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ARTICLE

Tissue-Engineered Neural Stem Cell Scaffolds in Spinal Cord Injury: Insights into Materials, Molecular Pathways, and Regenerative Applications

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ABSTRACT

Spinal cord injury (SCI) poses significant regenerative challenges because the central nervous system (CNS) has a limited intrinsic ability to repair itself after damage. The complex nature of SCI, including neuronal loss, glial scarring, and disrupted neural pathways, makes effective treatment difficult. In recent years, stem cell–based scaffolds have emerged as a promising therapeutic strategy aimed at facilitating functional recovery. These scaffolds provide a supportive three-dimensional (3D) structure that closely mimics the natural extracellular matrix (ECM) of the spinal cord. This biomimetic environment plays a crucial role in enhancing the differentiation of neural stem cells (NSCs). By guiding NSC behavior and integration into the injured spinal tissue, these scaffolds can help restore some degree of neural function. The synergy between stem cells and engineered scaffolds offers a multifaceted approach to spinal cord regeneration and holds substantial potential for clinical applications. A variety of biomaterials including natural and synthetic polymers, as well as hydrogels, have been developed for this purpose, often enhanced by growth factors, neurotrophic

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agents, and electrical stimulation to boost axonal regeneration and remyelination. Key signaling pathways like Notch, Wnt/β-catenin, Shh, and BMP play a role in guiding NSC differentiation and are being explored as therapeutic targets. Preclinical studies have shown functional improvements with scaffold-assisted cell delivery, and early clinical trials using collagen scaffolds with umbilical cord–derived MSCs show promising results. However, challenges such as immune response, scaffold degradation, and cost remain, highlighting the need for further research to ensure safe and effective clinical application.

Keywords: Neural Stem Cells; Stem Therapy; Neurotrophic Factors; Polyethylene Glycol; Collagen Scaffolds; Spinal Cord Injury Repair

1. Introduction

Stem Cell-Scaffold Therapies for Spinal Cord Injury Spinal cord injury (SCI) is a profoundly debilitating condition, associated with high rates of morbidity and mortality globally. Recent data indicate that the incidence of traumatic SCI is approximately 26.5 cases per million people, with a higher prevalence in males. Around half of all SCIs affect the cervical spine and are linked to increased mortality, particularly among the elderly. In the United States, leading causes of SCI include motor vehicle collisions, sports-related trauma, and accidental falls [1].

The development of SCI is believed to occur in two distinct phases: primary and secondary injury. The primary injury refers to the direct mechanical trauma to the spinal cord, whereas the secondary phase involves a series of acute and chronic processes such as immune system activation, neuroinflammation, and excitotoxicity. Key mechanisms underlying secondary SCI include lipid peroxidation, axonal damage and loss of myelin, elevated calcium influx, generation of free radicals, and abnormal remodeling of the extracellular matrix [2].

The extent of secondary injury is thought to significantly impact the overall severity of SCI, underscoring its importance as a potential therapeutic target. Nonetheless, further research is necessary to better understand the inflammatory responses in SCI and to determine which cellular and molecular players support or hinder the healing process. Neural regeneration after axonal damage is a highly intricate process that involves the coordinated activity of various proteins, signaling pathways, and gene expressions. The regeneration process begins with the rapid resealing of the damaged plasma membrane, followed by the development and stabilization of an axonal growth cone. Several neurotrophic factors, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and nerve growth factor (NGF), play a key role in promoting axonal regrowth by acting through tyrosine kinase receptors. Additionally, intraoperative electrical stimulation (ES) has emerged as a potential therapeutic strategy to support this regenerative process, with the potential to stimulate axonal regrowth [3,4].

Although neurons in the central nervous system have limited regenerative capacity following injury, restoring neurological function remains a primary objective in spinal cord injury (SCI) treatment. The current standard approach for managing acute SCI involves the use of pharmacological agents such as paracetamol, mild opioids, or non-steroidal anti-inflammatory drugs (NSAIDs) [5]. In certain situations, surgical procedures like spinal decompression may be required. However, the literature presents conflicting evidence regarding the impact of surgical timing on patient outcomes. One study reported that early decompression—within 8 to 12 hours post-injury—was linked to improvements in at least one grade on the American Spinal Injury Association Impairment Scale (AIS), irrespective of injury level or severity. Another study involving many patients similarly found that undergoing surgery within 24 hours was associated with better functional recovery. In contrast, other research suggested that surgical timing had no significant effect on AIS grade improvement six months after cervical SCI [6].

Consequently, SCI treatment protocols often vary based on individual patient factors and institutional guidelines. To enhance patient-specific outcomes, innovative therapeutic approaches for spinal cord injury (SCI) have

focused on the use of stem cell-based scaffolds. This technique involves constructing a three-dimensional framework that mimics the native extracellular matrix of the spinal cord tissue. These scaffolds provide a biocompatible environment that supports stem cell attachment, growth, and differentiation, offering a promising strategy for SCI repair [7]. Various types of scaffold materials have been investigated, including hydrogels, natural and synthetic polymers, and composite structures. However, most of these models are still in the experimental or preclinical phase, indicating a clear need for further research and development. Therefore, this review seeks to explore the current landscape of stem cell scaffold models for SCI and evaluate their potential for future clinical application [8].

2. Identifying the Research Gap and Authorial Perspective

Although numerous therapeutic strategies have been investigated for SCI ranging from neuroprotective pharmaceuticals and stem cell transplantation to surgical decompression these modalities have consistently fallen short in achieving durable neurological recovery [3]. A significant limitation of most existing treatments is their inability to concurrently offer both mechanical support and biologically active cues required for axonal regrowth and functional restoration [6]. Among emerging therapies, stem cell-based scaffolds represent a promising direction; however, comprehensive insight into the interplay between scaffold composition, neuroregenerative signaling pathways, and clinical translatability remains insufficient. This review endeavors to address this gap by integrating current knowledge on the design and application of stem cell-laden scaffolds, with particular emphasis on material selection, molecular guidance cues, and engineering strategies [8]. The authors advocate for a synergistic framework that combines advances in tissue engineering with cellular and molecular neuroscience to accelerate the transition of scaffold-based therapies from laboratory research to clinical application.

