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Review

Advancements in Silkworm-Derived Silk Fibroin Biomaterials for Peripheral Nerve Regeneration

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Abstract

Regenerating injured nerves is difficult because they have little spontaneous regeneration potential. Advances in tissue engineering and regenerative medicine have emphasized the possibility of biomaterial-based methods for nerve healing. Natural protein-based biomaterials have benefits over synthetic ones, such as biocompatibility, non-immunogenicity, and biodegradability. Silk fibroin, generated from mulberry and non-mulberry silkworms, is especially promising because of its abundance, simplicity of processing into nerve-like structures, adjustable biodegradability, and mechanical robustness. Furthermore, non-

mulberry silk fibroin contains the cell-affinitive RGD tripeptide, which enhances its ability to repair nerves. Studies using silk fibroin (SF)--based nerve conduits have demonstrated nerve regeneration rates of up to 80–90% compared to autografts, which remain the clinical gold standard. SF conduits exhibit outstanding mechanical properties, with tensile strengths up to 300 MPa and elastic moduli adjustable between kPa-MPa range, which closely mimic the native tissue and ensure durability in dynamic environments. This review explores the diverse types of silkworm silk fibroin (SSF) and their applications in biomaterial-based Peripheral Nerve Repair (PNR). It discusses the integration of SSF with other biopolymers and synthetic polymers, highlighting advancements in nerve guidance channels incorporating electro-conductive materials to enhance regeneration rates. The literature search was primarily conducted using the Web of Science database, employing relevant keyword combinations such as "silk fibroin + nerve repair," "silk fibroin + peripheral nerve repair," "silk + nerve repair," and "silk + nerve repair + electrical stimulation." As this review focuses on silkworm silk-based biomaterials, studies involving spider silk or recombinant silk-based biomaterials were excluded. The period considered began with the earliest relevant studies, with an emphasis on more recent advancements up to November 2024 to capture the latest developments in the field. Identified studies were categorized based on the biomaterial composition, including pure silk biomaterials, silk biopolymer binary composites, silk synthetic binary composites, and silk-hybrid composites. Key findings were synthesized to highlight the progress, challenges, and future directions in applying silk fibroin-based scaffolds and electrical stimulation technologies for nerve repair. The findings provide insights into the potential of SSF-based biomaterials and propose future directions for developing advanced nerve repair strategies.

Keywords
Nerve regeneration; nerve guidance channel; silk fibroin; non-mulberry silk; RGD tripeptide

1. Introduction

A famous Chinese proverb states, "Patience and the mulberry leaf become a silk robe". Similarly, with time and advances in science & technology, the traditional use of silk has transformed/spread from the textile industry into the biomedical applications/healthcare industry. There is additional evidence that natural silk was used for wound healing and surgical sutures in ancient times and at the dawn of modern healthcare [1]. Silkworm silk is a structured protein-based biomacromolecule generated by insects such as Lepidoptera and composed of two types of proteins-fibroin at the inner core (70-80 wt%), which is responsible for mechanical attributes) and sericin (20-30 wt%), a gooey outer that protects the fibroin [2].

Sericin, a protein found in silkworm silk, is controversial in biological applications owing to its dual nature. While it has antioxidant and antibacterial effects, it can also stimulate immunological reactions [3, 4], making it inappropriate for medicinal usage. The biocompatibility of sericin is debated, with some studies indicating potential adverse effects, including allergic reactions, immunogenicity, and the release of the tumor necrosis factor-alpha inflammatory marker [5, 6]. To

mitigate these concerns, sericin can be removed entirely or partially from silk fibroin via a procedure known as degumming [7], depending on the application requirements.

Silkworm silk fibroin (SSF) has a high concentration of β sheets or α helices with a domain of random coils at the center, which makes it crystalline and robust as compared to the other natural biopolymers. Additionally, biocompatibility, tunable biodegradability, and ease of processing into different formats have stemmed fromits exploration as promising biomedical materials. Silk-fibroin-based scaffolds break down in the body over time, producing mainly non-toxic and biocompatible by-products. These include amino acids such as glycine, alanine, and serine, which are metabolized or excreted without harm [8, 9]. Additionally, silk fibroin initially breaks down into peptides, which are further degraded into amino acids and safely processed by the body. Since sericin does not play any role in silk fibroin-based biomaterials, it will likely be removed during the processing stage [7].

Although autografts are considered the gold standard for PNR, they are associated with limitations, including donor site morbidity, limited availability, and inconsistent functional recovery. Despite advancements in biomaterial-based nerve guidance channels (NGCs), full functional recovery is still in question, with 50% of patients achieving complete recovery and up to 80-90% reporting sensory and motor deficits [10]. While the peripheral nervous system (PNS) has a natural regenerative capacity more significant than the central nervous system (CNS), complete recovery is rare, often misdirected, or associated with persistent neuropathic pain. More significant defects in the PNS require surgical intervention to connect proximal and distal nerve stumps directly or with biological or synthetic bridging grafts. Age-related structural and biochemical changes further hinder regeneration, as mature neurons cannot divide, significantly reducing the regenerative and reinnervation potential of nerve fibers [11]. Another major challenge is the prolonged denervation of the distal target tissue, as human nerve regeneration occurs slowly at approximately 1 mm/day. This delay often results in significant atrophy of the denervated tissue before regenerating axons can reach it. These challenges highlight the urgent need for innovative cell instructive biomaterial-based NGCs that mimic the native biological microenvironment of nerve tissue, support accelerated nerve regeneration, and effectively repair significant nerve defects to improve clinical outcomes in PNR.

Recent advancements in biomaterials research have offered compelling evidence supporting the replacement of autografts, the current gold standard for peripheral nerve repair (PNR), with innovative biomaterials composed of natural and synthetic polymers. As mentioned above, silk fibroin is a versatile nature-derived biomaterial offering unique advantages for peripheral nerve repair, including excellent biocompatibility, minimal immunogenicity, mechanical strength, flexibility, and tunable biodegradability that align with nerve regeneration timelines. A range of studies utilizing SSF-based biomaterials demonstrated enhanced proliferation of neural supporting cells and neuronal growth with significant potential for nerve repair [12-14].

Silk fibroin-based scaffolds can be designed into diverse morphologies such as hydrogels, nanofibers, and multichannel conduits, providing topographical cues that mimic native nerve structures and enhance directed axonal growth. Functionalization with bioactive molecules (e.g., RGD peptides, neural growth factors such as NGF, BDNF, GDNF) or integration with conductive materials enables enhanced bioactivity and electrical stimulation, promoting axonal elongation and functional recovery [12, 15]. With FDA approval and demonstrated efficacy in preclinical models, SF's degradable, scalable, and adaptable nature makes it a promising candidate for clinical translation in peripheral nerve repair.

Prof. David Kaplan from Tufts University, USA, a pioneer in biomaterials research, is most widely recognized for his groundbreaking work with silk-based materials in nerve regeneration. This work has significantly advanced the field by demonstrating the potential of silk fibroin due to its biocompatibility, mechanical strength, and versatility [16, 17]. Kaplan's research involves employing electrospun silk fibers as scaffolding to simulate the extracellular matrix, which improves nerve regeneration by directing neurite formation and promoting Schwann cell migration [18-20]. Additionally, biocompatible, biodegradable silk-based nerve conduits were designed to effectively bridge nerve gaps, offering a viable alternative to conventional nerve repair methods. Kaplan's study on functionalizing silk scaffolds with protein [21] and growth factors [22] revealed that these alterations can speed nerve healing by boosting neuronal survival and axonal extension. In addition, his team worked on overcoming the challenges of biocompatibility, degradation, and mechanical qualities to translate these silk-based breakthroughs from laboratory research to clinical applications [23, 24]. His efforts have established a strong foundation for applying silk-based biomaterials in peripheral nerve regeneration, emphasizing its potential as a viable option for conventional nerve repair methods.

Compared to previously published reviews on peripheral nerve repair (PNR) utilizing biomaterials, the current review brings several new insights and areas of focus. This review aims to address the limitations of current PNR methods by exploring the potential of SSF-based biomaterials, including mulberry and non-mulberry silk. It emphasizes silk fibroin's distinct features, including biocompatibility, non-immunogenicity, biodegradability, and mechanical robustness, making it an appealing alternative to more expensive macromolecules like collagen and fibronectin. It highlights SSF's unique features, such as the presence of the RGD tripeptide in non-mulberry silk, which enhances its efficacy for nerve repair. In addition, the current review also highlights the innovative usage of silk fibroin in combination with other polymers, as well as future thoughts on the fabrication of innovative nerve guidance channels, indicating a forward-thinking approach in this field. Here, we have aimed to highlight the potential of electrical stimulation technology utilizing silk-based conductive neural scaffolds for PNR. By identifying gaps in current strategies and presenting future directions, this review seeks to advance the application of silk fibroin in peripheral nerve regeneration and bridge the gap between laboratory research and clinical practice.

2. Neurobiology and Neural Tissue Engineering

2.1 Fundamentals of Neurobiology

The Central Nervous System (CNS) and Peripheral Nervous system (PNS) together comprise the human nervous system [Figure 1(a)] [25]. It is a specialized and quintessential part of our organ system and body physiology, which controls and coordinates among the various organs and organ systems, maintaining fine-tuning in communication and coordination. In general, it acquires information from different parts of our body, makes the responsive interpretation, and then transfers the effectory signal to the designated cells and organs to trigger a required response. While CNS mainly constitutes the brain, spinal cord, optic, olfactory, and auditory systems, PNS, on the other hand, includes quite extensive neural networking comprising spinal nerves that originate from cranial nerves of the brain, sensory nerve cell bodies known as dorsal root ganglia (DRG) and their processes. CNS communicates and is involved in signal processing for excitatory stimuli to PNS; PNS carry sensory inputs to CNS and signal transduction from CNS to various body parts. Together,

they orchestrate a perfect synergistic relationship in the nervous system. Multiple types of neurons in PNS along with their processes are shown in Figure 1 (b & c) [25].

