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Innovations in Bioink Materials and 3D Bioprinting for Precision Tissue Engineering

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Abstract

The progress in bioink materials and 3D bioprinting techniques has created new opportunities in tissue engineering. The goal is to develop cellular implants that closely resemble native tissues in structure, function, and microenvironment, addressing challenges such as nutrient transport and mechanical support for tissue regeneration. This literature review evaluates current trends in bioink development and 3D bioprinting methodologies, assessing their effectiveness for enhancing tissue-engineered constructs for clinical applications. Recent studies were comprehensively analyzed, focusing on novel bioink formulations, optimization of 3D bioprinting processes, and evaluate on of printed constructs' mechanical and biological properties. Various fabrication techniques and their implications for tissue integration were examined. The review shows significant progress in bioink compositions that enhance cell viability and nutrient diffusion within printed scaffolds. Constructs demonstrated improved mechanical properties and biological functionality, enabling better integration with host tissues. In vivo studies highlighted the potential of these bioprinted tissues to support cellular activity and regeneration, signifying significant advancements in clinical viability. The findings emphasize the crucial role of bioink materials and bioprinting technology in advancing tissue engineering. Continual innovation in bioink formulation and printing techniques is essential to overcome current limitations and achieve widespread clinical application. Future research should focus on personalized bioink development and expanding the range of tissues that can be effectively engineered, ultimately improving patient outcomes in regenerative medicine.

Keywords: Bioink; 3D Bioprinting; Tissue Engineering.

Introduction

In recent years, researchers have been growing interest in enhancing tissue-engineered constructs to create cellular implants that mimic native tissue in terms of structure, cell organization, and microenvironment. The goal is to facilitate efficient transport of nutrients for integration into the body's circulation, provide adequate mechanical support, and integrate various cell types for tissue regeneration. Tissue engineering, which seeks to maintain, restore, and improve the function of damaged or diseased tissues and organs, relies on continuously developing new biomaterials and scaffolds. These advancements have created more biomimetic tissues and significant progress in fabrication technologies, including programmed self-assembly and 3D bioprinting (Gang And Mani et al., 2022).

Three-dimensional printing (3DP) techniques have been used to create scaffolds with innovative designs at both small and large scales. This widely employed method in

tissue engineering and regenerative medicine offers precise material and cell placement within 3D constructs. There is a growing demand for functional tissue engineering using three-dimensional (3D) biological alternatives involving diverse scientific fields such as biomedical engineering, cellular and molecular biology, material science, and biochemistry. Stem cell-based therapies have been pivotal in traditional tissue engineering approaches, evolving since the concept emerged in the 1990s. The technology of 3D printing, also known as additive manufacturing, was first documented in 1989 by Emanual Sachs of MIT (Zhang et al., 2023).

The concept of 3D printing was initially introduced by David E. H. Jones in 1974. Subsequently, Hideo Kodama utilized photo-hardening thermoset polymers to pioneer the fabrication of 3D plastic models in 1981, marking the inception of early additive manufacturing (AM). In 1986, Charles W. Hull introduced *stereolithography*, a 3D printing technique where materials are deposited layer by layer and cured under ultraviolet (UV) light to form solid structures. This method later evolved to create sacrificial resin molds for producing 3D scaffolds with biological materials. Advances led to directly printing biomaterials into 3D frameworks, facilitating transplantation with or without seeded cells (Xie et al., 2020).

Advancements in nanotechnology, cell biology, and materials science have significantly advanced 3D bioprinting as a promising tool for tissue engineering, offering great potential for future medical progress. In 3D bioprinting, biomaterials, biochemicals, and living cells are precisely placed to create tissue-like structures, requiring specific technical approaches for mechanical and biological properties suitable for cell deposition and tissue restoration, such as biomimicry, autonomous self-assembly, and mini-tissue building blocks. Compared to traditional 3D printing for cell-free scaffolds, 3D bioprinting provides advantages such as precise cell distribution, high-resolution placement, scalability, and cost-effectiveness. However, there are still challenges for widespread adoption across industries, especially in medicine (Prem Ananth & Jayram, 2024).

Bioink materials are crucial in improving precision in tissue engineering. They provide a platform for creating tissues and organs with 3D bioprinting technology. Bioinks act as the ink in bioprinting processes, allowing for the controlled deposition of cells, growth factors, and other biologically relevant components layer by layer. This precise control over the scaffold architecture, composition, pore shape, size, and distribution helps researchers create highly structurally complex tissue tailored to specific requirements. Using bioinks enables incorporation of cells, extracellular matrix components, and other biomolecules into the bioprinted constructs, mimicking the native tissue microenvironment more accurately. This biological relevance is essential for promoting cell viability, proliferation, and differentiation within the engineered tissues. Additionally, the ability to design bioinks with specific mechanical properties, degradation rates, and functions contributes to the success of tissue engineering applications (Ji & Guvendiren, 2017).

Bioinks typically consist of biocompatible hydrogels containing living cells and can also include cell aggregates, microcarriers, or decellularized matrix components (Gungor-Ozkerim & Hospodiuk et al., 2017). The ideal bioinks should have mechanical, rheological, and biological properties that mimic the target tissues (Gungor-Ozkerim et al., 2018). The properties of bioinks before, during, and after gelation are crucial for printability, structural resolution, shape fidelity, and cell survival (Hölzl et al., 2016). In oncology research, bioprinted tumor microenvironments show promise for drug development and in vitro cancer modeling (Tiwari et al., 2021). Computational frameworks can predict tissue development and optimize bioprinting parameters (Hölzl

et al., 2016). Bioprinting, particularly extrusion-based bioprinting (EBB), has emerged as a promising technology for creating complex 3D tissue constructs (Mandrycky, Ozbolat & Hospodiuk, 2016).

