

REVIEW ARTICLE

Progress, challenges, and clinical prospects of hydrogel-based bioinks in spinal fusion applications

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Abstract

Traditional spinal fusion cages and autologous bone grafts face well-recognized limitations, creating a growing clinical need for next-generation fusion technologies. Research has accelerated the development of bioactive materials, with hydrogels emerging as a particularly versatile class for bone repair. These materials can provide structural support while facilitating tissue regeneration through the incorporation of therapeutics that promote integration with native bone. The convergence of hydrogel design and bioprinting technologies may enable customizable, biologically active fusion constructs that function with the body's repair mechanisms, allowing for more personalized and optimal treatment outcomes. This review summarizes current research on bioprinted hydrogel systems, focusing on materials selection, mechanical strength, degradability, bioink printability, and nanomaterial incorporation. It also discusses pre-clinical and computational approaches for assessing how scaffold design, material selection, printing parameters, printing strategies, and material flow affect mechanical behavior, material properties, and cell viability. Overall, the review highlights potential research avenues for developing new therapeutic platforms for spinal fusion applications.

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1. Introduction

1.1. Overview of spinal fusion

With the aging population, the prevalence of degenerative spinal diseases has been on the rise.¹ These diseases encompass a variety of conditions, including cervical disc disease, degenerative disc disease of the cervical and lumbar spine, spinal stenosis in the cervical and lumbar regions, and ossification of the posterior longitudinal ligament. When

conservative, non-surgical treatments prove insufficient for managing these pathologic changes—such as degenerative disc disease, spondylolisthesis, spinal fractures, spinal stenosis, and pars defects—surgical intervention becomes essential.²

One of the primary objectives of surgery in these cases is to relieve pain and restore function by decompressing the spinal cord and nerve roots and stabilizing the spine through spinal fusion procedures. The most common surgical options include anterior, posterior, transforaminal, oblique, and lateral lumbar interbody fusion techniques, as well as anterior cervical discectomy and fusion (ACDF).^{3–5} These procedures involve removing the damaged intervertebral disc to decompress neural elements, thereby relieving the pressure on the spinal cord and nerve roots caused by degenerative changes (Figure 1).⁶

During these surgeries, an intervertebral cage or spacer is typically implanted in the disc space to reconstruct the spinal alignment, restore disc height, and provide stability

to the affected spinal segment. This implant serves several purposes. First, it helps to maintain the natural spacing between vertebrae, preventing further nerve compression. Second, it stabilizes the spine by promoting fusion, where bone grows between vertebrae to create a solid mass, reducing the risk of future slippage or deformity in the affected segment. For optimal outcomes, these cages are often filled with bone graft materials to promote bone healing and fusion over time.

These surgical approaches have been developed to cater to specific patient needs and pathologies. Anterior approaches, such as ACDF, provide direct access to the disc and adjacent structures, minimizing disruption to surrounding musculature.⁷ In contrast, posterior and transforaminal approaches are advantageous in cases requiring extensive decompression or correction of spinal alignment. The oblique and lateral techniques allow for minimally invasive access to the spine with less disruption to major blood vessels and nerves, often resulting in

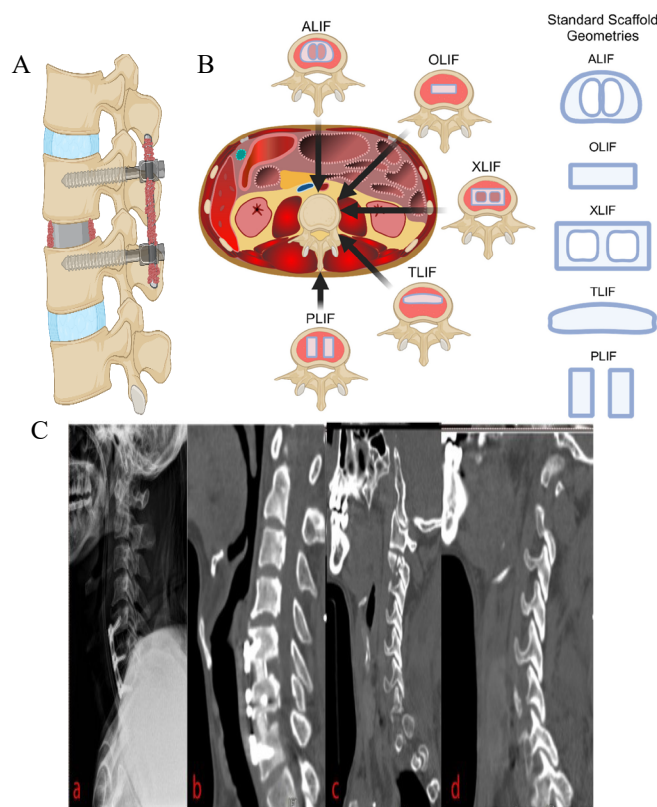


Figure 1. Overview of stabilization devices used in spinal fusion. (A) The main components of posterior stabilization are the interbody cage and bone graft. Created in BioRender.com. Yuanchen, Z. (2026) <https://BioRender.com/363lz41>. (B) Surgical approaches for lumbar interbody fusion and their respective interbody cage designs. Adapted from Cheers *et al.*⁸ with permission. Copyright © 2024 The Royal Society of Chemistry. (C) (a, b) Postoperative lateral X-ray and computed tomography scan showing ACDF performed at C5–6 and C6–7. Adapted from Zhu *et al.*⁹ with permission Copyright © 2024 The Authors.

Abbreviations: ALIF: Anterior lumbar interbody fusion; OLIF: Oblique lumbar interbody fusion; PLIF: Posterior lumbar interbody fusion; TLIF: Transforaminal lumbar interbody fusion; XLIF: Extreme lateral interbody fusion.

quicker recovery times.

1.2. Role of biomaterials in enhancing spinal fusion

Despite advancements in surgical techniques and instrumentation, there remain significant challenges. Current spinal fusion surgery techniques heavily rely on autologous bone grafts (ABG) or bone graft substitutes to achieve adequate bone healing and solid fusion.¹⁰ ABGs are preferred due to their inherent osteogenic potential, osteoinductivity, and osteoconductivity. Modern practice involves preparing ABG as morselized graft material to enhance integration within the intervertebral disc space and hollow cage regions.¹¹ Additionally, ABG is placed around screw-rod constructs to encourage posterior fusion between adjacent bone surfaces. The iliac crest is often used as a source of cancellous ABG; however, its use is limited by several factors, including the limited size and volume of available graft material, donor site morbidity (which can lead to persistent pain in up to 30% of patients post-harvest), and the presence of only a limited number of viable and bioactive cells within the graft.¹² Furthermore, after the age of 55, the number of stem cells present in ABG decreases significantly, impacting its regenerative capacity.¹³ The anatomical location from which ABG is harvested—whether from the iliac crest, iliac plate, or periosteal areas—also influences the composition of the graft and, subsequently, its regenerative ability.¹⁴ As a result, the rate of non-union after spinal fusion surgery ranges from 5% to 35%.¹⁵

Fusion surgeries can lead to complications, such as device subsidence, where the cage or implant sinks into the bone due to insufficient bone density, especially in osteoporotic patients.¹⁶ Additionally, the fusion process can put extra stress on adjacent segments,^{17,18} potentially leading to accelerated degeneration in these areas.¹⁹ Traditional intervertebral fusion cages are typically made from rigid materials such as stainless steel, titanium, or its alloys, and cobalt-chromium alloys. These materials offer excellent biocompatibility, corrosion resistance, and mechanical strength. Ansaripour *et al.*²⁰ compared the materials commonly used in total disc replacement in terms of long-term durability and ability to withstand mechanical stress, as detailed in Table 1. However, certain limitations of these materials can directly or indirectly compromise healing outcomes. Firstly, bones typically undergo stress and remodeling due to load-bearing. However, during the remodeling phase in the intervertebral space, the stress-shielding effect of rigid metals, with the device bearing most of the mechanical load, reduces the mechanical stimulus to the bone tissue surrounding the fusion cage. This reduction in mechanical stimulation leads to bone atrophy, which may delay the healing process and potentially result

in implant loosening and failure.²¹ Additionally, if the cage loosens or fractures, it can lead to localized pain or infection around the cage.²² Biodegradable polymers can effectively address some of the drawbacks of rigid metal implants. However, these materials also have limitations, particularly in terms of mechanical properties. Polylactic acid, one of the main polymers used in implants, has an elastic modulus and tensile strength of approximately 3 GPa and 50–70 MPa, respectively.²³ Furthermore, the acidic byproducts generated during polymer degradation can exacerbate local inflammation and accelerate bone resorption.²⁴

In the early 2000s, polyether ether ketone (PEEK) fusion cages gained popularity due to their elastic modulus, which is similar to that of bone and can reduce the rate of implant subsidence. However, PEEK's bio-inert nature limits its bone integration ability.²⁵ Furthermore, challenges such as the need for greater endplate preparation and the risks of over-distraction can affect its efficacy. Consequently, surface modifications and composite materials were introduced to enhance PEEK's properties. For instance, titanium-coated PEEK (Ti-PEEK) and bone morphogenetic protein-2 (BMP-2) coated PEEK were developed to combine PEEK's mechanical advantages with the biocompatibility of titanium and the osteoinductive properties of BMP-2. Advances in manufacturing techniques have also allowed for the creation of bionic structures with porosity and biomechanical properties similar to cortical bone.²⁶ However, issues such as coating delamination caused by wear and the resulting inflammatory reactions remain significant challenges.²⁷

In summary, current spinal fusion strategies present distinct trade-offs among biological performance, mechanical stability, and clinical feasibility. ABGs remain the gold standard due to their superior osteogenic, osteoinductive, and osteoconductive properties; however, their application is limited by donor site morbidity, restricted availability, and reduced regenerative capacity with aging. Rigid metallic cages provide excellent mechanical strength and long-term stability, but their high elastic modulus can induce stress shielding, leading to bone resorption and potential implant failure. Biodegradable polymer-based cages offer improved design flexibility and eliminate the need for graft harvesting, yet their relatively low mechanical strength and acidic degradation byproducts may compromise local tissue environments. PEEK cages achieve a better mechanical match with native bone and reduce subsidence risk, but their bio-inert nature limits osseointegration, often requiring additional surface modifications that introduce further complications. Collectively, these limitations highlight that no current

material simultaneously fulfills the requirements of optimal biomechanics, biocompatibility, and bioactivity. Therefore, there is a growing need for advanced biomaterials that can bridge this gap. In this context, hydrogel-based bioinks and bioprinting strategies have emerged as promising approaches, offering the potential to integrate tunable mechanical properties with biological functionality, thereby addressing the inherent limitations of existing grafts and cage materials.

