A Comprehensive Review of Bioinks and 3D Printing: Bridging the Gap Between Innovation and Practicality

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Abstract

Bioinks, polymer matrices printed alongside cells, simulate an extracellular matrix environment that supports cell viability and proliferation. These bioinks can be composed of hydrogels, synthetic polymers, or other specialized materials, each offering unique properties beneficial to tissue engineering and regenerative medicine. Various additive manufacturing techniques exist, but extrusion-based methods are favored for bioprinting due to their compatibility with various biomaterials. Direct Ink Writing (DIW) stands out for its precision and versatility. DIW process involves extrusion, solidification, and layer support, with theoretical underpinnings based on Deborah and Weissenberg numbers, which describe the flow behavior of the bioink. Improving nanoparticle and matric interaction yet considering cytotoxicity and cell viability during the extrusion process is a novel challenge that needs to be deeply studied. Types of particle-particle and matrix-particle forces within inks are discussed to introduce the critical concept of percolation threshold in nanomaterials and polymer matrices. This concept is essential for understanding and optimizing printed constructs' mechanical properties and functionality. Recent research highlights that viscosity modifiers enhance printability and shape fidelity but can adversely affect cell viability. Addressing these challenges is crucial for advancing the field of bioprinting and realizing its full potential in medical applications.

Keywords: Bioink, Additive manufacturing, Rheology, Shear Thinning, Extrusion Bioprinting, Printability

1 Introduction

Bioprinting, a novel type of Additive Manufacturing (AM) Technique, is used in tissue engineering and the repair of damaged tissues. The final product of this method can be utilized for repairing and replacing damaged tissues. By generating biological tissues, a 3D structure can be achieved that encapsulates the desired cells. Furthermore, this 3D structure can house living molecules, microtissues, and target organisms to form the desired tissue structure [1, 2]. Bioink, consisting of a polymer matrix, plays a crucial role in the bioprinting process alongside cells. Its significance can be delineated into two primary aspects. First, the direct exposure of cells to mechanical stress during the printing process can compromise cell viability and induce cell death. Bioink mitigates this by ensuring uniform stress distribution, thereby preventing excessive localized pressure on cell structures. Second, the polymer matrix endows the printed construct with enhanced robustness and stability, preserving cell integrity post-printing. This resilient structure, which closely replicates the mechanical properties of native human tissues, fosters an optimal microenvironment for cell growth and proliferation, facilitating the formation of functional tissue constructs. Furthermore, the polymer matrix can be engineered by incorporating nanoparticles with specific properties, such as magnetic responsiveness or biodegradability, to further augment cell growth and differentiation [3, 4]. On the basis of the study, we discuss the various components and different types of bioinks commonly used for biomedical engineering. Next, we explore different types of 3D bioprinting techniques, with a focus on the Direct Ink Writing (DIW) method, an extrusion-based technique that has proven superior for developing well-structured tissues required for bioprinting. We then employ a polymer engineering approach to thoroughly understand the parameters and mechanisms involved in this process. This is followed by an investigation into how the addition of viscosity modifiers can enhance bioink quality, impacting key parameters such as shape fidelity and cell viability during extrusion. Finally, we delve into the molecular interactions between nanoparticles and how the incorporation of these particles as viscosity modifiers can improve the polymer matrices. This approach is novel among review articles, bridging the gap between engineering and biological sciences through an engineering perspective.

1.1 Types of Bioinks based on Structure and Composition

1.1.1 Hydrogel-based Bioinks

The advantage of using hydrogels lies in their general biodegradability and biocompatibility, as well as their ability to store a large volume of water within their molecular structure. Additionally, some hydrogels have special binding sites where cells can attach and interact. Following these interactions, crosslinking throughout the hydrogel network becomes possible [5]. Collagen is an ideal material for creating such hydrogels due to its protein-based nature, which is inherent in the human body, making it both biocompatible and biodegradable [6]. Koch et al. formulated a collagen-based bioink with encapsulated a group of cells and fibroblasts to print multilayer 3D skin tissues. They discovered communication between different cell types in the skin grafts, indicating specific tissue functions, promising for creating complex tissue structures [7]. In other studies, a combination of collagen-based hydrogels with agarose was used to encapsulate a specific type of stem cell, forming desired tissues through extrusion-based printing. After cell differentiation, collagen alone provided a matrix allowing cells to disperse easily, contrasting with agarose-containing matrices which offered a highly robust structure, resulting in better final structure and mechanical strength. Collagen promoted softer tissue while agarose produced stiffer tissue with higher mechanical integrity [8].

1.1.2 Polysaccharide-based Bioinks

Alginate, or alginic acid, is a naturally-derived anionic polysaccharide that shares similarities with some molecules found in human tissues. Its advantages include biocompatibility, easy gelation process, and cost-effectiveness [9, 10]. Due to its rapid gelation process under moderate stresses that do not harm cell structures, it is widely used as a bioink. Alginate gelation occurs rapidly in the presence of calcium and barium ions, forming crosslinks between polymer chains. This rapid gelation process is crucial for printability, making alginate and its derivatives excellent choices for ink production in conjunction with other biocompatible materials [11, 12].

1.1.3 Bioinks Based on Synthetic Polymers

Polyethylene glycol (PEG) is a polymer derived from the polymerization process of ethylene oxide. It can be synthesized to form linear or branched structures. This material offers mechanical properties and tunability, making it a suitable option for bioinks; Although like alginates, one drawback of these materials is their weak interaction and bonding with cells, which can be enhanced by combining them with other hydrogels[13]. Rutz et al. demonstrated high mechanical properties in 3D structures using polyethylene glycol by introducing amino bonds in PEG structure. The final results demonstrated improved cell adhesion properties and promising cell differentiation [14]. Figure 1 schematically illustrates a PEG-based bioink and its preparation process for bioprinting.