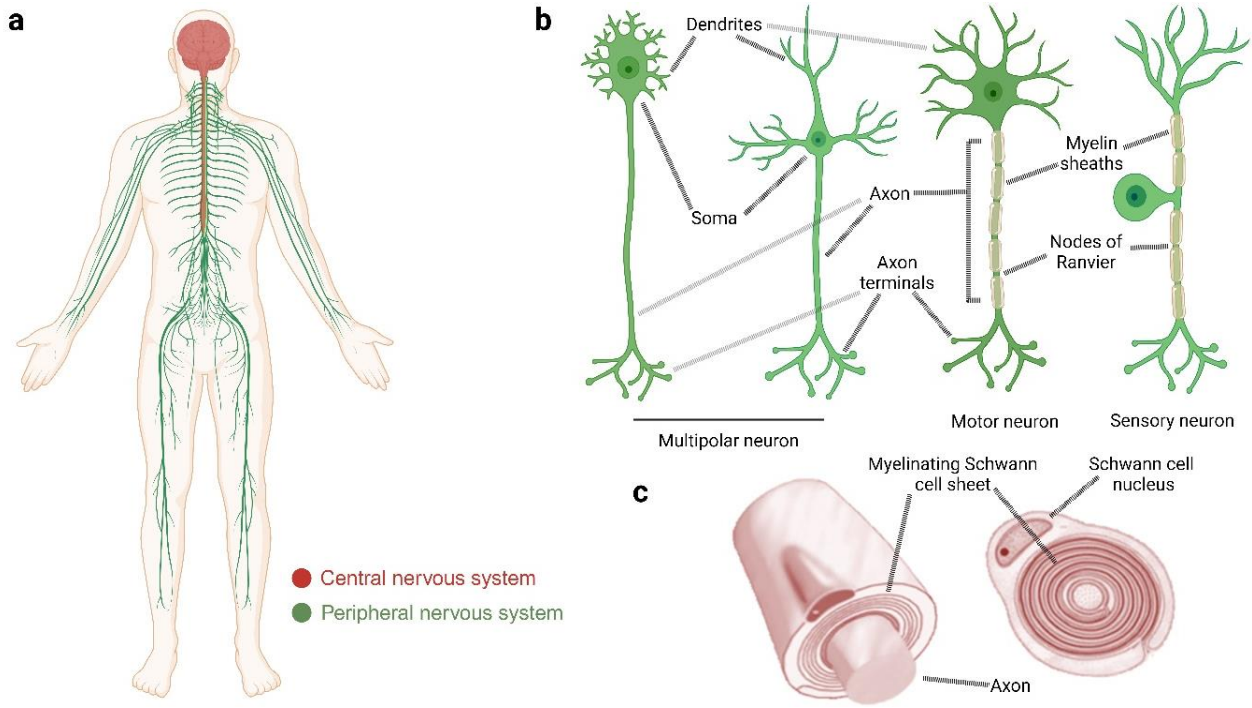

Figure 1 a. Human nervous system showing CNS (red) and PNS (green); b. Schematic illustration of the morphology of multi-polar neurons, motor neurons, and sensory neurons showing dendrites, soma (cell body), axon, axon terminals, and gap (Nodes of Ranvier) between myelin sheaths; c. 3D (right) and 2D (left) cross-sections of a myelinated axon formed by Schwann cells. Adapted and redrawn with slight modification with permission from López-Cebral et al. 2017 [25], Copyright © 2017 American Chemical Society.

While the peripheral nervous system (PNS) has some capability for self-repair, particularly with the help of external stimuli and Schwann cell migration generating Büngner bands, this regeneration ability reduces as the severity of the injury rises. Despite their limited regenerating capacity, severe PNS injuries need improved therapeutic approaches to optimize recovery and outcomes. After an injury in PNS, a series of biological activities occur, including modulation of the phenotype of affected neurons and Schwann cells, which determines cell survival and eventual regeneration. The remaining axons withdraw behind the damaged front, whereas the distal portions degenerate. This occurs 24-48 hours after damage, involving axon and myelin fragmentation. Macrophages and Schwann cells work together to clean and prepare the damaged site for regeneration. Depending on the severity of the damage, this might take months. In extreme situations, the absence of guidance signals, fibrin buildup, and extensive scarring may physically hinder axon regeneration. It is worth noting that mechanisms of PNR are controlled by different biochemical signals, where Schwann cells change their myelinating characteristic into a regenerating phenotype and guide neurotrophic factors and extracellular matrix molecules, thereby promoting regeneration.

2.2 Role of Biomaterial-Based Neural Tissue Engineering

Our nervous system is prone to several life-threatening injuries due to fatal conditions like trauma and car accidents, along with neurological disorders, such as Alzheimer's disease and Parkinson's disease, including strokes and brain tumors. Traumatic peripheral nerve injuries lead to motor and sensor disabilities, including neuropathic pain in the upper extremity, mainly radial, ulnar, and median nerves, extending to sciatic and peroneal nerves in the lower extremity. As a result of those injuries, the nerve gaps that occur are often complexto regenerate, usually leading to life-long disabilities in affected patients. Annually, around 2 million new cases of nerve injury are reported, which causes massive medical havoc [26]. Currently, in medical practice, direct end-to-end suturing is exceptionally suggested for short nerve injuries. However, its clinical efficacy is not satisfactory due to the reduced stretching capacity of end-to-end sutured nerves, which occurred due to Wallerian degeneration, intra and peri-neural fibrosis, and tissue adhesions [15]. Nerve grafts are more than essential for more significant nerve defects or gaps to provide a bridge between the proximal and distal nerve stumps. Autologous nerve transplant is currently regarded as the gold standard technique for these. Despite this, there are some downsides to this approach, including limited tissue availability, donor site morbidity, increased scar development, and differences in tissue architecture between the recipient and donor sites [27]. Xenografts and allografts were also evaluated, but their usage has been hampered mainly by the immunological responses that occur after graft transplantation, necessitating immunosuppressive techniques, and hence their success has been poor. Since axons also need physical guidance cues for directed growth, drugs and biologics alone are unsuitable. Additionally, axons grow more slowly than the other types of tissues at a rate of 1-3 mm/day, and studies have even demonstrated that regeneration does not always result in a desirable, fully working outcome [15]. Therefore, a biomaterial based neural tissue engineering approach is essential involving cells and growth factors, which can effectively address the existing shortcomings.

A biomaterial is an artificial extracellular matrix (ECM) that may be implanted or grafted into an injured or damaged tissue region of the human body. A biomaterial should be biocompatible, bioactive, biodegradable, immunocompatible, and mechanically robust, as well as flexible enough to design according to the tissue architecture for the desired application Repairing damaged nerves is a hefty affair, not only because it demands regeneration of nerves but also because the development of the nerves, connectivity, and structural plasticity should be equally addressed. All these factors equally and significantly depend on proper directional axonal growth. For this reason, biomaterial design should, therefore, encompass aligned topographical cues alongside relevant physiochemical and biological properties of the biomaterial. This design strategy is a valuable tool to mimic neural architecture to a certain extent. Furthermore, biomaterials combined with cell adhesive molecules and neurotrophic factors can better direct biochemical signals for neuron regeneration.

Several synthetic or natural biopolymers have been experimented for their utilization as artificial nerve grafts or nerve guidance channels till date, for example poly-glycolic acid (PGA) [28], poly-lactic acid (PLA) [29], poly(lactide-co-epsilon-caprolactone) (PLCL) [30], polyvinyl alcohol (PVA) [31], poly-3-hydroxy-butyrate (PHB) [32], conducting polymers [33-35], chitosan [36], collagen [37], alginate [38], and their composites or derivatives [39] (Table 1). They are excellent alternatives for sacrificing a healthy nerve in the event of autologous nerve grafts or subsequent surgical referrals.

However, there are only a few clinically approved biomaterials-based nerve conduits to date, out of which mostly are collagen-based (e.g., NeuroMatrix/Neuroflex, NeuroMend, NeuraGen, NeuraWrap), followed by PLCL-based NeuroTube, PDLLA-CL based NeuroLac and PVA based SaluBridge/SaluTunnel [15]. Most of these nerve grafts are effective only in cases of small nerve gaps with noncritical injuries, and they have demonstrated a maximum of up to 70% functional recovery. Lacunas, such as mechanical characteristics, processibility, and, most critically, efficiency with long-term safety, have not reached the bare minimum of a perfect artificial nerve graft. Due to its better inert and mechanical properties, silicone rubber has found widespread use in neural tissue engineering, initially in clinical settings. Still, this has seen limitations over the years primarily because of its non-degradability [40]. Also, upon completion of regeneration, there are chances of worsening or lethal conditions on target due to mechanical impingement and storming immune reactions. Among all natural biopolymers employed as nerve grafts, collagen has received FDA clearance in different formats. Yet, issues such as rigidity, high production cost, fast degradation rate, and handling difficulties during suturing are to be addressed. Hence, there has been a continuous research effort devoted to the search for perfect materials and compositions with appropriate designs to effectively address the shortcomings of the existing methods.

Table 1 An overview of several biopolymers, both natural and synthetic, that have been researched for use as artificial neural grafts or nerve-guiding channels.

