Strategies to Overcome the Triple Challenges in Spinal Bioprinting: Vascularization, Neural Integration, and Mechanical Stability

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Abstract. Spinal cord injury leads to irreversible loss of neurological function and imposes major health and economic burdens worldwide. Conventional interventions such as decompression, fixation, and grafting stabilize the spine yet provide limited functional restoration. Bioprinting has emerged as a promising strategy to integrate perfusion, axonal guidance, and load bearing within a single construct. Advances can be summarized across three critical domains. Vascularization approaches combine sacrificial ink networks and microfluidic perfusion modules to generate endothelialized conduits that sustain thick tissue viability. Neural integration leverages conductive hydrogels, axon guiding microarchitectures, and spatiotemporal delivery of neurotrophic factors to enhance neurite extension and electrophysiological reconnection. Mechanical stability is pursued through triply periodic minimal surface lattices that optimize permeability and stiffness, together with shape memory and composite scaffolds that adapt to cyclic spinal loading. These directions converge toward co design of structure, material, and function, aiming to overcome diffusion limits, restore signaling, and maintain long term durability. The purpose of this review is to provide a systematic framework linking design variables with reproducible validation metrics and to highlight priorities that may accelerate clinical translation of spinal bioprinting.

Keywords: Spinal bioprinting; vascularization; neural integration; mechanical stability; TPMS.

1. Introduction

Spinal cord injury is a serious neurological condition arising from traumatic incidents such as falls and traffic collisions, as well as from non-traumatic causes including tumors and degenerative disorders. Following injury, patients often experience loss of sensory and motor function below the affected region, which may present as complete or partial paralysis [1]. In current spinal repair surgeries, metallic materials like titanium alloys and stainless steel, together with certain medical-grade polymers, are commonly applied. While these materials provide excellent mechanical strength and structural stability, their elastic modulus is typically far greater than that of natural bone, making them prone to inducing the "stress shielding" effect. Under such conditions, the implant carries the majority of the mechanical load, while the surrounding bone tissue receives insufficient stress stimulation. Over time, this imbalance can result in osteoporosis, marginal bone resorption, and even implant loosening [2]. In addition, many of these conventional materials are biologically inert. When in contact with host bone cells, they struggle to establish a stable biological interface, limiting cellular adhesion and proliferation, and ultimately hindering osseointegration. Consequently, achieving both enhanced bioactivity and mechanical compatibility in implant design has become a central focus of current research [3].

Three-dimensional bioprinting, positioned at the intersection of tissue engineering and personalized medicine, has emerged as a transformative approach for fabricating intricate, functional tissue constructs. Unlike conventional single-material fabrication techniques, bioprinting enables the coordinated deposition of multiple materials within one construct, integrating cell-matrix composites, bioceramics, and polymeric scaffolds. This strategy not only ensures mechanical integrity but also establishes a biologically supportive environment [4]. Moreover, the technology affords precise control at the microstructural level. Through modulation of nozzle trajectories, printing parameters, and material formulations, it is possible to generate architectures such as graded pore distributions and biomimetic fiber orientations, which more effectively facilitate essential biological processes including cellular adhesion, migration, and nutrient transport [5]. Coupled with medical imaging-based patient-specific modeling, bioprinted constructs can be tailored to replicate individual anatomical characteristics, thereby enhancing the precision and functional fit of spinal repair.

Although three-dimensional bioprinting has made substantial advances in generating complex tissue constructs, its translation into spinal repair remains hindered by several critical challenges. The foremost limitation is insufficient vascularization, which restricts construct thickness and compromises long-term functionality; in larger grafts, the central regions frequently undergo necrosis due to inadequate nutrient and oxygen diffusion. A second obstacle is the limited efficiency of neural integration, which markedly impedes functional restoration. Current constructs generally lack structural cues to guide axonal regrowth, leading to suboptimal recovery of neural conduction. The third challenge concerns mechanical mismatch: most available biomaterials continue to exhibit considerable discrepancies in strength and stiffness compared with native bone, rendering them incapable of sustaining the spine’s long-term, complex loading environment. To overcome these issues, researchers have put forward a range of targeted strategies [6-9]. Against this backdrop, the present review seeks to systematically collate ongoing progress and highlight the principal technical pathways in spinal bioprinting, with a focus on vascularization, neural integration, and mechanical stability. Furthermore, it evaluates the feasibility and potential benefits of multi-strategy synergies, while outlining the remaining barriers and future priorities for clinical translation.