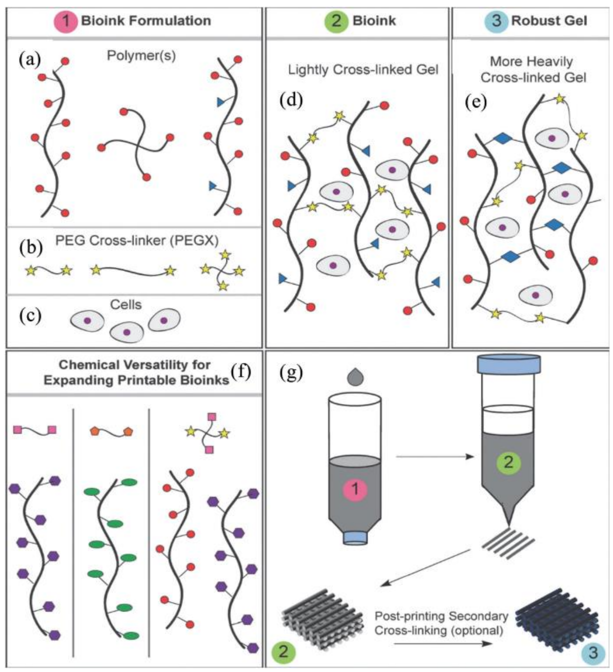

Figure 1. Structure of a PEG-based bioink and its preparation process for bioprinting [15]. Copyright 2020, Biomaterial Science

1.1.4 Composite bioinks

Nanomaterials possess numerous appealing characteristics, and researchers have integrated them into hydrogel biomaterials for use in bioinks. In one study, silver nanoparticles were used to 3D print ear tissue in alginate-based hydrogels. This research aimed to employ conductive nanoparticles and assess their impact on the structure of the formed tissue. Using electromagnetic wave tracking, it was observed that the cells were actively growing and dividing [16]. In another study, iron oxide nanoparticles were added to alginate-based bioink for enhancing printing speed and adjusting print orientations using a magnetic force. Although higher nanoparticle concentrations increased viscosity, cell viability initially dropped due to piston-induced stress but stabilized after 24 hours. Printing different viscosity matrices with equal nanoparticle content showed lower cell viability in viscous matrices, primarily due to increased pressure rather than the existence of nanoparticles themselves [17].

1.2 3D Bioprinting: Types of Processes

Bioprinting, a novel type of Additive Manufacturing technique, is mostly used in tissue engineering and the repair of damaged tissues [18]. 3D printing, or additive manufacturing, involves the construction of objects by sequentially layering cross-sectional slices. Initially designed using computer-assisted design, the model is sliced into layers stored as.STL files, which are compatible with industry-standard printers. Each layer is then printed sequentially. While adjustments may be required for different printing methods and materials, this approach enables versatile manufacturing, facilitating the production of identical parts across various printers [19]. 3D Printing methods offer cost-effectiveness, minimal waste, and faster production compared to traditional industrial processes or injection-mold manufacturing. Unlike traditional methods, 3D printing does not require tools like molds or indirect consumables, significantly reducing costs and lead times. Additionally, its low setup time and fixed costs make it economically viable for small production runs. Additionally, 3D printing allows for a significantly broader range of geometries compared to traditional methods. This includes printing simple solid objects like gears and complex systems with free-moving parts from the same machine [19]. Bioprinting is an advanced additive manufacturing technique where cells and biomaterials, referred to as 'bioink', are deposited simultaneously. This method bypasses the traditional challenge of seeding cells onto scaffolds in tissue engineering. It allows precise spatial distribution of different cell types within the printed structure, achieving high initial cell densities [20]. Different characteristics and parameters of bioinks, including rheological behavior, swelling properties, surface tension, and gelation kinetics, play a crucial role in determining its printability, especially in biofabrication techniques reliant on bioink dispensing. These parameters vary significantly depending on the specific bioprinting technique employed. Broadly categorized, these techniques include extrusion bioprinting (pneumatic and mechanical), orifice-free bioprinting (such as laser-induced forward transfer (LIFT) and printing by surface acoustic waves), and inkjet bioprinting (utilizing piezoelectric and thermal methods). Understanding and optimizing these properties are essential for advancing the precision and efficacy of bioprinted constructs in tissue engineering and regenerative medicine [21].

1.2.1 Inkjet bioprinting

In inkjet bioprinting, bioink droplets (10–50 μm diameter) are precisely deposited using piezoelectric or thermal inkjet systems. Piezoelectric printers use acoustic waves from crystals to eject bioink, while thermal printers vaporize bioink to expel droplets, without harming cells due to brief exposure (2 μs) to high temperatures (up to 300 °C). Surface tension influences droplet or jet formation; higher cell concentrations reduce surface tension by adsorbing cells to the liquid-gas interface. Gelation methods (physical, chemical, and photo-crosslinking) stabilize bioprinted structures, crucially occurring after nozzle exit to prevent blockages and maintain construct integrity. Maintaining low viscosity and cell densities ensures proper printing conditions, despite potential shear stress affecting cell viability. Inkjet bioprinters achieve high resolution, enabling precise cell deposition with various bioinks[21].

1.2.2 Orifice-free bioprinting

LIFT, also known as laser-assisted bioprinting, uses a pulsed laser beam to evaporate an absorbing layer beneath a bioink-coated donor substrate. This creates a high-pressure bubble that propels small bioink portions toward a collector platform, forming a temporary connection between substrates without nozzles or clogging issues. LIFT achieves resolutions of 10–100 μm, influenced by laser parameters, air gap, bioink viscosity (1-300 mPa s), and cell density (∼108 cells ml−1). Suitable for bioinks requiring fast crosslinking, it commonly employs ionic crosslinking in sodium alginate, temperature-dependent gelation in Matrigel, or enzymatic polymerization in fibrinogen. Surface acoustic wave bioprinting uses an acoustic ejector with interdigitated gold rings on piezoelectric substrates to eject bioink droplets from microfluidic channels, leveraging circular acoustic waves at the air-liquid interface for precise deposition [21].