While bioinks offer high reproducibility and precise control in tissue fabrication, further development is needed to address current limitations and expand their applications (Gungor-Ozkerim & Hospodiuk et al., 2017). One critical issue is the development of suitable bioinks that must support cell growth and function while maintaining printability and construct stability (Panwar & Tan, 2016). Current limitations include achieving high-resolution cell deposition, controlled cell distribution, vascularization, and innervation within complex tissues (Mandrycky et al., 2016). Additionally, the translation of bioprinting technologies to clinical applications is hindered by process engineering challenges, such as sterilization, scalability, and regulatory compliance (Angelopoulos et al., 2020). Overcoming these obstacles and realizing the full potential of bioprinting for tissue engineering and regenerative medicine requires future advancements in bioink development, printing techniques, and regulatory frameworks (Ozbolat, Hospodiuk & Angelopoulos, 2020).

Recent advancements in bioink materials have significantly improved the capabilities of 3D bioprinting, especially in tissue engineering and drug delivery applications. Biomaterials derived from marine sources, such as chitosan and alginate, provide sustainable and cost-effective options with excellent mechanical and biocompatible properties (Khiari, 2024). Chitosan-based hydrogels have emerged as promising bioinks due to their biodegradability, biocompatibility, and non-immunogenicity (Lazaridou et al., 2022). Bioinks are typically hydrogel-based materials that encapsulate cells. They can be natural, synthetic, or hybrid in composition (Gungor-Ozkerim et al., 2018). These materials undergo crosslinking to achieve shape fidelity and construct stability (Khoeini et al., 2021). Bioink formulations have incorporated nanomaterials, ceramics, and growth factors to enhance functionality (Mobaraki et al., 2020). Fritschen et al., (2003) noted that there often needs more clarity between the functionality and printability of bioinks u sed in tissue engineering. Materials such as collagen and fibrin provide a conducive environment for cells due to their adhesion properties. However, print resolution and geometric complexity may need to be improved. On the other hand, plant-based materials like agarose and alginate offer better printing properties, but they may lack the necessary cell adhesion motifs (Fritschen et al., 2023). The significance of bioink materials lies in their ability to fulfill both material and biological requirements for successful tissue engineering outcomes. Carefully designing bioinks for specific tissue engineering applications contributes to the precision and effectiveness of 3D-bioprinted tissue models (Fritschen et al., 2023). Therefore, the development and optimization of bioink materials play a critical role in advancing tissue engineering and regenerative medicine toward creating viable and functional tissue constructs (Ji & Guvendiren, 2017).

This systematic literature review aimed to evaluate the role of bioink in 3D bioprinting for precise tissue engineering. It provides a comprehensive overview of current advancements, materials, cell viability, printing technologies, applications, emerging trends, and existing challenges. The review highlights significant progress in developing bioinks with enhanced biocompatibility and mechanical properties, essential for achieving precision in tissue constructs. However, challenges remain in ensuring high cell viability during and after printing, emphasizing the need to optimize bioink formulations and printing parameters further. The discussion points to promising advancements in hybrid and multi-material bioinks, expanding the potential for creating more complex and functional tissues tailored to individual needs. Despite these

advancements, the review identifies vital gaps, including the lack of standardized bioink properties, the need for improved long-term stability of printed tissues, and the challenges of scaling up these technologies for clinical applications. This review emphasizes the importance of continued interdisciplinary research and innovation to overcome these challenges, ultimately advancing precise tissue engineering through more reliable and effective 3D bioprinting techniques.

Methods

In this study, we conducted a Systematic Literature Review (SLR) to gather and understand information about using bioink in 3D bioprinting for tissue engineering. We followed the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). We illustrated the screening process in Figure 1, following the PRISMA reporting format using the WATASE UAKE tool. We conducted the literature search using the Scopus database via the WATASE UAKE platform, a web-based system designed to facilitate collaborative research among researchers. It was launched in 2018 and further developed in 2020. This platform enabled efficient collaboration between researchers from various universities. Additionally, we used Zotero, a free and open-source application for managing references, organizing citations, references, and bibliographies, and streamlining the reference management process. We entered keywords such as 3D Bioprinting in Tissue Engineering and Bioink in 3D Bioprinting into the WATASE UAKE website to identify relevant articles. The search focused on publications from 2014 to 2024, producing 81 articles that analyzed bioink in 3D bioprinting over this decade. For this systematic literature review on bioink for 3D bioprinting in tissue engineering, clear inclusion and exclusion criteria were established to ensure the relevance and quality of the selected studies. The inclusion criteria focused on studies that addressed bioink development, composition, and application in 3D bioprinting for tissue engineering. These included those exploring interactions between bioink materials and cell viability, tissue integration, or functionality within bioprinted structures. Both original research articles and comprehensive review papers published between 2014 and 2024 were considered, with the language restricted to English and only full-text accessible articles included. Conversely, the exclusion criteria ruled out studies unrelated to bioink or 3D bioprinting, non-peer-reviewed publications, articles published before 2014 unless seminal, and studies are written in languages other than English or without full-text availability. These criteria ensured that the review focused on the most relevant, high-quality research. To refine the selection, we reviewed the abstracts of the 81 retrieved papers, focusing on their relevance to the topic. This process led to identifying 72 articles, of which we ultimately selected 30 that directly discussed bioink in 3D bioprinting for tissue engineering.

