*. *DNA Cell Biol*. 2004;*23*(3):149-154. doi:10.1089/104454904322964742
226. Kasoju N, Bhonde RR, Bora U. Preparation and characterization of Antheraea assama silk fibroin based novel non-woven scaffold for tissue engineering applications. *J Tissue Eng Regen Med*. 2009;*3*(7):539-552. doi:10.1002/term.196
227. Andiappan M, Kumari T, Sundaramoorthy S, Meiyazhagan G, Manoharan P, Venkataraman G. Comparison of eri and tasar silk fibroin scaffolds for biomedical applications. *Prog Biomater*. 2016;*5*:81-91. doi:10.1007/s40204-016-0047-5
228. Yazawa K, Tatebayashi Y, Kajiura Z. Eri silkworm spins mechanically robust silk fibers regardless of reeling speed. *J Exp Biol*. 2022;*225*(3):jeb243458. doi:10.1242/jeb.243458
229. Janzen DH. *Two Ways to Be a Tropical Big Moth: Santa Rosa Saturniids and Sphingids*. Department of Biology, University of Pennsylvania; 1984.

230. Sonwalker TN. *Handbook of Silk Technology*. New Age International Publisher; 1991.
231. Teshome A, Raina SK, Vollrath F. Structure and properties of silk from the African wild silkmoth *Gonometa postica* reared indoors. *J Insect Sci*. 2014;*14*:36. doi:10.1093/jis/14.1.36
232. Mhuka V, Dube S, Nindi MM. Chemical, structural and thermal properties of *Gonometa postica* silk fibroin, a potential biomaterial. *Int J Biol Macromol*. 2013;*52*:305-311. doi:10.1016/j.ijbiomac.2012.09.010
233. Ngoka BM, Kioko EN, Raina SK, Mueke JM, Kimbu DM. Semi-captive rearing of the African wild silkmoth *Gonometa postica* (Lepidoptera: Lasiocampidae) on an indigenous and a non-indigenous host plant in Kenya. *Int J Trop Insect Sci*. 2007;*27*(3–4):183-190. doi:10.1017/S1742758407883160
234. Yamada H, Kozo T. Characterization of silk proteins in the cocoon fibers of *Cricula trifenestrata*. *Int J Wild Silkmoth Silk*. 2001;*6*:47-51.
235. Hridya H, Guha L, Mazumdar M, Sarkar BN, Vijayakumar S, Borpuzari P. Probing the potentiality of the defoliator *Cricula trifenestrata* Helfer silk: a revisit. *Bull Natl Res Cent*. 2021;*45*(1):215. doi:10.1186/s42269-021-00669-w
236. Nindhia T, Knejzlik Z, Ruml T, Nindhia T. Tensile properties and biocompatibility of Indonesian wild silk *Cricula trifenestrata*: a preliminary study. *J Med Bioeng*. 2014;*3*:140-143. doi:10.12720/jomb.3.2.140-143
237. Li X, Yan S, Qu J, et al. Soft freezing-induced self-assembly of silk fibroin for tunable gelation. *Int J Biol Macromol*. 2018;*117*:691-695. doi:10.1016/j.ijbiomac.2018.05.223
238. Kadakia PU, Jain E, Hixon KR, Eberlin CT, Sell SA. Sonication induced silk fibroin cryogels for tissue engineering applications. *Mater Res Express*. 2016;*3*(5):055401. doi:10.1088/2053-1591/3/5/055401
239. Toprakcioglu Z, Knowles TPJ. Shear-mediated sol-gel transition of regenerated silk allows the formation of Janus-like microgels. *Sci Rep*. 2021;*11*(1):6673. doi:10.1038/s41598-021-85199-1
240. Lu Q, Huang Y, Li M, et al. Silk fibroin electrogelation mechanisms. *Acta Biomater*. 2011;*7*(6):2394-2400. doi:10.1016/j.actbio.2011.02.032
241. Chen P, Kim HS, Park C-Y, Kim H-S, Chin I-J, Jin H-J. pH-triggered transition of silk fibroin from spherical micelles to nanofibrils in water. *Macromol Res*. 2008;*16*(6):539-543. doi:10.1007/BF03218556
242. Hirlekar S, Ray D, Aswal VK, Prabhune A, Nisal A, Ravindranathan S. Silk fibroin-sodium dodecyl sulfate gelation: molecular, structural, and rheological insights. *Langmuir*. 2019;*35*(46):14870-14878. doi:10.1021/acs.langmuir.9b02402
243. Kim SH, Yeon YK, Lee JM, et al. Precisely printable and biocompatible silk fibroin bioink for digital light processing 3D printing. *Nat Commun*. 2018;*9*(1):1620. doi:10.1038/s41467-018-03759-y
244. Bessonov IV, Rochev YA, Arkhipova AC, et al. Fabrication of hydrogel scaffolds via photocrosslinking of methacrylated silk fibroin. *Biomed Mater*. 2019;*14*(3):034102. doi:10.1088/1748-605X/ab04e0
245. Piluso S, Flores Gomez D, Dokter I, et al. Rapid and cytocompatible cell-laden silk hydrogel formation via riboflavin-mediated crosslinking. *J Mater Chem B*. 2020;*8*(41):9566-9575. doi:10.1039/d0tb01731k
246. Whittaker JL, Choudhury NR, Dutta NK, Zannettino A. Facile and rapid ruthenium mediated photo-crosslinking of *Bombyx mori* silk fibroin. *J Mater Chem B*. 2014;*2*(37):6259-6270. doi:10.1039/c4tb00698d
247. Kim MH, Kim BS, Lee J, Cho D, Kwon OH, Park WH. Silk fibroin/hydroxyapatite composite hydrogel induced by gamma-ray irradiation for bone tissue engineering. *Biomater Res*. 2017;*21*:12. doi:10.1186/s40824-017-0098-2
248. Neo PY, Shi P, Goh JC, Toh SL. Characterization and mechanical performance study of silk/PVA cryogels: towards nucleus pulposus tissue engineering. *Biomed Mater*. 2014;*9*(6):065002. doi:10.1088/1748-6041/9/6/065002
249. Ghorbani M, Ai J, Nourani MR, et al. Injectable natural polymer compound for tissue engineering of intervertebral disc: in vitro study. *Mater Sci Eng C Mater Biol Appl*. 2017;*80*:502-508. doi:10.1016/j.msec.2017.06.007
250. Hofmann S, Hilbe M, Fajardo RJ, et al. Remodeling of tissue-engineered bone structures in vivo. *Eur J Pharm Biopharm*. 2013;*85*(1):119-129. doi:10.1016/j.ejpb.2013.02.011

How to cite this article: Croft, A. S., Spessot, E., Bhattacharjee, P., Yang, Y., Motta, A., Wöltje, M., & Gantenbein, B. (2022). Biomedical applications of silk and its role for intervertebral disc repair. *JOR Spine*, e1225. https://doi.org/10.1002/jsp2.1225