1.3. Focus of the review and methodology

Given the issues with traditional fusion cages and ABGs, there is a significant clinical demand for a new generation of fusion cages. Recent innovations in bioengineering have led to the development of bioactive materials, particularly a wide array of hydrogel materials for bone repair that have emerged in recent years. These materials not only provide some mechanical support but also promote tissue regeneration and integration with host bone. When combined with growth factors or stem cells for spinal fusion, these hydrogel materials may enhance the body's natural healing response, reduce the risk of implant subsidence, and promote faster and more stable fusion. Thus, the future of spinal fusion surgery may involve integrating hydrogels with bioprinting technology to work synergistically with the body's healing processes, ultimately improving surgical outcomes for patients with degenerative spinal diseases. This could serve as a promising alternative solution. This paper will provide a comprehensive overview of research into the potential application of bioprinted hydrogels in spinal fusion devices.

This review was conducted using a structured literature search to ensure comprehensive coverage of recent

advances in hydrogel-based bioinks for spinal fusion and related applications. Major scientific databases, including PubMed, Web of Science, and Scopus, were systematically searched for relevant studies published up to 2025. The search strategy combined keywords such as “hydrogel,” “bioink,” “3D bioprinting,” “spinal fusion,” “intervertebral disc,” “bone regeneration,” and “tissue engineering.” Boolean operators (AND/OR) were applied to refine the search and identify studies relevant to material design, bioprinting techniques, and preclinical or clinical evaluations. Inclusion criteria comprised (i) original research articles and review papers focusing on hydrogel-based bioinks or composite hydrogels, (ii) studies related to spinal fusion, bone regeneration, or intervertebral disc repair, and (iii) publications in English. Exclusion criteria included conference abstracts, non-peer-reviewed reports, and studies lacking sufficient methodological or experimental details. Relevant articles were further screened based on their relevance to key themes, including material properties, biofunctional modifications, mechanical performance, degradation behavior, and *in vitro/in vivo* validation models. Additional references were identified through citation tracking of key articles.

2. Hydrogel-based bioinks: Composition and properties

2.1. Hydrogels as bioink materials

Bioinks are specialized hydrogel materials formulated to support cell growth, differentiation, and tissue formation within printed constructs. They serve as fundamental building blocks in tissue engineering, integrating biocompatibility, printability, and bioactivity to promote cellular functions.²⁸ The field of bioinks is rapidly evolving,

Table 1. Mechanical and wear-related properties of selected alternative biomaterials

Material	Young's modulus (GPa)	Tensile strength (MPa)	Fracture toughness (MPa√m)	Compressive strength (MPa)	Wear rate
Zirconia toughened alumina	338	350	6	2758	Low
Cobalt chromium alloy	220	1,403	100	1,296	Moderate
Ti-6Al-4V	114	940	91	1,172	Moderate
Stainless steel 316L	193	485	95	620	High
Ultra-high-molecular-weight polyethylene	1	21.4	6.41	13.8	Very high
Polyether ether ketone	3.6	80	6.76	124	Very high

Note: Data were reproduced from Ansaripour *et al.*²⁰

driven by the need for advanced solutions to address the diverse challenges of tissue repair, regeneration, and organ replacement.²⁹

The role of bioinks in tissue engineering extends beyond providing structural support. They are engineered to mimic the extracellular matrix (ECM) of native tissues, enabling cell adhesion, migration, and nutrient transport.³⁰ In this context, the customization of bioinks has emerged as a critical area of research. Tailoring the composition, mechanical properties, and bioactivity of bioinks to meet patient-specific needs is pivotal for achieving optimal tissue regeneration. This personalization is increasingly achievable thanks to advances in multi-material three-dimensional (3D) printing, which allow for the creation of structures with unprecedented complexity and functionality.

Hydrogels, as the primary matrix component of bioinks, are particularly attractive due to their high-water content and similarity to natural ECM.³¹ However, their intrinsic limitations, such as low mechanical strength and rapid degradation, pose significant challenges. Researchers are addressing these issues by exploring hybrid bioinks that combine natural and synthetic polymers.

2.2 Overview of common hydrogel polymers for bone and spinal applications

Several naturally derived hydrogels—including gelatin, collagen, hyaluronic acid (HA), chitosan, alginate, and chondroitin sulfate (CS)—have demonstrated potential as bone regenerative matrices due to their inherent bioactivity and ability to support cellular adhesion, proliferation, and differentiation (Table 2).

Gelatin, derived from collagen through partial hydrolysis, retains arginine-glycine-aspartic acid sequences, which facilitate cell adhesion and proliferation. In addition to its excellent biocompatibility, gelatin exhibits low immunogenicity, making it a favorable material for biomedical applications.³² Its retained bioactive motifs not only promote cell attachment but also provide signaling cues that influence cellular behavior. Gelatin-based hydrogels can be tailored to optimize the microenvironment for specific cellular responses, such as promoting osteogenic differentiation of stem cells *in vitro*.³³ However, gelatin exhibits rapid degradation and low mechanical strength, which restricts its direct application in load-bearing bone or spinal tissues.³⁴

Collagen, the most abundant structural protein in mammals, is a key component of the ECM. Commonly used types in tissue engineering include collagen types I, II, and III, which are known to support cell adhesion, proliferation, and differentiation. Type I collagen, in

particular, serves as the structural basis of connective and load-bearing tissues. While unmodified collagen hydrogels provide essential biological cues, their intrinsic mechanical weakness and poor long-term stability limit their application in regenerative medicine. To address these challenges, recent studies have explored strategies that enhance collagen's mechanical and biological functionality. For instance, Zhou *et al.*³⁵ utilized 3D printing technology to fabricate scaffolds with aligned collagen fiber structures, which not only improved the mechanical properties of the construct but also significantly enhanced osteogenic performance. The oriented collagen fibers guided cell alignment along the fiber direction and promoted cell proliferation, differentiation, and mineralization. Similarly, Buxton *et al.*³⁶ demonstrated that dense collagen matrices serve as effective stimulators for osteoblast differentiation *in vitro* and provide a supportive environment for cell survival and differentiation, even in the presence of matrix metalloproteinase inhibitors. These findings underscore the potential of structurally engineered collagen-based hydrogels in promoting targeted tissue regeneration.

Hyaluronic acid, the simplest glycosaminoglycan present in the ECM, plays vital roles in numerous biological processes, including the regulation of cell proliferation, differentiation, and migration. It contributes to tissue hydration, lubrication, and elasticity, owing to its high water-binding capacity, intrinsic viscosity, and viscoelastic properties.³⁷ Moreover, HA is characterized by its high biocompatibility, biodegradability, and non-immunogenic nature, making it highly attractive for biomedical applications. Cui *et al.*³⁸ demonstrated that the incorporation of HA into bone cement formulations not only improved the compressive strength of the composite but also significantly enhanced its osteoinductive capacity. However, native HA is prone to rapid enzymatic degradation by hyaluronidases *in vivo*, which restricts its long-term utility in tissue regeneration.³⁹

Chitosan, a cationic polysaccharide derived from chitin, consists of β -(1 $\rightarrow$ 4)-linked D-glucosamine and N-acetyl-D-glucosamine. Its structural similarity to glycosaminoglycans and inherent antibacterial properties make it an attractive material for wound healing and injectable formulations.⁴⁰ However, its poor water solubility and mechanical performance limit its applicability in spinal fusion.⁴¹

Alginate is a natural polysaccharide derived from brown seaweed, consisting of β -D-mannuronic acid and α -L-guluronic acid. Alginate hydrogels form through ionic crosslinking with divalent cations like Ca^{2+} , Zn^{2+} , and Cu^{2+} , offering simplicity and speed in gel formation. However, their weak mechanical properties and poor cell

adhesion, attributed to the absence of cell-adhesive motifs, limit their applications. Moreover, chelating agents or high concentrations of sodium or magnesium ions (Mg^{2+}) can destabilize the gel.

Chondroitin sulfate, a sulfated glycosaminoglycan comprising D-glucuronic acid and N-acetyl-D-galactosamine, is abundant in connective tissues such as cartilage, skin, and tendons. It supports physiological processes such as cartilage elasticity, bone formation, and anti-inflammatory responses.⁴² Despite its promising biocompatibility and biodegradability, unmodified CS lacks the mechanical robustness required for advanced biomedical applications such as high-stress environments like spinal fusion. Studies have shown that CS hydrogels exhibit poor mechanical properties and undergo premature degradation within one week, compromising their structural and functional stability.⁴³

Due to their inherent limitations, including poor mechanical strength, uncontrolled degradation rates, and insufficient structural integrity, these native hydrogels require functional modification to meet the mechanical and biological demands of spinal fusion.

3. Functionalization of commonly used hydrogels for printing

To overcome these limitations of native hydrogels, various modification strategies have been developed, including methacrylation, incorporation of nanoparticles, and integration with cells and bioactive molecules. These approaches improve hydrogel mechanical properties, biocompatibility, degradation control, and printability, thereby enhancing their applicability in bioprinting for spinal fusion.