Results and Discussion

The results and discussion of this study provide a critical examination of the current landscape of bioink research in 3D bioprinting for tissue engineering. The review represents a curated selection of 30 high-quality studies and employs a systematic approach rooted in PRISMA guidelines. Here the Tabel 1. Comprehensive Overview from the Review. The review synthesizes critical findings across a decade of research and identifies significant trends, challenges, and opportunities within the field. The analysis sheds light on the various strategies and materials explored in bioink development and their implications for cell viability, tissue integration, and the mechanical properties of bioprinted constructs. In the discussion, we delve into the implications of these findings, examining how different bioink compositions and bioprinting techniques influence the

outcomes of tissue engineering applications. By contextualizing these results within the broader scientific discourse, we aim to provide a nuanced understanding of the current state of the art and highlight areas where further research is needed to advance the field. The discussion also addresses the gaps identified in the existing literature. It proposes potential directions for future investigations to overcome current limitations and pave the way for more effective and reliable bioprinting solutions.

Tabel 1. Comprehensive Overview of the Review

Category	Research Focus	Key Findings	References
Materials	Natural Polymers (e.g., collagen, alginate, decellularized extracellular matrix.)	High biocompatibility but limited mechanical strength and printability.	(Isaeva et al., 2021) (Fatimi et al., 2022) (Zhe et al., 2023) (H. Liu et al., 2023)
	Synthetic Polymers (e.g., PEG, PCL, carboxymethyl cellulose.)	Enhanced mechanical properties but often less biocompatible than natural polymers.	(Zennifer et al., 2021)
	Composite Bioinks (e.g., carboxymethylcellulose-based hydrogels, clay minerals, nano composit.)	Combining natural and synthetic materials to balance biocompatibility and mechanical strength.	(Mallakpour et al., 2021) (Di Marzio et al., 2020) (García-Villén et al., 2021) (Kakarla et al., 2022)
Cell Viability	Post-printing Cell Survival	Viability is influenced by bioink composition, printing parameters, and crosslinking methods.	(Jian et al., 2021)
	Impact of Shear Stress During Printing	High shear stress can reduce cell viability; optimizing viscosity and printing speed is critical.	(Züger et al., 2023)
Printing Techniques	Extrusion-based Bioprinting	Widely used but may suffer from low resolution and cell viability issues due to shear stress.	(Ding et al., 2023) (Masri et al., 2022)
Applications	Tissue Engineering Neural Tissue Engineering Skeletal Muscle Tissue Engineering	Bioinks used to create functional tissue constructs for regenerative medicine.	(Gupta & Bit, 2022) (Saini et al., 2021) (Lee et al., 2018) (Huang et al., 2017) (Ostrovidov et al., 2019)

	Cardiac Tissue Engineering		(N. Liu et al., 2021) (Wu et al., 2023) (Kozaniti et al., 2021) (Wang et al., 2021)
	Articular Cartilage Tissue Engineering		(O'Connell et al., 2017)
Emerging Trends	Hybrid Bioinks	Development of bioinks that integrate multiple materials to improve performance.	(Xie et al., 2020)
	Multi-material Bioprinting	Exploring the use of multiple bioinks in a single construct to mimic complex tissue structures.	(Sodupe-Ortega et al., 2018)
	Personalized Bioinks	Customizing bioink.	(Alonzo et al., 2020) (Ji & Guvendiren, 2017b)
Challenges & Gaps	Standardization of Bioink Properties	Need for standardized protocols to assess printability, mechanical properties, and biocompatibility.	(Yu et al., 2020)
	Scale-up for Clinical Applications	Bridging the gap from research to applied clinical practice.	(Cavallo et al., 2023)

1. Materials

a. Definition and Role of Bioink Materials In 3d Printing

The term bio ink refers to the placement of cells or cell aggregates within biomaterials in a three-dimensional manner. It is important to distinguish between bio-inks containing cells and biomaterial inks that do not. Biomaterials in bio-inks must act as carriers for cell delivery during the formulation and bioprinting processes. In contrast, biomaterial inks can be printed but only infused with cells after printing. Therefore, biomaterial inks do not meet the criteria to be classified as bio-inks because cells are usually introduced into the bioprinted biomaterial scaffold through a separate seeding process. However, this approach helps to mitigate the biological constraints that affect the properties and behavior of the ink (Fatimi et al., 2022).

Bioink refers to a cell-laden, biocompatible material intended for three-dimensional bioprinting (García-Villén et al., 2021). These materials are used in 3D bioprinting to replicate native tissue structures, providing a framework for cell growth and interaction and enabling the development of complex tissue formations. The bioink

utilized in the research by Alonzo and the team is a combination of alginate and gelatin. Alginate is a naturally occurring polymer that can be readily crosslinked with multivalent cations, while gelatin offers cell attachment sites on specific residues within the polymer (RGD). Bioinks are crucial in 3D bioprinting, serving as the medium for layer-by-layer cell deposition to create tissue-like constructs and provide a supportive environment for cell proliferation and differentiation. Therefore, the selection of bioink material is crucial as it directly impacts the bioprinting process, cell viability, and the overall functionality of the printed structures. The composition and characteristics of bioinks significantly influence the success of bioprinting endeavors and the resulting tissue functionality (Alonzo et al., 2020).

Bioinks consist of biomaterials, biological molecules, and cells processed using bioprinting technologies. They are essential in 3D bioprinting as they serve as the materials deposited layer by layer to construct intricate tissue structures containing living cells, growth factors, and other biomolecules that are necessary for tissue regeneration and engineering. The properties of bioinks, including viscosity, biocompatibility, and printability, are crucial for the success of bioprinting processes. These bioink materials, which typically comprise hydrogels and may incorporate biological molecules or cells, are used in 3D bioprinting to create cellularized constructs for tissue engineering and regenerative medicine applications. They provide structural support and an environment for cell growth, differentiation, and tissue development within the printed structures. It is essential in determining the printability, biocompatibility, and functionality of the final 3D bioprinted constructs, making bioinks integral to biofabrication (Di Marzio & Sodupe-Ortega et al., 2018).

