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# Design and Chemical Engineering of Polymeric Biomaterials: Functionalization, Crosslinking, and Characterization Strategies for Advanced 3D Bioprinting

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## Abstract

Three-Dimensional (3D) bioprinting of polymeric biomaterials has emerged as a transformative platform in tissue engineering, enabling the fabrication of patient-specific scaffolds with precise spatial control. The performance of these constructs is fundamentally governed by the chemical architecture of the constituent polymers and the mechanisms of crosslinking that dictate their rheological behavior, mechanical integrity, and degradation kinetics, which collectively determine print fidelity and biological functionality. This review synthesizes current advances in natural and chemically modified synthetic polymers, elucidates the physicochemical principles underlying physical, ionic, and covalent crosslinking modalities, and highlights the intricate structure-property relationships that shape the behavior of printable bioinks. Moreover, contemporary characterization methodologies, recent material innovations, persistent challenges, and emerging directions aimed at enhancing biocompatibility and functional maturation are critically examined. Collectively, this work provides a rigorous framework to guide the rational design, optimization, and translational development of polymer-based biomaterials for next-generation regenerative medicine.

**Keywords:** Three-dimensional bioprinting, Polymeric biomaterials, Bioinks, Hydrogels, Tissue engineering.

## 1 | Introduction

Tissue engineering, aimed at the regeneration, repair, and replacement of damaged tissues and organs, has emerged as one of the most prominent interdisciplinary fields in life sciences and engineering [1]. This field integrates fundamental principles of cellular biology, materials science, chemical engineering, and advanced fabrication technologies to develop systems capable of recapitulating the complex microenvironment and native functionality of tissues. The increasing demand for alternative therapeutic strategies, the limited availability of transplantable tissues, the risk of immune responses following allogeneic transplantation, and the rising prevalence of tissue-degenerative diseases have positioned tissue engineering as a central research focus in regenerative medicine.

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Simultaneously, the advent of Three-Dimensional (3D) bioprinting has enabled the fabrication of constructs with complex, precisely controlled architectures, overcoming the limitations of conventional tissue engineering approaches, such as molding and electrospinning [2]. Among the critical factors influencing bioprinting performance, polymeric biomaterials play a pivotal role. Both natural and synthetic polymers, such as alginate, chitosan, gelatin, Polycaprolactone (PCL), and Polyethylene Glycol (PEG), serve as bioinks that support cell viability, provide suitable rheological properties for printing, and recreate Extracellular Matrix (ECM)- like microenvironments [3]. The tunable nature of these polymers with respect to mechanical properties, permeability, and degradation behavior renders them suitable candidates for regenerative applications. Accordingly, the rational selection and engineering of polymeric biomaterials are key aspects of bioprinting research [4], [5].

Beyond the type and nature of the biomaterials, polymer chemistry and crosslinking mechanisms critically dictate print quality and long-term stability of bioprinted constructs [4]. Crosslinking strategies, including ionic, photoinduced, enzymatic, and chemical approaches, directly impact hydrogel properties such as mechanical behavior, structural integrity, gelation kinetics, and biocompatibility. Precise control over crosslink density and inter-chain interactions not only improves print fidelity and geometry retention but also influences biological processes such as cell migration, proliferation, and differentiation [6]. Therefore, recent advances in crosslinking technologies have paved the way for the development of smart biomaterials with enhanced biological performance [3], [7], [8].

This review aims to provide a comprehensive framework for understanding the role of chemistry in the design of polymeric bioinks. Initially, the fundamental principles of polymer chemistry and the key characteristics of bioinks suitable for 3D printing are discussed. Subsequently, the existing challenges and limitations in materials, crosslinking strategies, and biological performance are analyzed. Finally, strategies and future directions for the development of smart, multi-material bioinks are addressed. The structure of this review is designed to guide readers through the interplay among material chemistry, printing processes, and biological functionality in a stepwise, coherent manner.

# 2 | Chemically Engineered Polymeric Biomaterials for Three-Dimensional Bioprinting

Chemically engineered polymeric biomaterials are a cornerstone in advancing next-generation bioinks, critically influencing printability, rheological behavior, structural stability, and the overall biocompatibility of printed constructs [9]. Through precise chemical modifications, including functionalization, copolymerization, and the establishment of controlled crosslinked networks, these biomaterials can be rationally designed to achieve properties specifically aligned with the requirements of the target tissue [4], [5].

## 2.1 | Chemically Modified Natural Polymers

Natural polymers have garnered considerable attention in bioprinting owing to their inherent biocompatibility, structural resemblance to the ECM, and biodegradability. Despite these advantages, their limited mechanical strength, structural instability, and low viscosity pose challenges for direct printing, necessitating targeted chemical modifications [10], [11]. Such modifications typically involve the introduction of reactive functional groups, the synthesis of crosslinkable derivatives, or conjugation with cell-adhesive peptides, collectively enhancing network stability, cell-material interactions, and printability [3], [7], [12].

### 2.1.1 | Alginate and ionically crosslinkable derivatives

Alginate is widely employed as a bioink due to its rapid gelation in the presence of divalent cations, such as $Ca^{2+}$ [13]. However, its intrinsically low cell adhesive properties and limited mechanical tunability necessitate chemical modification [14]. Derivatives such as methacrylated alginate (AlgMA) and peptide-conjugated alginates enable controlled ionic or photo crosslinking, simultaneously improving printability, bioactivity, and

structural fidelity. These modifications support the mechanical integrity and load-bearing capacity of the initial printed constructs [11], [14].