3.1. Methacrylation

Methacrylation is a widely used chemical modification that introduces methacrylate groups into natural polymers, enabling photo-crosslinking to form stable hydrogel networks. This process enhances mechanical strength, structural fidelity, and degradation tunability. Gelatin methacryloyl (GelMA), collagen methacrylate (ColMA), and HA methacrylate (HAMA) are typical examples. The introduction of methacrylate groups enhances structural integrity, allows tuning of mechanical properties by varying the degree of substitution, and maintains biological functionality due to preserved bioactive sequences.⁴⁴⁻⁴⁶

For instance, Liu *et al.*⁴⁷ utilized GelMA, known for its excellent biocompatibility and tunable mechanical properties, to address localized tissue defects following discectomy, aiming to restore the mechanical stability of the spine. The photocrosslinkable nature of GelMA

enabled precise conformation to irregular defect sites and rapid in situ solidification, thereby providing immediate mechanical support to the spinal structure. Similarly, Ullah *et al.*⁴⁸ developed a ColMA hydrogel that integrates the bioactivity of collagen's native triple-helix structure with the crosslinking ability of methacrylate groups. This composite system significantly enhanced cell-matrix interactions and mechanical strength. When applied to a rat femoral defect model, the ColMA hydrogel promoted higher bone mineral density and more mature bone formation, demonstrating its superior osteogenic potential. Furthermore, Xing *et al.*⁴⁹ synthesized methacrylated chitosan hydrogels with adjustable degrees of substitution of methacrylic acid, allowing fine-tuning of their physicochemical properties, cytotoxicity, and antibacterial activity. The optimized hydrogels provided a dual function: maintaining a biocompatible environment for osteogenesis while offering antibacterial protection and preventing soft tissue invasion into bone defect sites, thus preserving the spatial structure required for effective bone regeneration.

3.2. Incorporation of nanomaterials

The addition of nano-scale inorganic or organic fillers can significantly enhance the mechanical, osteoconductive, and functional properties of hydrogels. These nanomaterials, due to their high surface area, unique physicochemical properties, and ability to form strong interactions with the hydrogel matrix, serve as reinforcing agents or bioactive cues within the hydrogel networks.

For example, Santana *et al.*⁵⁰ enhanced GelMA hydrogels by integrating graphene, achieving uniform dispersion of graphene particles at higher concentrations. Combined with low-temperature 3D printing, this approach facilitated efficient crosslinking of GelMA, resulting in a molecular network with significantly improved compressive strength, increasing mechanical performance to 36 kPa. Similarly, Gao *et al.*⁵¹ developed an injectable nanocomposite hydrogel by doping chitosan nanoparticles into dynamic polymer networks of oxidized sodium alginate and gelatin in the presence of borax. The synergistic effects of multiple reversible interactions, including dynamic covalent bonds (imine and borate ester bonds) and non-covalent interactions (electrostatic and hydrogen bonding), provided effective energy dissipation and high fatigue resistance. This dynamic hydrogel exhibited exceptional mechanical properties, including a compressive strength of 2.43 MPa, fracture strain of 493.91%, and toughness of 89.54 kJ/m³, highlighting its capacity to endure cyclic loading and high fatigue resistance. In another approach, Kaur *et al.*⁵² developed a thermoresponsive, injectable hydrogel by integrating carboxylated single-walled carbon nanotubes (COOH-SWCNTs) into chitosan and collagen. Inspired

by the mechanical performance of COOH-SWCNTs and the bioactivity of natural polymers, they created a highly porous and aligned crystalline structure that significantly enhanced mechanical strength from the kPa to MPa range—comparable to natural bone. β -glycerophosphate and temperature were critical for achieving physiological gelation. Furthermore, the presence of COOH-SWCNTs promoted osteoblast proliferation and accelerated hydroxyapatite (HAP) adsorption, indicating enhanced bioactivity.

The incorporation of nanomaterials also plays a crucial role in promoting osteogenesis. For instance, Montanari *et al.*⁵³ developed a bioink based on Mg^{2+} -modified nano-HAP incorporated into GelMA to improve both the printability and biological functionality of the hydrogel. The Mg^{2+} -modified nano-HAP not only enhanced the structural fidelity and printability of GelMA-based constructs but also significantly influenced the expression of osteogenic markers at both the RNA and protein levels.

In addition to enhancing mechanical strength and promoting osteogenesis, nanomaterials can also impart controlled drug release capabilities to hydrogel systems. By incorporating drug-loaded nanocarriers such as liposomes, hydrogels can achieve sustained and localized delivery of therapeutic agents, which is particularly valuable for long-term bone regeneration. For example, researchers have developed a composite hydrogel by embedding liposome-encapsulated osteoinductive drugs within a GelMA matrix. This strategy enabled prolonged drug release over several weeks, maintaining a therapeutic concentration in the target site. The sustained release of bone-promoting agents significantly enhanced osteogenic differentiation, ECM mineralization, and bone tissue formation, highlighting the potential of nanomaterial-assisted delivery systems in spinal fusion and bone defect repair.⁵⁴

3.3. Bioactive molecule and ion loading

Loading growth factors, peptides, or therapeutic ions into hydrogel matrices allows controlled and localized release of bioactive cues that direct stem cell differentiation and tissue regeneration. This strategy is particularly valuable in spinal fusion applications, where targeted osteoinduction is essential for successful bone integration and repair. Li *et al.*⁵⁵ demonstrated the effective application of GelMA as a carrier for BMP-2 and bone marrow-derived mesenchymal stem cells in promoting spinal fusion. In an intervertebral fusion model, the GelMA-based scaffold exhibited strong osteoinductive properties, effectively facilitating intervertebral fusion and bone regeneration. The hydrogel provided a supportive microenvironment for the sustained release of BMP-2, enhancing the differentiation of bone

marrow-derived mesenchymal stem cells into osteoblasts while maintaining biocompatibility and structural integrity. In a similar approach, Guo *et al.*⁵⁶ developed a composite material by incorporating graphene oxide into GelMA hydrogels, along with bone-forming peptide-1, to enhance the expression of osteogenic-related genes such as *Alpl*, *Runx2*, *Bglap*, and *Spp1*, as well as proteins such as RUNX2 and OCN, indicating that this material could significantly contribute to improving intervertebral bone fusion by promoting bone formation and accelerating the healing process.

Beyond biomolecules, ionic modification of hydrogels with osteoinductive ions offers another promising strategy. Incorporation of ions such as Sr^{2+} , Mg^{2+} , or Zn^{2+} can effectively stimulate osteoblast activity and modulate local immune responses.^{53,57} For instance, Sr^{2+} -loaded hydrogels have been shown to not only promote osteogenic differentiation but also inhibit osteoclast-mediated bone resorption. Similarly, a more recent study demonstrated that Mg^{2+} can simultaneously promote osteogenesis and suppress osteoclast differentiation, highlighting its dual regulatory role in bone remodelling.⁵⁸ This dual action makes them particularly beneficial for spinal fusion in osteoporotic patients, where bone loss and immune imbalance are critical concerns.⁵⁹

3.4. Composite hydrogel systems

Combining different natural or functionalized hydrogels into a hybrid system is an effective strategy to integrate the advantages of multiple components while compensating for their individual limitations. Such composite hydrogels can offer enhanced mechanical integrity, tunable degradation, improved printability, and a more favorable microenvironment for cell behavior.

Gelatin is often combined with alginate to form dual-crosslinkable bioinks, as alginate provides ionic crosslinking with calcium ions for immediate structural stability. Benefiting from the thermosensitivity of gelatin and the dual-crosslinking mechanism, this dual-network system enables precise printing with high shape fidelity and supports cell encapsulation during bioprinting.⁶⁰ Wang *et al.*⁶¹ developed a novel bioink composed of GelMA, methacrylated alginate (AlgMA), and HAP nanoparticles, in which each component played a distinct and complementary role. GelMA provided a cell-adhesive and photopolymerizable matrix that promoted cell proliferation and long-term structural stability, while AlgMA offered rapid ionic crosslinking and tunable mechanical support. In addition, bacterial cellulose, particularly when modified (e.g., maleic acid-treated), has been incorporated into gelatin-based composite

bioinks to enhance printability, mechanical strength, and *in vivo* bone regeneration potential. The incorporation of HAP nanoparticles endowed the hydrogel with osteoconductivity and self-mineralization capability, closely mimicking the natural bone ECM.⁶² Using this advanced bioink, complex bone ECM-like structures were fabricated to support the long-term culture and gradual maturation of bioprinted bone organoids. Moreover, the synergistic effects of these components facilitated multicellular differentiation and enhanced bone repair both *in vitro* and *in vivo*, offering valuable insights into the early stages of bone formation and regeneration.⁶³ Similarly, HAP and GelMA are frequently combined to improve both biological activity and mechanical strength. HAP enhances hydrophilicity and mimics ECM viscoelasticity, while GelMA reinforces mechanical properties and introduces photo-crosslinkability. HAP/GelMA composite hydrogels developed in several studies have demonstrated high porosity, excellent cell adhesion, and, notably, superior mechanical stability. These hydrogels exhibit shape-memory behavior under compression and can revert to their original form after water retention. Moreover, they effectively promote the osteogenic differentiation

of mesenchymal stem cells, making them highly suitable for applications in spinal and bone tissue engineering.^{64,65} Combining chitosan with collagen or gelatin is also a widely used strategy. Chitosan provides antimicrobial and hemostatic properties, while collagen supplies cell-adhesive peptide sequences that support cellular attachment and proliferation. For example, Bharadwaj *et al.*⁶⁶ developed a thermosensitive chitosan/gelatin hydrogel capable of rapid gelation at body temperature. This hydrogel exhibited excellent biocompatibility without the need for additional chemical crosslinkers, and it promoted both the proliferation and osteogenic function of bone marrow-derived mesenchymal stem cells.