The role of bioink materials in 3D printing, include (Masri et al., 2022):

1) Providing a scaffold for cell growth and organization.
   Bioinks act as a supportive matrix for the encapsulated cells, allowing them to proliferate, differentiate, and organize into functional tissue structures.
2) Facilitating cell viability and functionality.
   Bioinks are designed to maintain the viability and functionality of the encapsulated cells during and after the printing process, ensuring successful tissue formation.
3) Allowing for spatial control and customization.
   Bioinks enable precise spatial control over cell placement and distribution, allowing for the creation of complex tissue architectures tailored to specific applications.
4) Supporting tissue maturation and integration.
   Bioinks promote the maturation of printed tissues by providing a conducive microenvironment for cell interactions, extracellular matrix deposition, and tissue development.
5) Enabling biocompatibility and biodegradability.
   Bioinks are formulated to be biocompatible with the host tissue, minimizing immune responses, and can be designed to degrade over time as the engineered tissue matures and integrates with the surrounding environment.

Overall, bioink materials play a fundamental role in 3D bioprinting by serving as the vehicle for delivering cells and bioactive factors to create functional tissue constructs for various biomedical applications, including skin regeneration and wound healing.

b. Basic Characteristics and Requirements of Bioinks

Bioinks are crucial in 3D bioprinting for creating functional tissue models. When choosing bioinks, it is important to consider their printability and cell biological properties. The matrix material used in bioinks is vital for developing biomimetic tissue and organ models. The ideal bioink should have mechanical and rheological properties suitable for bioprinting and be compatible with cell behavior, including viability,

proliferation, and morphology. The microstructure, rheology, and chemical composition of bioinks affect cell functions and printability in bioprinting. When selecting bioinks, it is important to consider properties such as gelation kinetics, mechanical stability, cell adhesion motifs, and handling. Choosing the right bioink based on its strengths and weaknesses is crucial for achieving high printability, stability, and cell interaction in 3D bioprinted tissue models. Thoroughly characterizing bioinks and considering specific application requirements can lead to the development of optimized bioprinted tissue models. Standardized characterization and evaluation procedures for bioinks ensure the selection of the most suitable materials for bioprinting applications (Fritschen et al., 2023). The basic characteristics and requirements of bioinks include (Ji & Guvendiren, 2017):

1) Material Requirements:

a) Printability
The bioink should have suitable viscosity, surface tension, and cross-linking properties to ensure proper processing and printing fidelity.

b) Mechanics
The bioink should have the necessary stiffness to create self-supporting constructs and control cellular behavior.

c) Degradation
The bioink should be designed for gradual degradation to enable tissue integration and replacement by cells.

d) Functionalizability
The bioink should allow the incorporation of biochemical cues to direct specific cellular behaviors.

2) Biological Requirements:

a) Biocompatibility
The bioink should be compatible with living tissues and support cell growth and function.

b) Cytocompatibility
The bioink should not harm the cells encapsulated within it and should support their viability.

c) Bioactivity
The bioink should possess bioactive properties to influence cellular adhesion, migration, and differentiation.

Bioinks are crucial in 3D bioprinting and are vital in creating biological structures. These materials are derived from decellularized extracellular matrix (dECM) components and closely mimic the characteristics of native tissue. In order to support cell growth and tissue regeneration, bioinks must have specific qualities, such as mechanical strength, degradability, and biocompatibility. Mechanical properties are fundamental as they influence cell behavior and proliferation and can be adjusted to optimize tissue regeneration potential. Additionally, biodegradability is vital to ensure gradual breakdown, allowing for cell integration with the extracellular matrix (ECM) microenvironment. DECM-based bioinks can incorporate cells, cytokines, and chemicals by enhancing functionalization. Taking advantage of these bioinks in 3D bioprinting enables the recreation of tissue properties, facilitating the development of in vitro disease models and tissue substitutes (H. Liu et al., 2023).

c. Types Of Bioink Materials (Natural, Synthetic, Hybrid)

Bioinks are designed to mimic target tissues' extracellular matrix (ECM), promoting cell proliferation and differentiation. They need to be printable, with rheological properties being crucial. However, bioinks with high polymer concentrations

can be easily printable but may not be suitable for cells, as they can restrict cell spreading, migration, proliferation, and matrix remodeling. There is a shift in bioink formulation for tissue engineering towards lower polymer concentrations to enhance tissue development. Additionally, bioinks should ensure even cell distribution in suspension to prevent aggregation and deposition, particularly for larger constructs, which can extend bioprinting time. Both natural and synthetic polymers are used in bioink development to bioprint skeletal muscle constructs. Among natural polymers, rapidly cross-linked hydrogels like calcium alginate and fibrin are used directly or as support polymers during printing to maintain the shape of less stable bioinks. Natural hydrogel bioinks such as alginate, collagen, and gelatin are widely used to provide physical support and cell-supportive functionalities for engineered tissues (Ostrovidov et al., 2019).