2.1. Specificity of Neural Stem Cells (NSCs)

The process of neural stem cell (NSC) differentiation begins when these stem cells develop into either neural or glial progenitor cells. Neural progenitors have the potential to further specialize into various types of mature neurons, while glial progenitors give rise to oligodendrocytes and astrocytes. In contrast, microglia originate from hematopoietic lineage rather than neural stem cells. Each stage of differentiation is marked by specific surface markers, which can be utilized to track the progression of NSC differentiation into distinct neural cell types. This differentiation process is tightly regulated by multiple signaling pathways and transcription factors that play key roles in both embryonic neural development and adult neurogenesis (**Figure 1**). Among the most critical pathways involved are Notch, Wnt/β-catenin, Sonic Hedgehog (Shh), and bone morphogenetic protein (BMP) signaling cascades [9,10].

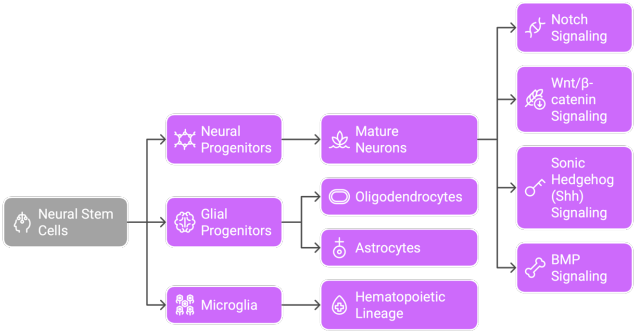

Figure 1. Neural stem cells differentiate into neurons or glial cells via regulated pathways, excluding microglia, marked by surface markers and signaling.

The Notch signaling pathway serves a critical function in inhibiting the differentiation of neural stem cells (NSCs). This regulatory mechanism begins when ligands bind to Notch receptors located on the surface of NSCs. This binding initiates a proteolytic cleavage of the receptor, resulting in the release of the Notch intracellular domain (NICD) into the cell's cytoplasm [11]. The NICD then translocates into the nucleus, where it binds to the DNA-binding protein RBPj, forming the NICD-RBPj complex. This complex activates the transcription of basic helix-loop-helix (bHLH) repressors, such as members of the hairy and enhancer of split (Hes) family. These repressors inhibit the expression of key pro-differentiation transcription factors, preventing the progression of NSCs into specialized neural lineages. Associated with spinal cord injury (SCI), the Notch signaling pathway becomes activated, which may contribute to the inability of neural stem cells (NSCs) to differentiate into fully functional neurons at the injury site. Consequently, targeting and inhibiting Notch

signaling presents a promising therapeutic approach to enhance neurological recovery and alleviate SCI-related symptoms [12].

Various strategies for inhibiting Notch signaling have been explored, including electroacupuncture, transplantation of oligodendrocyte precursor cells, and bone marrow-derived mesenchymal stem cell transplantation. Blocking Notch signaling has been associated with increased neuronal proliferation and differentiation at the lesion site, normalization of disrupted protein expression levels, and reduced activation of neurotoxic astrocytes [13]. Therefore, therapies aimed at inhibiting the Notch pathway may offer significant benefits in the management of SCI. Wnt/β-Catenin Signaling The canonical Wnt/β-catenin signaling pathway plays a crucial role in promoting the differentiation of neural stem cells (NSCs) during both embryonic development and in the adult nervous system. Wnt proteins are secreted and bind to the Frizzled receptor, along with the co-receptor low-density lipoprotein receptor-related protein 5/6 (LRP5/6), forming a receptor complex at the cell membrane. This interaction results in the inhibition of glycogen synthase kinase-3β (GSK-3β), a key enzyme involved in diverse biological processes [14]. Under normal conditions, without Wnt signaling, GSK-3β activity leads to β-catenin phosphorylation, tagging it for degradation. However, when GSK-3β is inhibited by Wnt signaling, β-catenin becomes stabilized and translocates into the nucleus, where it binds to T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) transcription factors.

This interaction drives the expression of several genes that support NSC differentiation. Studies have shown that Wnt signaling is upregulated at the site of spinal cord injury (SCI) following trauma. Given its pivotal role in stem cell differentiation, therapies that stimulate this pathway may hold therapeutic promise for SCI recovery. For instance, enhancing the expression of miR-124, a gene that regulates NSC differentiation and proliferation, has been shown to promote recovery in SCI models by activating the Wnt/β-catenin pathway [15]. Similarly, compounds like salvianolic acid B, sirtuin-1, and rapamycin have shown the ability to enhance neurological function and stimulate the differentiation and proliferation of neural stem cells (NSCs) by activating the Wnt/β-catenin signaling pathway. Collectively, these results highlight the therapeutic promise of modulating this pathway in the treatment of spinal cord injury (SCI). Shh Signaling Sonic hedgehog (Shh) signaling plays a critical role in various developmental and regenerative processes, including limb formation, early development of the central nervous system (CNS), and the differentiation of adult neural stem cells (NSCs) [16].

In the adult brain, Shh signaling is known to influence NSC differentiation and migration within the subventricular zone adjacent to the lateral ventricles. This signaling cascade is initiated when Shh ligands bind to the Patched (Ptch) receptor, which in turn activates the G protein-coupled receptor-like protein Smoothened (Smo). Activation of Smoothened triggers downstream signaling involving transcription factors from the Gli family, which promote NSC proliferation and differentiation. Although direct evidence linking Shh signaling to spinal cord injury (SCI) is currently limited, emerging research suggests that upregulation of Shh signaling may have therapeutic benefits in other neurological conditions, such as traumatic brain injury and cerebral ischemia. These findings indicate that enhancing Shh requires further investigation to confirm its application in SCI [10]. BMP Signaling in SCI Evidence has shown that BMP signaling is pathologically upregulated at spinal cord injury (SCI) sites, contributing to poor functional recovery, lipid peroxidation, increased cellular apoptosis, extracellular matrix disruption, and limited axonal regeneration (**Figure 2**).

Due to these detrimental effects, targeting BMP signaling for inhibition has emerged as a potential therapeutic strategy for SCI. This concept has been explored mainly in mouse models using noggin, a naturally occurring BMP antagonist. Studies have shown that noggin-mediated BMP inhibition leads to improved locomotor performance and substantial corticospinal tract regeneration after spinal contusion. Other research demonstrated that early BMP suppression via noggin enhanced remyelination, oligodendrocyte formation, and short-term neurological recovery in rats with SCI [13]. However, it is worth noting that noggin administration did not lead to sustained long-term recovery, suggesting its therapeutic benefit may be limited to the acute phase of SCI. Despite this limitation, the results support the therapeutic potential of BMP inhibitors in restoring neurological function and promoting axonal repair following SCI [17].