In addition, composite hydrogels often possess favorable tunable degradation properties. Due to the relatively fast degradation rate of GelMA and the slow degradative properties of HAMA, they have been used in several studies for their tunable degradation rates, achieved by mixing them in different ratios, varying crosslinking density, and incorporating enzyme-responsive linkages. This adaptability ensures that the scaffold gradually degrades as new tissue forms, facilitating a seamless transition.⁶⁷⁻⁶⁹

Table 2. Common hydrogels used for 3D printing applications

Hydrogel	Key properties	Main drawbacks	Modified product	Modification advantages	Photo-crosslinking time	Mechanical properties	Degradation time	Typical applications
Gelatin	Contains bioactive sequences, promotes cell adhesion and proliferation	Fast degradation, low mechanical strength	GelMA	Photo-crosslinkable, tunable mechanical properties and degradation rates	Seconds to minutes (UV or visible light, dependent on degree of methacrylation)	Tunable stiffness 2–35 kPa (5–15% w/v), depending on polymer concentration and crosslinking density ⁷⁰	7–28 days ⁷⁰ (depends on crosslinking density and enzymatic degradation)	3D bioprinting, drug delivery, tissue engineering
Collagen	Supports cell adhesion, proliferation, and differentiation	Low mechanical strength and stability	ColMA	Enhanced mechanical strength, controlled degradation, improved stability, reduced immunogenicity	Minutes (requires optimization for structural integrity)	2–20 kPa (0.6–2.5% w/v), tunable by crosslinking conditions and species-based variations ^{71,72}	Days to weeks (improved compared to unmodified collagen)	Wound healing, drug delivery, organ-on-chip, tissue engineering

(cont'd...)

Table 2. Common hydrogels used for 3D printing applications

Hydrogel	Key properties	Main drawbacks	Modified product	Modification advantages	Photo-crosslinking time	Mechanical properties	Degradation time	Typical applications
Hyaluronic acid	Tissue hydration, lubrication, elasticity	Rapid enzymatic degradation, poor stability	HAMA	Enhanced mechanical properties, tunable degradation, good biocompatibility	Seconds to minutes (dependent on methacrylation degree)	2–100 kPa (2–20% w/v), but tunable with crosslinking density ⁷²	1–38 days ⁷² (enzymatically degradable)	Cartilage repair, controlled drug delivery, injectable hydrogels
Chitosan	Antibacterial, glycosaminoglycan-like, suitable for wound healing	Poor solubility, low stability	ChiMA	Photo-crosslinkable, improved mechanical properties, slower degradation, controlled swelling	Minutes (requires higher light intensity for efficient crosslinking)	Improved stiffness compared to unmodified chitosan; tunable with modifications (14–80 kPa) ⁷³	Weeks to months (controlled swelling and slower degradation)	Regenerative medicine, drug delivery, antibacterial scaffolds
Alginate	Forms hydrogels via ionic crosslinking, rapid gelation	Weak mechanical properties, poor cell adhesion, instability in an ionic environment	AlgMA	Enhanced cell adhesion, improved mechanical stability, controlled degradation	Seconds to minutes (dependent on methacrylation and ionic interactions)	30–230 Pa (2.5–5% w/v); ⁷⁴ tunable with polymer concentration	Weeks (more stable than unmodified alginate)	Drug delivery, nerve repair, tissue scaffolding
Chondroitin sulfate	Supports cartilage elasticity, bone formation, and anti-inflammatory responses	Low mechanical strength, unstable standalone scaffold	CSMA	Improved mechanical properties, photo-crosslinkable, mimics the native tissue microenvironment	Seconds to minutes (dependent on degree of methacrylation)	3–39 kPa (20% w/v, degree of methacrylation: 13–22%); ⁷⁵ tunable with polymer concentration and methacrylation degree	Weeks to months (depends on crosslinking and enzymatic degradation)	Cartilage repair, 3D cell culture, targeted drug delivery

3.5. Ideal bioink in spinal fusion

An ideal bioink for spinal fusion should achieve a balance between mechanical support and biological functionality. From a mechanical perspective, the bioink should provide sufficient initial stiffness to withstand physiological loading conditions (typically ranging from hundreds of kPa to several MPa depending on the surgical site⁷⁶) while maintaining an elastic modulus comparable to native bone to minimize stress shielding. In terms of degradation, the bioink should exhibit a controlled degradation profile that aligns with the stages of bone healing: it should remain stable during the early inflammatory phase (~2 weeks) and subsequent angiogenesis, then gradually degrade following

the onset of osteoinduction (2–3 weeks post-implantation) to support new bone formation, and finally enable dynamic replacement during osteoconduction and remodeling phases (spanning months to ~1 year).⁷⁷ In terms of bioactivity, the bioink should support cell adhesion, proliferation, and differentiation, while promoting osteoinduction and angiogenesis through the incorporation of bioactive molecules. Additionally, it should maintain high biocompatibility, low immunogenicity, and minimal inflammatory response. Finally, from a manufacturing perspective, the bioink must possess suitable rheological properties for precise bioprinting, enabling the fabrication of patient-specific constructs with high structural fidelity.

4. Bioprinting techniques for spinal fusion devices

4.1. 3D bioprinting technologies

Three-dimensional bioprinting is an advanced manufacturing technology that employs various printing techniques, such as inkjet, extrusion-based, and laser-assisted, to fabricate 3D biological structures using cell-encapsulated biomaterials known as bioinks. These biofabrication techniques enable the creation of physiologically relevant tissue constructs, providing promising solutions for regenerative medicine, tissue engineering, and drug testing.³¹

Inkjet bioprinting operates on the principle of droplet-based bioink deposition, relying on different actuation mechanisms, such as thermal, piezoelectric, electrostatic, acoustic, hydrodynamic, or microvalve-based methods, to generate and deposit bioink droplets with precise spatial control.³⁰ This method is well-suited for low-viscosity bioinks and allows for high-speed printing, but it may face challenges in maintaining structural stability post-printing due to the low mechanical integrity of the printed constructs.⁷⁸

Extrusion-based bioprinting, in contrast, typically requires bioinks with higher viscosity. This technique employs direct mechanical or pneumatic dispensing systems to extrude bioinks containing cells, enabling the fabrication of continuous filaments that form complex 3D structures.⁷⁹ The viscosity and gelation kinetics of bioinks play a crucial role in determining the resolution and structural fidelity of the printed constructs. While highly concentrated polymeric bioinks offer better printability and shape retention, they often compromise cell viability by restricting cell diffusion, migration, proliferation, and ECM remodeling.²⁹ In response, hydrogel-based bioinks have gained significant attention due to their ability to mimic the native ECM, thereby providing a supportive microenvironment for cell proliferation and differentiation.⁸⁰

Laser-assisted bioprinting utilizes laser pulses to induce the evaporation of a thin metal film, propelling bioink droplets toward a receiving substrate. This technique is characterized by its high precision, rapid deposition speed, and superior cell viability, although its implementation remains costly.³¹ Additionally, digital light processing-based 3D bioprinting enables the fabrication of complex structures with microscale resolution, further expanding the potential applications of bioprinting.⁸¹

4.2. 3D bioprinting in spinal fusion

Three-dimensional bioprinting offers significant

advantages over traditional spinal fusion techniques, particularly in material biofunctionalization and patient-specific implant customization. This technology enables the combination of biomaterials with excellent biocompatibility, osteoconductivity, osteoinductivity, and antimicrobial properties with living cells while allowing for the fabrication of personalized intervertebral fusion cages based on a patient's spinal anatomical parameters. [Figure 2](#) provides an overview of 3D bioprinting on spinal fusion. This customization enhances the fit between the implant and adjacent vertebral bodies, effectively addressing the mismatch issues commonly associated with conventional spinal fusion devices, thereby improving implant stability and fusion efficacy.⁸² Currently, titanium and its alloys are the predominant materials used for 3D-printed spinal implants. However, traditional titanium fusion devices exhibit a significantly higher Young's modulus (110–115 GPa) compared to natural bone (10–30 GPa), leading to stress shielding effects where mechanical loads are primarily borne by the implant rather than the surrounding bone tissue.⁸³ This results in reduced mechanical stimulation for bone remodeling, which may contribute to implant loosening over time. Through 3D printing, customized porous titanium implants can be fabricated with optimized mechanical and structural properties, allowing for precise control over porosity, connectivity, and pore size to achieve a more bone-mimetic elastic modulus while minimizing stress shielding. Additionally, porous structures with pore sizes exceeding 300 μm have been shown to facilitate bone ingrowth and vascularization, further enhancing implant stability and biological integration.⁸⁴ Studies indicate that 3D-printed titanium scaffolds not only maintain spinal stability following bone graft fusion but also enhance bone integration and guide bone ingrowth within their porous architecture. The increased surface area of porous structures improves the bone-implant interface, promoting deeper infiltration of bone and vascular tissues, which in turn reduces the risk of implant subsidence, a common complication associated with conventional titanium mesh cages.⁸⁵ However, despite the numerous advantages of 3D-printed porous titanium implants in spinal fusion, the lack of inherent bioactivity remains a key challenge for achieving optimal bone integration. To address this issue, various surface modification strategies have been explored to enhance osseointegration and improve implant performance. One such strategy is the use of HAP coatings, which mimic the mineral composition of native bone, to enhance bone bonding.⁸⁶ Additionally, silicon-functionalized HAP and strontium coatings have demonstrated the ability to promote both osteogenesis and angiogenesis.⁸⁷ Furthermore, the incorporation of BMP-2 onto implant surfaces has been shown to accelerate

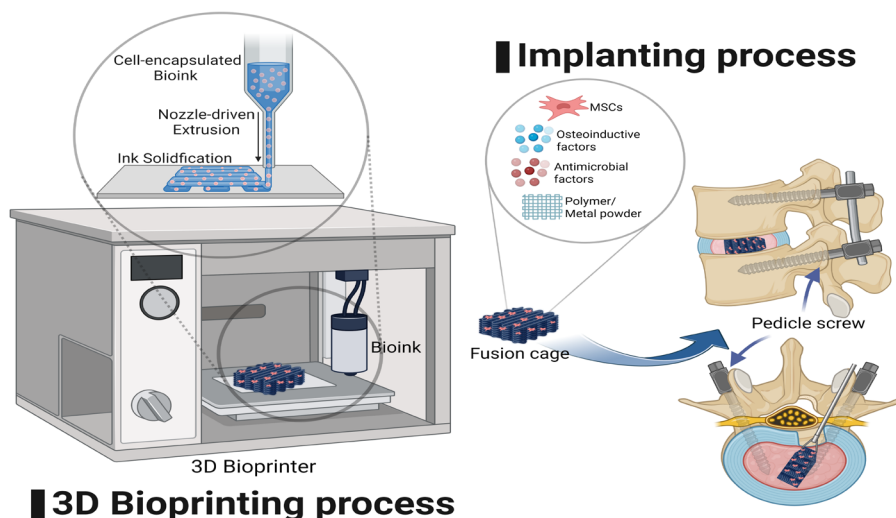


Figure 2. Schematic diagram of a 3D-bioprinted spinal fusion cage and its surgical implantation process. Created in BioRender. Yuanchen, Z. (2026) <https://BioRender.com/1w327rh>.