The materials currently used in 3D bioprinting include natural polymers such as collagen, gelatin, laminin, fibronectin, alginate, chitosan, fibrin, and hyaluronic acid (HA), which are often derived from animal or human tissues, as well as synthetic polymers. The advantages of using natural polymers lie in their similarity to the native extracellular matrix (ECM) and their inherent bioactive properties, which have well-established interactions with cells. Recent advancements in extracellular matrix decellularization provide a promising method for obtaining intact decellularized extracellular matrices (dECM) for integration into bioprinting. On the other hand, synthetic polymers, which are chemically synthesized, can be precisely tailored with specific chemical and mechanical properties to meet various bioprinting needs. Pluronics, which are categorized as ABA-type triblock copolymers with a hydrophilic polyethylene glycol (PEG) A block and a hydrophobic polypropylene glycol (PPG) B block, have advantageous gelation temperatures and excellent printability but lack bioactivity and are not intended for long-term cell viability support (Ostrovidov et al., 2019). Although the biocompatibility of synthetic hydrogels may slightly lag behind that of naturally purified hydrogels, their strong mechanical characteristics, ease of manipulation, low immunogenicity, and lack of batch variability have garnered significant interest across various complex tissue engineering fields, including cardiovascular regeneration (N. Liu et al., 2021).

Pluronics are commonly used as sacrificial layers in 3D bioprinting. PEG is widely used in various compositions for 3D bioprinting, either in hydrogel fabrication or in creating crosslinkable polymers after functionalization with diacrylate or dimethacrylate groups. Another synthetic polymer, Poly (N-isopropyl acrylamide) (PNIPAAM), with a low solidification temperature of 30–37°C, is employed in 3D bioprinting, often in combination with natural polymers such as HA or alginate to enhance biocompatibility. The selection of cells is crucial in fabricating tissues and organs via 3D bioprinting, requiring multiple cell types with specific biological functions to closely mimic native tissues or enable stem cell proliferation and differentiation post-printing. Ideally, cells chosen for 3D bioprinting should accurately emulate physiological states in vivo and retain their in vivo functionalities within optimized microenvironments. Cells obtained from patients are preferred for clinical applications to mitigate potential immune responses. However, limitations such as finite lifespan and challenges in isolating and culturing primary cell types hinder their utility for bioprinting long-term tissue structures. In contrast, stem cells, including those from bone marrow, fat, and perinatal sources such as amniotic fluid, can proliferate and differentiate into required cell types, holding promise for autologous applications (Xie et al., 2020).

Hybrid biomaterials combine the cell-supportive characteristics of natural polymers with the mechanical attributes and adjustability of synthetic polymers. Instances include combining alginate with gelatin methacrylate or with polyvinyl alcohol in bio-

inks. Multi-material constructs provide advantages such as enhancing structural intricacy, modulating growth factors, incorporating diverse cell types in distinct areas to emulate natural cellular diversity and function, and simultaneously depositing biomaterials with diverse physical and chemical traits, helpful in crafting tissues with varying regional properties (Wu et al., 2023).

2. Cell Viability

Jian and his team conducted research that resulted in a primary finding a specialized 3D printing system, along with a specific bioink, successfully created a scaffold that closely resembles the native meniscus in terms of structure, strength, components, and environment. This development significantly improves the quality and effectiveness of meniscal scaffolds for tissue engineering. However, the study also acknowledges some limitations, such as the difference in hardness between the materials used and the loss of specific properties in the meniscal extracellular matrix after processing. It highlights the need for further advancements in materials and printing technologies to achieve more accurate biomimetic results. The materials used in the 3D bioprinting process include polycaprolactone (PCL) for constructing the meniscal frame, gelatin methacrylate (GelMA) as part of the bioink, meniscal extracellular matrix (MECM) to enhance the bioink's properties, and meniscal fibrocartilage chondrocytes (MFCs) to promote tissue regeneration. These materials were combined to achieve a similar structure and strength to the native meniscus and to create a suitable environment for cell viability and tissue formation. Cell viability was assessed using a Live/Dead Viability/Cytotoxicity Kit, which showed cell viability rates exceeding 90% for single-nozzle and dual-nozzle printing techniques. The constructs were cultured for 1 and 14 days, and the cell viability remained above 90% even after extended culture periods, demonstrating the materials' compatibility. Cell proliferation was notably better in the single-nozzle model than in the dual-nozzle model, possibly due to a reduced surface area for substance exchange in the dual-nozzle setup (Jian et al., 2021).

The study focuses on creating an innovative, cost-effective, and versatile bioink for 3D bioprinting in tissue engineering. This bioink is primarily made of gelatin and methylcellulose. This combination allows for the independent optimization of rheological properties, which enhances printability and helps achieve desirable tissue engineering outcomes. Bioink has shown high cytocompatibility and the ability to produce scaffolds with variable stiffness, making it suitable for various tissue types, including myocardial and neural tissues. The research found that although the cell viability within the 3D-bioprinted constructs was initially high, at approximately 86% on Day 1, it decreased over time, dropping to around 68% on Day 3 and further declining to 33% in thicker regions of the construct by Day 5. The decrease in cell viability in thicker crossing structures was attributed to nutrient diffusion limitations within the constructs. The bioink significantly impacts 3D bioprinting, especially in terms of printability, structural stability, and cell viability. Its rheological properties, characterized by low viscosity during cell suspension and printing, minimize shear stress on cells, while an increase in viscosity after printing ensures structural stability during incubation, which is crucial for maintaining the shape and integrity of the printed constructs. Furthermore, the bioink's stiffness can be adjusted through enzymatic crosslinking using transglutaminase (TG), providing the appropriate mechanical environment required for optimal cell function and differentiation in different tissues. The bioink has also demonstrated high printability, with uniform pore structure and shape fidelity in the printed constructs. These are essential for creating accurate and reproducible 3D structures that mimic natural tissues. Despite the observed decrease in cell viability over time, particularly in thicker regions, the overall performance of the

bioink supports its potential use in tissue engineering applications. The design and properties of this bioink were crucial to the study's success, enabling the production of viable, structurally stable tissue-like constructs (Züger et al., 2023).