1.2.3 Extrusion-based bioprinting

Extrusion bioprinting is a widely used method for creating 3D cell-laden constructs, where bioink is extruded through disposable plastic syringes using pneumatic or mechanical (piston- or screw-driven) systems onto a substrate. Unlike inkjet or LIFT bioprinters, extrusion bioprinters deposit larger hydrogel filaments (150–300 µm diameter). Piston-driven systems offer precise flow control, while screw-driven systems handle high-viscosity bioinks but may harm cells due to pressure drops. Pneumatic systems adjust well to various bioink types (30 _ $6*10^7$ mPa s) and high cell densities, enabling the incorporation of cell spheroids into 3D scaffolds [21]. Figure 2 illustrates all three types of bioprinting discussed in this section. One of the drawbacks of this technique is the application of shear stress on cells.

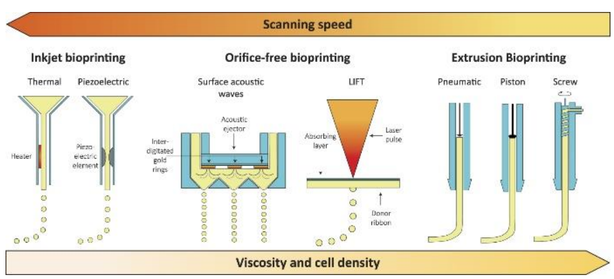

Figure 2. Overview of the most prevalent bioprinting techniques and the key parameters critical for material printability[21]. Copyright 2016, Biofabrication

While direct shear stress produces continuous lines of bioink, essential for creating an environment for cell growth and differentiation, it is detrimental to cells during the extrusion process, especially near the wall due to the shear stress profile and its maximum level in that area. Optimizing the induced pressure to fabricate tissues while maintaining cell viability during extrusion remains a challenge. Figure 3 briefly illustrates various bioprinting processes based on extrusion techniques [22].

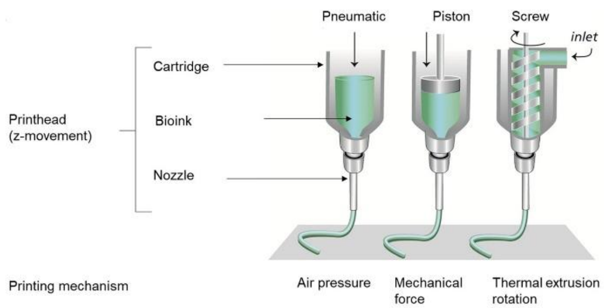

Figure 3. Schematic illustration of extrusion printing techniques: pneumatic, piston, and screw-driven printing [23]. Copyright 2020, Chemical Reviews

1.3 Direct Ink Writing Method: Developing a Roadmap and Emerging Challenges

DIW is an innovative extrusion-based technique that has gained attention due to its ability to create diverse geometric shapes of materials referred to as inks. The layer-by-layer printing approach and minimal heat transfer involved in most DIW processes make it suitable for printing sensitive materials such as bioinks, biomolecules, and metal-organic framework-based materials [24]. Extrusion-based bioprinting is a particular type of DIW method in which cells are added to a polymer matrix to create a desired product. For a deeper understanding, in this section, DIW method will be discussed. The DIW process generally consists of three main sub-functions which will be discussed below [25].

1.3.1 Extrusion

Extrusion serves as the primary stage in DIW techniques. During this process, ink and material within a cylinder (extruder in miniature dimensions) undergo stress, applying pressure gradients and shear forces to the polymer matrix, inducing its fluidization. Most polymers experience significant viscosity reduction under stress, a phenomenon known as Shear Thinning. It is essential for the ink within the piston to exhibit Shear Thinning behavior to ensure a reasonable pressure differential within the system, typically reported between 2-30 Psi for bioinks and below 130 Psi for inks in general [25, 26] From an engineering and molecular perspective, as stress and strain are imposed on the polymer matrix, the ink initially experiences stress and must reach its minimal yield point, known as Yield Stress, to initiate flow. With increasing stress and achieving the minimum yield stress, the Deborah number decreases significantly, indicating a transition from solid-like to liquid-like behavior in the polymer chains. During this stage, it is crucial for the ink within the piston to reduce its Deborah number adequately to prevent nozzle blockages and ensure smooth extrusion. Moreover, escalating the applied stress rate elevates the Weissenberg number within the system, where the number correlates directly with the applied stress rate. A higher Weissenberg number results in the opening of polymer chain coils, aligning them parallel to the applied stress direction. In other words, intermolecular and surface tension forces enhance with the rise of these parameters in the system, which is vital to maintain the original shape and external appearance of the ink post-extrusion and prevent ink from clotting and spreading within the substrate [27].

1.3.2 Solidification

At the end of the extrusion process, polymer layers are deposited either as the initial layer on the surface or on previously printed layers. This phase follows extrusion and the cessation of applied stress. During this state, the ink remains at rest and in relaxation, effectively experiencing zero external stress. In other words, the Deberah number dramatically increases, showing solid-like behavior, while the Wiesenberg number leads to zero due to zero-induced shear stress. At this stage, intermolecular and surface tension forces play a crucial role, essential for maintaining the overall shape and integrity of the printed line. Essentially, the only external force acting on the printed line is the ink mass in each layer. Due to gravitational forces, if intermolecular forces and surface tension are inadequate, structural failure and flow occur [25, 26].

1.3.3 Layer Support

In this stage, the structural integrity of the printed line and the presence of strong intermolecular and chain forces against the weight force of the printed line. The printed material line is either placed as the first layer on the surface and printing bed or subsequently on previously printed material lines. This stage is crucial because, besides its own force, the printed line must withstand the pressure exerted by the weight force of upper layers. Strong intermolecular and chain forces and desirable surface tension help the printed line withstand the weight of additional layers. Increased pressure from the upper layers may cause the structure to undergo complete sagging and collapse, resulting in an undesirable shape. Semi-empirical formulas proposed by Yu and Oi suggest critical points for deformation and apparent deformation of the structure [26].