Polymer [Type]	Outcome		Impact on Clinical Importance	Ref
	In vitro	*In vivo*		
Polyglycolic Acid (PGA) [Synthetic]	Promotes Schwann cell adhesion and proliferation.	Fosters the regeneration of nerves, however, they degrade too fast, which results in inflammation	FDA-approved for clinical usage; often used in nerve guidance channels, but requires a combination with other materials for best outcomes.	[41, 42]
Polycaprolactone (PCL) [Synthetic]	Easy to fabricate and promotes Schwann cell expansion;	Long degradation duration: nerve regeneration is possible with necessary modification.	Often used in blends to regulate degradation rate, promising for long-term usage in nerve transplants; enhanced neurite outgrowth	[43]
Polylactic Acid (PLA) [Semi synthetic]	Improved alignment, topography, and mechanical properties; increased cell adhesion	Supports axonal regeneration, Slow degradation	Biocompatible and extensively researched, it is often employed with other polymers for nerve repair.	[44, 45]
Poly(lactic-co-glycolic acid) (PLGA) [Semi synthetic]	Good biocompatibility Supports Schwann cell attachment and neuronal growth.	Effective nerve regeneration with controlled degradation.	FDA-approved; used in nerve guiding conduits; combines the advantages of PGA and PLA.	[46, 47]

Chitosan [Natural]	Excellent support for cell adhesion and proliferation. The regeneration-supportive properties	Promotes functional recovery and axonal regeneration in models of nerve damage.	Clinically employed in various tissue engineering applications, particularly promising in nerve transplants.	[36, 48, 49]
Silk Fibroin	Promote neurite outgrowth and Schwann cell proliferation; Superior mechanical and permeable properties.	Promotes axonal regrowth and functional recovery; biocompatible and biodegradable.	Because of its versatility and favorable mechanical properties, it has a high potential for therapeutic applications.	[50]

3. Silkworm Silk Fibroin for Peripheral Nerve Repair

SSF has received a lot of interest as an affordable biomaterial for PNR due to its natural origin and widespread availability. Although SSF has demonstrated significant advantages, these materials have many drawbacks compared to other natural biomaterials, such as spider SF (Table 2). From peripheral neural tissue engineering perspectives, SSF possesses tunable biodegradation kinetics, robust mechanical stability with flexibility to process in formats mimicking peripheral nerve architecture alone or in conjunction with other synthetic or natural materials, cells, and growth factors, non-immunogenicity, and tunable porosity for transportation of nutrients and essential water-soluble metabolites [1, 2, 51]. Some SSF varieties even contain cell instructive peptide sequences, which promote cellular adhesion [52]. SSF can be mulberry or non-mulberry, depending on dietary needs, ecology, and climatic conditions. In the following section, different varieties of SSF having their characteristic features, which make them suitable for nerve regeneration approach, are briefly discussed to appreciate their vast untapped utility in the neural tissue engineering field.

Table 2 Advantages and disadvantages of silkworm SF compared to other natural biomaterials such as spider SF.

Silkworm SF		Spider SF		References
Advantages	Disadvantages	Advantages	Disadvantages	
Enhances the interaction with cells	Extremely slow self-gelation (depending on the concentration)	Large availability	Cells are usually loosely attached to the hydrogel surface	[13, 53-55]
Formation of β-sheets due to Glycine-Alanine repeats	Impractical for cell encapsulation	Rapid gelation	Formation of cell aggregates	
Relevant for self-assembly		Influenced assembly in the presence of the cations	No influence on cellular adhesion	

The heavy chain of SF mainly directs the formation of β-sheets Excellent ability to synthesize silk	Smaller yield production

3.1 Mulberry Silk

Mulberry silk isobtained from Lepidoptera silkworms of the *Bombycidae* family, known as *Bombyx mori* [1, 2]. Due to its domesticated nature, it is available almost worldwide. It produces tiny fibroin strands encased in sericin. *Bombyx mori* silk fibroin (BmSF) consists of a heavy (H, ~350 kDa) and a light (L, ~26 kDa) polypeptide chain linked by a glycoprotein, P25 (~ 30 kDa) [2]. Poly-(glycine-alanine) repeats, i.e., Gly-Ala-Gly-Ala-Gly-X (X stands for Ser or Tyr), make up ~55% of BmSF and have the tendency to get into stable anti-parallel β sheet conformations, enhancing its tensile strength. It also makes BmSF insoluble in water, which somehow to an extent limits its widespread applications. L-chain, on the other hand, contains a high concentration of leucine, isoleucine, valine, and acidic amino acids. BmSF is negatively charged under physiological conditions, having an isoelectric point of 4.32.

Ideally, BmSF is extracted from the silk glands of the worm or by dissolving spanned fiber proteins through a degumming process, which requires heating and mild acidic or alkaline solution, for example, sodium carbonate and/or using detergents. Sericin elicits pro-inflammatory reactions. Hence, it is removed from natural silk fibers. BmSF is dissolved in 9.2 M LiBr aqueous solution as a first step to obtain regenerated SF, followed by dialysis to remove LiBr [24]. Figure 2 presents the isolation methods for mulberry and non-mulberry SF and includes images of BmSF larvae, their cocoons, degummed fibers, and regenerated SF solution. Specifically, Figure 2A illustrates the BmSF extraction process, while Figure 2B shows the extraction methods for non-mulberry SF. BmSF is an excellent biocompatible and immuno-compatible material for neural tissue engineering and is of immense use in regenerative medicine. It has been the subject of extensive biological study with few clinically approved tissue-engineered products in the healthcare sector. Subsequent bio-erosion of BmSF leads to byproducts, which are anti-inflammatory and have remarkable antioxidant properties. Vastly advantageous physicochemical facets include its dielectric behavior and tunable rheological properties. Alongside, this material can also be used as a delivery platform forthe controlled release of neurotrophic factors, as well as an efficient methodology to encapsulate and implant exogenous stem and progenitor cells, having their added therapeutic benefits.

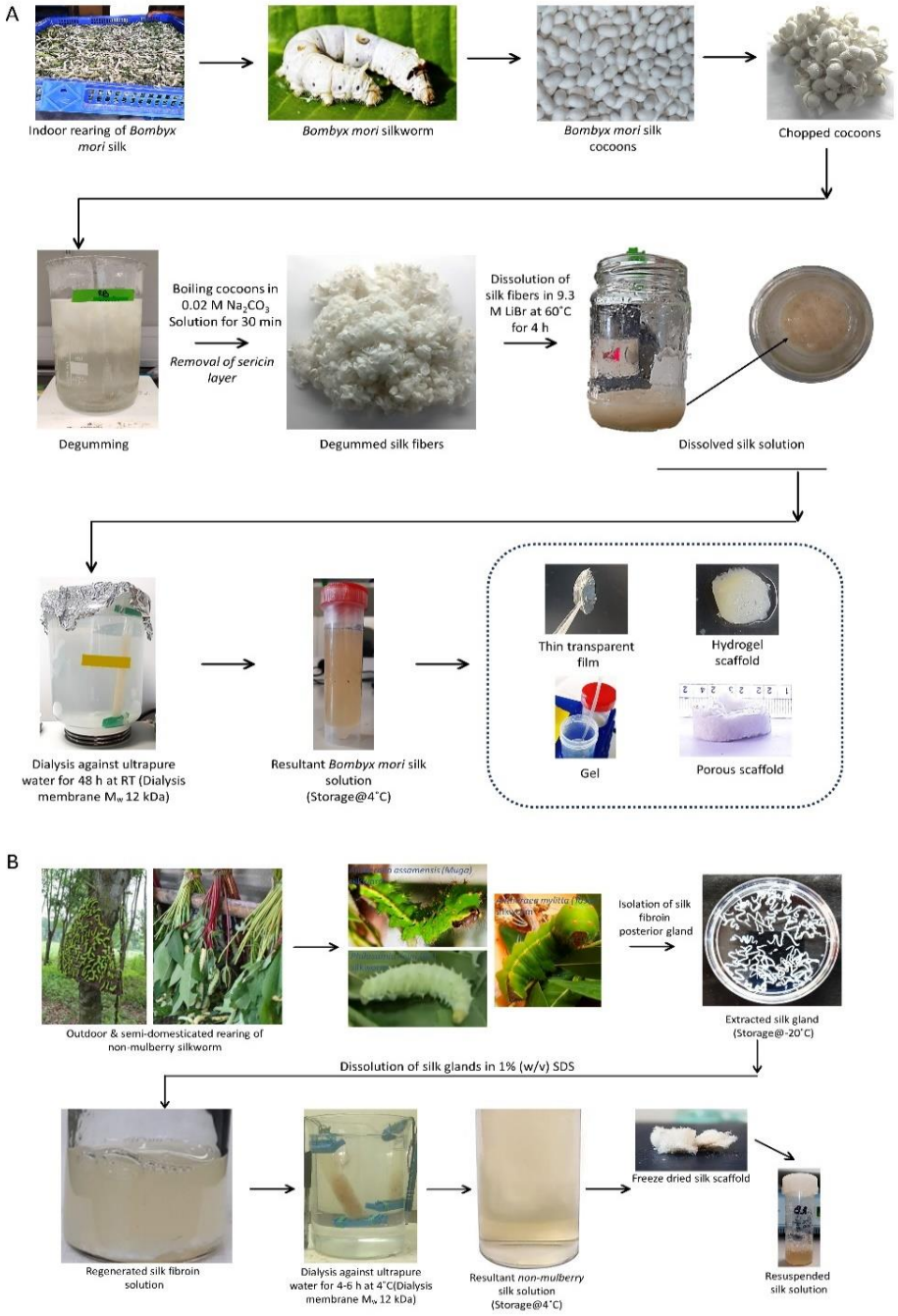

Figure 2 Mulberry and non-mulberry SF isolation methods. A. Extraction process of mulberry Bombyx mori silk fibrin (BmSF) in a step-by-step process beginning from rearing in an indoor environment, collection of silk cocoons, degumming, dissolution, and purification. Purified BmSF proteins can be processed into gel, transparent thin film, hydrogel, and porous scaffold for tissue engineering applications. B. Non-mulberry SF (*Antheraea Assam, Antheraea mylitta*, *Philosamia ricini*) extraction process from the posterior glands of the 5th instar matured larvae followed by dissolution in sodium dodecyl sulfate (SDS), purification through dialysis at 4°C, resulting in SF solution, which *is* used for further experimentation. (Figures including all the raw images/photographs are acquired or generated by the authors, while the BmSF extraction protocol is presented as reported by Rockwood *et al.* 2011 [18], and non-mulberry SF extraction methods are presented as reported in previous studies [56-60].

3.2 Non-mulberry Silk

Non-mulberry silk is generated by wild silkworms of the *Saturniidae* family, which have diverse eating habits and a wide geographic range. Non-mulberry silk fibroin, unlike mulberry silk, lacks L-chain and P25 proteins [2] and has polypeptide H-chains ranging from 197-230 kDa, which contribute to its crystallinity and mechanical robustness. Non-mulberry silk varieties such as *Antheraea assama* (Muga silk) and *Antheraea pernyi* have been researched for their potential in neural regeneration due to the presence of RGD tripeptides, which aid in neuronal cell attachment and proliferation [61]. These silks have tremendous potential as a matrix for drug delivery systems and tissue engineering. These silk-based biomaterials are extensively used in drug delivery for their versatility, chemical modifiability, and controllable degradability [62]. Drug loading on filamentous proteins can be accomplished via physical electrostatic adsorption or chemical grafting, depending on the application, drug properties, and protein features. Physical adsorption is non-covalent, based on charge interactions, and is simple and reversible, although it might result in weak and uncontrolled drug binding. Chemical grafting includes covalent bonding, which results in more stable and regulated drug release but also necessitates more sophisticated methods and may compromise protein or drug function. Chemical modification relies heavily on reactive groups on the protein, such as amino, carboxyl, thiol, and hydroxyl groups [63].