2. Vascularization

For engineered tissues with a thickness of 200-300 µm, vascularization dictates early cell survival and the durability of function. In spinal-related tissues, the vascular network must concurrently satisfy hierarchical branching and organ specificity, perfusability, and compatibility with the local mechanical milieu. These criteria can be framed as a “structure-function-mechanics” coupling standard that underpins the design and evaluation of bioprinted constructs [10].

The integration of controlled shear and stable perfusion in chip-based microfluidics with the spatial assembly capacity of multi-material bioprinting has become a key strategy for building perfusable vascular networks. Performing the sequence of “tubulogenesis-endothelialization-perfusion” within one construct improves early oxygen and glucose delivery and supports subsequent anastomosis with host circulation [11]. Recent reviews group this approach into reusable modules such as direct writing or coaxial extrusion, microfluidic molding, and hybrid channel-chamber systems, which can be combined with bone-like scaffolds. In parallel, sacrificial-ink methods using gelatin, Pluronic, and carboxymethyl cellulose are widely employed. After removal by thermal or solvent triggers, these inks leave continuous, endothelializable channels. The rheological characteristics of the ink, including shear-thinning, yield stress, and elastic recovery, remain critical for printing accuracy and channel stability [7].

At the macroscopic scaffold level, gradient porous structures and triply periodic minimal surfaces (TPMS) with radial and axial mechanical gradients can balance pore size, permeability, and strength. Within the range of 570-1440 µm, TPMS scaffolds with such gradients can achieve permeability on the order of 10^{-12}-10^{-11} mm^2 and compressive strength between 3.0 and 9.3 MPa, values comparable to cancellous bone. These characteristics help establish a bone-like microenvironment that is more permissive for mass transport mediated by both convection and diffusion [12]. Comprehensive reviews in the fields of bioprinting and regenerative medicine further provide methodological guidance for the integration of modular microfluidics, sacrificial-ink strategies, and gradient TPMS designs [4, 6].

Despite these advances, several technical barriers remain. Microfluidic architectures subjected to long-term perfusion are prone to thrombosis and biofouling, necessitating early-stage risk assessment and validation of anti-clogging strategies, alongside evaluation of anticoagulant and antifouling surface treatments. Sacrificial-ink systems are sensitive to both printing temperature and processing time, and their batch-to-batch reproducibility often limits scalability. Therefore, stringent quality management is required, encompassing raw-material control and process-capability verification. Likewise, highly interconnected topologies such as TPMS enhance convective transport but can reduce local stiffness, requiring trade-offs between regional mechanics and overall structural stability [6, 7, 12].

Considering the load-bearing characteristics and postural variations of the spine, a combined strategy is advisable: microfluidic interfaces with stable perfusion, hierarchical channels formed by sacrificial inks, and bone-like scaffolds with radial or axial mechanical gradients. These address early perfusion, geometric hierarchy, and long-term patency and load-bearing, respectively. For in vitro validation, long-term perfusion fatigue, end-to-end perfusion pressure drops, and endothelial continuity are recommended as combined evaluation endpoints [10-12].

3. Neural Integration

Neural integration is not only concerned with the “geometric connectivity” of axons traversing the lesion site, but also with reconstructing the electrophysiological microenvironment. To this end, scaffolds must simultaneously provide geometric guidance, biochemical cues, and continuous electrical pathways, while remaining compatible with long-term challenges such as inflammation and scarring [13]. Current strategies focus on conductive scaffolds, multimodal cue structures, and the extraction of transferable parameter sets from peripheral nerve conduit designs, which can serve as a methodological library to complement structural guidance in spinal cord applications. For example, PEDOT:LS-doped GelMA/HAMA hydrogels exhibit conductivity comparable to white matter and a storage modulus within the kilopascal range of soft tissues. Such scaffolds have been shown to enhance the neuronal differentiation of neural stem cells, attenuate glial scarring, and improve hindlimb motor recovery in complete spinal cord transection models [8].

Multimodal cue structures further advance axonal guidance by incorporating microgrooves and fiber orientation into the printing trajectory. In parallel, localized delivery of NGF, BDNF, and SDF-1 establishes chemical gradients that, when combined with low-amplitude electrical stimulation, create a geometric-chemical-electrical tri-modal system. This integrated approach markedly enhances synaptic reconnection and increases the probability of functional neural conduction [13]. In addition, geometric parameters, growth factor release kinetics, and the selection of supportive cells derived from peripheral nerve conduit research can be adapted as a transferable parameter library, thereby supplementing spinal cord-specific guidance strategies [4, 8].