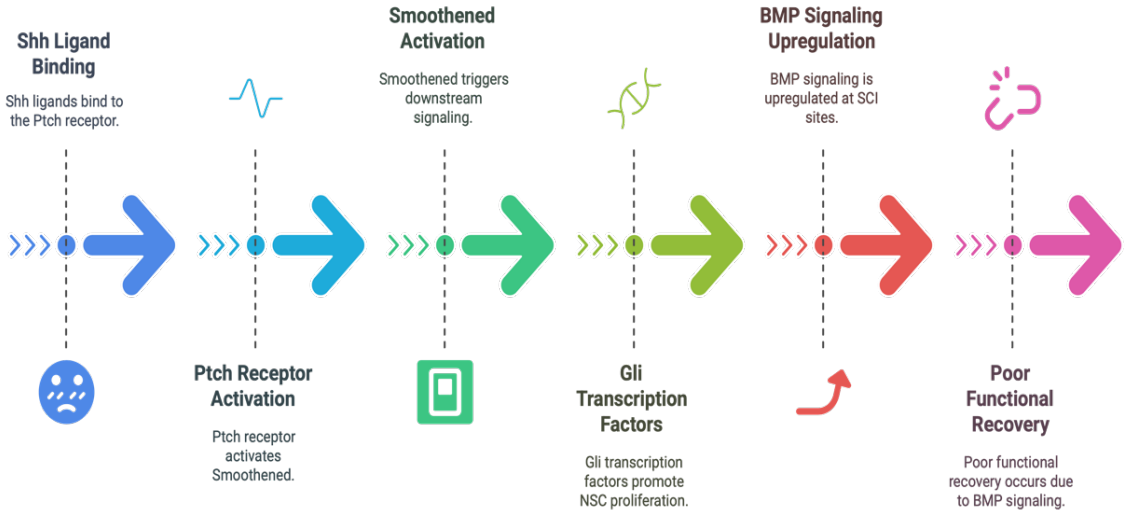

Figure 2. Shh signaling promotes adult NSC differentiation, potential SCI therapy. BMP signaling in SCI impairs recovery through apoptosis, disruption, and degeneration.

2.2. Axonal Regeneration After Injury

Axonal regeneration in three dimensions (3D) following injury is a highly intricate process influenced by a range of genes, signaling pathways, proteins, and components of the extracellular environment. Successful axonal regrowth begins when the primary neuron becomes physically separated from its distal target. Once this disconnection occurs, the proximal segment of the axon—still attached to the neuronal cell body—enters a regenerative phase. During this phase, an axonal growth cone is formed [4].

This dynamic, motile structure navigates the extracellular environment using available signaling molecules and growth factors to guide axonal elongation and direct regrowth. Plasma Membrane Sealants Following axonal injury, rapid repair of the damaged plasma membrane is crucial to prevent intracellular contents from leaking out or harmful extracellular substances like calcium ions (Ca^{2+}) from entering the cell [18]. The sealing process occurs naturally in vivo through physical mechanisms such as line tension generated by hydrophobic interactions at the lipid edges, along with membrane tension shaped by cytoskeletal arrangement and curvature of the membrane. However, research has shown that this repair can be accelerated using external agents like hydrophilic polymers (e.g., polyethylene glycol) or surfactants such as poloxamers. Polyethylene glycol (PEG) has demonstrated the ability to artificially seal severed axons and circumvent calcium-dependent processes to facilitate the fusion of adjacent cells [19].

The addition of methylene blue to PEG has been found to enhance this sealing effect, suggesting that methylene blue may also be used to evaluate the safety and performance of PEG-based scaffolds in clinical trials. Moreover, PEG-induced axonal fusion has led to improved behavioral outcomes and reinforced neuromuscular structures in rat models of spinal cord injury (SCI). These findings underscore the therapeutic potential of PEG in plasma membrane repair after axonal damage [7]. However, the effects of other stem cell scaffold types—such as those made from natural polymers, synthetic materials, or composites—on membrane sealing after injury are not yet well understood. Further investigations are needed to explore how these scaffolding materials contribute to membrane repair, which will enhance our understanding of their safety, effectiveness, and functional capacity in SCI treatment. Formation and Stabilization of the Growth Cone Following successful plasma membrane repair, axonal regeneration progresses with the development of the growth cone—a dynamic, specialized structure similar to that observed during early neurodevelopment. This motile structure adopts a fan-like shape and plays a crucial role in navigating the regenerating axon by responding to extracellular signals. It achieves this through intricate interactions between the cytoskeletal components, namely actin filaments and mi-

crotubules [20].

Structurally, the growth cone comprises a central core of microtubules and a peripheral region dominated by actin filaments. Microtubules are key players in driving axonal extension and are essential for steering the axon toward external cues, such as growth factors and signaling molecules—a process referred to as axonal turning. Similarly, the backward movement of the actin cytoskeleton relative to the microtubules generates traction, enabling more accurate and regulated axonal extension [21]. This interaction not only supports axon elongation but also plays a critical role in maintaining the stability of the growth cone. Stability is essential during axonal regeneration, as instability in microtubule structures often leads to axonal retraction and the formation of retraction bulbs—structures that halt the regeneration process (**Figure 3**). Introducing external agents that enhance microtubule stability could support the proper formation and directionality of growth cones during axonal repair. For instance, paclitaxel (taxol), a well-known chemotherapy drug, stabilizes microtubules and shows promise in promoting growth cone integrity [22].

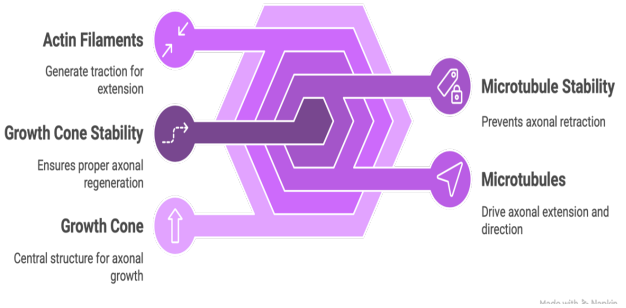

Figure 3. Growth cone structure relies on microtubules and actin; stability supports axon growth. Paclitaxel may aid axonal regeneration by stabilizing microtubules.