These silks also have excellent mechanical qualities, making them ideal for applications such as spinal cord repair and nerve regeneration. The filament has superior stiffness properties capable of the extent of spinal cord repair as it supports neural mechano-biology. The stiffness of filamentous membranes or silk-based filaments may be controlled in various ways, including chemical or physical crosslinking, blending with other polymers, regulating crystallinity, and altering fiber diameter or hydration levels. Beta sheets determine the mechanical strength of silk fibroin-based biomaterials. A lower concentration of silk fibroin results in a highly porous, softer silk scaffold, while a higher concentration produces a stiffer scaffold that remains flexible [64]. Additionally, degummed silk fibers (which consist primarily of silk fibroin with negligible sericin) are mechanically more muscular than the silk fibroin obtained after the dissolution and dialysis steps [24]. Silk fibroin obtained through this process is referred to as regenerated silk fibroin.

These approaches enable stiffness to be adjusted throughout a wide range, from soft and flexible to rigid and strong, depending on the application. Young's modulus ranges from ~1 MPa to over 10 GPa, making it appropriate for soft and hard tissue engineering. These changes allow for customization for unique tissue needs.

4. Morphologies of Different Silk Based Biomaterials

Neurons, especially the axons, followthe contact guidance phenomenon. Hence, various morphological forms of fabricated silk fibroin (SF) are now explored in the neural tissue engineering domain, like hydrogels, sponges, films, and conduits, integrated with modification strategies to incorporate macro/micro/nanoscale features to the biomaterial-based nerve scaffold to provide appropriate physical guidance for axonal regeneration [15, 17, 65] have fabricated various silk based electroconductive scaffolds in the form of micro channeled and porous tubular construct and aligned microfibers [15].

4.1 Films

The common standard methodology to obtain films is the dry casting method, which is relatively simple, cost-effective, and has lower invasiveness [12]. However, for proper formation, induction of beta-sheets is required by further treatment steps which involve cross-linking. Their fundamental applications include but are indeed not limited to drug delivery, axonal growth and guidance, and neural electrodes covering [66-69]. Film rigidity is an essential characteristic of biomaterial fabrication. There is a positive correlation between this characteristic and mechanical stiffness, which influences the activity of several important signaling molecules for neuronal regeneration [70].

4.2 Hydrogels

Hydrogels are morphologically porous semisolid microstructure, having hydrophilic polymeric networks containing approximately 30-90% water of the dry weight, and their formation occurs by SF self-assembly through gelation. Various factors like temperature changes, sheer mechanical stresses, ultra-sonication, pH modifications, and chemical cross-linkers aid in the gelation process [71]. Their formation in an aqueous solution can also happen by the following processes such as cross-linking, hydrogen bonding, and photo-polymerization. One obvious advantage of hydrogels over film morphology, is that there is no need for induction of beta-sheet in subsequent steps. A distinguished feature of hydrogels is that they have injectable properties, which means neurotrophic factors or other therapeutic compounds can precisely reach the area of nerve damage, and there is no need for suturing.

4.3 Conduits

Nerve guidance conduits (NGCs) are the scaffolds having the closest morphological features to peripheral nerves [72]. Li *et al*. have developed a water-based strategy to develop water-insoluble silk fibroin (SF) nerve conduits with morphological features closely resembling those of peripheral nerves (Figure 3) [73]. Single hollow tube configurations were initial designs in NGC's arena, often leading to aberrant targeted re-innervation or poly-innervation. To address the above concerns, filling materials like gels, sponges, filaments, and fibers are now increasingly introduced into the NGC lumen architecture as topological cues for better-guided neural regeneration [74]; however, they have permeability issues. In recent years, complex material designs have become a more holistic approach for NGC's fabrication, together with multiple intraluminal channels [75, 76]. Apart from obtaining NGCs mostly through a freeze-drying approach, several recent fabrication techniques have emerged, such as extrusion-based, inkjet-based, and laser-based 3D bio-printing technologies [77]. For extrusion and inkjet-based techniques, the lack of suitable bio-inks to produce larger 3D constructs is still a challenge, though.

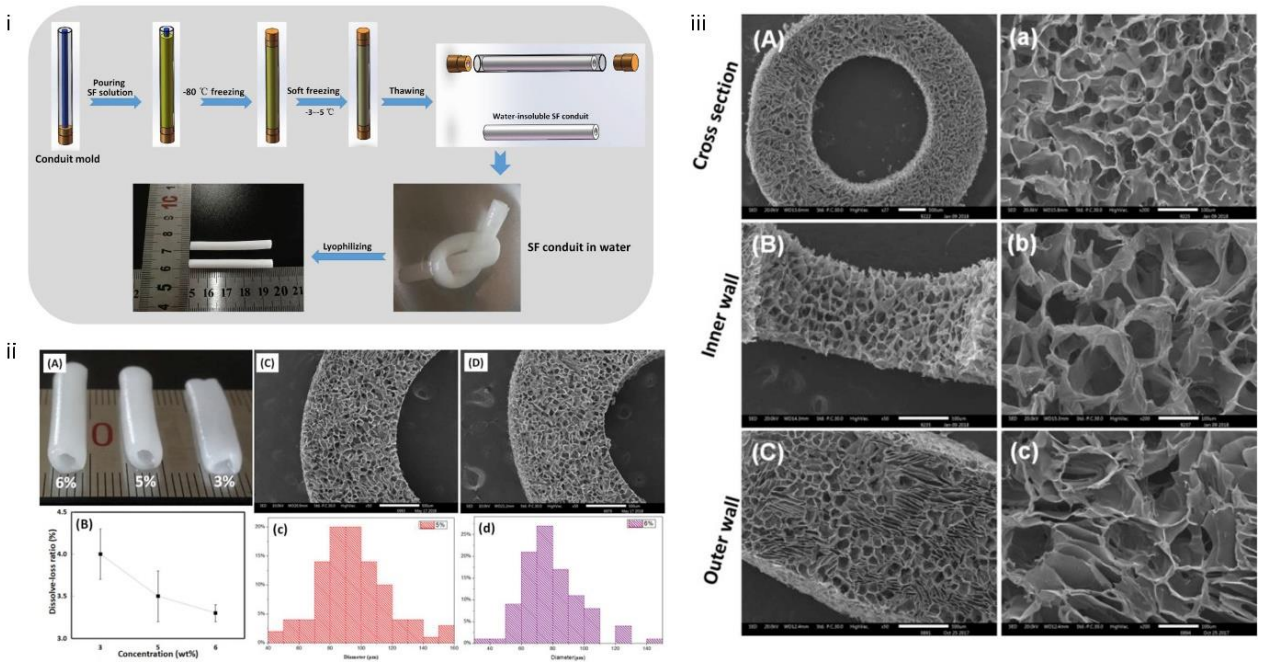

Figure 3 (i) Schematic illustration showing the preparation of stable water-stable nerve conduit using the soft freezing method. (ii) (A) Aesthetic appearance of SF conduits constructed using various concentrations, (B) water solubility test showing solubility decreases with increasing SF concentration, (C, D) cross-sectional SEM images of SF conduits depicting their surface morphology and their corresponding (c & d) pore size distribution. (iii) SEM images showing the morphological characteristics of SF conduits prepared from 6% SF aqueous solutions from different angles, as indicated. Scale bars: (A, B, C) 500 µm, (a, b, c) 100 µm. Reprinted and adapted from Li *et al*. 2019 [73], Copyright Elsevier (2019).

4.4 Nanofibrous Scaffolds

This particular scaffold class has been immensely studied owing to its structural and morphological similarity with the fibrous architecture of native ECM. Some of the standard methodologies adapted for their fabrication are electrospinning [78, 79] (Figure 4), phase separation, and self-assembly [80].