### 2.1.2 | Gelatin and Gelatin methacrylate

Gelatin contains bioactive motifs that facilitate cell adhesion, proliferation, and migration. Nonetheless, its thermoreversible gelation limits print fidelity and construct stability. Chemical modification of gelatin with methacrylate groups produces Gelatin Methacrylate (GelMA), enabling controlled crosslinking, enhanced structural stability, and improved mechanical strength [3]. GelMA allows the fabrication of complex, geometrically stable scaffolds while maintaining high cell viability, making it a versatile choice for bioprinting [15], [16].

### 2.1.3 | Hyaluronic acid and methacrylated hyaluronic acid

Hyaluronic Acid (HA) is a principal ECM component that provides bioactive cues to support cellular functions and tissue repair [10]. Native HA possesses low mechanical strength and viscosity, limiting its suitability for direct bioprinting. Methacrylation of HA generates Hyaluronic Acid and Methacrylated Hyaluronic Acid (HAMA), which permits controlled crosslinking and tunable scaffold stiffness and degradation rates [7]. These characteristics render HAMA particularly suitable for soft tissue applications, including brain, vascular, and cartilage constructs [17].

### 2.1.4 | Chitosan and other functionalized polysaccharides

Chitosan, recognized for its antibacterial properties and biocompatibility, is a promising candidate for bioink formulation. Its limited solubility and weak gelation necessitate chemical modification. Methacrylated chitosan or PEG conjugated derivatives improve network stability, rheological properties, crosslinking efficiency, and print fidelity [18]. Similarly, other functionalized polysaccharides, such as modified starch and dextran, exhibit enhanced printability and mechanical performance, expanding the utility of natural polymers in advanced bioprinting applications [6].

## 2.2 | Synthetic biodegradable polymers

Synthetic biodegradable polymers are a cornerstone in the development of advanced bioinks and 3D-printed scaffolds, owing to their precisely tunable mechanical properties, degradation kinetics, and structural integrity [19]. Compared to natural polymers, they offer superior mechanical stability, controllable biodegradability, and the potential for targeted chemical modifications, enabling the rational design of scaffolds tailored to the specific requirements of the intended tissue [1], [20].

### 2.2.1 | Polylactic acid and its copolymers

Polylactic Acid (PLA) and its copolymers, such as Poly(Lactic-co-Glycolic Acid) (PLGA), are among the most extensively utilized biodegradable polymers in tissue engineering [1]. The degradation rate of PLGA can be finely tuned by adjusting the lactide-to-glycolide ratio, allowing scaffolds to be engineered with temporally defined profiles for tissue replacement. The inherent mechanical strength, processability, and compatibility of PLA and PLGA with load-bearing structures render them ideal for applications in hard tissue engineering, including bone and cartilage scaffolds [1], [19].

### 2.2.2 | Polycaprolactone and aliphatic polyesters

PCL is widely employed due to its low melting point, thermal flexibility, ease of processing, and long-term stability in physiological environments. Its slow degradation kinetics make PCL particularly suitable for long-term scaffolds and tissues that require sustained structural support [20]. The incorporation of PCL with other aliphatic polyesters, such as Poly(Dioxanone) (PDO) or Poly(Butylene Succinate) (PBS), facilitates the fabrication of multiphasic scaffolds with enhanced mechanical performance and tunable degradation profiles [1].

### 2.2.3 | Polyethylene glycol and polyethylene glycol-based networks

PEG and its functional derivatives, including PEGDA and PEGMA, are widely used for constructing photo-crosslinkable hydrogel networks [6]. PEG offers high hydrophilicity, excellent biocompatibility, and the ability to regulate network stiffness and permeability finely, enabling the creation of hydrogels with controlled biological responses. By modulating the degree of crosslinking, PEG-based hydrogels can be applied across diverse contexts, including soft tissue engineering, injectable bioinks, and drug delivery systems. At the same time, their physical properties and stability remain precisely tunable [8].

### 2.2.4 | Degradability control via copolymerization and terminal group modification

A critical strategy in designing synthetic biodegradable polymers is the fine-tuning of degradation kinetics and swelling behavior through copolymerization and terminal-group functionalization. Block, random, branched, and star-shaped copolymers provide precise control over mechanical properties, hydrophilicity, and network degradation rates. Terminal group modifications further enable modulation of cell-material interactions, water uptake, chemical stability, and degradation behavior [19], [21]. Collectively, these approaches facilitate the development of scaffolds with tailored, tissue-specific performance and temporally regulated functionality [1], [19].

## 2.3 | Hybrid polymer-nanostructured systems

Recent advances in bioprinting have underscored the critical role of hybrid polymeric and nanostructured systems in simultaneously enhancing the mechanical, rheological, and biological performance of bioinks [4]. These systems typically combine natural and synthetic polymers with nanoparticles, composites, or bioactive ceramic phases, enabling the fabrication of scaffolds that more closely replicate the hierarchical architecture and functional properties of native tissues. The precise design of polymer networks and surface engineering are pivotal factors that determine both structural stability and functional efficacy [22–24].

### 2.3.1 | Polymer-ceramic hybrid scaffolds

Integrating biocompatible polymers with bioactive ceramics, such as Hydroxyapatite (HAP) and β-Tricalcium Phosphate (β-TCP), provides an effective strategy for producing scaffolds with enhanced mechanical strength, bioactivity, and osteoconductivity. The incorporation of ceramic phases improves load-bearing capacity, mitigates premature degradation, and promotes cellular adhesion and bone formation. Critical parameters, including the polymer-to-ceramic ratio, nanoparticle size, and uniform distribution within the matrix, significantly influence stress transfer, mechanical integrity, and biological functionality, making these composites particularly suitable for bone and dental tissue engineering applications [1], [17].