1.4 Developing a Novel Roadmap to Address Challenges

Due to its desirable characteristics, DIW has garnered significant attention in various bioprinting applications. The roadmap must encompass a general strategy to classify and categorize inks without considering their specific chemical compositions. Generally, inks are classified into two categories based on their yield stress. The first category, time-dependent inks, requires additional processes such as UV exposure or thermal curing to achieve sufficient structural integrity. In contrast, Yield Stress Inks, another group of inks, maintain their initial structure with minimal applied stress, allowing reversible transitions between solid and liquid states. This distinction highlights Yield Stress Inks' physical and reversible nature compared to the irreversible phase transitions observed in Time-dependent Inks post-UV exposure or curing processes [25, 26]. To devise a comprehensive roadmap, all sub-functions and aspects must be carefully considered. During the extrusion stage, the desired ink properties should exhibit high Shear Thinning behavior and prompt recovery of the matrix structure immediately after deposition. Therefore, conducting rheological studies and analyzing data from rotational tests, dynamic tests within the linear viscoelastic range, and shear recovery measurements are crucial. These tests yield comprehensive insights into viscosity variations influenced by parameters such as applied shear stress and strain rate, providing valuable information for optimizing the extrusion process [25, 26]. During the Solidification and Layer Support phase, inks are not subjected to shear stress, and intermolecular and surface tension forces play a significantly better role, as shear stresses make it difficult to maintain intermolecular and chain forces. Therefore, these forces need to withstand the pressures caused by their own weight of the printing line or upper layers. Thus, this stage requires testing for stress that may predict changes in solid-liquid behavior or vice versa. The rheological investigations, such as oscillatory dynamic measurements to determine the linear viscoelastic region and analysis of ink rheology after UV curing, are suitable tools for completing the roadmap. The results of the Large Amplitude Oscillatory Sweep (LAOS) test serve as an invaluable tool for delineating the emergence of linear viscoelastic and nonlinear regions. Moreover, the intersection of the storage (elastic) modulus and the loss (viscous) modulus in dynamic tests indicates the phase transition of the ink from solid-like to liquid-like behavior. Evaluating the ratio of these two moduli provides comprehensive insights into the behavior of time-dependent inks. In such inks, upon exposure to UV light, the formation of final networks and the gelation phenomenon induce a solid-like behavior, reflected by an increase in the dynamic storage modulus. The gel point, characterized by the intersection of these moduli, defines the critical stress at which the ink undergoes solidification. This stress level determines the structural integrity of the cured ink. Figure 4 illustrates data drives from LAOS analysis and evaluating gelation point for TDS inks [25, 26].

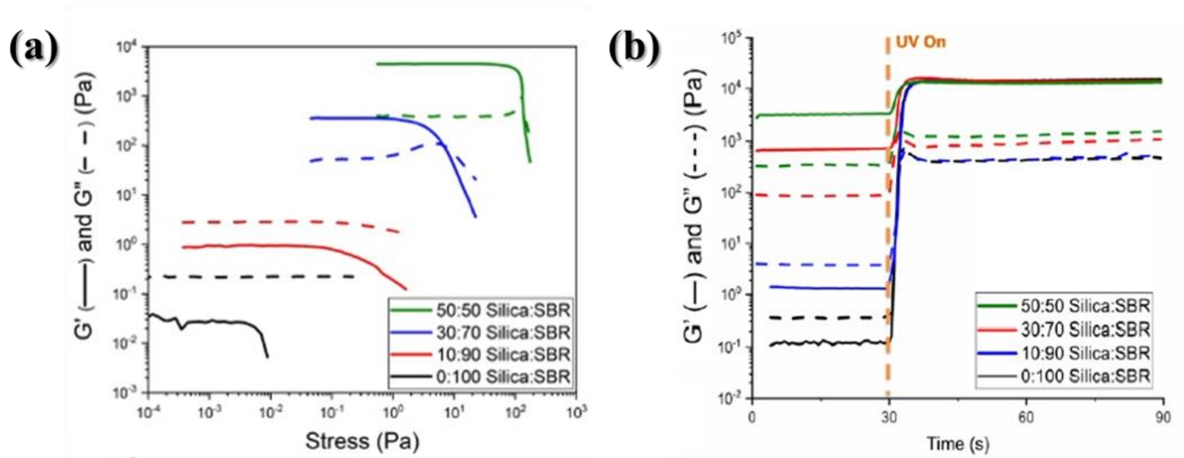

Figure 4. (a) Representative oscillatory strain sweeps plotted against stress. The blue and green formulations. (b) Illustration of UV curing of a combined solidification ink [28].Copyright 2018, Additive Manufacturing

Response tests such as Step Strain Sweep can simulate the extent of polymer matrix recovery. As previously mentioned, once the polymer exits the nozzle and the external shear stress is removed, the polymer can restore its structure. This test serves as a valuable benchmark for quantifying these properties, as it examines step responses; any delay in structural recovery indicates weak bonds that fail to maintain the overall shape integrity. Also, measuring how quickly a DIW ink resolidifies involves assessing changes in viscosity or stress when the applied shear rate is altered. When the syringe plunger is pressed, the shear rate rapidly increases, causing a significant rise in stress. As the ink is extruded, the shear rate quickly decreases, and the stress drops to zero. This behavior is demonstrated using the three-interval thixotropy test (3ITT) Thixotropy refers to time-dependent rheological behaviors that occur on different timescales than those associated with viscoelasticity. In thixotropic materials, a rapid decrease in viscosity occurs when shear is applied, followed by a time-dependent recovery in viscosity when the shear rate is reduced [27]. Figure 5 shows recovery behavior of inks simulating extrusion process.