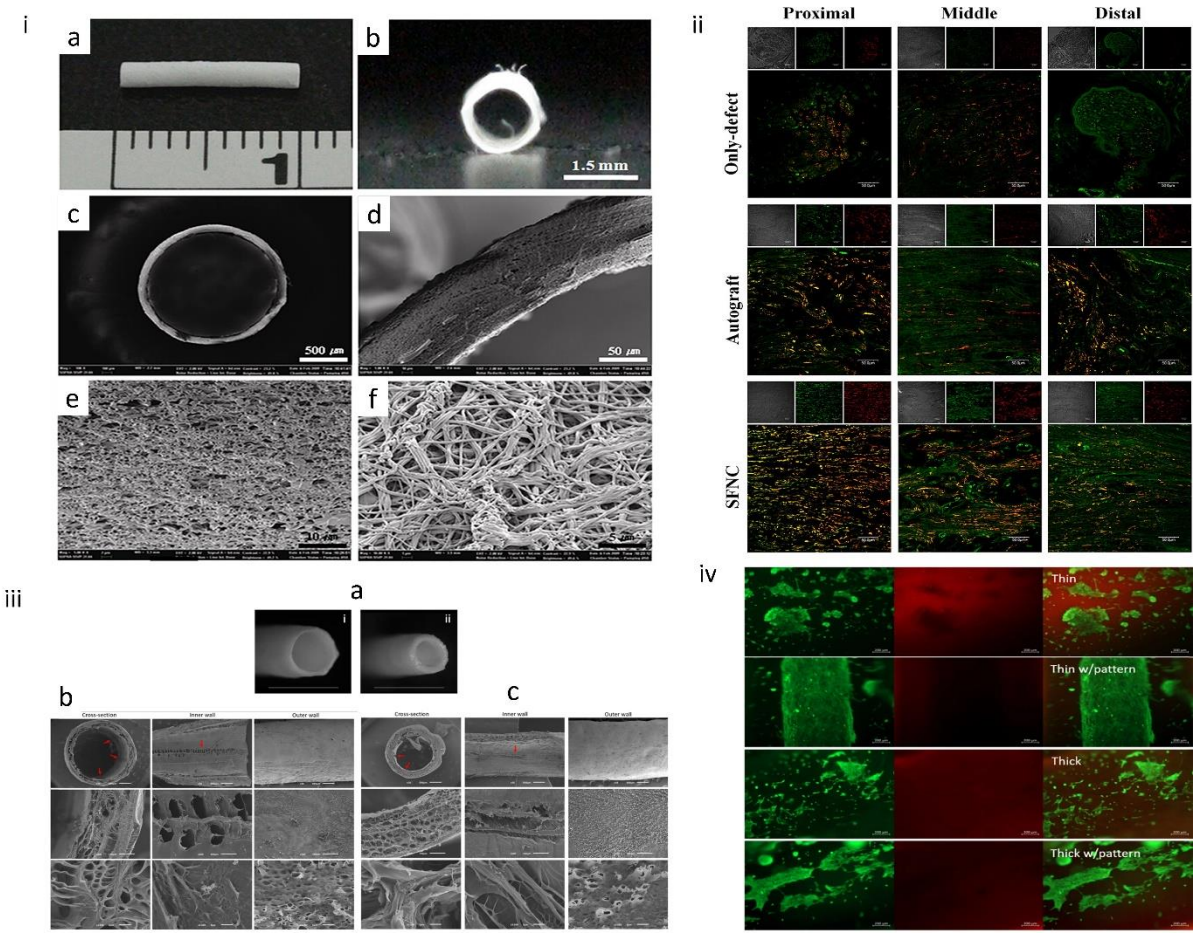

Figure 4 (i) Digital photographs (a & b) and SEM micrographs (c & d) of electrospun SF conduit. (e & f) show the high-magnification images with the nanofibrous network. (ii) Immunostaining of sciatic nerve regenerated using the electrospun SF conduit after 10 weeks; axonal neurofilament (green) and myelin essential protein (red) expression compared with autograft and defect-only group indicating comparable nerve repair by the electrospun SF conduit. [(i) & (ii)] Reprinted and adapted from Park *et al. 2015,* [78] Copyright © 2012 John Wiley & Sons, Ltd. (iii) Stereomicroscope images of SF conduits with (a1) thin (~200 mm) and (2) thick (~400 mm) wall (Scale bar = 1 cm); Microstructures of SF conduits with (b) thinner wall and (c) thicker wall thickness. (iv) Viability of Schwann cells on thin (patterned and unpatterned) thick (patterned and unpatterned) walled SF conduits after 24 h of culture, indicating myelination potential of the conduits. [(iii) & (iv)] Reprinted and adapted from Escobar *et al.* 2023 [79] under the terms of the Creative Commons CC BY license.

5. Silk Fibroin Based Biomaterials for Neural Tissue Engineering

From age-old centuries, silk has been in use as a compatible suture material during surgeries, and so, it is one of the oldest materials which have been medically relevant. Several research groups, with their profound insights and experimentations, have dubbed silk an almost perfect biomaterial for peripheral nerve regeneration. No doubt, it has FDA approval too. Herein, different silk-based materials have been categorized based on the composition, such as pure silk (only SF, one or more varieties), silk/biopolymer (SF composites with natural polymers), silk/synthetic material (SF

composites with synthetic polymers) and finally silk/hybrids (SF composites containing both natural and artificial materials) have been discussed to have a holistic view of their potential in neural regeneration.

5.1 Pure Silk Biomaterials

As stated earlier, SSF has the advantage of fabricating biomaterial scaffolds alone or in combination with other materials. However, only non-mulberry BmSF is suitable to process into desired neural architecture formats due to its mechanical robustness without supporting other materials [81]. Yang *et al.* explored purified SSF's feasibility in line with artificial nerve grafts by assessing its biocompatibility with peripheral nerve tissues and cells [14]. For that purpose, DRG from rats were cultured on SSF-derived substrate, wherein they observed DRG's cell outgrowth through light and electron microscopy, also utilizing immunocytochemistry technique. Not limiting their investigation with DRGs, Schwann cells from rat sciatic nerves were also cultured; however, they were cultured in silk fibroin extract fluid. RT-PCR and Western blot analysis together with immunocytochemistry showed that there was even no difference in the expression of nerve-related factors like nerve growth factors (NGF), brain-derived neurotrophic factor (BDNF), and S-100 secreted by Schwann cells in extraction fluid or L15 medium. They substantially concluded that silk fibroin showed desirable biocompatibility results with DRG and is also essential for Schwann cell survival without rendering any cytotoxic effects. Importantly, before this report, very few articles were published regarding silk-based or silk-coated materials for peripheral nerve repair. Hence, the study was an experimental foundation in this arena. The same group subsequently reported the development of eggshell-like microstructure SSF-based nerve grafts having biomimetic design containing oriented SF filaments [50]. It had superior mechanical strength and permeable properties required for neural regeneration. SSF graft was tested for bridge implantation along a 10 mm rat sciatic nerve defect. Post 6 months of implantation, electrophysiological experimentations, FluoroGold retrograde tracing, and histological studies showed promotion of peripheral nerve regeneration.

Glial cell line-derived neurotrophic factor (GDNF), and NGF was utilized synergistically by Madduri *et al.* for loading onto SSF nerve conduits, having both aligned and non-aligned SF nanofibers for effective topographical functionalization [82]. GDNF participates in multiple signaling cascades, among which it is involved in activating multiple kinase proteins and has an immense role in Schwann cell migration, as it acts as a stimulant in that line. At the same time, NGF is credited with increased survival and axonal outgrowth of sensory neurons. Axonal outgrowth rates parallel to aligned nanofibers and an augmented length were observed in chicken embryos derived from DRG sensory neurons and spinal cord motor neurons. Interestingly, DRG glial cells were ahead of the outgrowing axons in terms of their proliferation and migration, which, however, didn't complement non-aligned fibers. The SSF nerve conduits contained both DRG and SC explants in their luminal architecture and hence exhibited unidirectional orientation of axo-glial outgrowth. Future studies should concentrate on improving the regulated release of GDNF and NGF to maintain localized delivery, investigating Schwann cell integration for improved nerve healing, and overcoming issues with non-aligned fibers to boost coordination between axonal growth and glial cell migration. Incorporating additional bioactive molecules like brain-derived neurotrophic factor

(BDNF) and ciliary neurotrophic factor (CNTF) alongside GDNF and NGF into SSF scaffolds could improve nerve repair outcomes.

Simultaneous loading of BDNF and vascular endothelial growth factor (VEGF) was carried out on electrospun fabricated regenerated silk fibroin (RSF) aligned scaffolds by Liu *et al.* to see their combinatorial therapeutic efficacy towards peripheral nerve damage [83]. The release of dual factors from the scaffolds was measured through an ELISA assay, which was well-regulated for up to 2 weeks. SEM and Laser scanning confocal microscopy (LSCM) revealed Schwann cell morphologies on the scaffolds after their seeding procedure. Furthermore, with MTT assay of those seeded cells, the scaffolds were subcutaneously implanted in a mouse model to primarily evaluate in-vivo angiogenesis coupled with neural regeneration, for which histological and immune-histochemical (IHC) experiments were performed post 4, 8 weeks of the implantation. Positive endothelial markers (von Willebrand factor, vWF) and innervation markers (S-100 protein) were observed in retrieved scaffolds, which supported innervation and vascularization, also denoting non-chronic inflammatory response. The sole aim of neural regeneration through a dual-factor loaded designed scaffold was met as the material exhibited nerve regeneration compared to the control group. Varone *et al.* demonstrated that degummed *Antheraea pernyi* filaments (DAPF) didn't activate microglia and surrounding immune cells, both *in vitro* and *in vivo*, when implanted in a cord [61]. A nanofibrous silk-based nerve conduit prepared by the electrospinning method was evaluated for its efficacy by Ebrahimi *et al.* for peripheral nerve regeneration in rats [84]. There were tubes with or without loaded Schwann cells implanted into the sciatic nerve defect of the rats in a 10 mm gap. Macroscopic and histological assessments were done for proper monitoring of regenerated nerves post four months after the surgical procedure. Grafts containing Schwann cells could reconstruct the sciatic nerve trunk, including myelination, and the histological data showed the presence of both Schwann and glial cells. Future studies should look into synergistic combinations of bioactive substances such as CNTF or FGF with BDNF and VEGF to boost nerve regeneration and vascularization. Furthermore, optimizing scaffold design, performing long-term in vivo research, adding supportive cell types, and moving forward with clinical trials will be critical for enhancing peripheral nerve regeneration results and assessing the translational potential of these therapies.

BmSF-based nerve conduit - SilkBridge™ was manufactured by Alessandrino *et al*., which had a novel 3D architecture consisting of two electrospun layers inside and outside of the conduit wall, along with a middle textile layer [85]. The device exhibited high compression strength, which typically meets clinical needs. This study was particularly unique because it considered several cells/cell lines, viz., glial RT4-D6P2T, schwannoma cell line, and a mouse motor neuron NSC-34 cell line, to see their interactions with developed conduit. Glial RT4-D6P2T cells showed glial-like morphology, and NSC-34 cells had significant neurite length. Female Wistar rats were used for conducting *in-vivo* pilot assays to test the efficacy of conduit in a median nerve 10 mm long gap, which was repaired with 12 mm SilkBridge™. The cell types colonized the lumen two weeks post-operation, and at the proximal level, the concomitant presence of regenerated myelinated fibers having a thin myelin sheath was seen. A preclinical validation study for translational peripheral nerve regeneration of the above-mentioned SilkBridge TM was conducted, and in the following year, it was reported by Fregnan *et al.* [86]. The conduit (12 mm) was tested against a 10 mm long gap in the median nerve of a rat model, which eventually got repaired, based on periodic observation of 4

weeks, 12, and 24 weeks. The nerve recovery was quite similar to the reference autograft nerve reconstruction procedure.