These elements are interdependent and require several trade-offs. Conductive materials may show long-term instability in conductivity and inconsistent immune compatibility. Growth factor dosage and timing must be calibrated to balance regeneration with immune regulation, while excessive electrical stimulation can induce abnormal excitability. These factors define the boundary between neural and vascular modules. In spinal repair engineering, continuous conductive networks (such as conductive polymers or nanoconductors) within axially aligned channels, combined with early short-term release of neurotrophic factors, are recommended. Neural and vascular modules may be advanced in parallel, but compatibility of materials and printing conditions is critical to maintain cell viability and scaffold integrity. Validation should employ composite endpoints, including conduction velocity, threshold, axonal orientation distribution, and inflammatory markers, to comprehensively assess recovery and safety [8, 13].

4. Mechanical Stability and Long-Term Load Bearing

Spinal segments are constantly exposed to cyclic compressive, shear and torsional forces during daily activities. When scaffolds are too stiff, they may induce stress shielding and marginal bone resorption, whereas scaffolds that are too soft often fail early under fatigue loading. Therefore, it is crucial to achieve a balance among strength, stiffness gradients, fatigue resistance and permeability networks [2]. Recent strategies attempt to translate hierarchical biological structures into engineering parameters, aiming to create a closed-loop link between structure and function. Features of the extracellular matrix and trabecular bone such as porosity, pore size, connectivity and anisotropy can be parameterized and reproduced by three-dimensional printing to generate biomimetic structural gradients. This mapping of structure and function allows scaffolds to meet the combined needs of mechanical stability, mass transport and immune regulation [5].

Gradient TPMS scaffolds demonstrate this concept by maintaining permeability while providing compressive strength and energy dissipation values that are close to cancellous bone, thereby supporting coordinated bone and vascular responses [12]. Shape memory composites also contribute to this approach. PLA/PCL/β-tricalcium silicate ternary systems with pore sizes around 550 micrometers and porosity above 50 percent can achieve compressive strengths of about 50 MPa and shape recovery close to 84 percent. These properties facilitate minimally invasive shaping and interfacial conformity and at the same time reduce the risk of excessive rigidity associated with conventional fixation [14].

Despite these advances, important challenges remain. The evolution of pore structures under manufacturing tolerances and cyclic fatigue requires long-term observation. The stability of recovery and fragmentation of shape memory systems under repeated loading have not been systematically characterized. After implantation, stiffness matching with host bone can vary, leading to over- or under-matching depending on the surgical procedure [2, 3]. To address these issues, gradient TPMS scaffolds, which balance permeability and strength, may be combined with shape memory composites that provide initial stability and improve interfacial conformity. Validation should be conducted together with vascularization and neural integration modules in an integrated construct. For evaluation, cross-disciplinary testing frameworks are recommended, including long-term perfusion fatigue, cyclic mechanical loading, and electrophysiological tracking, to reduce bias from single indicators [14]. Future research should also explore multi-scale computational modeling combined with artificial intelligence-based optimization to better predict scaffold failure under complex physiological loading.

5. Conclusion

The core challenge in spinal bioprinting is to integrate vascularization, neural integration, and mechanical stability within a unified design framework. For vascularization, constructing hierarchical channels that sustain perfusion enables thick tissues to obtain convective oxygen supply and early endothelialization, improving patency and anticoagulant function. Sacrificial-ink and microfluidic systems have repeatedly demonstrated the feasibility of this approach. At the scaffold scale, gradient porosity and TPMS architectures provide a measurable balance between permeability, pore connectivity, and compressive strength, thereby reducing stress shielding and limiting bone resorption from interfacial mismatch. From a manufacturing standpoint, complex topologies increase demands on process tolerance and fatigue resistance, making it essential to establish process windows around permeability, pressure drop, and strength retention, complemented by traceability and in situ monitoring to mitigate scale-up risks. Coupling these strategies creates a pathway from early survival to long-term integration, while reserving structural and temporal margins for later vascular connection. Neural integration requires both axonal guidance and excitability. Microgrooves and fiber networks can direct axonal growth, and in combination with conductive hydrogels and mild electrical stimulation, they enhance neurite extension, conduction reliability, and functional recovery in spinal models. Mechanical stability relies on stiffness matching and interfacial adhesion under sustained loading, where gradient anisotropic designs and shape-memory composites improve conformity, reduce micromotion, and delay loosening. To avoid reliance on single endpoints, a comprehensive validation framework is recommended, incorporating perfusion pressure drop, endothelial continuity, electrophysiological thresholds, conduction velocity, strength retention, and pore evolution under continuous flow and cyclic loading. Reproducible quantitative indicators aligned with clinical scenarios should be prioritized, providing the foundation for parameterized modeling and multi-objective optimization that link material selection, structure, and stimulation strategies into a traceable evidence chain supporting regulatory approval and clinical translation.

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