Research has shown that low concentrations of taxol increase neurite outgrowth in an environment mimicking spinal cord injury and reduce the occurrence of retraction bulbs, which often hinder regeneration. Therefore, incorporating microtubule-stabilizing compounds like taxol into stem cell scaffolds may significantly enhance growth cone development and stability. For example, collagen-based neural stem cell scaffolds fortified with taxol have been shown to support neural repair, demonstrating the potential of scaffold-based strategies to improve axonal regeneration outcomes [9]. Neurotrophic Factors in Axonal Regrowth and Directionality The axonal growth cone formation and stabilization in regeneration are essential in the regulation of the axonal guidance process. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and nerve growth factor (NGF) play a vital role in supporting neural growth, cell survival, and directional axon regeneration [23].

These molecules act through specific tyrosine kinase receptors—trkA, trkB, and trkC—each of which activates key downstream signaling pathways including the mitogen-activated protein kinase (MAPK) and phospholipase C-γ (PLC-γ) pathways to facilitate axonal regrowth. For example, NGF specifically binds to trkA, triggering its dimerization and activation, which in turn initiates the MAPK, PLC-γ, and phosphatidylinositol 3-kinase (PI3K) signaling cascades. Once this NGF/trkA complex is internalized via clathrin-mediated endocytosis or pincher-mediated micropinocytosis, it undergoes retrograde transport and promotes axonal repair and regeneration [22]. BDNF binds to trkB, leading to localized translation of actin mRNA and directing axonal growth cone movement through chemoattraction. Applying BDNF externally to injured axons has been shown to enhance actin transport, support growth cone formation, and encourage forward extension. NT3 binds primarily to trkC, stimulating gene expression that supports neural stem cell (NSC) survival and differentiation. Although its main receptor is trkC, NT3 can also interact with trkA and trkB, albeit with lower affinity [24].

The logical starting point of exploring neurotrophic factors for promoting axonal regrowth is their proven ability in both in vitro and in vivo settings to support neural survival and guide regeneration. However, their use must be carefully controlled, as excessive or misdirected neural growth could potentially impair rather than enhance function [3]. Future research should focus on examining how stem cell scaffolds influence the concentration and biological activity of neurotrophic factors to enhance the safety and effectiveness of existing and novel scaffold-based treatments for spinal cord injury. Matrix Vehicles for Axonal Regeneration While neurotrophic factors alone are effective in supporting axonal regeneration, their impact is significantly amplified when delivered within a matrix vehicle. These matrices offer a controlled environment that not only provides targeted access to growth factors for the

regenerating growth cone but also restricts their diffusion, thereby minimizing the risk of abnormal or misplaced neural growth [25].

For instance, type I collagen filaments—originating from the extracellular matrix involved in axonal repair—have demonstrated the ability to support axonal reconnection across gaps as long as 30 mm. Collagen is believed to function both as a scaffold for new blood vessel formation and as a physical guide for axonal extension during regeneration. Contemporary matrix vehicles such as polyethylene glycol (PEG), fibrin, and peptide-based hydrogels are engineered to deliver essential growth factors and stem cells directly to the site of spinal cord injury in a biocompatible and individualized manner. For example, PEG can be chemically cross-linked to form hydrogels capable of carrying bone marrow mononuclear cells and growth factors directly to the lesion site [26].

These hydrogel matrices can be further optimized by incorporating proteins and neural stem cells tailored to the patient's specific biochemical environment, thereby enhancing the overall regenerative outcome [8]. Biomimetic, self-assembling peptide hydrogels such as Fmoc-DIKVAV, enriched with neural stem cells, have been shown to create a supportive microenvironment for axonal regeneration and lead to improvements in motor function in rodent models. Additionally, hydrogels incorporating fibrin—a fibrous protein formed from fibrinogen—have shown potential in enhancing axonal regrowth and promoting functional recovery in rodents following spinal cord injury. However, it is important to emphasize that most studies involving hydrogel-based matrix vehicles are still in the preclinical research stage, indicating a clear need for further investigation before such approaches can be adopted in clinical settings [25].

3. Axonal Growth Stimulation as a Strategy

Electrical stimulation (ES) has demonstrated the potential to support axonal regeneration, although the underlying mechanisms are not yet fully understood. It is believed that ES mimics the retrograde flow of intracellular calcium waves directed toward the neuronal soma, a process crucial for action potential transmission and axonal growth. Additionally, ES has been shown to enhance the expression of recombination activating genes (RAGs) within the neuron, which are essential for axonal elongation and regrowth, by promoting the production of brain-derived neurotrophic factor (BDNF) (**Figure 4**). This calcium-dependent increase in BDNF expression simultaneously boosts the levels of Tα1 tubulin and growth-associated protein-43, while also inhibiting Rho signaling—all contributing to improved cytoskeletal organization [27].

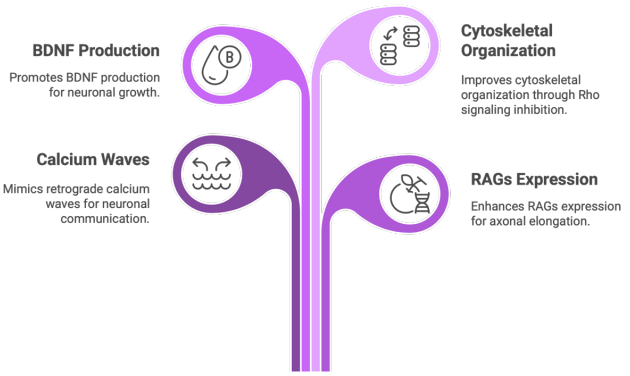

Figure 4. Electrical stimulation promotes axonal regeneration by enhancing calcium signaling, BDNF expression, cytoskeletal proteins, and inhibiting Rho signaling for growth.

Moreover, pathways such as the p38 mitogen-activated protein kinase (MAPK) cascade may further assist neurite extension by activating the cAMP response element-binding protein (CREB). Numerous murine studies have examined the impact of electrical stimulation (ES) on axonal regeneration. For instance, one hour of intraoperative ES at 20 Hz has been shown to significantly enhance regeneration and branching of dorsal root ganglia neurons in mice with complete femoral nerve transections. This stimulation is also linked to increased expression of growth-associated protein 43 (GAP-43) mRNA two days post-repair [28].