To study re-myelination physiology, Liu *et al.* co-cultured DRG neurons and Schwann cells in an electrospun 3D silk fibroin membrane [87]. SEM visualization showed tightly wrapped myelin formations around axons. Also, at different time points, DRG neuron's gene expression levels were analyzed based on real-time quantitative PCR, which demonstrated higher mRNA levels of N-cadherin, laminin, and fibronectin, all belonging to the class of extracellular matrix (ECM) proteins. Straining flow spinning (SFS) technique was used for the very first time by Mercado *et al.* to obtain high-performance regenerated silk fibers as scaffolds [88]. The SFS fibers led to the formation of highly interconnected cellular spheroid-like tissues, further leading to neuronal projections, which was a positive transition from spontaneously organized dissociated cortical primary cells. SSF films exhibiting different anisotropic microgroove/ridge architectures were fabricated by micro-patterning technology to see the effect of surface topology on peripheral nerve regeneration [70]. SSF film with 15% concentration and groove width of 30 µm demonstrated guided and directional DRG nerve fiber growth, which later led to nerve fiber bundle formation. Carvalho *et al.* incorporated growth factors in an advanced SSF conduit, which aided neural regeneration after injury [89]. Cross-linking and absorption methods were utilized for respective incorporations of NGF and GDNF, while ELISA was done to analyze their release profile. DRGs were used for the bioactivity assay of the factors. The GDNF-loaded silk fibroin conduits offered retrograde neuroprotection in a 10 mm sciatic nerve defect implantation, which was at par with autografts. To enhance axonal growth and myelination, future studies should concentrate on improving electrospun silk fibroin scaffolds by modifying fiber alignment, concentration, and surface topology. The bioactivity and effectiveness of nerve regeneration may also be enhanced using a broader spectrum of growth factors and extracellular matrix proteins, such as laminin, fibronectin, and N-cadherin.

Using silk nanofiber fillers for tuning into hierarchical anisotropic architectures with micro-porosity in silk based hollow conduits, Lu *et al.* had successfully demonstrated the conduit assembly [90]. Schwann and PC12 cells proliferated well in those conduits and BDNF also got secreted. These results were in corroboration with the functional recovery of rat sciatic nerve defect. Recently, in 2021, pure SF hydrogels exhibiting aligned micro-grooved topographies having 10, 30, and 50 µm as width dimensions were developed by Gu *et al.*, which showed aligned growth of Schwann cells [26].

5.2 Silk-Biopolymer Composites

Zhang *et al.* loaded mecobalamin into an aligned SF scaffold through a facile strategy wherein aligned SF fibers were cross-linked with regenerated SF solution combining drying and ethanol treatment procedures to see neural growth promotion and survival, which yielded convincing results of Schwann cells and DRG neurons uniform migration along the scaffold, including characteristic high strength property of the material [91]. To study the growth of neural progenitor cells, Li *et al.* prepared aligned and random electrospun regenerated SF scaffolds, on which isolated NPCs from embryonic mouse hippocampus were cultured, along with laminin-coated SF mats [92]. Aligned and random RSF showed increased NPC proliferation and neuronal differentiation compared to controls. Electrospun silk fibroin-aligned nanofibers were obtained through an optimized procedure controlling the surface linear velocity of a rotating drum to provide

biochemical and topographical cues for long neural regeneration, wherein functionalization was achieved by binding silk nanofibers with laminin covalently [93]. Further, PC 12 cell proliferation and neurite outgrowth assays were done to investigate directional axonal growth on the aligned nanofibers. The results suggested enhanced neuronal regeneration on the laminin-immobilized silk fibroin nanofibers. Melanin was utilized in combination with SF, which eventually led to the formation of random and aligned nanofibrous composite scaffolds, which were fabricated by electrospinning [94]. The neurogenic potential of the scaffolds was assessed using human neuroblastoma cells for cell viability, proliferation, adhesion, and neural differentiation. SH-SY5Y-neuroblastoma cells differentiated sufficiently into neurons with axis orientation along scaffolds, suggesting potential for neural regeneration. Hyaluronic acid (HA) is a natural biopolymer found mainly in the ECM of tissues. It was explored with SF for neuronal applications [95]—Roca *et al.* fabricated tubular conduits containing a blended HA and SF matrix. Rat Schwann cells were cultured onto those conduits, which showed their increased proliferation and the subsequent formation of a tight cell layer. Future research should improve scaffold functionalization by integrating ECM proteins such as laminin to enhance cell adhesion and motility. Additionally, including bioactive compounds such as neurotrophic factors and hyaluronic acid might improve neuronal regeneration.

5.3 Silk-Synthetic Binary Composites

SSF contains several reactive amino acids like lysine, tyrosine, serine, aspartic acid, and glutamic acid, enabling it to be easily blended with a range of synthetic polymers [96]. Hu *et al.* reported the fabrication of electrospun BmSF nanofibers with a diameter of 305 ± 24 nm and demonstrated the efficacy of the scaffolds for neurite growth *in vitro* [97]. They found the scaffold beneficial for adherence, proliferation, and migration of mice Schwann cells without any cytotoxic effects downstream. Electrospinning allows an effective larger surface area for neural cell attachment and outgrowth [97-100]. Based on their findings, they concluded that the nanofibers helped in the formation of bands of Bunger, which enhances nerve regeneration. Zhao et al. demonstrate that polypyrrole/silk fibroin (PPy/SF) conductive scaffolds, created using 3D bioprinting and electrospinning, enhance neural regeneration. Electrical stimulation of Schwann cells on these scaffolds improved cell viability, proliferation, and neurotrophic factor expression, promoting axonal regeneration and remyelination *in vivo*. The findings underscore the clinical potential of PPy/SF scaffolds for nerve repair [101]. Further, Moroder et al. evaluated polycaprolactone fumarate–polypyrrole (PCLF–PPy) scaffolds for conductive nerve conduits, finding excellent mechanical stability, flexibility, and electrical conductivity [102]. Electrical stimulation (ES) enhanced neurite growth, with neurites aligning to the applied current. The results suggest that PCLF–PPy scaffolds are promising for treating severe nerve injuries.

Although non-mulberry SSF-based neural scaffolds have been minimally explored for nerve tissue engineering, the available evidence is highly encouraging. Non-mulberry SSF-based biomaterials possess superior mechanical properties compared to mulberry SSF, such as higher toughness and elongation at break, making them suitable for applications requiring mechanical robustness. As mentioned, non-mulberry SSF contains natural RGD sequences that promote enhanced cell adhesion, proliferation, and differentiation without external modification. Its biodegradability can be tailored to match the regeneration timeline of the target tissue, providing sustained structural support while avoiding inflammatory responses, making it a promising material

for advanced regenerative applications. This has been extensively reviewed elsewhere [81]. In this direction, Wang et al. showed *Antheraea pernyi* silk fibroin (ApSF) based electrospun nanofibers blended with poly (L-lactic acid-co-caprolactone) (P(LLA-CL)) with a higher elastic modulus than the pure P(LLA-CL) electrospun scaffold [103]. The study showed that increasing the ApSF content improved the hydrophilicity of the scaffolds while the mechanical properties were adjustable by modifying the blend ratio. Notably, the ApSF: P(LLA-CL) ratio of 25:75 demonstrated optimal performance by significantly enhancing Schwann cell proliferation and alignment compared to other compositions and controls. These findings highlight the potential of ApSF/P(LLA-CL) nanofibrous scaffolds as promising materials for peripheral nerve regeneration.

Naskar *et al.* were the first group to use a "Green aqueous based methodology"- solvent evaporation technique to functionalize and disperse carbon nanofibers (CNF) within regenerated *Antheraea mylitta* silk fibroin (AmSF) [104]. CNF is economically feasible has lower toxicity, and can be easily functionalized. Due to CNF fabrication, the films showed excellent conductivity up to 6.4×10^{-6} Mho/cm, 3 orders of magnitude of pure AmSF matrix. Regarding mechanical behaviour, superior tensile modulus up to 1423 MPa was noted. The films showed better fibroblast cell growth characteristics and proliferation. This study didn't consider neural cells in experimentations. However, it hinted towards a multifunctional biomaterial platform using silk having superior conductivity and tensile strength, which can be evaluated for a neural regeneration approach. To address peripheral nerve repair in spinal cord injury, Hu *et al.* developed a 3D porous silk fibrous scaffold (3D-SF) having formic acid decomposition followed by lyophilization [105]. Reprogramming of rat dermal fibroblasts into neurons – chemically induced neurons through small molecular combination CFLSSVY (CHIR99021, Forskolin, LDN193189, SB431542, SP600125, VPA, and Y27632) was achieved to see their growth on to 3D-SF scaffolds which were convincing. The neural scaffolds were transplanted into the 2 mm transected spinal cord stumps of rats, wherein the tissue got repaired substantially, due to reduced cavity areas, decrease in GFAP expression and functional axonal regeneration and myelination. In addition, hind-limb movement and motor-nerve conductivity also improved, as indicated by a higher Basso-Beattie-Bresnahan (BBB) score. A 45° inclined grid test was also conducted to observe the alternative movement of the two hind limbs. Crucial analytical experimentations included walking track analysis, morphogenesis, vascularization, axonal regrowth and myelination. An innovative strategy has been elucidated by Moysenovich *et al.*, whereby they produced a photo-cross-linked methacrylated silk fibroin (FBMA), which showed increased mechanical stiffness [106]. The photo-crosslinking methodology increased the rigidity of fibroin protein from the reported 25 kPa to 480 kPa. SH-SY5Y neuro-blastoma cells differentiated well onto the scaffolds, and there was a concomitant increase in the length of neurites along with increased levels of neural differentiation markers MAP2 and β-III-tubulin. To address the difficulties in manufacturing electrically conductive biomaterials for tissue regeneration, Das et al. developed a microfibrous scaffold composed of graphene nanoplatelets (GNPs) coated on Bombyx mori silk fibroin (BmSF). Using l-ascorbic acid (l-Aa) as a reducing agent, the electrical conductivity of the scaffold was optimized; a 48-hour reduction period dramatically enhanced conductivity and promoted axonal development in PC12 cells under electrical stimulation [107] (Figure 5). To improve conductivity, strength, and cell contact, future studies should focus on optimizing silk fibroin scaffolds by including bioactive materials like graphene oxide or carbon nanofibers. To promote neurite formation and increase scaffold stiffness, photo-cross-linking approaches have to be investigated.