Abbreviation: MSCs: Mesenchymal stem cells.

osteogenic differentiation and new bone formation,⁸⁸ while silver (Ag) coatings can release Ag⁺ ions, providing antimicrobial properties to prevent postoperative infections and improve implant safety.⁸⁹ As advancements in 3D bioprinting and biomaterial science continue to progress, the development of spinal fusion implants with optimized mechanical properties, enhanced bioactivity, and personalized structural designs is becoming increasingly feasible. By integrating porous architecture with advanced surface modification strategies, 3D-printed spinal implants have the potential to revolutionize spinal fusion surgery, improve clinical outcomes, and accelerate patient recovery.

4.3. 4D bioprinting technologies

Looking ahead, the integration of hydrogel-based bioinks with 4D bioprinting holds immense promise. In 2014, Tibbitts⁹⁰ from MIT introduced the concept of 4D bioprinting, integrating shape memory polymers with 3D printing and introducing the dimension of time into the process. Unlike traditional 3D bioprinting, which typically produces static, inanimate structures, 4D bioprinting enables printed scaffolds to dynamically respond to internal or external stimuli through intrinsic mechanisms, allowing them to adapt to the dynamic nature of biological tissues. This innovation holds significant potential for personalized bone regeneration, as 4D-printed spinal fusion implants could be designed to respond to external stimuli such as water, heat, pH changes, light, electric fields, and magnetic fields, thereby altering their material properties to better

meet individual patient needs.⁹¹ More importantly, this emerging technology paves the way for the fabrication of functional, living tissues and organs.

Through programmable crosslinking or structural reorganization, the mechanical properties of implanted scaffolds can be dynamically tuned, enabling the design of self-folding microtubes that regulate the spatiotemporal distribution and release of bioactive cells.⁹² This approach facilitates the regeneration of bone, vascular, and neural tissues, establishes a long-term biomimetic microenvironment, and enhances the osteogenic differentiation of stem cells.^{93,94}

Additionally, 3D bioprinting can be considered a specific subset of 4D printing, as many 3D-printed scaffolds exhibit dynamic changes over time. These changes primarily stem from cellular remodeling during tissue maturation and biodegradable materials degrading *in vivo*. However, in some cases, biodegradation alone does not qualify as a 4D effect, as the printed structure merely serves as a template for the desired shape and function, and its degradation products no longer retain the original integrity of the scaffold.⁹⁵ Studies have reported that biodegradable dynamic fixation systems and traditional titanium alloy fixation systems in lumbar fusion models demonstrate comparable fusion outcomes, with safe *in vivo* degradation.⁹⁶

Since its introduction, 4D bioprinting technology has made significant advancements, leading to innovative applications in regenerative medicine and tissue

engineering. For instance, Lin *et al.*⁹² developed two types of shape-memory vascular stents with negative Poisson's ratio structures using 4D printing, which exhibited excellent shape-memory properties *in vitro*, making them highly promising for vascular stenosis treatment. Similarly, Zhang *et al.*⁹⁷ fabricated biocompatible polylactic acid/Fe₃O₄ composite filaments via 4D printing, which were actuated by a 27.5 kHz magnetic field, demonstrating a composite shape-memory structure mimicking spinal bone morphology, highlighting their potential in bone tissue repair. Moreover, Rana *et al.*⁹⁸ leveraged complementary aptamer sequences hybridization to regulate the release kinetics of vascular endothelial growth factor, developing a programmable bioink that facilitates the dynamic presentation of vascular endothelial growth factor to guide vascular morphogenesis within bioprinted structures.

5. Challenges for spinal fusion applications: Linking material properties to functional demands

Hydrogel-based bioinks hold significant promise for spinal fusion due to their unique ability to mimic the ECM and support critical regenerative processes. However, their clinical translation requires addressing several key challenges, including mechanical integrity, controlled degradation, printability, and bioactivity/biocompatibility. These challenges are closely tied to the selection and modification of polymeric materials, which ultimately dictate the functional performance of the bioinks. In addition, reproducibility-related issues, including batch-to-batch variability in bioink composition, sensitivity to printing parameters, and discrepancies between *in vitro* and *in vivo* performance, further complicate the reliable evaluation and clinical translation of these systems.⁹⁹

5.1. Mechanical Strength

In spinal fusion procedures, implant materials must provide sufficient mechanical support while maintaining bioactivity, as they are required to withstand significant physiological loads generated by body weight and motion. Although studies have shown that posterior lumbar fusion (PLF) combined with pedicle screw fixation significantly reduces intervertebral pressure, the region still experiences stress levels around 300 kPa postoperatively.¹⁰⁰ In contrast, ACDF procedures involving titanium screws and PEEK cages can result in initial stress levels approaching 30 MPa due to differences in fixation methods and spinal biomechanics.¹⁰¹ Such high-stress conditions far exceed the mechanical limits of conventional hydrogels—for instance, 10% gelatin hydrogel exhibits an elastic modulus of less than 1 MPa, which is inadequate for supporting the

mechanical demands of interbody fusion devices.¹⁰²

5.2. Degradability

For successful spinal fusion, the degradation rate of hydrogel must synchronize with new bone formation to ensure proper support and gradual replacement by native tissue. A scaffold that degrades too quickly may fail to provide sufficient mechanical support for the developing bone, while one that persists too long can hinder regeneration and integration with native bone. In contrast to conventional spinal implants such as PEEK, which exhibit no degradation behavior due to their hydrophobic nature,¹⁰³ hydrogel-based scaffolds offer the advantage of tunable degradability.¹⁰⁴ This property is critical for spinal fusion applications, where the regeneration and stabilization of bone tissue typically require a period of 3 to 6 months.¹⁰¹ Hydrogels can be engineered with varying degradation rates by adjusting polymer composition, crosslinking density, and incorporating enzyme-sensitive linkages.^{69,105} This allows the scaffold to gradually degrade in synchrony with new tissue formation, providing temporary mechanical support while avoiding long-term foreign body reactions.

5.3. Printability

The success of bioprinting in spinal fusion relies on the precise deposition of bioinks to fabricate patient-specific implants. Printability is influenced by polymer properties such as molecular weight, degree of functionalization, and concentration.²⁸ According to Mancha Sánchez *et al.*'s¹⁰⁶ review of hydrogels used for bioprinting in recent years, although unmodified natural hydrogels are favored for their excellent biocompatibility and affordability, their rheological properties limit their printability, resulting in printed constructs whose mechanical performance only meets the requirements of certain applications. Natural hydrogels tend to reach their technical and biological limits easily, thus gradually failing to meet the high-performance demands of complex bioprinting. For example, gelatin exhibits poor rheological properties, while chitosan suffers from low mechanical strength and slow gelation, making these natural hydrogels rarely used alone in recent years.

To address these material and performance-related challenges, robust evaluation strategies are essential. The following section explores how hydrogel-based bioinks for spinal fusion are assessed across multiple levels, including *in silico* modeling, *ex vivo* systems, and clinical studies. These complementary approaches enable systematic investigation of mechanical behavior, degradation profiles, printability, and biological performance, helping to bridge the gap between material design and clinical translation.

6. *In silico* models: Assessment of mechanics and bone implant integration

To reduce reliance on costly animal and cadaveric studies, computational modeling approaches are increasingly being applied to the field of tissue engineering and regenerative medicine to establish: (i) the mechanical performance of these engineered devices, scaffolds, and hydrogels within patient-specific anatomies and (ii) optimize the manufacturing process for creating these structures to ensure cell viability. Fundamentally, creating validated computational models provides a significant improvement on tedious experimental iterations for optimizing bioink and the desired mechanical properties of these 3D-printed structures, reducing the quantity of animal testing and producing results in a time and cost-effective manner.¹⁰⁷⁻¹⁰⁹ Developing validated models requires the development of a virtual model of the implant and the relative mechanical properties of the tissue and the implant, in which these are generally obtained from experimental tests. Once validated, the relevant contact, loading, and boundary conditions can be implemented and subsequently simulated to assess the biomechanics of the tissue and the influence the implant has on the surrounding structures. Furthermore, *in silico* models offer distinct and underutilized advantages for hydrogel development when compared to pre-clinical and clinical investigations. Computational approaches can enable simulation of hydrogel behavior across temporal and spatial scales, allowing prediction of crosslinking dynamics, degradation profiles, and cell-material interactions under controlled conditions. Moreover, they support parametric optimization of bioink formulations and printing conditions, reducing experimental variability and accelerating design iteration.

Therefore, the following section highlights *in silico* modeling not as a supplementary tool, but as a central framework that complements pre-clinical and clinical studies, helping bridge the gap between material design and clinical translation. Several computational techniques are of particular interest for assessing the mechanical properties of 3D-printed structures, the mechanics and influence of the device, the effects of printing parameters, and the effects of bioink compositions on cell viability. [Figure 3](#) provides an overview of the standard assessment workflow, and these techniques will be discussed in Sections 6.1 and 6.2.

6.1. Finite element analysis

Finite element analysis (FEA) is an *in silico*-based technique used to simulate and predict how physical systems behave under various conditions, for example, thermal and mechanical conditions, and is widely used in the field of

engineering to understand how structures, components, and different materials respond under these loading conditions. Previously, FEA has been used extensively as an important tool in characterizing spinal biomechanics and scaffold implant after spinal fusion.¹¹⁰⁻¹¹⁴ In this review, we will summarize the effect of implant geometric design and material composition on its mechanical strength and the influence that bioink composition and printing parameters have on its mechanics.