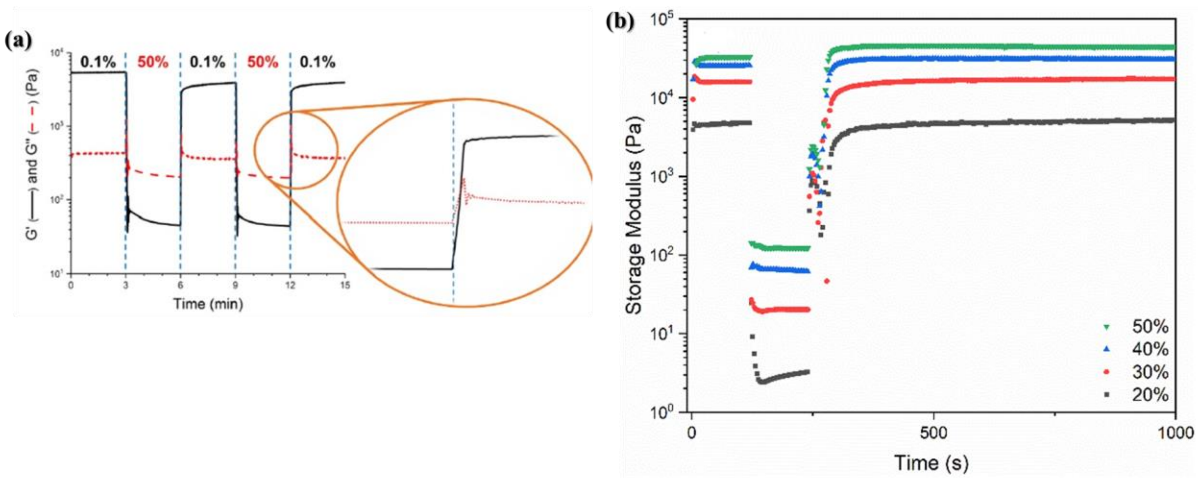

Figure 5. (a) Strain step-change experiment evaluating the time for the yielding and reconstructor of the internal structure [28]. Copyright 2018, Additive Manufacturing (b) 3ITT investigation showing changing structures of Pluronic solutions and glass inks[29]. Copyright 2015, Food Research International

1.5 Enhancing Polymer Matrices: Developing Network Structure with Nanoparticles

The utilization of nanoparticles to enhance the physical properties of inks and polymer matrices has been extensively studied. Various investigations have shown that increasing nanoparticle content improves the properties of polymer matrices. Based on molecular approaches, nanoparticles' dispersion state and distribution within the polymer system form a network that restricts the movements and rotations of polymer chains, leading to significant structural alterations in the matrix [27, 30]. As nanoparticle content increases and gets close to a particular concentration, there is an exponential increase in the rheological properties. The formation of this threshold is influenced by critical parameters such as nanoparticle dispersion, size and distribution, and environmental conditions, including temperature. Generally, identification of the Percolation Threshold is accomplished through oscillatory dynamic sweeps. The percolation thresholds of various polymeric systems have been extensively investigated by incorporating loadings of multiwall carbon nanotubes (MWCNTs) and cellulose nanocrystals. Figure 6 shows that increasing nanoparticle loading promotes dynamic moduli and in a specific mass ratio the nanoparticle makes a strong network which is equivalent to the percolation threshold point [30].

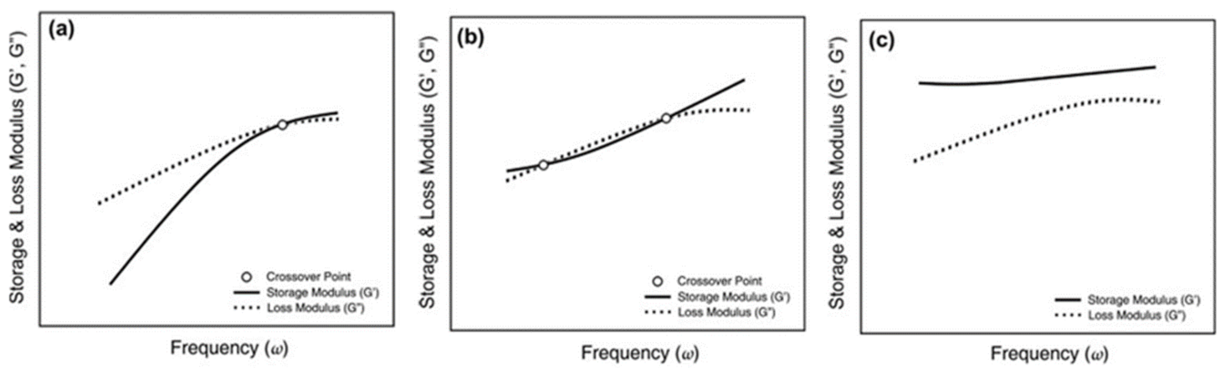

Figure 6. Storage and loss moduli of polymer/CNT composites with varying CNT content vs. frequency: (a) pure polymer, (b) low CNT, (c) high CNT [31]. Copyright 2014, Carbon

As previously discussed, extrusion-based bioprinting is a type of DIW method used to print cells alongside a polymer matrix to create a suitable environment for cell growth. Consequently, the incorporation of nanoparticles to develop a robust network structure is an area of significant interest. Current research on 3D printing of bioinks indicates that the functionality of the bioink is influenced by three critical factors: (a) printability, (b) shape fidelity, and (c) biocompatibility [30]. These factors are essential for ensuring the effectiveness of the printed constructs in tissue engineering applications. Increasing viscosity enhances printability and shape fidelity but necessitates higher applied extrusion pressure. This increased pressure can be detrimental to the living cells within the bioink, a factor often overlooked in the bioink optimization process. Habib et al. utilized a hybrid hydrogel composition, consisting of Alginate and medium viscosity carboxymethyl cellulose (CMC). The pressure exerted on the bioink during extrusion processes was analytically measured, and this data was incorporated into the bioink's rheological design. The impact of different CMC concentrations on rheological behavior with respect to shear rate was determined through the rheological investigations shown in Figure 7. All compositions exhibit shear-thinning properties, with viscosity decreasing as shear rate increases. The addition of CMC increases both viscosity and shear stress.