### 2.3.2 | Polymer-nanofiller composites for rheological optimization

Embedding nanofillers, such as nanosilica, nanoclays, carbon nanotubes, and graphene oxide, within polymer matrices can substantially enhance mechanical reinforcement while finely tuning the rheological behavior of bioinks [22], [23]. These nanomaterials confer shear-thinning properties, improve post-printing structural fidelity, and enable precise control over gelation kinetics. Collectively, these modifications enable the production of high-resolution constructs with stable geometries and reproducible mechanical performance, which are essential for reliable scaffold fabrication and subsequent cellular organization [23], [25].

### 2.3.3 | Interface chemistry and network architecture in hybrid systems

Surface chemistry manipulation, interphase compatibility, and multi-scale network architectures are fundamental strategies for developing advanced hybrid scaffolds [26]. Optimizing crosslink density, polymer chain distribution, and hierarchical structuring, including multi-layered or gradient networks, directly impacts scaffold stiffness, swelling behavior, permeability, and long-term stability. By precisely controlling these parameters, hybrid scaffolds can achieve superior biological performance, enhanced cell-material interactions, and improved integration with host tissues [14], [24].

# 3 | Crosslinking Strategies in Three-Dimensional Printable Polymeric Biomaterials

Crosslinking is a fundamental determinant of the physicochemical and biological performance of 3D-printable polymeric biomaterials. It governs critical attributes such as mechanical strength, structural integrity, rheological behavior, and long-term scaffold stability [4]. The selection of an appropriate crosslinking modality directly influences print fidelity, shape retention, cellular interactions, scaffold permeability, and the efficiency of tissue regeneration processes. Designing polymer networks that effectively balance printability with biological functionality is essential for achieving scaffolds with predictable and reproducible performance. In the context of 3D bioprinting, crosslinking strategies encompass a range of approaches, including physical interactions, ionic bonding, advanced covalent chemistries, and dynamic dual-network systems [21]. The integration of multimodal and hybrid crosslinking mechanisms enables the fabrication of scaffolds that more closely recapitulate the mechanical and biological properties of native tissues while maintaining stability under complex physiological conditions. Given the strong dependence of bioink behavior on network architecture, rational and intelligent design of crosslinking mechanisms remains a central focus in the development of next-generation polymeric biomaterials [5].

## 3.1 | Physical and Ionic Crosslinking

Physical crosslinking is a predominant strategy in many natural polymers, offering mild, reversible, and nonreactive interactions that facilitate cell encapsulation without exposing cells to potentially cytotoxic chemical or photoinitiated agents [27]. Although constructs formed through physical crosslinking generally exhibit lower mechanical strength than covalently crosslinked networks, they are essential for maintaining the initial scaffold architecture and providing immediate structural integrity. Moreover, these networks can be strategically reinforced via secondary ionic or covalent crosslinking to enhance ultimate mechanical performance and long-term stability.

### 3.1.1 | Hydrogen bonds and hydrophobic interactions

Hydrogen bonding, van der Waals forces, and hydrophobic interactions constitute the primary mechanisms underlying physical network formation in natural polymers. These interactions are critical for gelation in materials such as gelatin, chitosan, poly(Vinyl Alcohol) (PVA), and agarose [28]. Notably, physical networks can form under physiological conditions and mild temperatures, enabling seamless integration of encapsulated cells while minimizing cellular stress. However, the inherently weaker nature of these interactions limits mechanical robustness. Strategies to augment structural stability include modulation of temperature, ionic strength adjustments, salt incorporation, polysaccharide complexation, or nanoparticle reinforcement, which collectively increase network density and preserve scaffold geometry.

### 3.1.2 | Thermoresponsive Gelation

Thermoresponsive gelation is widely employed in biofabrication due to its ability to support extrusion in a liquid state and to stabilize the gel at physiological temperatures. Temperature-sensitive polymers, including Pluronic F127, gelatin, and Poly(N-Isopropylacrylamide) (PNIPAAm), exhibit fluid-like behavior at lower temperatures, promoting printability, and transition into cohesive, stable networks as the temperature approaches physiological levels [29]. This mechanism enables gelation without the need for chemical or photoinitiated crosslinkers, making thermoresponsive polymers particularly suitable for cell-laden bioprinting. Nonetheless, thermally induced networks often lack sufficient mechanical strength for load-bearing or long-term applications, necessitating reinforcement through covalent, ionic, or photoinitiated crosslinking strategies to ensure structural stability and functional performance.

## 3.2 | Covalent and Photocrosslinking Strategies

Covalent crosslinking is a fundamental approach for constructing mechanically robust, structurally stable networks in 3D-printable biomaterials [26]. This strategy involves the formation of covalent bonds between polymer chains, providing precise control over mechanical stiffness, permeability, gelation kinetics, and long-term stability of the printed constructs. Engineered biomaterials such as HAMA, GelMA, and PEGDA predominantly rely on covalent mechanisms, enabling the fabrication of scaffolds with predictable mechanical properties and high structural fidelity.