6.1.1. Assessment of the effects of implant geometric design and material composition on mechanical strength

First, computational approaches have been used to assess the effects of varying geometric structures and material composition on mechanical strength.¹¹⁵⁻¹²² Ali *et al.*¹²³ showed that higher porosity in Ti6Al4V alloy for both gyroid- and lattice-based rectangular architectures reduces the elastic modulus and compressive strength. Furthermore, they demonstrated that lattice-based rectangular scaffolds exhibited higher elastic modulus and compressive strength than the gyroid structures at the same porosity level, making them more suitable for load-bearing applications. Gryko *et al.*¹²⁴ simulated scaffolds of different geometries (sphere, octagonal prism, cube, and triangular prism) with different pore sizes and porosity, assessing a range of metals such as Ti6Al4V alloy, cobalt-chromium, and 316L stainless steel. They observed that higher porosities reduced mechanical strength, while lower porosities increased the stiffness of scaffolds that exceeded bone stiffness, potentially suggesting that these could contribute to stress shielding and bone resorption. Interestingly, Schipani *et al.*¹²⁵ combined 3D printing with FEA models to investigate the design of polycaprolactone (PCL) scaffolds with varying fiber diameters, spacing, and laydown patterns (aligned, single offset, double offset). They also found that increasing fiber spacing or reducing the fiber diameter increased scaffold porosity but reduced the compressive modulus, with higher porosity scaffolds experiencing greater permanent deformation under compressive strain. Overall, the design and material composition have a significant influence on the mechanical properties of the implant created and must be optimized for its particular use case.

6.1.2. Effect of bioink composition and printing setup on mechanics

Although previous studies show what is expected of different structural designs and materials used for scaffolds, they do not investigate the effect that bioink composition and printing setup have on the mechanics of the created 3D-printed scaffold. Castilho *et al.*¹²⁶ explored GelMA

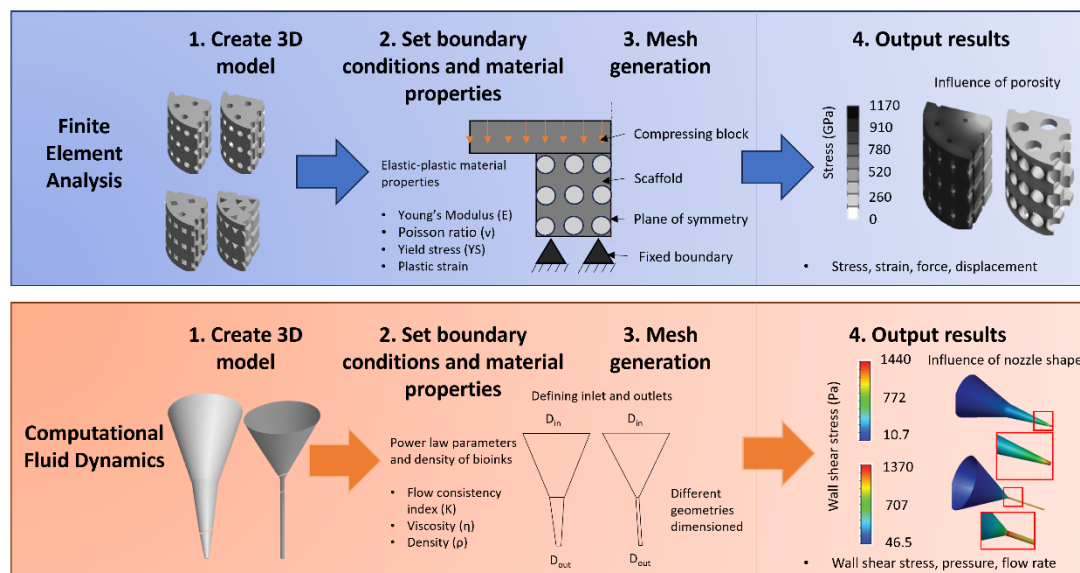


Figure 3. Standard *in silico* workflow for evaluating scaffold mechanics, bone–implant integration, and cell viability using finite element analysis (adapted with permission from Gryko *et al.*¹²⁴ Copyright © 2022, Elsevier Ltd.) and computational fluid dynamics (adapted with permission from Chand *et al.*¹³²)

hydrogels reinforced with melt-electrospun PCL fibers. They observed that lateral expansion of the hydrogel placed the fibers under tension, with this confinement mechanism being more prominent at lower fiber volume fractions. To assess these experimental results, a continuum model was developed to capture the hydrogel confinement, and a micro-finite element model was used to analyze stress distributions and deformation under compression. They observed that the continuum model effectively captured the hydrogel confinement mechanism by observing the axial area expansion seen experimentally. Furthermore, stress distributions differed significantly between scaffold alone and scaffold hydrogel composites, with the stiffness increasing when combined with the hydrogel. Ansari *et al.*¹²⁷ investigated the effect of 3D printing on the morphological and mechanical properties of PCL filament and scaffolds. They observed that printing processes, such as residence time and solidification time, in conjunction with printing parameters such as pressure, print speed, and printing path length, influence the filament properties. Furthermore, by increasing the filament diameter, a significant increase in the compressive elastic modulus of the scaffold can be achieved. To extend on this work, Soufivand *et al.*¹²⁸ further investigated the mechanical behavior of 3D-bioprinted tissue-engineered scaffolds. By assessing four different inner geometries: lattice, wavy, hexagonal, and shifted microstructures, they observed that FEA predicted the mechanical properties of each scaffold, with hexagonal providing the highest stiffness. Scocozza *et al.*¹²⁹ focused on the scaffold that combines PCL for

mechanical strength and an alginate-based hydrogel for the gradual release of lysozyme for osteoinductive and osteoconductive purposes. They found that honeycomb infill patterns significantly improved the scaffold stiffness when compared to linear patterns, with the inclusion of alginate slightly reducing the mechanical strength. These observations were validated by FEA simulations. More recently, Vila Pouca *et al.*¹²⁰ simulated the printed process of sodium alginate hydrogels crosslinked with calcium chloride and predicted the expected mechanical deformations due to printing and how these deformations affect the final properties of the printed scaffold. They observed that slower speeds (5 mm/s) led to higher deformation observed in the scaffold when compared to the faster speed (10 mm/s), attributing this difference to gravity and viscoelastic creep during layer buildup.¹²⁰ Interestingly, the geometrical differences between the two scaffolds at different speeds had a noticeable deformation in the lower layers, further supporting this assessment of viscoelastic creep being one of the reasons the scaffold is deforming. Moreover, the simulation also captured this response, observing the bending of the filaments and bottom-layer compression.¹²⁰ Overall, these studies highlight the effect printing parameters have on the overall mechanical strength of scaffolds created with different materials, showing similar trends. Furthermore, the structure of the scaffold significantly influences its behavior under loading, and this must be considered depending on the application of interest.

6.2. Computational fluid dynamics assessment of cell viability and bioprinting strategies

Computational fluid dynamics (CFD) is an *in silico*-based technique that is fundamentally grounded in fluid mechanics and uses numerical approaches to simulate and predict how fluids behave within an environment. In order to assess the bioprinting process and determine the optimal printing conditions, CFD offers the opportunity to compare and predict the empirical and operational conditions of the 3D printer, the material that is used, the influence of flow on the permeability and wall shear stress (WSS) within the scaffold, and the overall printing environment to enhance cell viability.¹²³ To complement its structural analysis, Ali *et al.*¹²³ firstly showed that increased permeability, which is desirable for cell survival and proliferation, is observed with increases in porosity and pore size, with Rec-L scaffolds exhibiting higher permeability than gyroid scaffolds at the same porosity. Secondly, they observed that the WSS, which is beneficial for promoting cell differentiation, decreases as porosity increases due to the larger pore size and reduced velocity gradient of the fluid. Overall, the results in this study suggest there must be a trade-off to optimize both permeability and WSS in the design of the scaffold, with certain characteristics being more desirable depending on the specific tissue engineering requirement. Saatchi *et al.*¹³⁰ simulated 3D-printed scaffolds with different strand orientations inside a perfusion reactor at different inlet flow rates, observing the fluid velocity, shear stress, and WSS. They observed that with 30-degree strand orientation, there was higher fluid velocity, shear stress, and WSS, suggesting possible increased osteogenic differentiation and mineralization of mesenchymal stem cells. Emmermacher *et al.*¹³¹ also performed CFD simulations along with analytical calculations to predict flow parameters such as shear stress, flow velocities, and pressure gradients for extrusion of a bioink comprising alginate, methylcellulose, and agarose. They observed that the bioink exhibited a plug-like flow due to the shear-thinning properties, protecting the cells from extreme shear stress. Furthermore, significant gradients were observed in the cartridge and needle with elastic effects influencing the flow for bioinks. Lastly, they observed that the maximum shear stress at the needle outlet was proportional to the mass flow rate, but increased less significantly due to the shear thinning behavior of the bioink. Chand *et al.*¹³² performed a robust CFD investigation into the effects of bioprinting parameters such as nozzle geometry and diameter, inlet pressure, bioink rheology, and printing speed on the maximum WSS and cell viability. They observed that a cylindrical nozzle geometry has the lowest maximum WSS but exposes cells to shear stress for a longer portion of the nozzle length, reducing cell viability. Increasing the nozzle

diameter reduces maximum WSS and increases mass flow rate, improving cell survivability but reducing printing resolution. Lee *et al.*¹³³ performed a CFD assessment to evaluate the internal flow characteristics of bioinks within a screw-based dispenser system. Similar to previous studies, they examined velocity distribution, internal pressure, and WSS. They observed that the highest velocities occurred near the screw and nozzle outlet, whereas lower velocities were observed in the reservoir region. The outlet velocity increased with rotational speed, whereas the mass flow rate decreased as the nozzle size decreased. Internal pressure was notably higher in both the screw and nozzle regions, with an increase observed with higher bioink viscosity. Furthermore, WSS was highest in the screw region due to the shear-thinning properties of the bioink. Overall, these studies highlight the importance of characterizing the optimal printing parameters and conditions, bioink rheological properties, and assessment of the WSS within these systems to ensure cell viability after printing.