Similarly, other studies observed greater axonal density, enhanced nerve regeneration, and elevated macrophage recruitment in rats treated with 16 Hz ES for one hour. In another study involving mice with complete tibial nerve resections, just 10 minutes of 16 Hz intraoperative ES significantly promoted axonal regrowth and functional recovery. Supporting these findings, notable improvements in motor function were observed following 10 minutes of intraoperative ES in rats treated with isografts for sciatic nerve transections. Several clinical trials are currently underway to assess the effects of intraoperative ES on axonal regeneration in human subjects. Given the regenerative potential of stem cell scaffolds, it is plausible that a com-

binatorial approach involving ES followed by scaffold implantation could synergistically enhance spinal cord injury (SCI) recovery. Future investigations should focus on identifying the optimal parameters of timing, duration, and frequency in ES and determine the types and scaffold materials prior to clinical translation [29,30].

4. Stem Cell Scaffolds

Stem cell scaffolds are artificially engineered 3D frameworks that replicate and regulate essential features of the natural extracellular matrix (ECM). The scaffolds serve as a supportive platform for stem cell adhesion, proliferation, and differentiation. The major classification types include hydrogels, natural polymers, synthetic polymers, and composite materials. A critical advantage of stem cell scaffolds is their adaptability for targeted uses. Key modification strategies discussed here include integrating growth-regulating compounds, cell-adhesion surface molecules, and electrical stimulation techniques. A fundamental role of scaffolds is to create a supportive three-dimensional structure for stem cell growth [31].

Following spinal cord injury (SCI), this framework safeguards developing cells and axons from mechanical stresses. Porosity—the density of pores within the scaffold material—represents a critical design parameter as it directly influences available surface area for cellular attachment. Research indicates that elevated porosity levels correlate with improved stem cell proliferation and differentiation outcomes. Current literature has not yet established optimal pore dimensions for SCI applications [29].

However, considering that corticospinal tract axons measure 1–22 μm in diameter (with most between 1–4 μm), scaffolds require minimum pore sizes of 22 μm to accommodate all axonal types. Furthermore, additional space may be necessary to ensure proper nutrient/waste exchange and to permit regeneration of larger neural structures including pyramidal tract axons, Purkinje cell projections, and Aα/Aβ sensory fibers. Notably, the porosity enhancements must be balanced against corresponding reductions in mechanical properties like compressive strength and elastic modulus. An equally important scaffold characteristic involves controlled biodegradation that produces minimal toxic byproducts. This gradual breakdown enables natural ECM replacement while mitigating chronic inflammation, promoting continued tissue regeneration, and facilitating sustained release of therapeutic growth factors [18].

The biologically derived molecules of polymer scaffolds are fabricated to offer superior cell adhesion and biocompatibility compared to synthetic alternatives. Their natural abundance also makes them more cost-effective and easier to produce. In spinal cord injury (SCI) applications, these scaffolds have demonstrated potential in restoring motor function and stimulating axonal regeneration. Common materials for natural polymer scaffolds include collagen, chitosan, gelatin, fibrin, and alginate [30].

Among these, collagen, fibrin, and gelatin have been identified as particularly effective for stem cell scaffolding in rat SCI models. As the primary structural protein in the extracellular matrix (ECM), collagen consists of α-chains that assemble into triple-helical fibrils. This natural polymer offers exceptional biocompatibility and complete biodegradability, with versatile fabrication methods including electrospinning, 3D printing, and hydrogel formation. Through denaturation, collagen yields gelatin—a derivative that maintains comparable biodegradability and biocompatibility but loses the triple-helix configuration [31].

This structural alteration enables random α-chain polymerization into gel networks with enhanced hydrophilicity and tunable gel densities compared to native collagen. Gelatin has demonstrated particular advantages for SCI applications through cost-effective 3D printing of implantable microsphere scaffolds with high reproducibility, suggesting strong potential for clinical translation. Fibrin, the natural clotting matrix formed from thrombin-mediated fibrinogen polymerization, also demonstrates remarkable regenerative capabilities.

Fibrin scaffolds seeded with mesenchymal stem cells have been shown to significantly enhance axonal regeneration, remyelination, and motor recovery in SCI models, positioning it as a leading candidate for neural repair applications. Scaffolds of Synthetic Polymer Stem cell scaffolds can be created using various synthetic materials, such as poly(lactic-co-glycolic acid), polycaprolactone, polyethylene glycol, and others. These synthetic polymers offer significant advantages, including a wide array of customizable mechanical properties like improved strength and durability. Additionally, synthetic scaffolds are well-suited for mass production through advanced manufacturing tech-

niques such as 3D printing (inkjet, stereolithography, fused deposition modeling) and micro-extrusion [32].

In the context of spinal cord injury (SCI), these scaffolds can effectively incorporate complex biomaterials, such as superparamagnetic iron oxide nanoparticles [13]. The sophisticated nature of synthetic polymer scaffolds supports enhanced nerve fiber regeneration and the recovery of motor function after SCI, potentially offering benefits beyond those of natural polymer scaffolds. However, a challenge lies in the fact that the materials used for synthesis might harm, break down, or diminish the effectiveness of any therapeutic agents embedded within them. Therefore, it is crucial to identify the best polymer composition before proceeding with experiments in living organisms (in vivo). Hydrogels Hydrogels, composed of water-attracting polymers that may be either synthetic or natural, offer several benefits as scaffolding materials for stem cells. They are typically characterized by high permeability, compatibility with biological systems, and the ability to break down naturally over time [22].

A key advantage of hydrogels is their capability to transition from a liquid to a gel state after being injected into a damaged site—referred to as injectable hydrogels. This feature makes them an appealing option for therapeutic applications due to their ease of administration. Additionally, hydrogels often create environments that support cell survival and growth, which is essential for developing neural stem cell (NSC) scaffolds for spinal cord injury (SCI) treatment. Nevertheless, one of the main challenges with hydrogel-based scaffolds in living systems is the potential toxicity from certain substances used during their production [33].

These substances—such as stabilizers, initiators, organic solvents, and emulsifiers—can harm or kill healthy cells near the injury site. Recent advancements have focused on developing hybrid or composite scaffolds by combining two or more biomaterials, allowing researchers to harness the strengths of each material and optimize scaffold performance. For instance, incorporating a gelatin-based hydrogel with the synthetic polymer polycaprolactone has demonstrated enhanced structural support and guidance for axonal regeneration [17]. Likewise, embedding a conductive polymer within a photocrosslinkable gelatin/polyethylene glycol matrix improves scaffold stability and enables more efficient delivery of neural stem cells to SCI sites. Because composite scaffolds consist of varied components, their synthesis, compatibility with biological systems, and biodegradability can differ significantly depending on their formulation. Nonetheless, the strategy of merging multiple biomaterials into a single scaffold offers advantages over traditional scaffolds made from a single natural or synthetic polymer [19].