### 3.2.1 | Radical polymerization of (meth)acrylate functionalized polymers

Radical polymerization of acrylate and methacrylate groups is the most widely adopted method for generating printable hydrogels. Upon light irradiation in the presence of a photoinitiator, radical reactions are initiated, covalently linking polymer chains to form a stable network [16]. This mechanism underpins many commonly used bioprinting hydrogels, including GelMA, AlgMA, HAMA, and PEGDA. Key advantages include rapid gelation, tunable mechanical properties, and the ability to fabricate complex geometries with high spatial resolution.

### 3.2.2 | Photoinitiators, light sources, and biocompatibility considerations

Photoinitiators critically influence both the efficiency of network formation and cell viability, making their selection pivotal in bioprinting applications [30]. Irgacure 2959, historically the standard, exhibits limited reactivity under visible light, prompting the adoption of alternatives. Recent photoinitiators, such as LAP, activated by blue light, offer higher photoreactivity, lower cytotoxicity, and accelerated polymerization. For highly sensitive cell types, initiators such as Eosin Y, activated by visible light, offer biocompatible alternatives. Key parameters, including wavelength, light intensity, solubility, cytotoxicity, and tissue penetration, must be carefully optimized to ensure efficient crosslinking while preserving cellular viability [7], [16].

### 3.2.3 | Click chemistry and addition reactions

Click chemistry, encompassing thiol-ene reactions, Michael additions, and Diels-Alder cycloadditions, has emerged as a versatile tool in bioprinting due to its high reaction rates, selectivity, mild reaction conditions, and compatibility with near physiological environments [31]. Networks engineered via click reactions allow precise tuning of mechanical stiffness, integration of functional domains, and spatially controlled scaffold architectures. This strategy has recently enabled the development of scaffolds with programmable biological functions and enhanced responsiveness, advancing the potential of next-generation tissue-engineered constructs [16].

## 3.3 | Dual and Dynamic Network Architectures

Dual network architectures have emerged as an advanced class of printable biomaterials that integrate the favorable rheological properties of physically crosslinked systems with the mechanical robustness and long-term structural stability of covalent networks [5]. Owing to this synergistic combination, dual network hydrogels have attracted considerable attention for the fabrication of constructs that must simultaneously exhibit high mechanical resilience, flexibility, softness, and biological functionality.

### 3.3.1 | Hybrid physical-covalent networks

In hybrid dual network systems, an initial physically crosslinked matrix ,commonly formed from gelatin or ionically crosslinked alginate ,is established to provide immediate structural integrity and printability. Subsequently, a secondary covalent network, such as GelMA or AlgMA, is introduced to reinforce the construct and enhance its mechanical durability [14]. This hierarchical architecture produces hydrogels characterized by favorable viscoelastic behavior, adequate shear-thinning for extrusion-based bioprinting, high compressive strength, and prolonged stability under physiological conditions. Such properties render

these networks particularly suitable for the fabrication of large-scale, load-bearing, and architecturally complex bioprinted tissues [15], [27].

### 3.3.2 | Dynamic networks based on reversible and supramolecular interactions

Dynamic hydrogels rely on a diverse set of reversible interactions that impart adaptive mechanical behavior, autonomous self-healing, and responsiveness to biochemical or physical stimuli [32]. The most commonly employed dynamic chemistries include:

I. Schiff base linkages: between amines and aldehydes, offering rapid formation and reversibility under mild conditions.

II. Boronate diol complexes, which exhibit reversible association modulated by pH changes or glucose concentration.

III. Metal ligand coordination: enabling tunable stiffness and intrinsic self-repair capacity.

IV. Host-guest interactions: typically cyclodextrin-based systems that assemble and dissociate selectively and reversibly.

These reversible interactions give rise to hydrogels with high structural dynamics, excellent self-healing capacity, and tunable mechanical properties, attributes that make them especially advantageous for bioprinting soft, structurally delicate tissues, such as neural tissue, adipose constructs, or tumor microenvironments [18], [21].

### 3.3.3 | Decoupled control of printability and long-term mechanical reinforcement

A contemporary design paradigm in hydrogel engineering aims to decouple rapid gelation for printability from secondary crosslinking mechanisms responsible for long-term mechanical reinforcement [33]. Representative strategies include:

I. Ionically crosslinked alginate combined with photo-crosslinkable GelMA: enabling high resolution printing followed by enhanced final stiffness.

II. Thermoresponsive hydrogels, which provide rapid phase transition during extrusion and are subsequently stabilized through slower covalent crosslinking.

III. PEG thiol networks incorporating dynamic Diels Alder reactions: offering reconfigurable network architecture alongside sustained mechanical stability.

Such multi-stage crosslinking approaches facilitate the creation of hydrogels with precise structural fidelity, reliable mechanical performance, and tunable viscoelastic profiles. These characteristics are essential for next-generation bioprinting and tissue engineering applications [5], [34]. The schematic below (*Fig. 1*) provides a concise representation of the sequential workflow underlying the formulation of natural polymer-based bioinks and the overall 3D bioprinting process.