7. Pre-clinical and clinical assessment of hydrogels for spinal fusion

Assessing the *in vivo* efficacy of hydrogels in promoting spinal fusion requires the use of appropriate animal models; however, no single model can fully replicate the complex mechanics and healing processes of the human spine. Small animal models, such as rats, are generally unsuitable for direct spinal fusion studies due to their limited body size, which restricts surgical manipulation and implant placement. Rabbits are more commonly used in posterolateral and dorsolateral lumbar fusion models, but their relatively small size still imposes technical challenges and requires considerable surgical expertise. Large animal models, such as goats and sheep, are often preferred because their vertebral dimensions are comparable to those of humans, allowing human surgical techniques to be directly applied in constructing cervical and lumbar fusion models.¹³⁴ Nevertheless, the use of large animal models presents a major limitation, as it is nearly impossible to replicate the natural biomechanics of the human spine in these systems. Most large animal models are quadrupeds, and their spinal loading patterns, posture, and mechanical environment differ substantially from those of the bipedal human spine, thereby restricting their translational relevance despite their anatomical similarities.

7.1. Assessment of mechanics and bone implant integration

Although all the above-mentioned pre-clinical models have been used to assess the therapeutic potential of the engineered hydrogels and scaffolds, a question that is still

up for debate is the mechanical validity of these models and the influence of bone implant integration on the biomechanics of the spine. Rodents are typically used due to their cost-effectiveness and feasibility for therapeutic studies; however, mechanically, the anatomical sites commonly used in these studies, the coccygeal vertebrae and lumbar vertebrae, do not replicate the anatomy, size, and human loading conditions and magnitudes, with reported stiffnesses in the range of 90–180 N/mm, which is a poor mechanical model.^{135,136} For the assessment of bone-implant integration, this is not ideal either, as the size of the implanted device would be smaller than its expected use case. Larger animals such as rabbits and canines have higher stiffnesses trending toward that of human bone, in the ranges of 500–850 N/mm and 700–950 N/mm, respectively, but are still insufficient for the assessment of spinal mechanics and are more difficult to use due to both their size and ethical approval issues, also limiting their capacity to assess bone-implant integration.^{137–139} Sheep/goat/porcine models further move towards the stiffness exhibited by human bone, in the ranges of 1,100–2,000 N/mm and 1,000–3,000 N/mm, respectively.^{140–143} Table 3 provides a summary of the reported stiffnesses. However, a limitation for all these animal models is that they are quadrupeds, so axial loading conditions and spinal kinematics are significantly different from humans, limiting their capability of being used as a mechanical model of the human spine.¹⁴⁴ Cadaveric human spines would be deemed to be the gold standard in terms of mechanical comparability due to having the patient anatomy, including muscles and

ligament attachments, geometry and geometrical variance, load transfer characteristics, and minimal effect of the fixation media on the mechanical properties of bone.¹⁴⁵ However, interdonor variability in bone quality and the possible presence of disease, anatomical variance, and degeneration reduce the reproducibility of the mechanical tests, and being poor for any therapeutic-based assessment reduces its feasibility as a model for device assessment. Furthermore, the testing conditions, with and without intervertebral discs, ligaments, and endplates, can influence the observed stiffness and failure mechanisms.^{137,138,145} Overall, a compromise must be made on the mechanical and therapeutic assessment approaches when using these models.

7.2. Therapeutic capacity

Due to these inherent limitations, the current practice is to employ alternative animal models to indirectly validate the potential of biomaterials in promoting spinal fusion. To date, *in vivo* validation of hydrogels primarily focuses on the use of animal models to assess their wound healing, antibacterial, osteogenic, and angiogenic properties, which are particularly relevant for spinal fusion applications. Notably, mechanically enhanced synthetic hydrogels, such as polyvinyl alcohol/polyacrylamide systems, have been shown to promote tissue repair not only through structural support but also by modulating immune responses and enhancing angiogenesis via paracrine signaling.¹⁴⁶ The antibacterial and wound-healing capabilities of hydrogels are commonly evaluated

Table 3. Pre-clinical models used for the assessment of spinal mechanics and bone implant integration

Model	Mechanical relevance	Reported segment stiffnesses	Bone-implant integration	Strengths	Limitations
Rat (rodents)	Low	Axial compressive stiffness (90–180 N/mm)	Moderate	Cost-effective, good for early testing	Non-load bearing, poor biomechanical mimicry, small size
Rabbit	Moderate	Axial compressive stiffness (500–850 N/mm)	Good	Easy handling, decent bone remodeling	Small size, limited biomechanical mimicry
Canine	Moderate–high	Axial compressive stiffness (700–950 N/mm)	High	Similar anatomical scale depending on breed	Ethical issues, quadrupedal gait
Sheep/goat	High	Axial compressive stiffness (1,100–2,000 N/mm)	High	Good mechanical analog, fast bone remodeling	Quadrupedal species, differences in spine anatomy
Porcine	High	Axial compressive stiffness (1,000–3,000 N/mm)	High	Similar anatomical scale and size	Quadrupedal species
Cadaveric human lumbar spine	High	Axial compressive stiffness (250–3,000 N/mm)	High	Human spine anatomy	Fixation limits therapeutic study and inclusion of soft tissue, access difficult, level of degeneration present

using animal wound infection models, including both small and large animal models. In small animal models, the full-thickness wound infection model in rats is widely used, where wound healing is assessed by monitoring tissue redness, swelling, and the ratio of wound closure to the initial defect area.¹⁴⁷ In large animal models, the sheep tibial infection model is frequently employed to simulate bone infection conditions. Moriarty *et al.*¹⁴⁸ developed a chronic tibial infection model in Swiss Alpine sheep by implanting intramedullary nails and inoculating the bone with methicillin-sensitive or methicillin-resistant *Staphylococcus aureus*, while Boot *et al.*¹⁴⁹ used this model to assess the *in vivo* antibacterial efficacy of antibiotic-loaded hydrogels. The osteogenic and angiogenic potential of hydrogels is often investigated using critical-sized bone defect models in both small and large animals. In small animal models, rat calvarial defect and femoral condyle defect models are commonly used, where micro-computed tomography (CT) analysis measures new bone volume, bone mineral density, and trabecular structure, while histological staining, immunohistochemistry, and immunofluorescence staining assess the expression of osteogenic and angiogenic markers.⁵⁴ To bridge the gap between preliminary research and clinical applications, small animal models such as rats and rabbits are often chosen for their advantages, including ease of handling and housing, flexible anesthetic management, relative resistance to infection, and cost-effectiveness for early-stage studies. However, interbody fusion requires large, predictable, and parallel endplates, which are only present in larger animals. Small animals cannot provide such anatomical conditions and are therefore mainly limited to transverse process fusion models.¹³⁴ Consequently, large animal models such as goats and sheep are preferred for spinal fusion studies, as they not only share physiological, immunological, and anatomical similarities with humans but also offer vertebral dimensions suitable for constructing clinically relevant interbody fusion models. Among these, goat and sheep models are widely used because of their comparable bone remodeling rates, low aggression, and ease of handling. The sheep humeral/femoral defect model is extensively applied in bone tissue engineering, as demonstrated by Liu *et al.*,¹⁵⁰ who used it to investigate the bone regeneration potential of calcium phosphate scaffolds doped with surface or internally incorporated BMP-2. Another widely used model is the goat iliac crest defect model, which structurally resembles the metaphyseal region of long bones as it consists of both cortical and cancellous bone and is non-load-bearing, making it comparable to long bone defects stabilized with fixation devices.¹⁵¹ de Silva *et al.*¹⁵² employed this model to evaluate critical-sized bone defects in the cubic centimeter

range and validated the feasibility of endochondral ossification-mediated bone regeneration. In the context of interbody fusion models, most studies reported in the literature have relied on large animals such as goats and sheep; rabbit models are rarely employed to evaluate the efficacy of biomaterials in interbody fusion.¹⁵³ Lee *et al.*¹⁵⁴ developed an injectable HAP/ β -tricalcium phosphate hydrogel combined with stromal vascular fraction and low-dose recombinant human BMP-2 to enhance osteogenic differentiation and angiogenesis (Figure 4). They evaluated bone fusion and the quality of newly formed bone using a rat PLF model and a minipig oblique lateral interbody fusion model, the latter of which closely resembles human spinal fusion surgery and allows for the assessment of the clinical applicability of combination therapies. When rabbits are used, the models are typically dorsolateral or posterolateral fusion approaches, which do not adequately capture the role of biomaterials within the intervertebral space. This limitation highlights the need for alternative strategies to assess the mechanical properties and biological performance of biomaterials in spinal fusion. By integrating findings from both small and large animal models, researchers can systematically assess hydrogel-mediated wound healing, antibacterial activity, osteogenesis, and angiogenesis, providing valuable preclinical evidence for their potential application in spinal fusion and bone regeneration therapies.

7.3. Clinical models for spinal fusion

Current clinical trials for spinal fusion devices remain largely centered on comparisons between conventional materials, particularly 3D-printed titanium alloy cages and PEEK cages, rather than emerging bioengineered constructs such as hydrogel-based systems. Most existing clinical trials evaluate postoperative outcomes using standardized metrics, including fusion rate, cage subsidence, and range of motion, which serve as key indicators of biomechanical stability and functional recovery. For instance, Zhai *et al.*¹⁵⁵ conducted a systematic review and meta-analysis comparing 3D-printed porous titanium and PEEK interbody cages in ACDF. Their findings demonstrated no significant difference in fusion rates between the two groups. However, the porous titanium cages exhibited several clinical advantages, including reduced operative time, decreased intraoperative blood loss, shorter hospital stays, and fewer postoperative complications. Additionally, they were more effective in maintaining cervical lordosis and intervertebral height, alleviating pain, and promoting faster functional recovery. Similarly, Mayer *et al.*¹⁵⁶ reported that 3D-printed titanium cages achieved fusion rates and clinical outcomes comparable to conventional PEEK cages.