Composite scaffolds can incorporate a broader array of functional materials to better support axonal repair and offer more tailored treatment options for SCI patients. Moreover, they hold the potential to fulfill both the mechanical and biological requirements necessary for safe and effective use in living organisms. That said, composite scaffolds come with challenges. Their complexity often makes them more costly and less efficient to manufacture compared to simpler, single-material scaffolds. Additionally, the intricate nature of their design may require extended development time before they are ready for in vivo testing [34].

5. Growth-Modulating Factors

Incorporating growth-modulating factors into stem cell scaffolds presents a powerful approach to enhance neural, axonal, and vascular regeneration and integration. This strategy offers multiple benefits. One major advantage is that as the scaffold gradually degrades, it can release a controlled and sustained amount of growth factors directly at the injury site. This localized delivery is especially beneficial since growth factors typically degrade rapidly within biological tissues. Additionally, because the growth factors are delivered locally and in smaller quantities, the risk of systemic side effects is reduced compared to full-body treatments [30].

Brain-derived neurotrophic factor (BDNF) is one such growth factor that supports the survival and differentiation of neural stem cells. Other factors—such as glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor 1 (IGF-1), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF)—are also known to promote blood vessel formation and tissue repair after injury (**Figure 5**). Recently, neurotropin has drawn attention for its potential role in SCI recovery due to its ability to regulate cytokine activity and reduce cell death (ap-

optosis). Another promising factor, neuregulin-1, supports recovery by engaging the Nrg-1/ErbB signaling pathway, which plays a role in NSC differentiation, neuronal migration, and myelin formation. Overall, due to their critical roles in supporting axonal growth and repair, integrating growth-modulating factors into stem cell scaffolds could significantly enhance the effectiveness of various scaffold designs for spinal cord injury treatment [35].

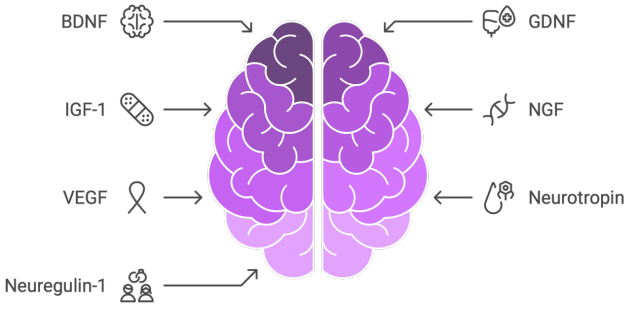

Figure 5. Growth factors like BDNF, GDNF, IGF-1, NGF, and VEGF aid NSC survival, tissue repair, and axonal growth; neurotropin and neuregulin-1 show SCI potential.

6. Latest Application

Previous attempts at SCI repair have spanned a wide range of strategies, including pharmacological interventions, surgical decompression, and cell-based therapies [33]. Pharmacological approaches, such as the administration of corticosteroids like methylprednisolone, aimed to reduce inflammation and limit secondary damage, but their long-term efficacy and safety have been questioned. Surgical interventions, including early spinal decompression, have shown mixed results, with some studies suggesting benefits in neurological recovery when performed within a narrow time window post-injury [15]. Early stem cell therapies, using mesenchymal stem cells or olfactory ensheathing cells, demonstrated modest improvements in motor function but often faced challenges related to cell survival, integration, and immune rejection. Moreover, efforts involving biomaterial scaffolds without cellular components provided mechanical support but lacked the biological cues necessary for true regeneration (**Table 1**). These limitations have driven the evolution toward more sophisticated tissue-engineering strategies, such as neural stem cell–loaded scaffolds, which aim to combine structural support with regenerative signaling to better mimic the natural healing environment of the spinal cord [30].

Table 1. Comparative Overview of Prominent SCI Treatment Strategies.

Treatment Approach	Mechanism of Action	Delivery Method	Clinical Stage	Advantages	Limitations
Stem Cell–Loaded Scaffolds	Combines structural support and regenerative signalling for axonal regrowth and remyelination	Surgical implantation at injury site	Preclinical to Early Clinical Trials	Supports NSC survival, guides axonal growth, controlled release of growth factors	Invasive, costly fabrication, immune response, degradation timing must be optimized
Stem Cell Injections	Promotes regeneration via paracrine signalling, immune modulation, and neuronal replacement	Intrathecal or intravenous injection	Early to Mid-Clinical Trials	Minimally invasive, autologous sources available, modulates inflammation	Poor engraftment/survival, limited control over localization, short-term benefits
Neuroprotective Drugs	Reduces inflammation, excitotoxicity, and oxidative stress post-SCI	Systemic (oral or IV)	Approved or in Advanced Trials	Rapid administration, widely available, low cost	Limited regeneration capacity, time-sensitive efficacy, systemic side effects
Electrical Stimulation	Enhances neural plasticity, axonal regeneration, and growth factor expression	Intraoperative or external application	Early Clinical Trials	Non-pharmacological, enhances other therapies' effects	Requires precise timing/dosing, unclear long-term effects, equipment-dependent
Scaffold Alone (Acellular)	Provides mechanical support and guidance for axons	Surgical implantation	Preclinical	Biocompatible materials, customizable architecture	Lacks biological cues, no active regenerative signalling

Due to the promising therapeutic potential of scaffold-based strategies for spinal cord injury (SCI), a wide range of preclinical studies have explored different scaffold constructs for their effectiveness in SCI repair. Collagen—the most prevalent protein in mammals—is essential for forming connective tissues like skin, bone, muscle, tendons, and cartilage. Its favourable properties, including high biocompatibility, hydrophilicity, abundance in body tissues, and strong cell adhesion capabilities, make it an ideal material for scaffold fabrication [36]. Consequently, collagen-based stem cell scaffolds have been extensively investigated in the context of SCI treatment. These scaffolds have demonstrated the ability to attract and safeguard embryonic neural stem progenitor cells (NSPCs) at the injury site, while also promoting neural stem cell (NSC) adhesion, proliferation, and differentiation. When infused with NSPCs, the effectiveness of collagen scaffolds is significantly enhanced—leading to improved axonal extension, neural regeneration at the lesion, NSPC maturation, and functional integration into the existing neural circuitry [28].