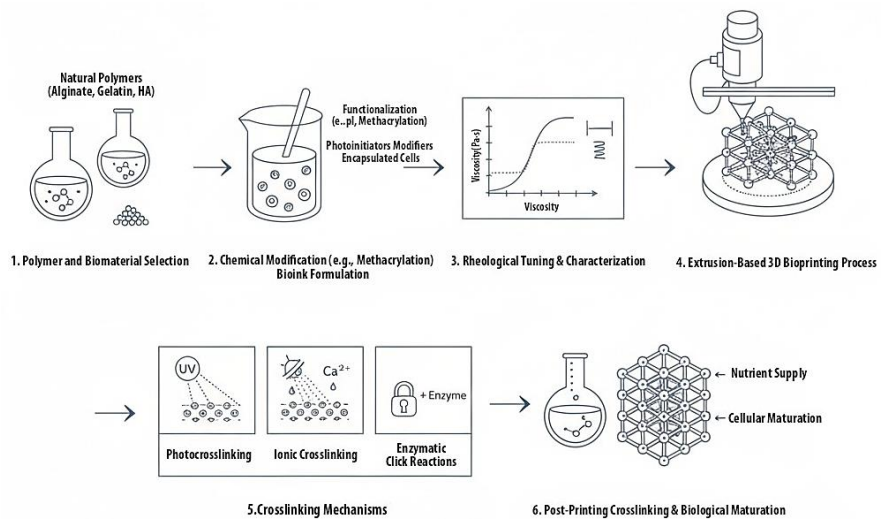


**Fig. 1. Schematic overview of the complete three-dimensional bioprinting process, including bioink preparation, printing, crosslinking, and tissue maturation.**

# 4 | Structure Property Relationships in Three-Dimensionally Printable Polymeric Biomaterials

A comprehensive understanding of the structure-property relationships in three-dimensionally printable polymeric biomaterials is essential for predicting and optimizing their performance during bioprinting. The interplay among molecular architecture, rheological behavior, mechanical response, degradation profile, and chemical functionality plays a decisive role in determining printability, structural fidelity, and the final quality of fabricated scaffolds [35]. By elucidating these correlations, it becomes possible to design biomaterials with enhanced print performance rationally, improved structural stability, and favorable biological interactions suitable for tissue regeneration [4], [5].

## 4.1 | Rheology and Printability

Appropriate rheological behavior is an essential prerequisite for the 3D printing of polymeric biomaterials, as the material's flow characteristics determine whether the bioink can be stably extruded from the nozzle, maintain its predefined shape, and allow successive layers to be accurately deposited [33], [35].

### 4.1.1 | Molecular weight, chain architecture, and viscosity

The molecular weight of the polymer and the chain architecture, whether linear, branched, or star-shaped, play a direct and critical role in defining the viscosity and viscoelastic behavior of the hydrogel. Polymers with higher molecular weight typically form denser physical networks, resulting in greater resistance to deformation during extrusion. Conversely, branched or star-shaped architectures enhance flowability while simultaneously improving the structural stability of the deposited printed layers [4], [33].

### 4.1.2 | Shear thinning behavior, yield stress, and filament formation

Shear-thinning behavior is a key characteristic of bioinks based on natural polymers, as reduced viscosity under shear enables smoother, more uniform extrusion through the nozzle [35]. An appropriate yield stress ensures that the material resolidifies after extrusion and retains its 3D structure. Furthermore, stable filament formation during extrusion depends on the balance between the elasticity of polymer chains and the rate of structural recovery following shear. This balance directly influences print line quality and prevents filament breakage [11].

### 4.1.3 | Influence of crosslink density on flow behavior

Crosslink density is a major factor governing the flow characteristics of hydrogels. Increasing the density of crosslinks elevates viscosity and enhances resistance to deformation [36]. Although high crosslink density can hinder extrusion and reduce flowability, it simultaneously improves post-printing structural stability and the ability to maintain geometric fidelity. Therefore, printable formulations are typically designed so that the material exhibits low crosslink density during deposition, followed by a stabilizing step, such as photoinduced or ion-mediated gelation, to increase the crosslink density after layer formation [33], [37].

## 4.2 | Mechanical Performance and Degradation Behavior

The mechanical characteristics and degradation profiles of printable biomaterials are critical determinants of scaffold stability, post-printing shape fidelity, and functional compatibility with the target tissue [27]. These properties ultimately govern the scaffold's performance within the biological environment and its ability to maintain structural integrity throughout natural degradation processes.

### 4.2.1 | Elastic modulus, strength, and toughness of printed scaffolds

The elastic modulus of hydrogels and printed scaffolds is primarily influenced by polymer molecular weight, concentration, and the nature of the network bonds [38]. Highly dense covalent networks exhibit increased stiffness, enhanced mechanical strength, and superior toughness, as greater energy is required to break the crosslinks. In contrast, physically or ionically crosslinked networks with lower crosslink densities exhibit more compliant, flexible behavior, making them better suited for replicating soft tissues. Consequently, scaffolds designed for bone or cartilage regeneration require high modulus, mechanically robust networks. In contrast, hydrogels with lower stiffness, which more closely replicate the mechanical environment of soft tissues such as brain, liver, or adipose tissue, are preferred for these applications [4], [27].

### 4.2.2 | Influence of bond type and water content on mechanical behavior

The nature of bonds within the polymer network, including physical, ionic, covalent, and supramolecular interactions, plays a decisive role in defining mechanical behavior, particularly stiffness, deformation recoverability, and fracture resistance [25]. Increased water content generally reduces modulus and strength while enhancing toughness, as water acts as a plasticizing agent, softening the network and allowing large deformations without catastrophic failure. Accordingly, high-water-content hydrogels often exhibit mechanical responses similar to those of native soft tissues [32].