3. Printing Technique

The research thoroughly investigates how bioinks, particularly those based on hyaluronic acid (HA) hydrogels, affect extrusion bioprinting. It emphasizes the importance of the bioink's viscosity in ensuring smooth extrusion and maintaining the printed structure's shape. A too high viscosity can cause clogging, while one that is too low can result in poor structural integrity, leading to sagging or collapsing layers. It is preferred that the bioink exhibit shear thinning properties, meaning it maintains high viscosity at rest and flows easily under shear stress during extrusion, facilitating smooth printing. The bioink's viscoelastic nature is crucial for supporting subsequent layer deposition and maintaining the 3D structure's integrity. To enhance mechanical strength and stability, various crosslinking strategies, such as double-crosslinking, are employed to improve mechanical stability and achieve tunable swelling properties necessary for robust tissue scaffolds. Mixing HA with materials like chitosan, alginate, or nanoparticles in composite bioinks further enhances mechanical properties, including stiffness and strength, making the bioink more suitable for extrusion bioprinting. The printability of HA-based bioinks is influenced by their rheological properties and the crosslinking methods employed, ensuring precise layer-by-layer deposition and stability of self-supporting structures. Biocompatibility is a critical factor, with HA-based hydrogels demonstrating excellent support for cell viability during and after printing by mimicking the natural extracellular matrix (ECM), which promotes cell adhesion, proliferation, and differentiation. It is also essential to control the degradation rate of the bioink to match tissue regeneration, ensuring that the scaffold supports new tissue formation before gradually degrading. The study identifies the challenges of balancing mechanical strength, viscosity, and biocompatibility and notes that modifying HA or blending it with other polymers can optimize these properties. Functional additives, such as growth factors or nanoparticles, can further enhance the bioink's functionality for specific tissue regeneration processes. Overall, the success of extrusion bioprinting with HA-based bioinks depends on finely tuning these properties to meet the demands of the printing process and the intended tissue engineering application (Ding et al., 2023).

This research examines how the composition of bioink affects extrusion bioprinting, with a specific focus on hybrid gelatin-polyvinyl alcohol (GPVA) bioinks for creating 3D-bioprinted skin models. The GPVA bioink blends natural gelatin with synthetic polyvinyl alcohol (PVA) and uses genipin (GNP) as a crosslinking agent to enhance mechanical stability and biocompatibility. Adding GNP significantly improves the mechanical properties of the bioprinted structures, enhancing their structural integrity and pore size distribution, which is crucial for supporting cell growth and tissue formation. The viscosity of the bioink, influenced by the PVA content, ensures smooth extrusion through the nozzle but may need optimization to prevent clogging. Crosslinking with GNP results in larger pore sizes and increased interconnectivity, which benefits cell migration and nutrient diffusion. However, adding PVA slightly reduces pore size, potentially affecting cell behavior. The GPVA bioink, especially when crosslinked with GNP, shows excellent biocompatibility, supporting high cell viability and proliferation-the higher cell densities within the bioink lead to improved cell viability and more robust cellular interactions. Furthermore, the crosslinking process enhances the mechanical strength of the bioink, making the printed scaffolds more durable and stable. Overall, the hybrid GPVA bioink, with genipin crosslinking, balances printability, mechanical

stability, and biocompatibility, making it practical for extrusion bioprinting and suitable for 3D-bioprinted models and other tissue engineering applications (Masri et al., 2022).

4. Applications

Combining a hybrid scaffold made of polycaprolactone (PCL) and fibronectin decellularized extracellular matrix (dECM) has shown promising results for bone regeneration. Researchers have utilized a combination of Gelatin Methacryloyl (Gel-MA) with human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (hMSCs) to create vascularized bone scaffolds. It has been found that hybrid bioinks containing dECMs can promote both angiogenesis and osteogenesis. Additionally, hybrid bioinks have shown potential for applications in tissue and organ repair, organoid construction, and the development of disease models. It is also important to note that hybrid bioinks can be personalized by incorporating cells, peptides, and cytokines to improve tissue repair (Huang et al., 2017).

Different tissue types may require specific bioink formulations to replicate the natural extracellular matrix and provide the necessary cell growth and differentiation signals. For instance, decellularized extracellular matrix bioink is used for printing tissue analogues, while tunable bioinks offer biodegradability, biocompatibility, and desired mechanical properties. In tissue engineering, researchers and practitioners often customize bioink formulations to meet the specific needs of the engineered tissue. It involves considering factors such as cell type, mechanical properties, and bioactive cues necessary for successful tissue regeneration (Xie et al., 2020).

The different types of tissues require specific bioink formulations for 3D bioprinting with a focus on high biocompatibility and biodegradability. The bioinks should be capable of encapsulating cells while preserving their viability and structural stability. In cardiac tissue engineering, natural hydrogels, such as biocompatible methacrylic anhydride gelatine (GelMA), are commonly used as the primary bioink to encapsulate cells and create functional cardiac tissue. Maintaining the stable and continuous expression of cardiac maturation markers is crucial, ensuring cell viability and structural stability and achieving comprehensive and uniform expression of mature smooth muscle markers in cardiac tissue engineering (N. Liu et al., 2021).