In rat models of complete spinal cord transection, this strategy has shown significant improvements in hindlimb motor function, nerve repair, and neural outgrowth. Collagen scaffolds used for spinal cord injury treatment can be further enhanced by incorporating patient-derived bone marrow mononuclear cells or mesenchymal stem cells (MSCs). In animal studies involving mice and dogs with complete spinal cord transections, collagen scaffolds seeded with MSCs from neonatal umbilical cord tissue led to improved motor function and a reduction in the size of the injury site. Building on these findings, a phase I clinical trial involving 40 patients with acute, complete cervical SCI was conducted to assess the safety and effectiveness of this treatment approach [37].

While preclinical studies using animal models have provided valuable insights into SCI repair, these models present inherent limitations when extrapolating to human applications. Differences in spinal cord anatomy, immune response, and injury mechanisms between rodents and humans can result in variable therapeutic outcomes, making it difficult to predict clinical efficacy. Furthermore, many animal studies involve acute injuries, whereas human SCI patients often present with chronic conditions that are more complex and less responsive to regenerative interventions [35]. Another critical factor influencing long-term outcomes is scaffold degradation. Although biodegradable scaffolds are designed to be gradually replaced by native tissue, inconsistent degradation rates or the release of cytotoxic byproducts can hinder regeneration and provoke inflammatory responses [32]. Balancing scaffold stability with controlled biodegradation is essential to ensure sustained support for neural repair without compromising biocompatibility or therapeutic effectiveness over time [20].

One year after transplantation of the collagen scaffold embedded with human umbilical cord MSCs at the site of injury, patients exhibited new nerve fiber growth and improved electrophysiological responses in nearby neurons. Additionally, enhancements were observed in patients' daily living scores, American Spinal Injury Association (ASIA) assessments, and bowel and urinary function. These findings are particularly important as few clinical studies to date have reported outcomes in human subjects regarding the use of stem cell–loaded scaffolds for SCI therapy. Further support comes from a follow-up study, which evaluated the long-term outcomes (2–5 years) of collagen scaffolds infused with either patient-specific bone marrow mononuclear cells or human umbilical cord MSCs. This study also reported encouraging results, including improved bladder and bowel sensation, restored voluntary walking in some patients, and enhanced finger mobility. Incorporating proteins naturally expressed by mesenchymal stem cells can further improve the performance of collagen scaffolds in SCI treatment. For instance, one study explored the use of a linearly aligned collagen scaffold enhanced with N-cadherin—a protein found in mesenchymal cells [38–40].

This modification resulted in better neural stem progenitor cell adhesion to the scaffold. When implanted into rats with complete spinal cord transections, the N-cadherin-modified scaffolds attracted more NSPCs to the injury site, and animals in this group showed significantly better motor recovery compared to control groups. Collagen scaffolds loaded with MSCs can also be optimized with additional materials such as silk fibroin or heparan sulfate. Silk fibroin, a natural fibrous protein found in silk and spider webs, possesses excellent biocompatibility and mechanical strength, making it a suitable candidate for regenerating tissues like skin, bone, and fat. Compared to unseeded col-

lagen/silk composites, collagen/silk scaffolds seeded with MSCs support enhanced nerve regeneration, improved remyelination, and faster formation of synaptic connections at the injury site. Human umbilical cord-derived MSCs placed onto these collagen/silk fibroin scaffolds have been shown to promote functional improvement and better motor behaviour in rat models of complete SCI [41].

Likewise, heparan sulfate—a polysaccharide that plays a role in cell growth, inflammation control, and blood vessel formation—can further boost scaffold effectiveness. Collagen scaffolds combined with heparan sulfate and MSCs have demonstrated significant gains in locomotor performance, motor-evoked potentials, urinary function, and inflammatory cytokine regulation in canine models with complete spinal cord transections. Advancements in 3D bioprinting have significantly improved the design and effectiveness of stem cell scaffolds for treating spinal cord injuries [42]. This technology allows for the precise fabrication of biomimetic scaffolds that can be customized to match the specific anatomical dimensions of an individual and can be produced rapidly to meet therapeutic timelines. For example, a 3D-printed scaffold composed of sodium alginate and gelatine, embedded with neural stem progenitor cells and oligodendrocytes, has been shown to enhance nerve regeneration and restore hindlimb motor function in rodent SCI models. In a similar approach, scaffolds bioprinted with induced pluripotent stem cells (iPSCs) derived from human urine have demonstrated therapeutic potential in mouse models of SCI [43].

The effectiveness of 3D bioprinted scaffolds can be further improved by incorporating small bioactive molecules. One such innovation involves the integration of OSMI-4, an inhibitor of O-GlcNAc transferase, into the scaffold to promote targeted differentiation of NSCs at the injury site, leading to more efficient spinal cord repair. This enhanced scaffold promoted axonal regeneration and neural repair, resulting in notable improvements in motor function in rats with SCI. These findings underscore the value of 3D bioprinting as a tool for optimizing stem cell scaffold construction and therapeutic application in SCI treatment. Conclusion Injuries to the central nervous system (CNS) pose a significant treatment challenge due to the limited regenerative capacity of CNS neurons. However, stem cell scaffolds offer promising potential to restore neurological function by supporting axonal repair [44].

These scaffolds can be customized using various stem cell types, emphasizing their adaptability and patient-specific design. This flexibility enables clinicians to tailor scaffold-based treatments according to the type, location, and severity of spinal cord injury (SCI). While stem cell scaffolds represent a promising therapeutic avenue for SCI management, most studies are still in the preclinical phase. Further research is essential to fully understand the biological mechanisms behind the success of these scaffolds in animal models and to evaluate their safety and effectiveness in humans [45]. Critical factors that need exploration include identifying the most suitable cell types for specific SCI scenarios, as well as determining the best dosage, timing, and delivery method for transplantation. For instance, intravenous delivery may offer a less invasive and more practical option for patients compared to direct application at the injury site, particularly when repeated treatments are required. These formulation and dosing considerations may pose hurdles for translating experimental findings into routine clinical use. Additionally, combining stem cell scaffolds with emerging therapies—such as biomaterials and extracellular matrix (ECM) modifiers—could enhance outcomes. However, the use of advanced synthetic polymers in scaffold construction could significantly raise treatment costs, potentially limiting patient access [46].