### 4.2.3 | Hydrolytic and enzymatic degradation mechanisms

Biodegradable synthetic polymers containing ester linkages, such as PLA, PCL, and PLGA, undergo hydrolytic degradation under physiological conditions through cleavage of ester bonds [19]. In contrast, many natural polymers, including gelatin, hyaluronan, and alginate, are predominantly degraded enzymatically by tissue-specific enzymes such as collagenase, hyaluronidase, or alginate lyase [7]. The rate and pattern of degradation are governed by factors such as crosslinking density, polymer crystallinity, water content within the network, and permeability to water molecules and enzymes. Rational design of these parameters enables precise synchronization between scaffold degradation and the tissue regeneration timeline [9].

## 4.3 | Surface Chemistry and Interaction with the Biological Environment

The surface chemistry and properties of polymeric scaffolds are critical determinants of their interactions with the biological environment [34]. The scaffold surface serves as the first interface with proteins and cells, thereby playing a pivotal role in biocompatibility and tissue function. This superficial layer directly influences cell adhesion, organization, and, ultimately, the scaffold's tissue-level performance. Therefore, controlled surface design constitutes a key step in the engineering of printable biomaterials [9], [24].

### 4.3.1 | Functional groups, surface charge, and hydrophilicity/hydrophobicity

The chemical composition of the scaffold surface, including the type and density of functional groups, dictates surface charge, polarity, and the degree of hydrophilicity or hydrophobicity. Functional groups such as -COOH, $-NH_2$, -OH, and $-SO_3H$ modulate the surface charge and the network's hydrophilic nature. Hydrophilic surfaces generally exhibit lower protein adsorption and are more suitable for soft-tissue applications, whereas hydrophobic surfaces enhance adhesion to specific cell types [24]. Controlling these parameters allows precise modulation of biological responses in vitro and in vivo [4], [9].

### 4.3.2 | Protein adsorption and initial cell-scaffold interactions

Proteins adsorbed within the initial seconds on the scaffold surface play a crucial role in mediating cell-material interactions. Early adsorption of serum proteins, such as albumin and fibronectin, dictates cell attachment, proliferation, and differentiation pathways. Surface modifications using adhesive peptides, such as RGD, or ECM proteins, enhance cellular adhesion and organization. These modifications are especially important for scaffolds that are inherently nonadhesive or highly hydrophilic, including PEG- or alginate-based materials [12], [24], [39].

### 4.3.3 | Surface modification strategies for bioactivation

Various strategies are employed to enhance scaffold bioactivity, including plasma treatment, graft polymerization, incorporation of bioactive factors, and enzymatic conjugation. These approaches directly improve cell adhesion, proliferation, differentiation, and localized signaling [9]. Such modifications enable the fabrication of scaffolds that not only provide structural support but also actively guide cellular behavior and tissue regeneration [5], [34].

# 5 | Physicochemical Characterization Methods for Three-Dimensional Printable Polymeric Biomaterials

## 5.1 | Chemical Structure and Network Analysis

### 5.1.1 | Fourier transform infrared and Nuclear magnetic resonance for confirmation of functionalization and crosslinking

Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy are fundamental tools for evaluating successful chemical modification or functionalization of polymers and the formation of physical or covalent networks in hydrogels [28]. In FTIR analysis, changes in the intensity of characteristic bands, such as C=O, C=C, and other functional groups, indicate the formation of new bonds and crosslinking. NMR provides both qualitative and quantitative information on the chemical arrangement, the extent of introduced functional groups, and the verification of repeating unit structures, enabling precise assessment of chemical modifications (*Fig. 2*). Recent studies have demonstrated that combining FTIR and NMR analyses allows for accurate determination of reaction efficiency and uniformity of the polymeric network [3], [7].

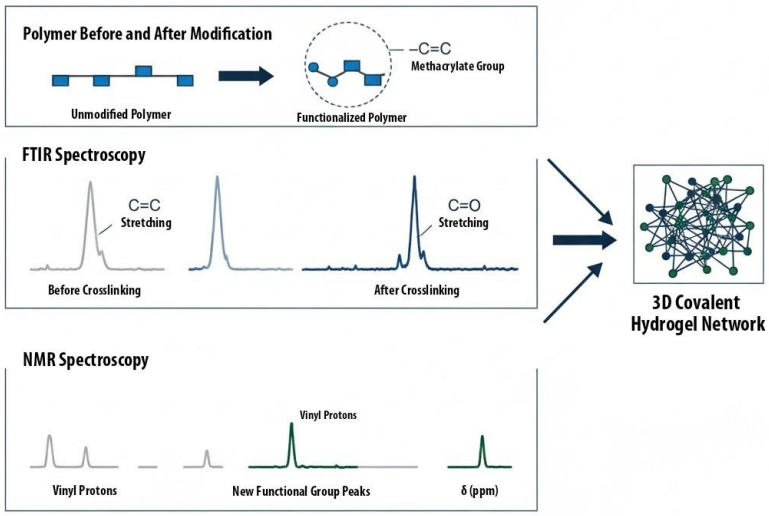


**Fig. 2. Spectroscopic confirmation of polymer functionalization and hydrogel crosslinking through Fourier transform infrared band shifts and nuclear magnetic resonance peak changes.**

### 5.1.2| determination of degree of substitution and crosslink density

In functionalized polymers, measuring the Degree of Substitution (DS) and crosslink density enables the prediction of swelling behavior, mechanical properties, and degradation patterns. DS is typically quantified via quantitative NMR, chemical titration, or analysis of FTIR band intensities. Crosslink density is estimated using a combination of equilibrium swelling data, elastic modulus measurements, and theoretical network models [40]. An increase in crosslink density generally results in reduced swelling and enhanced stiffness and strength, whereas a higher DS provides greater reactivity and a more regular polymer network [32], [41].