In contrast, the clinical translation of hydrogel-based

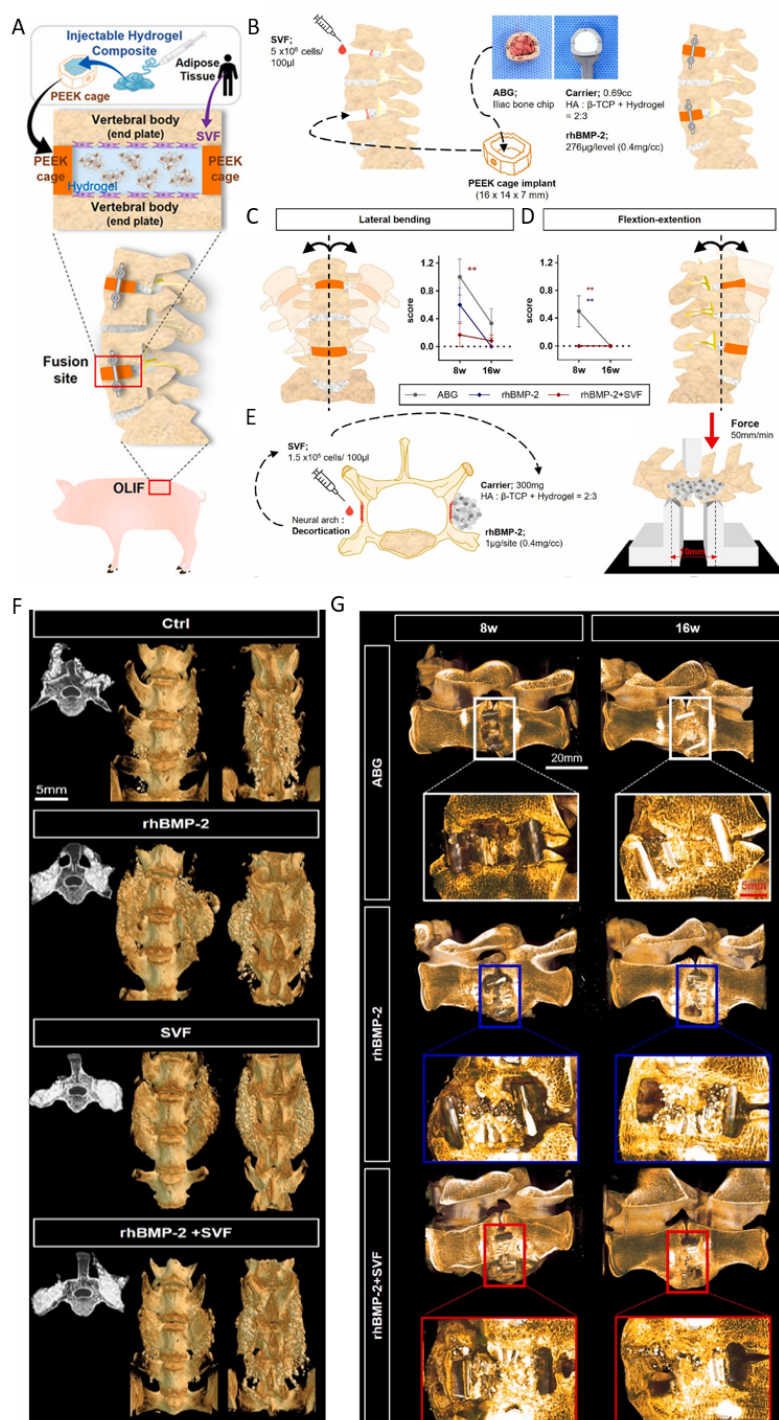


Figure 4. Experimental design and fusion assessment in minipig OLIF and rat PLF models. (A) Study design schematic. (B) Schematic image of transplantation conditions in the minipig OLIF model. (C) Lateral bending test of fused bone at 8 and 16 weeks. (D) Flexion–extension of fused bone at 8 and 16 weeks. Free movement in lateral bending and flexion–extension was recorded as 2 points, and no movement was recorded as 0 points. (E) Schematic image of the transplantation conditions in the rat PLF model. (F) Micro-computed tomography (CT) image of the rat PLF model for each experimental group, 2D and 3D reconstruction. (G) Micro-CT imaging of the fusion site, centered on the 3D-reconstructed cage implantation site. Adapted from Lee *et al.*¹⁵⁴ with permission. Copyright © 2025, Elsevier.

Abbreviations: ABG: Autologous bone graft; PEEK: Polyether ether ketone; OLIF: Oblique lateral interbody fusion; PLF: Posterior lumbar fusion; rhBMP-2: Recombinant human BMP-2; SVF: Stromal vascular fraction.

materials for spinal fusion remains extremely limited. Although hydrogels have been extensively explored in preclinical studies, particularly in intervertebral disc repair, where numerous *in vivo* animal studies have demonstrated promising regenerative and biomimetic properties,^{157,158} there is still a notable lack of human clinical trials. This translational bottleneck may stem from stringent regulatory approval requirements, concerns regarding long-term mechanical stability, and the inherently cautious nature of clinical adoption in spinal surgery, as well as the complexity and lack of clearly defined regulatory pathways. In the United States, the Food and Drug Administration (FDA) classifies 3D-printed and bioprinted products into medical devices, biologics, or drugs depending on their characteristics, with oversight provided by different centers such as the Center for Devices and Radiological Health, Center for Biologics Evaluation and Research, and Center for Drug Evaluation and Research. Approval pathways are determined based on risk classification (Class I–III), including routes such as 510(k), Premarket Approval, or Investigational Device Exemption.¹⁵⁹ However, the FDA has not yet established dedicated regulatory guidelines for bioprinted products containing living cells or complex biological architectures, and current evaluations largely rely on existing frameworks on a case-by-case basis, with stringent requirements for design control, manufacturing consistency, and performance validation.¹⁶⁰ In Europe, the European Medicines Agency (EMA) regulates such products under the Medical Device Regulation (MDR, Regulation (EU) 2017/745) and the Advanced Therapy Medicinal Products (ATMPs) framework. Bioprinted constructs containing cells or bioactive components are typically classified as tissue-engineered products or combined ATMPs, requiring rigorous standards for quality control, preclinical testing, and clinical evaluation.¹⁶¹ Although the EMA provides scientific advisory pathways for high-risk products, the overall approval process remains complex and time-consuming, with strict requirements for long-term safety and product consistency.¹⁶² Notably, no truly bioprinted spinal fusion devices have yet received regulatory approval for routine clinical application, further highlighting the uncertainty in this field. Consequently, a clear gap exists between the rapid advancement of hydrogel-based bioinks in experimental research and their limited adoption in clinical practice, reflecting significant regulatory barriers in the translation from bench to bedside. Future research should therefore incorporate regulatory considerations at the early stages of material design and promote the establishment of standardized evaluation frameworks to accelerate clinical translation.

8. Conclusion and future perspectives

The advancement of hydrogel-based bioinks for spinal fusion applications represents a transformative step toward developing more effective and biocompatible intervertebral fusion devices. Traditional spinal fusion techniques, which rely heavily on ABGs and rigid metallic implants, often face limitations such as stress shielding, implant subsidence, and insufficient osseointegration. In contrast, hydrogel-based bioinks offer enhanced biocompatibility, tunable degradation, and the ability to support cellular adhesion, proliferation, and differentiation, making them promising candidates for next-generation spinal implants.

Recent developments in 3D bioprinting and biomaterials engineering have enabled the fabrication of personalized, patient-specific implants with optimized mechanical, biological, and structural properties. Hydrogels, particularly those modified with bioactive molecules such as BMP-2 or reinforced with HAP or graphene-based nanomaterials, have demonstrated the ability to promote osteogenesis and vascularization, essential for successful spinal fusion. Additionally, hybrid bioinks that integrate synthetic and natural polymers have improved mechanical properties, ensuring structural stability while maintaining a favorable microenvironment for tissue regeneration.

However, challenges remain, particularly regarding the mechanical strength of hydrogel scaffolds, their long-term stability *in vivo*, and the need for controlled degradation rates that align with bone remodeling processes. Innovations such as dynamic crosslinking strategies, nanoparticle reinforcement, and bioactive coatings continue to improve the functionality of hydrogel-based bioinks, moving them closer to clinical translation.

With these advancements, 4D bioprinting is poised to revolutionize tissue engineering, offering intelligent, stimuli-responsive implants that adapt to the biological environment in real time, providing new possibilities for regenerative medicine, spinal fusion, and beyond.

Computational modeling approaches have typically been used to support experimental findings and guide optimization of the scaffold structure and printing parameters; however, several limitations remain in their application to FEA and CFD analysis of 3D-printed scaffolds. Many models use idealized geometries and simplified material assumptions; therefore, constitutive parameters should be calibrated against experimental data, and *ex vivo* imaging techniques (micro-CT) should be used to reconstruct scaffold geometry. Swelling or shrinkage of hydrogels is also difficult to capture without

complex user-defined material subroutines, yet these changes in geometry and stiffness significantly affect stress distributions, WSS, and cell viability predictions and should be included. To optimize the efficiency of these simulations, design of experiments and optimization strategies that include response surface modeling are recommended. Furthermore, fluid-structure interaction simulations, rather than separate FEA and CFD, could also provide more holistic insights into scaffold design. Overall, computational modeling offers a powerful means of evaluating scaffold mechanics and optimizing printing conditions to enhance bioink performance and cell viability, while reducing reliance on costly, time-intensive experiments.

Lastly, relevant pre-clinical and clinical models provide critical insight into the biomechanical performance and therapeutic efficacy of hydrogel-based bioinks and the constructs they form. *Ex vivo* systems such as cadaveric spinal segments enable controlled assessment of mechanical stability, load distribution, and early-stage integration under physiologically relevant conditions, while minimizing biological variability. *In vivo* pre-clinical models, including small and large animal studies, are essential for evaluating host responses, vascularization, bone regeneration, and long-term functional outcomes in a complex biological environment. Clinical studies, in turn, remain the definitive benchmark for assessing safety, efficacy, and translational feasibility in human patients. Importantly, the selection of an appropriate model should be guided by the specific research question and stage of development. For instance, early-stage studies may prioritize *in silico* and *ex vivo* approaches to optimize material properties and reduce variability, whereas *in vivo* models are more suitable for investigating biological integration and functional performance. Ultimately, a tiered and complementary use of *in silico*, *ex vivo*, pre-clinical, and clinical models is necessary to comprehensively evaluate hydrogel systems and ensure their successful translation to spinal fusion applications.

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Conflict of interest

The authors declare they have no competing interests.

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