Therefore, affordability must be prioritized to ensure widespread accessibility to these therapies [47]. Neural stem cells (NSCs), which can differentiate into multiple mature cell types, rely on specific signalling pathways for this process. Since scaffold biomaterials are engineered to deliver growth factors critical for axonal regeneration over time, they may also influence these differentiation pathways. Future investigations should focus on how different scaffold platforms modulate NSC differentiation and develop tools to monitor these cellular processes within the scaffold environment. Despite the need for further validation, current findings and ongoing trials underscore the exciting potential of stem cell scaffolds to transform SCI treatment [48–50].

Despite promising outcomes in preclinical studies, the translation of neural stem cell-based scaffolds into clinical practice faces several challenges **(Table 2).** Variability in stem cell sources, scaffold biocompatibility, long-term safety, and patient-specific factors can impact therapeutic

outcomes [51]. Moreover, standardization of protocols for stem cell isolation, expansion, and scaffold fabrication remains limited across research institutions. Regulatory hurdles and the lack of large-scale, multicentre clinical trials also impede the pathway to approval and adoption. Addressing these barriers through rigorous safety profiling, harmonized clinical protocols, and real-world validation will be essential for effective clinical translation [52].

The ethical landscape surrounding stem cell scaffold therapies is complex, particularly regarding the use of embryonic or foetal-derived stem cells. Issues related to donor consent, potential immunogenicity, and equitable patient access to advanced therapies must be thoroughly considered [53]. There is also a growing need for transparent regulatory frameworks that balance innovation with safety, especially as stem cell-based interventions become more personalized and technically sophisticated. Public engagement and ethical oversight are imperative to ensure responsible development and deployment of these regenerative strategies [54].

Technological advances such as 3D bioprinting and artificial intelligence (AI) are poised to revolutionize neural tissue engineering. 3D bioprinting enables the fabrication of patient-specific, anatomically precise scaffolds that can incorporate multiple cell types, vascular structures, and bioactive molecules in a spatially organized manner [55]. AI-driven modeling and machine learning algorithms can further optimize scaffold composition, predict stem cell behavior, and enhance the design of regenerative microenvironments. The integration of these technologies could accelerate discovery, reduce experimental variability, and facilitate personalized treatment strategies for spinal cord injury [56,57].

Table 2. Limitations of NSC-Based Therapies for Spinal Cord Injury and Emerging Solutions.

Limitation	Description	Recent Advancements Addressing the Limitation
Low cell survival post-transplantation	NSCs often undergo apoptosis or fail to engraft in the injury microenvironment	Biomaterial scaffolds providing protective niches; PEG hydrogels with enhanced oxygen diffusion
Poor differentiation control	NSCs may not reliably differentiate into desired neuronal subtypes	Use of molecular cues (e.g., Shh, Wnt agonists, microRNA-124); signaling-modulating scaffolds
Immune rejection and inflammation	Host immune system may attack transplanted cells or exacerbate injury	Immunosuppressive scaffolds; use of autologous or iPSC-derived NSCs
Limited functional integration	New neurons may not form functional synapses or integrate with host circuitry	3D-bioprinted scaffolds with spatial guidance; N-cadherin-enhanced matrices
Short therapeutic window	Optimal timing for NSC delivery is narrow due to evolving injury microenvironment	Injectable hydrogels allow minimally invasive, timed delivery
Tumorigenic potential	Risk of uncontrolled proliferation in pluripotent-derived stem cells	Pre-differentiation before implantation; gene editing to suppress oncogenic pathways
Scalability and clinical translation	Difficulties in large-scale NSC expansion and standardization for human use	Advances in GMP-grade NSC production; AI-guided scaffold optimization

7. Conclusions

In conclusion (**Figure 6**), stem cell–based scaffolds offer a groundbreaking avenue for spinal cord injury repair by providing structural support and promoting neural regeneration. The use of natural and synthetic biomaterials, integrated with growth factors and bioactive cues, enhances stem cell function and facilitates axonal growth. While preclinical and early clinical results are promising, significant challenges such as long-term safety, immune response, and cost remain. Continued research is essential to refine scaffold design, improve delivery strategies, and ensure effective clinical outcomes. With further development, this regenerative approach holds great potential to revolutionize SCI therapy and significantly improve patient recovery and quality of life.

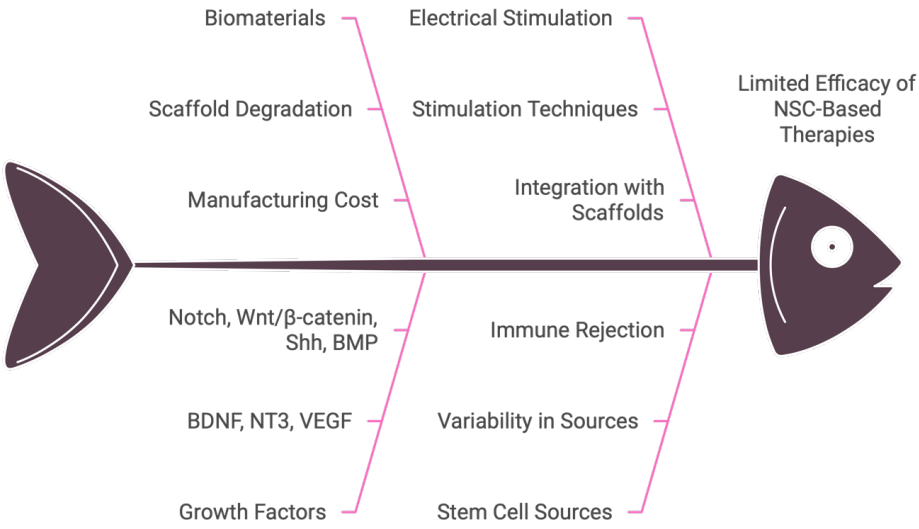

Figure 6. Graphical abstract / Conclusion. Stem cell–based scaffolds offer a promising strategy for spinal cord injury repair by combining structural support, molecular signaling, and regenerative cues to enhance neural regeneration and functional recovery.

Author Contributions

Planned designed and final review of the manuscript, A.S.A.; data collection, J.S. and M.M.R.; data analysis, J.S., M.M.R., A.S.K., E.M.H. and L.S.M.; discussion of results, J.S., M.M.R. and A.S.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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