### 5.1.3|Correlating spectroscopic data with macroscopic properties

For the rational design of printable biomaterials, spectroscopic data should be correlated with macroscopic properties, including elastic modulus, mechanical strength, stability, and swelling behavior. Variations in the intensity of functional bands are typically associated with increased stiffness or decreased swelling. For example, an increase in the intensity of covalent bond-related FTIR bands usually corresponds to higher stiffness, mechanical stability, and thermal resistance of the printed scaffold [41]. Recent studies have shown that integrating spectroscopic data with macroscopic modeling enables precise prediction of material behavior under practical conditions [4], [8].

## 5.2|Thermal and Mechanical Characterization

### 5.2.1|Differential scanning calorimetry and thermogravimetric analysis for thermal transitions and stability

Thermal analyses, particularly Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA), play a crucial role in evaluating the thermal behavior and stability of 3D printable polymeric biomaterials. DSC enables the identification of the glass transition temperature (Tg), melting temperature, degree of crystallinity, and thermogelation behavior in polymeric systems. Conversely, TGA provides precise information on thermal stability, residual moisture content, and the proportion of organic/inorganic constituents by recording mass changes under controlled heating conditions [28] (*Fig. 3*). These techniques are essential for assessing the effects of additives, network type, and chemical modifications on thermal performance. Studies have demonstrated that increasing crosslink density typically elevates the decomposition temperature and reduces the rate of mass loss [27].

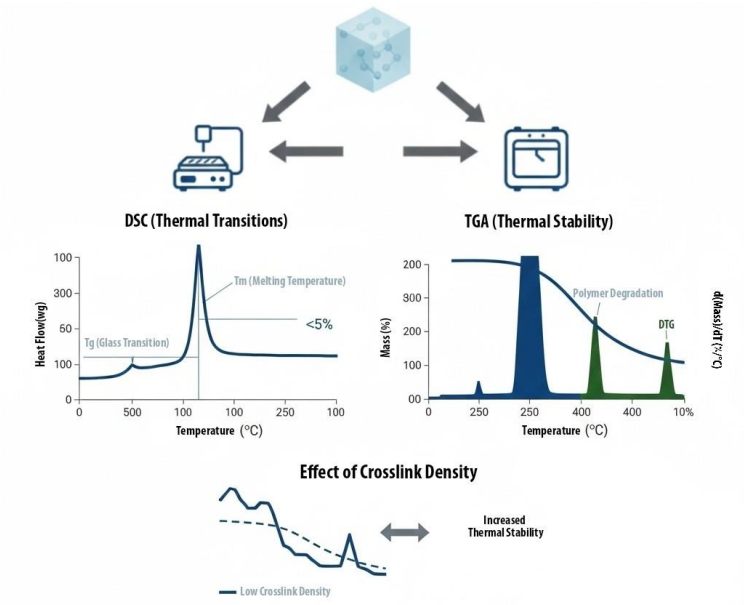


**Fig. 3. Thermal analysis of polymeric biomaterials using differential scanning calorimetry and thermogravimetric analysis to evaluate transition temperatures, thermal stability, and the effects of crosslinking density.**

### 5.2.2 | Tensile, compressive, and bending tests of printed scaffolds

Mechanical evaluation of 3D-printed scaffolds is critical to ensuring structural functionality in biological applications [8]. Tensile, compressive, and bending tests directly assess parameters such as the elastic modulus, ultimate strength, strain at break, and the material's elastic-plastic behavior. In hydrogel networks, higher crosslinking density or the incorporation of semi-crystalline polymers generally enhances mechanical strength while reducing deformation. Performing mechanical tests under hydrated conditions is particularly important, as it closely simulates the physiological environment and provides a more accurate representation of in vivo performance [20], [34].

### 5.2.3 | Viscoelastic assessment of hydrated networks

Hydrogel networks exhibit viscoelastic behavior, and their dynamic, time-dependent response makes viscoelastic characterization essential [42]. Measurements of storage modulus (G') and loss modulus (G'') in dynamic rheological tests provide insight into the network's response to variations in frequency, strain amplitude, and environmental conditions. These data allow determination of the gel point, relative stiffness, and recovery behavior following applied stress. In printable biomaterials, a direct correlation exists between rheological properties, gelation kinetics, and shape fidelity after extrusion [15], [37].

## 5.3 | Online Monitoring of Crosslinking and the Printing Process

### 5.3.1 | Rheometry during gelation and printing

Precise control over the printing process and the structural stability of scaffolds requires in situ rheometry. This approach enables real-time monitoring of viscosity, dynamic moduli, and gelation points during network formation. Such real-time data are particularly critical for photopolymerizable and thermogel systems, as they allow fine-tuning of printing speed, extrusion pressure, light intensity, and curing rate. Empirical studies have demonstrated that in-line rheological monitoring during printing substantially improves filament stability, shape fidelity, and uniformity of the final constructs [37].

### 5.3.2 | Online spectroscopic monitoring for reaction kinetics

Spectroscopic techniques such as ATR-FTIR, Raman, and UV-Vis provide essential real-time insights into the kinetics of reactive group conversion and crosslinking. These methods deliver instantaneous information

on double-bond conversion, consumption of reactive groups, and bond formation, thereby enabling precise evaluation of curing uniformity and printed-layer quality. ATR-FTIR, in particular, allows detailed monitoring of the initiation, propagation, and completion stages of photopolymerization or click reactions, facilitating optimization of curing time and minimizing structural defects [7], [12].