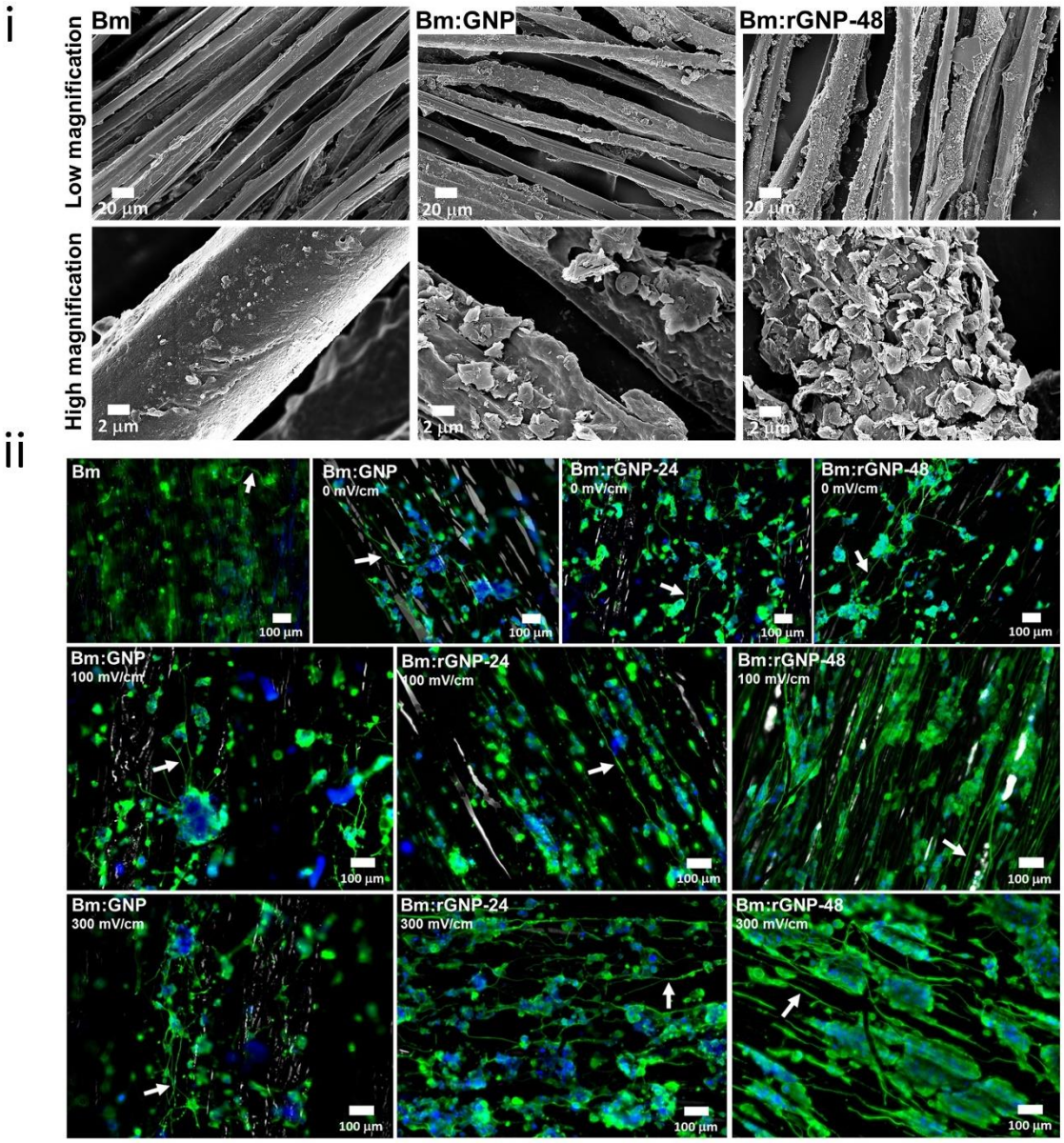

Figure 5 Aligned BmSF fibers were coated with graphene nanoplatelet (GNP) and reduced for 24 (Bm:rGNP-24) and 48 h (Bm:rGNP-48) for electrically stimulated axonal growth of PC12 cells. (i) FESEM Bm scaffold, Bm:GNP and Bm:rGNP-48 as indicated; Upper panel: low magnification images showing the overall surface morphology of the scaffolds (Scale bar = 20 µm) and lower panel: high magnification images (Scale bar = 2 µm) showing the surface morphology of the individual fibers. (ii) Electrically stimulated enhanced axonal growth; Immunostaining of PC12 cells after 10 days of culture to confirm their neuronal differentiation by β (III) Tubulin neuronal marker, counterstained by DAPI (nucleus staining), which were subjected to pulsed ES of frequency 50 Hz, pulse width 1 ms with amplitude of 100 and 300 mV/cm for 2 h/day till three consecutive days starting from Day 1 to Day 3. A. Representative fluorescent images of the differentiated PC12 cells with axonal projections (indicated with white arrow) on different scaffolds as labeled. Scale bar: 100 µm. Reprinted and adapted from Das *et al. 2024* [107] under the terms of the Creative Commons CC BY license.

5.4 Silk-Hybrid Composites

Silk-hybrid materials consist of a combination of three or more components, integrating both synthetic and natural materials along with SSF. While synthetic materials have the advantage of mechanical strength, they lack their sites for cell adhesion. Natural materials, although address the concern of cell adhesion, they are susceptible to rapid degradation, and hence, the combination of both types of materials, along with silk fibroin, is a sensible strategy for optimal material fabrication. PLGA-silk fibroin-collagen (PLGA-SF-COL) bio-composite fiber matrices were studied by Wang *et al.* [108]. They studied such hybrid scaffold fabrication for neural regeneration applications. Different weight ratios of PLGA-SF-COL were prepared via an electrospinning procedure. The fiber matrices were highly porous and hydrophilic, with high tensile strength. Among the various prepared variations of the bio-composites, they found 50% PLGA, 25% Silk fibroin, and 25% Collagen more suitable for nerve tissue engineering. These findings were evident by SEM and MTT results of Schwann cells which got attached and proliferated well in the scaffolds. Jiao *et al.* introduced silk fibroin (SF)-based neurobridge containing alginate, as a scaffold with/without NGF to see its viable therapeutic potential for spinal cord repair [109]. The results affirmed an increase in surviving neuron numbers. 3D hierarchical biomimetic nerve conduit exhibiting a fascicle-like structure was fabricated by Wang et al. through nanofiber dispersion, template molding, freeze-drying and cross-linking methods, taking into account Antheraea pernyi SF/(Poly(L-lactic acid-co-caprolactone))/graphene oxide (GO) (ApSF/PLCL/GO) [76]. They were successful in their efforts to make parallel multi-channel, having biomimetic fibrous fragments as the surrounding material. This kind of biomaterial design resulted in the *in vivo* repair of sciatic nerve defect in a rat model, similar in functionality to auto-grafts.

To fabricate conduits for the repair of peripheral nerve injury (PNI), Escobar et al., developed innovative poly(3,4-ethylenedioxythiophene) (PEDOT) nanoparticles [110]. These nanoparticles were synthesized by the mini-emulsion process and incorporated into SF. These PEDOT-loaded SF conduits demonstrated good conductivity and mechanical properties, as well as resistance to bending and suturing, and were not harmful to Schwann cells (Figure 6). Cheng et al. investigate using electrical stimulation with a polypyrrole-coated polycaprolactone/silk fibroin scaffold to promote sacral nerve regeneration [111]. Electrical stimulation enhanced nerve growth and polarized macrophages toward the pro-regenerative M2 phenotype, with bioinformatics analysis showing that it regulated STATs to boost M2-related gene expression, highlighting the importance of macrophages in the regeneration process. Another study introduces a novel poly(3,4-ethylenedioxythiophene): poly(4-styrene sulfonate) (PEDOT: PSS) conductive silk conduit for peripheral nerve regeneration, featuring ultrasound-triggered NGF release [112]. The conduit combines silk fibroin scaffolds, a thermosensitive hydrogel, and PEDOT: PSS to enhance nerve repair. In vivo experiments show that the conduit accelerates nerve regeneration by promoting neuron outgrowth and NGF delivery, demonstrating its potential for clinical application.