The bioinks for other tissue types may vary based on the specific structural and functional characteristics required for each tissue type (N. Liu et al., 2021). Examples of tissue types and their specific bioink requirements are discussed, including developing a functional liver carcinoma model using 3D bioprinting technology. Specifically, the study focuses on bioinks composed of agarose, gelatin, collagen, and their blends for this application. Different hydrogel properties, such as microstructure, rheology, and chemical composition, enhance cell functions and printability in liver cancer models. The bioinks are characterized for their mechanical and rheological properties and albumin diffusivity to determine their suitability for creating biomimetic tissue models. The importance of selecting suitable matrix materials for tissue models fabricated with 3D-bioprinting technology is to improve cell functions and printability. In another example, the study mentions the choice of bioink components critical to the success of bioprinted tissue models, especially when considering non-cancerous cell types such as primary hepatocytes, myoblasts, or fibroblasts. It emphasizes the need to study cell-material interaction to optimize bioink selection for various tissue types. Additionally, it discusses contradictions often observed between biofunctionality and printability of bioinks for different tissue types. For instance, extracellular matrix proteins like collagen and fibrin provide an attractive cell environment. However, they may have limitations in printing resolution and geometric complexity, while plant-based materials like agarose and

alginate offer superior printing properties but may lack cell adhesion motifs. These examples demonstrate the importance of considering tissue-specific requirements when selecting bioink components for 3D-bioprinting applications (Fritschen et al., 2023).

The properties of bioink play a crucial role in 3D bioprinting for tissue engineering. It is important to develop hydrogels with specific rheological properties such as shear-thinning for easy cell extrusion, post-printing structural stability, and elastic moduli relevant to the physiological environment for optimal tissue regeneration. The research specifically focuses on creating a bioink combining gelatin and methylcellulose (MC) to enhance 3D printability. This bioink aims to minimize shear force on cells during printing, facilitate cell adherence and infiltration into printed structures, and support tissue remodeling and regeneration to recover injured tissue completely. By optimizing the properties of the bioink to mimic specific tissue types, it can be used for a wide range of applications in regenerative tissue engineering and enable the printing of cell-laden scaffolds with long-term shape fidelity (Züger et al., 2023). Key points regarding the importance of bioink properties include (Cavallo et al., 2023):

a. Architectural Mimicry
   Bioink properties play a crucial role in mimicking the native tissue architecture. By carefully selecting and optimizing the bioink composition, researchers can replicate the complex structure of skin tissue, including the dermal and epidermal layers, to create a biomimetic skin equivalent.
b. Biomechanical Properties
   The mechanical properties of the bioink, such as stiffness and elasticity, are essential for maintaining the structural integrity of the printed tissue construct. Proper mechanical properties ensure that the bioprinted skin equivalent can withstand physiological forces and mimic the mechanical behavior of native skin.
c. Printability and Homogeneity
   Bioink properties, such as viscosity and shear-thinning behavior, influence the printability of the material. A bioink with excellent printability allows for precise deposition of cells and biomaterials, forming well-defined tissue structures with homogeneous cell distribution.
d. Biocompatibility and Cell Viability
   The bioink must be biocompatible to support cell growth, proliferation, and differentiation within the printed tissue construct. Optimizing bioink properties ensures a suitable cell microenvironment, promoting high cell viability and functionality in the bioprinted skin equivalent.
e. Degradability and Tissue Integration
   Bioink properties related to biodegradability and tissue integration are crucial for the long-term success of the bioprinted skin equivalent. A bioink that degrades at an appropriate rate and promotes tissue integration allows for the remodeling and maturation of the printed tissue over time.
f. Bioactivity
   Bioinks may incorporate bioactive molecules, growth factors, or signaling cues to promote specific cellular responses, such as differentiation or tissue regeneration. Bioactive bioinks can enhance the functionality and therapeutic potential of the printed constructs (Ding et al., 2023).
g. Clinical Translation
   Bioink properties are also important for clinical translation of 3D-bioprinting technologies. Ensuring the bioink meets regulatory standards, such as safety and efficacy requirements, is essential for advancing tissue engineering applications toward clinical trials and eventual patient use (Isaeva et al., 2021).

By carefully tailoring the properties of the bioink, researchers can create a conducive environment for cell growth and tissue development, ultimately leading to the successful fabrication of functional and biomimetic skin equivalents using 3D bioprinting technology.

5. Emerging Trends

Hybrid bioinks combine natural and synthetic polymers used in 3D bioprinting. The main goal of these bioinks is to leverage the advantages of different biomaterials while minimizing their drawbacks. Using hybrid bioinks improves cell compatibility, enhances mechanical characteristics, and speeds tissue regeneration. Carbon nanotubes and gold nanorods are often included in hybrid bioinks. These bioinks can be easily printed independently or through a microfluidic system (Wang et al., 2021). A scaffold made of hybrid microfibrillated PCL-collagen is used to mimic skeletal muscle structure. This scaffold is part of a biohybrid robot with a 3D-printed resin skeleton and hydrogel sheets filled with myoblasts. The robot can perform large movements and remains active for a long time. These bio-bots have an asymmetrical design powered by skeletal muscle strips. They are created using a combination of skeletal muscle cells and other cell types in co-cultures to produce hybrid tissues (Ostrovidov et al., 2019).