# 6 | Current Challenges and Future Directions in Biomaterial Chemistry for Three-Dimensional Bioprinting

Recent advances in biomaterial chemistry have significantly transformed the field of 3D bioprinting. However, limitations in crosslinking strategies, the need for stable, biocompatible polymers, and the need to develop smart materials indicate that this technology still faces considerable challenges before clinical translation [4]. These issues emphasize the critical importance of continued research and investment in biomaterial chemistry to enhance printing fidelity, optimize biological performance, and ultimately enable the successful application of 3D bioprinting in tissue engineering and regenerative medicine [5].

## 6.1 | Limitations of Current Crosslinking Chemistries

Common crosslinking strategies, including radical photopolymerization, ionic bonding, click reactions, and enzymatic mechanisms, each present distinct limitations [30]. For instance, although photocrosslinking offers high control, it is constrained by limited light penetration, the potential cytotoxicity of photoinitiators, and incompatibility with sensitive cell types. Ionic crosslinking, while rapid and straightforward, often compromises long-term mechanical stability and network uniformity. Similarly, strong covalent bonds enhance mechanical strength but are frequently incompatible with mild cellular conditions [7], [41].

Future research is directed toward the development of mild orthogonal reactions, visible-light photochemistry, and dual crosslinking strategies that enable initial shape formation during printing, followed by covalent network formation for final stabilization [4], [41]. These approaches aim to overcome current limitations and advance the translation of 3D bioprinting technologies to clinically relevant applications.

## 6.2 | Need for Biocompatible and Stable Polymeric Systems

The increasing demand for bio-based and environmentally friendly materials has made the development of renewable, biodegradable polymers a primary focus in 3D bioprinting [24]. Natural polymers and their derivatives, such as cellulose, alginate, chitosan, and polyhydroxyalkanoates, have attracted considerable attention due to their high biocompatibility, potential for chemical derivatization, and tunable rheological and mechanical properties. Despite these advantages, limitations such as structural heterogeneity, sensitivity to processing conditions, and difficulty in precisely controlling material properties remain [9], [40].

Future directions in this field include designing engineered bio-based polymers with controlled degrees of substitution, molecular weight, and degradability, as well as using green chemistry approaches in polymer synthesis and modification to produce safer, more sustainable bioinks [4], [24].

## 6.3 | Smart and Responsive Biomaterials for Three-Dimensional Bioprinting

Smart biomaterials that respond to stimuli such as temperature, pH, enzymatic activity, light, or electric and magnetic fields constitute the foundation of advanced 4D bioprinting and dynamically adaptive scaffolds [29]. These materials can perform functions such as controlled drug release, temporally programmed shape changes, and tissue structural reorganization. However, integrating responsive properties with the necessary rheological behavior for 3D printing while maintaining cellular viability remains a significant challenge. Moreover, controlling multiple stimuli simultaneously and maintaining stable material behavior in complex physiological environments are critical hurdles for broader clinical applications. Future research focuses on developing hybrid hydrogels that combine dynamic and covalent networks, incorporating nanostructures to enhance responsiveness, and creating multi-stimuli systems compatible with stable printing [18], [26], [41].

## 6.4 | Integration of Multi-Material and Gradient Structures in Chemical Design

Integrating multi-material structures and generating precise gradients in printed scaffolds is a crucial strategy for mimicking the complex heterogeneity of natural tissues [26]. Many tissues, including bone, cartilage, tendon, and skin, exhibit gradual variations in composition, density, mechanical properties, and biological activity. To replicate such structures, precise control over rheology, crosslinking kinetics, and chemical compatibility between different materials is required [20], [27]. Although multi-material printing technologies and microfluidic systems have enabled the fabrication of gradient patterns at the micron scale, challenges such as chemical incompatibility, differences in flow behavior, and mismatched curing windows and layer strength still hinder the production of integrated structures [34], [37]. One effective solution is the use of orthogonal chemistries that can operate without interference or unwanted reactions in a shared environment, thereby facilitating the formation of uniform and stable gradient structures. Designing bioinks with compatible viscosities and curing mechanisms, developing multi-channel printers with precise mixing control, and integrating real-time structural monitoring collectively define the future trajectory of this field [43].

# 7 | Conclusion

## 7.1 | Key Principles of Chemical Design in Three-Dimensional Printable Polymeric Biomaterials

Designing polymeric biomaterials for 3D bioprinting requires a careful balance between molecular structure, rheological behavior, and the final scaffold properties. The selection of polymer type, molecular weight, chain architecture, and functional groups must ensure proper flow and shape fidelity during printing while providing controlled mechanical strength, toughness, and degradation post-printing. Additionally, the choice of crosslinking strategy should be compatible with cellular viability, network formation kinetics, and long-term stability. Surface chemistry also plays a crucial role in cell adhesion and scaffold tissue integration [44].

## 7.2 | Outlook on the Synergy Between Chemistry, Printing Process, and Biological Response

The future of bioprinting relies on the seamless integration of material chemistry, printing technology, and cellular behavior. The development of smart materials, multi-stage networks, and gradient scaffolds enables the fabrication of more complex and functional constructs. Simultaneously, advances in multi-material printing techniques and real-time process monitoring enhance control over scaffold architecture and curing. Combining these technological advancements with a deeper understanding of cellular requirements paves the way for producing transplantable tissues and advanced functional biomaterials

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