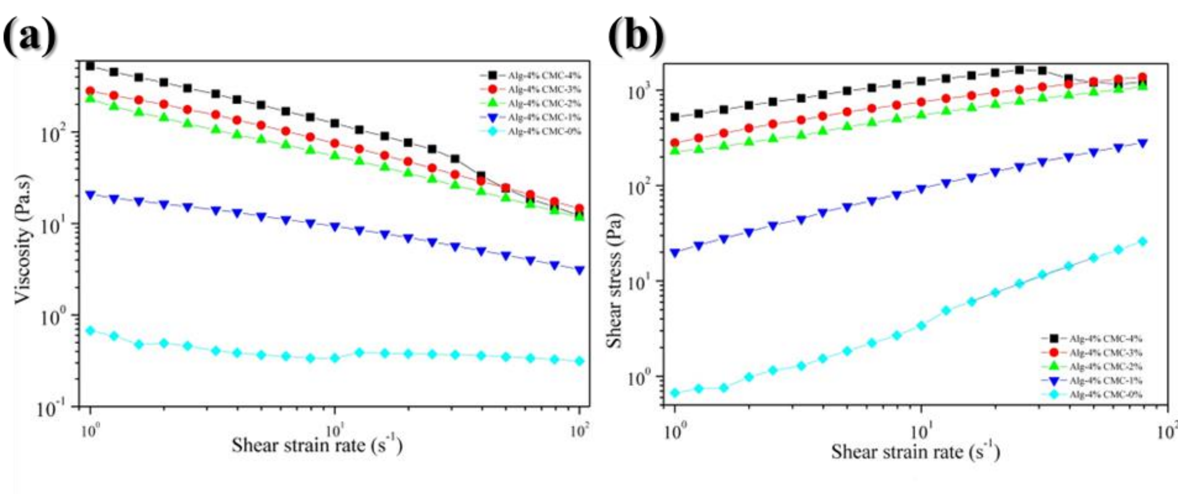

Figure 7. Assessment of shear-thinning in hydrogels: (a) Viscosity vs. shear rate and (b) Shear stress vs. shear rate [22]. based on increasing viscosity modifier mass ratio. Copyright 2022, Biotechtoday

The LAOS results for hydrogel compositions, represented by the G' and G" vs. shear strain (%) outcomes in Figure 8, indicates that increasing CMC percentage transforms the hydrogel from a liquid-like to a solid-like state. For 0-2% mass ratio of CMC, G" dominates G' at all shear strains, maintaining a liquid-like state. However, with 3% and 4% CMC, G' dominates G" up to a certain shear strain, defining the linear viscoelastic range (LVR), beyond which both moduli decrease, with G" becoming dominant, indicating a return to a liquid-like state. This intersection point marks the LVR, where the suspension resists permanent deformation and resembles solid-like behavior.

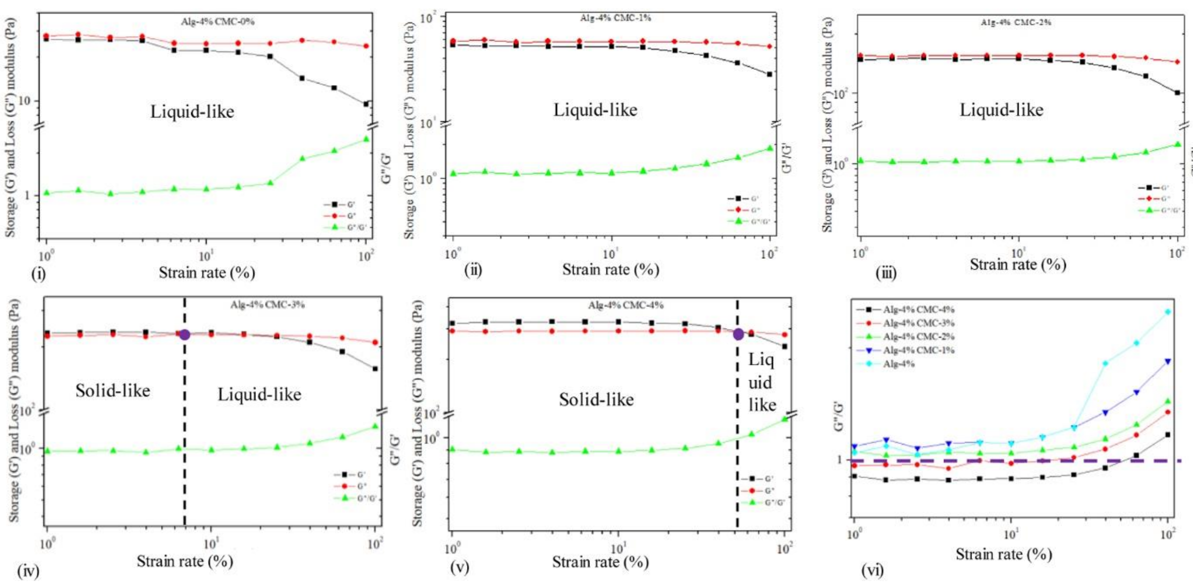

Figure 8. Dynamic strain sweep test for hybrid hydrogels: Transition from liquid-like to solid-like behavior by increasing of nanoparticle Mass ratio[22]. Copyright 2022, Biotechtoday

Although increasing nanoparticle loading improves printability, final shape fidelity, and rheological properties, the higher pressure induced in the cylinder during the extrusion process is detrimental to cells. Reduction in cell viability results from increased stress applied to the cell matrix. It has been observed that higher applied pressure and shear stress from ink movement in the nozzle increase dead cell accumulation in the region near the wall, where shear stress peaks. Consequently, the thickness of the layer formed also increases with pressure. Therefore, while increasing nanoparticles enhances polymer and rheological properties, cell viability, which is a critical challenge, requires optimization of the weight percentage to avoid severe damage to cells during the extrusion process. Figure 9 Demonstrates the concentration of dead cell increases as the pressure increases during the extrusion process [30].