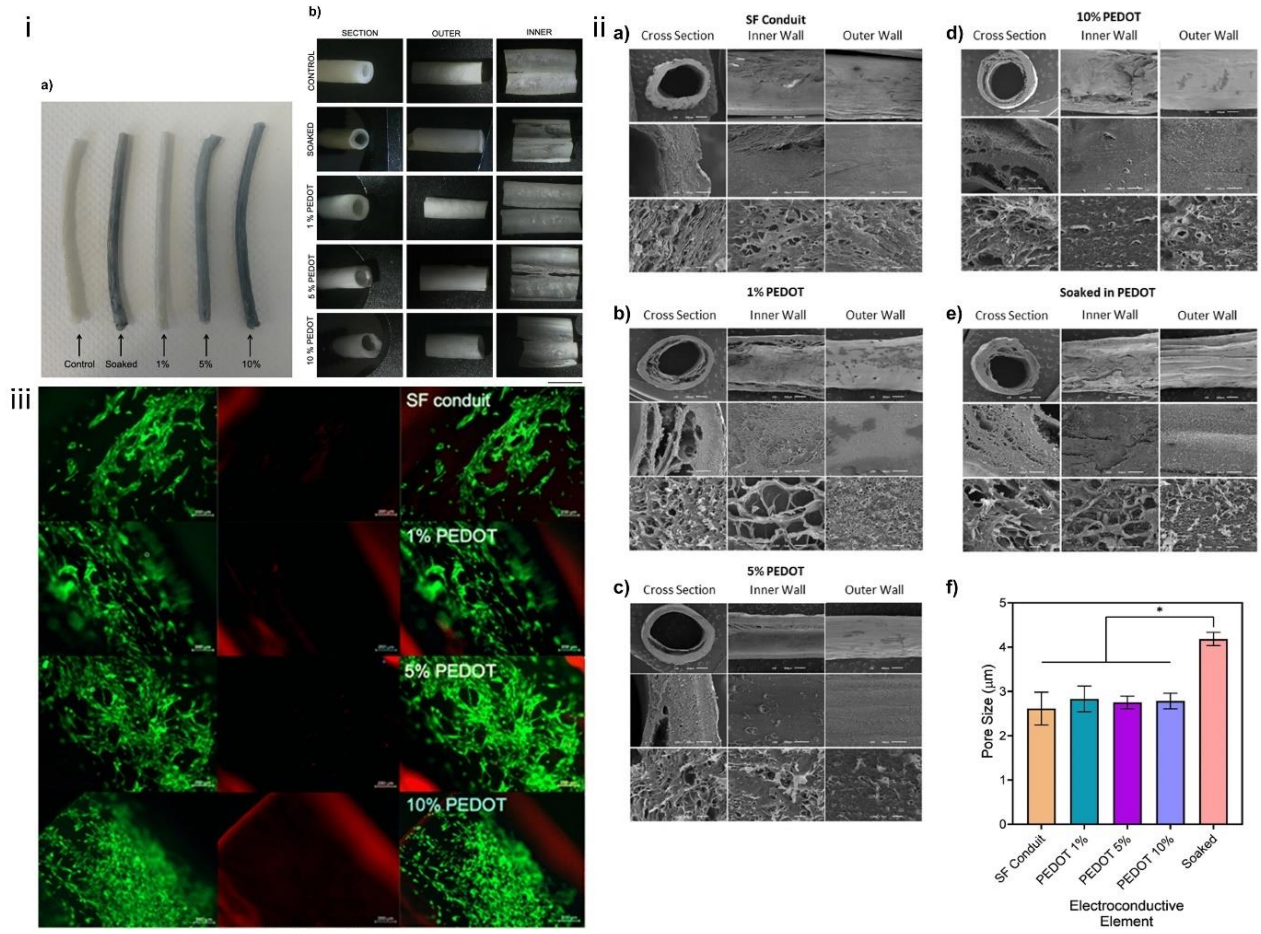

Figure 6 Electrically conductive PEDOT/SF conduits capable of mimicking the natural bioelectrical environment of nerve tissue. (i) Digital photographs of (a) (from left to right) pure SF conduit, SF conduit soaked in PEDOT NPs and 1, 5, and 10% PEDOT nanoparticles (NPs) loaded conduits; the various SF-based conduits with (b) close up view from a different angle. (ii) SEM micrographs of the various SF-based conduits showing their porous microstructures as labeled. (iii) Live/dead staining results showing the viability of Schwann cells for potential application of these in nerve repair. Reprinted and adapted from Escobar *et al*. 2023 [110] under the terms of the Creative Commons CC BY license.

Furthermore, they effectively prevented fibroblast infiltration, which reduced the possibility of the formation of scar tissue. These properties make the conduits promising biomaterials for PNR. Wang et al. incorporated GO with nanofibrous scaffolds of ApSF/P(LLA-CL), which demonstrated improved hydrophilicity and mechanical strength and enhanced Schwann cell migration, proliferation, and myelination *in vitro* [113]. These scaffolds significantly increased NGF secretion from SCs and promoted PC12 cell differentiation via focal adhesion kinase (FAK) upregulation. *In vivo*, GO/ApSF/PLCL scaffolds effectively repaired a 10 mm sciatic nerve defect in a rat model, showing comparable functional and morphological recovery to autografts, including enhanced nerve conduction velocity and reduced muscle atrophy. The same group employed further an advanced strategy to incorporate a reduced form of GO (rGO) into the ApSF/P(LLA-CL) scaffold. It evaluated the neural differentiation outcome under electrical stimulation using PC12 cells [114]. *In vivo*, rGO/ApSF/P(LLA-CL) NGC supported the regeneration of a 10 mm rat sciatic nerve defect, demonstrating functional recovery, enhanced nerve conduction velocity, and improved muscle

reinnervation, comparable to autografts. Recent evidence from our group highlights the development of a biohybrid aligned microfibrous scaffold composed of polypyrrole and Bombyx mori silk fibroin (BmSF), further functionalized with non-mulberry Antheraea assamensis silk fibroin (AaSF), which is rich in the RGD cell-adhesion tripeptide and shows significant potential for low-power electrically stimulated nerve repair [115]. The study demonstrated that incorporating non-mulberry SSF enhanced not only the biological functionality but also the electrical and electrochemical properties, such as conductivity and charge transfer efficiency, which are essential for low-power electrical stimulation.

Further research into natural and synthetic material combinations, such as silk fibroin with PLGA, collagen, or alginate, is needed to improve mechanical characteristics, degradation rates, and cell adhesion for nerve tissue engineering applications.

6. Fabrication Methods for Silk Based Materials for Nerve Repair

Having the advantage provided by 3D biomaterial scaffolds by supporting cellular adhesion, proliferation, recruitment, and differentiation as the native ECM, most of the studies fabricated SSF-based neural scaffolds by conventional methods, viz., freeze-drying/lyophilization, fiber bonding, self-assembly solvent casting, gas foaming, salt leaching, and electrospinning techniques as elaborately described elsewhere [12, 15]. SSF-based 3D hydrogel scaffolds can also be designed for PNR using various gelation techniques assisted by mechanical stimulation (sonication, vortexing), temperature, ion or acid incorporation, etc. [18, 116]. In addition, rapid prototyping methods like 3D printing, microfluidic extrusion, molding, and lithography techniques are variably used based on the intended physical features needed for biomaterial fabrication [117]. Material fabrication through photolithography allows obtaining micro-grooved patterns through sequential reactive ion etching technique, followed by micro-patterning on quartz or silicon wafers, and finally, transferring the patterns onto the biomaterials by solvent casting and/or compression molding. This methodology, however, incurs high costs while going for mass production. Advanced manufacturing technologies such as 3D printing and microfluidic extrusion will allow for customizable scaffolds while optimizing 3D hydrogel scaffolds using various gelation and mechanical stimulation approaches will promote nerve regeneration. Tailoring these manufacturing procedures to individual injuries to nerves can improve overall outcomes.

7. Conclusions and Future Perspectives

Due to their limited capacity to bridge and repair gaps with complete functional recovery, currently, authorized nerve conduits for peripheral nerve restoration have only assisted a small percentage of PNI patients. Those commercially accessible nerve conduits are optimal for tiny injury defects, and tubular structures with additional features viz., filled conduits, physical guiding signals, growth stimulants, etc., may enhance the efficacy. In recent years, there has been substantial development in the clinical translation of silk-based nerve conduits. However, the lack of appropriate comparisons with the existing methods demands standardization of gap defect length and effective use of controls across animal experiments. More importantly, future clinical trials should be correlated with the benchmark nerve autograft. SSF-based nerve scaffolds should be optimized with an inclusive approach involving cells and suitable biologics to improve Schwann and neuronal cell activity. However, the influence of neurotrophic growth factors on the silk scaffold,

including dosage and long-term effect, and whether neurotrophic factors might accomplish the same benefits as cell-loaded structures are also to be evaluated for a clear picture to establish the advantage of SSF-based scaffolds over the existing strategies. To completely utilize their potential in silk-based materials, additional study is necessary to exploit their cost-benefit balance properly. Integrating biomolecules also limits the use of high temperatures and strong chemicals during processing, causing difficulty in scaffold creation. Another option is to regulate protein adhesion and release kinetics by immobilizing or embedding such biomolecules. However, FDA has yet to approve any of these medicines for use in nerve restoration.

Electroactive moieties inside SSF-based scaffolds might be used as passive electrical stimulators, which could help with nerve regeneration. Although SSF-based electroconductive nerve scaffolds can be designed, concerns about degradability and toxicity need to be addressed to determine their appropriateness. There is excellent scope for further studies on silk-based electroconductive scaffolds utilizing varieties of SSF with or without conjunction with external electrical stimulation. Moreover, NmSF having cell affinitive RGD motifs is yet to be explored extensively for PNR.

To fully exploit the potential of silk-based materials, especially silkworm silk fibroin (SSF), in peripheral nerve regeneration, several key research areas and issues must be addressed. Long-term research is required further to understand SSF's biodegradation and biocompatibility within the body, assuring its safety over time. SSF's mechanical characteristics must be optimized to mimic natural nerve tissues better, and cost-effective production methods must be developed for large-scale clinical use. The fabrication of electroconductive SSF composites may boost nerve signal transmission, and the integration of bioactive molecules, such as growth factors, into SSF scaffolds, may augment nerve healing. Another hurdle is complying with regulations, which necessitates extensive safety profiles and clinical testing. Further promising developments include the design of biological signal-responsive smart nerve guiding channels and customizing SSF materials for each patient. Ultimately, for SSF-based products to be used in clinical settings, it will be crucial to address ethical and regulatory issues early in the development process. These research areas will help make SSF viable options for widespread use in nerve regeneration by balancing cost-benefit, performance, and regulatory requirements.

In conclusion, while significant research is being conducted in the field of SSF for PNR, and new technologies are constantly emerging, increased efforts on novel strategies would promote the development of SSF-based scaffolds that can address the biology of the regenerating nerve. Furthermore, further study is needed to overcome the present regulatory constraints of bringing an SSF-based nerve conduit into the clinic before a tissue engineering nerve conduit technique can replace the gold standard of nerve autografts. It should be remembered, however, that the wounded location is only one of many parts of the neurobiology of the nerve injury–repair process, and additional measures should be considered for patients to restore complete capability (i.e., rehabilitation therapy).

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Author Contributions

Jitu Mani Das: Writing original draft and Editing, Conceptualization, Literature Search, Formal Analysis; Isha Behere: Writing and Editing, Revision, Literature Search, Formal Analysis; Jnanendra Upadhyay: Writing original draft; Rajiv Borah: Writing original draft and Editing, Formal Analysis, Conceptualization, Literature Search, Formal Analysis, Funding acquisition; Ganesh Ingavle: Writing original draft and Editing, Literature Search, Formal Analysis, Conceptualization, Formal Analysis, Funding acquisition, Supervision.

Competing Interests

The author has declared that no competing interests exist.

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