Di Marzio and colleagues state that hybrid bioinks are formed by combining traditional hydrogels with nano-biomaterials to create advanced bioink formulations with improved properties. Incorporating nano-biomaterials into bioinks makes it possible to adjust the mechanical and structural characteristics and regulate the release of biomolecules close to cells. Moreover, hybrid bioinks can be designed to self-assemble into nanostructures, like nano-fibers or nano-pores, further enhancing their functionality. These hybrid bioinks have demonstrated potential in promoting cell adhesion, differentiation, and overall tissue regeneration, making them an important area of focus in biofabrication (Di Marzio et al., 2020). Hybrid scaffolds have applications in the field of tissue engineering to regenerate cartilage. It entails the merging of a polycaprolactone (PCL) framework with an alginate hydrogel that has been encapsulated with chondrocyte cells. A combination of nanofibrillated cellulose (NFC) and alginate fabricates cartilage tissue. Moreover, an alginate sulfate-nanocellulose material is utilized to spread and proliferate cells. Lastly, the fabrication of cartilage is achieved by amalgamating nano cellulose and alginate with human chondrocytes and hMSCs (Gupta & Bit, 2022).

The bioink composition includes poloxamer hydrogels, gelatin-alginate, and bioinks that emit fluorescence. Human adipose-derived stem cells (hASCs) were integrated by combining them with the bioink. The bioink was dispensed through a tapered nozzle with a gauge size of 25G. Various combinations of bioinks were used to create multi-material constructs. Highly intricate vascular networks and elaborate multi-material 3D models were produced the cell viability in the bioinks after printing was confirmed to have favorable outcomes. Future studies will focus on creating more intricate tissue constructs using different biomaterials and cell types. This proposed method could be advantageous compared to other bioprinting technologies (Sodupe-Ortega et al., 2018). The study used a combination bioink made of gelatin-polyvinyl alcohol (GPVA). This bioink was crosslinked using genipin (GNP) as the crosslinking agent. The resulting GPVA-GNP hydrogels showed improved biocompatibility and mechanical properties. The combination bioink was used to create a three-dimensional in vitro skin model (Masri et al., 2022).

The study conducted by Kakarla and their team utilized a hybrid bioink consisting of gelatin, alginate, and boron nitride nanotubes (BNNTs). BNNTs are a type of nanomaterial that improves the bioink. The hybrid bioink showed improved structural

stability and printability. Living cells were successfully integrated into the hybrid bioink and extruded. BNNTs have unique characteristics that promote cell growth and specialization (Kakarla et al., 2022). A hybrid stimulus-responsive bioink was used for 3D bioprinting. The bioink consisted of norbornene-modified CMC and carbic-modified CMC. Mesenchymal stem cells and fibroblasts were incorporated into the bioink for printing. UV irradiation crosslinking was used to stabilize the printed structures (Zennifer et al., 2021). Research has been conducted on hybrid cell printing methods. The bio-ink GelMaHAMa, a combination of gelatin and methacryloylhyaluronic acid, is known for creating core-shell structures. Gelatin macromers, chemically modified with methacrylate, retain their mechanical strength. Fibrin, a protein involved in blood clotting, is also used in printing. The speed of printing affects the efficiency of creating biostructures (Fatimi et al., 2022).

Research has shown that a hybrid scaffold made of polycaprolactone (PCL) and fibronectin decellularized extracellular matrix (dECM) is effective for bone regeneration. Scientists have combined Gelatin Methacryloyl (Gel-MA) with human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (hMSCs) to create vascularized bone scaffolds. It has been discovered that hybrid bioinks containing dECMs can promote both blood vessel growth (angiogenesis) and bone tissue formation (osteogenesis). Additionally, hybrid bioinks have shown promise for repairing tissues and organs, constructing organoids, and creating disease models. It is important to note that hybrid bioinks can be customized by adding cells, peptides, and cytokines to improve tissue repair (Ding et al., 2023).

6. Challenges And Gaps

The study has revealed significant challenges and gaps in the standardization of bioink properties for 3D bioprinting. A major issue is the lack of universal standards, particularly in the rheological characteristics of bioinks, such as viscosity and shear-thinning behavior. These characteristics ensure consistent bioprinting outcomes across different laboratories and applications. Additionally, the mechanical properties of bioinks, such as stiffness and elasticity, are challenging to standardize but are essential for replicating the physical environment of various tissues. The variability in crosslinking methods used to solidify bioinks further complicates this issue, leading to differences in printed structures' mechanical properties and stability. These challenges underline the necessity of developing standardized protocols and materials to ensure consistency and reliability in tissue engineering applications (Yu et al., 2020).

The study highlighted challenges and gaps in scaling up 3D bioprinting technology for clinical applications. These include the difficulty in achieving higher printing speeds needed for replicating clinically relevant sizes. Although there has been significant progress, current bioprinting technologies still need to be fully optimized for large-scale production required for clinical settings. New methods are needed to maintain cell viability and functionality during the printing process and subsequent maturation of tissues on a larger scale. Additionally, the need for standardized protocols and the complexity of creating vascular networks within bioprinted tissues present significant obstacles to scaling up for clinical use (Cavallo et al., 2023).

Conclusion

Advancements in bioink materials and 3D bioprinting techniques have greatly improved the potential for creating functional tissue-engineered constructs that closely resemble native tissues. Integrating various scientific fields such as biomedical engineering, cellular and molecular biology, and materials science has resulted in better

scaffold designs and more efficient cell distribution. As a result, these technologies are opening up new possibilities for innovative tissue regeneration and transplantation approaches. However, challenges still exist in the widespread adoption of 3D bioprinting in clinical applications. Future research should concentrate on enhancing the mechanical and biological properties of bioinks, refining the printing processes, and ensuring the viability of cells after printing. Addressing regulatory obstacles and standardizing protocols will also be critical in translating 3D bioprinted tissues from the lab to real-world medical applications. Further exploration of nanotechnology and the development of new biomaterials will be crucial in overcoming current limitations. Collaborations across multidisciplinary teams will drive innovation, resulting in breakthroughs that could revolutionize regenerative medicine and improve patient outcomes.

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