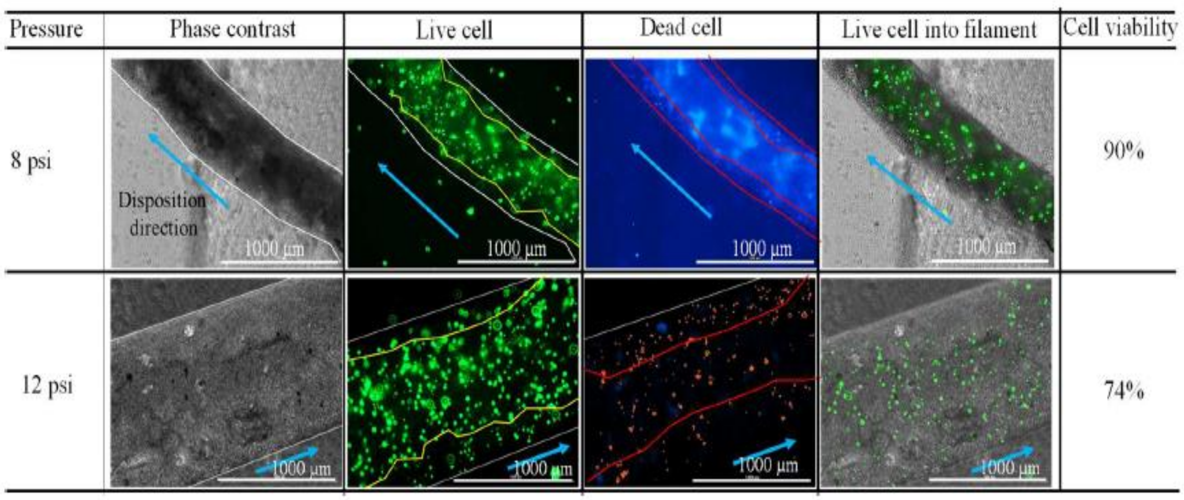

Figure 9. Demonstration of live/dead cell distribution in deposited filaments under induced pressures of 8 psi and 12 psi [22]. Copyright 2022, Biotechtoday

To better understand the molecular perspective of the impact of nanoparticles on the polymer matrix structure, we first address the various forces arising from nanoparticle-nanoparticle interactions. Tadros classified the factors contributing to interparticle friction into four types: (i) hard-sphere interactions, (ii) electrostatic interactions from double-layer repulsion, (iii) steric repulsion based on adsorbed layers, and (iv) Van der Waals forces [32]. According to hard-sphere interaction, In the context of DIW inks, particles are assumed to be non-deformable hard spheres with no spatial overlap. In this hard-sphere model, repulsive and attractive forces are neglected, and the rheology of the suspension is governed by Brownian diffusion and hydrodynamic forces. Particles are treated with a hard-sphere radius (R_{HS}), where no interaction occurs at distances greater than R_{HS}. However, when the distance between two particles approaches $2R_{HS}$, interactions increase significantly, approaching infinity [27]. In the second model, factors influencing the chemical functionality of particles involve "soft" interactions, which deviate from hard-sphere behavior. These factors include electrostatic forces arising from surface charges on particles, such as ionized surface groups or adsorbed ionic surfactants. In such cases, counterions aggregate on the particle surface, forming a double layer, with co-ions then aggregating onto the counterion layer. This phenomenon results in an increase in the effective radius of the particles (R_{eff}). When particles with the same charge approach each other, their double layers overlap, causing increased repulsion. Additionally, steric effects further enhance the Steric effects occur when particles have strongly adsorbed (or covalently attached) nonionic species, such as nonionic surfactants or polymer chains, forming a layer with thickness. In matrices where these species are well-soluble, interactions between surface species are unfavorable. Consequently, at interparticle distances $D > (R + r)$, particles repel each other, and steric repulsion intensifies. When $D = R$, the interaction energy becomes infinite, akin to the hard-sphere model. In cases where adsorbed species can interact via intermolecular forces or due to high particle concentrations, sufficient shear force is required to overcome these interactions and induce flow [27]. Finally, van der Waals attraction plays a crucial role in determining the extent of interparticle interactions within solid-liquid suspensions. These forces encompass dipole-dipole interactions, dipole-induced dipole interactions, and London dispersion forces (induced dipole-induced dipole interactions).

Interactions related to dipoles are significant for polar compounds, whereas London dispersion forces originate from fluctuations in electron density and apply to both polar and nonpolar compounds. As depicted in Fig. 10 (d), van der Waals attractive forces exhibit a sharp increase below a critical distance known as the "capture distance." At this distance, all particles are strongly attracted to each other and tend to flocculate. Considering van der Waals forces provides a more realistic view of the system. For instance, the presence of functional groups on the surface of nanoparticles can lead to repulsion or, in some cases, attraction between particles. Moreover, this model provides a better understanding of the shear-thinning behavior of polymers under high stresses. The presence of repulsive or attractive forces contributes to increased friction and improved distribution of nanoparticles within the polymer matrix [27, 33]. Figure 10 illustrates all types of nanoparticle-nanoparticle interactions discussed earlier.

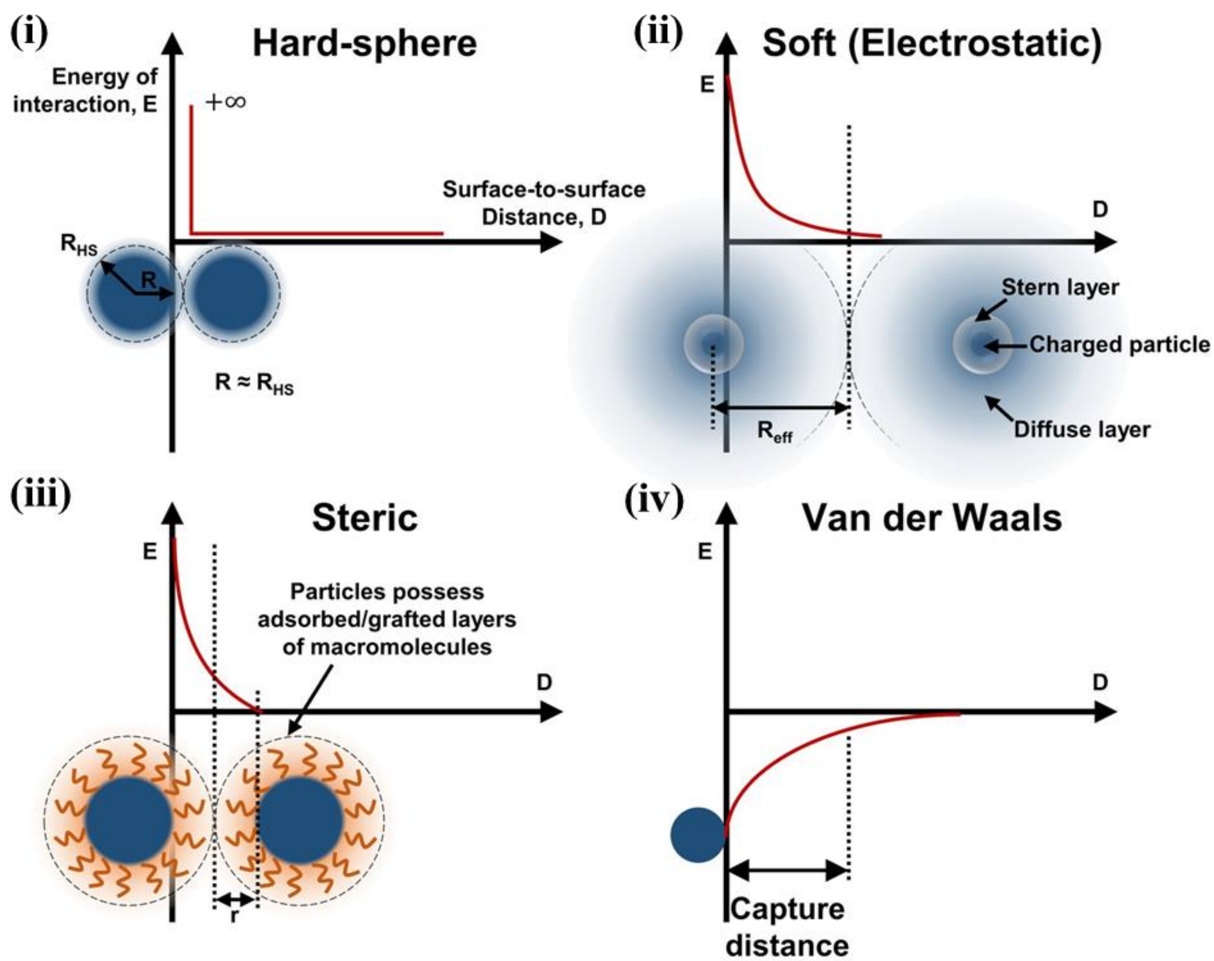

Figure 10. Types of particle-particle interactions in suspensions and inks: (i) "hard-sphere"; (ii) "soft" (electrostatic); (iii) steric; (iv) van der Waals[27]. Copyright 2023, Applied physics

Another significant class of interactions involves the binding between nanoparticles and polymer chains, which enhances the overall properties of the matrix. Increasing the specific surface area plays a pivotal role in augmenting these characteristics within polymer matrices. Additionally, the presence of chemically functional groups on nanoparticle surfaces profoundly influences the enhancement of these interactions. Furthermore, the utilization of "Low Dimensional Particles" merits attention, referring to nanoparticles that exhibit at least one or two dimensions at the nanoscale level [33-35]. These particles, which typically possess dimensions smaller than nano-scale in one, two, or three dimensions, contribute significantly to property enhancement due to their amplified surface area. For instance, carbon nanotubes, characterized by elongated structures with dimensions larger than conventional nanoparticles along their longitudinal axis, and two-dimensional materials like graphene oxide, which form planar sheets and exceed nano-scale dimensions in two axes, exemplify effective strategies for maximizing surface area. Moreover, the introduction of functional groups such as hydroxyl groups can induce van der Waals interactions in polar polymer matrices, further enriching the interfacial dynamics and overall performance enhancement of composite materials [36, 37].

2 Conclusion and Outlook

Bioprinting stands at the forefront of advancing tissue engineering and regenerative medicine. Bioinks, comprising hydrogels, synthetic polymers, or specialized materials, function as scaffolds that mimic the extracellular matrix, crucial for supporting cell viability and proliferation in controlled environments. Among bioprinting techniques, DIW excels due to its compatibility with diverse biomaterials and precise fabrication of complex tissue structures. These rheological characteristics are particularly relevant for the 3D printing of nanocomposites using DIW technique, which necessitates the ink's ability to fully recover its structure after the extrusion process. Such insights are critical for optimizing the formulation and processing conditions to achieve desired printability and structural integrity in the final printed constructs. Previous researches have demonstrated that the incorporation of nanoparticles into a polymer matrix significantly enhances structural properties. This enhancement is attributed to the formation of nanoparticle-matrix interactions and the development of an interconnected network. However, integrating nanoparticles into bioinks presents unique challenges, balancing enhanced mechanical properties with concerns over cytotoxicity and cell viability during printing. Understanding particle-particle and matrix-particle interactions, particularly the concept of percolation threshold, is critical for optimizing the mechanical strength and functional performance of bio printed constructs. Recent advances underscore the role of viscosity modifiers in improving printability and maintaining shape fidelity while recognizing their potential impact on cellular health

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