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A Review of Current State of Bioinks

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ABSTRACT

3D bioprinting has revolutionized the field of tissue engineering and regenerative medicine by enabling the precise fabrication of complex biological constructs. Central to this transformative technology is the bioink, a specialized material serving as the scaffold for 3D structures housing living cells. This review provides a comprehensive overview of the current state of bioinks, offering insights into their biocompatibility, rheological properties, cell viability, printability, gelation properties, mechanical attributes, biodegradability, release capabilities, and scalability. A wide array of bioinks, encompassing natural and synthetic materials, is examined in detail, including hydrogels (collagen, gelatin, alginate), cellulose, Polycaprolactone (PCL), chitosan, Polyethylene Glycol Diacrylate (PEGDA), thermoresponsive hydrogels, fibrin, Gelatin Methacryloyl (GelMA), and silk. Each bioink type is evaluated in terms of its strengths and limitations, emphasizing the intricate balance required to meet specific tissue engineering and regenerative medicine needs. Despite the ongoing quest for the ideal bioink that balances biocompatibility, mechanical strength, and printability, researchers continue to refine formulations and explore novel materials to advance the field's potential in creating functional, transplantable tissues and organs.

Keywords: 3D Bioprinting • Tissue Engineering • Regenerative Medicine • Bioink • Hydrogel

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INTRODUCTION

Bioprinting represents a cutting-edge technology that harnesses the power of 3D printing techniques to fabricate intricate biological constructs, including cells and tissues, with remarkable precision and control [1]. At the heart of this transformative process lies the pivotal role of bioinks, hydrogel-based materials meticulously formulated to serve as the scaffolding upon which living cells are strategically placed [2]. Bioinks play a multifaceted role in bioprinting, serving as the structural foundation, the protective matrix for enclosed cells, and the conduit for vital nutrients and oxygen. As the CAD-designed model takes shape within the 3D printer, bioinks are dispensed with precision, layer by layer, enabling the assembly of intricate tissue structures with the utmost fidelity to the original design [2].

The significance of tissue engineering and regenerative medicine becomes abundantly clear in the realm of bioprinting. Tissue engineering and regenerative medicine emerge as the driving forces behind this innovation. These fields provide the foundational knowledge and techniques necessary to design bioinks, hydrogel-based materials that serve as the scaffold for living cells in bioprinting. The synergy between tissue engineering, regenerative medicine, and bioprinting exemplifies the dynamic evolution of this transformative field. By seamlessly integrating these disciplines, we can aspire to create patient-specific tissues and organs, addressing the pressing need for organ transplantation and pioneering personalized medical solutions. This interdisciplinary collaboration holds the promise of reshaping

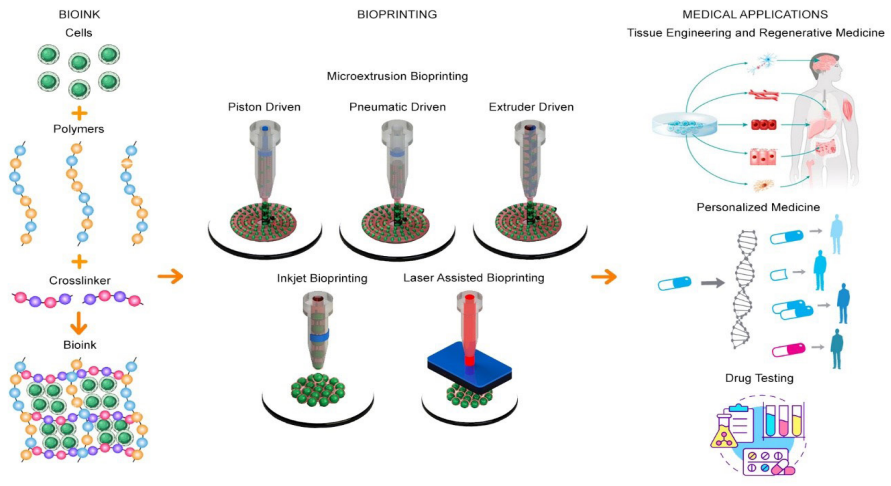

Figure 1: *A flow chart of bioprinting methods with biomedical applications.*

healthcare, revolutionizing pharmaceutical development, and advancing tissue engineering, all of which have the potential to redefine the landscape of regenerative medicine.

The necessity for bioinks goes beyond mere structural support. These hydrogel matrices serve as nurturing environments, providing the essential conditions for the survival and proliferation of the encapsulated cells. The selection of an appropriate bioink is, therefore, a critical decision in bioprinting, as it must balance the need for structural integrity with the imperative to create a milieu that promotes cell viability and functionality [3]. Researchers delve into a spectrum of bioink formulations, each tailored to specific applications, whether it be for engineering cardiac tissues with contractile properties or constructing skin grafts with exceptional regenerative capabilities. Moreover, the journey of bioprinted structures does not conclude at the printer's output. Following the precision-driven fabrication process, these bioink-laden constructs are delicately transferred to incubators, carefully calibrated to replicate the physiological conditions conducive to optimal cell growth and development [3]. Ensuring the maturation of bioprinted tissues into functional and viable structures is a crucial aspect of this nurturing phase, enabling them to fulfill their intended purposes. These purposes can range from disease modeling and drug screening to therapeutic transplantation [4]. The correlation becomes evident when examining the (Figure 1), which highlights the precise alignment of distinct bioprinting methods with specific bioink formulations. Each combination serves a unique and indispensable role within the domains of tissue engineering, regenerative medicine, and pharmaceutical research [1-4]. Furthermore, this symbiotic relationship between bioprinting technology and bioink sophistication reflects the dynamic evolution of the field. Advancements in bioprinting drive the demand for increasingly sophisticated bioink formulations, raising expectations for precision, viability, and therapeutic efficacy. This progression holds the potential to revolutionize the biomedical landscape, ushering in new frontiers in regenerative medicine, pharmaceutical development, and tissue engineering.

Bioprinting Methods

The field of bioprinting encompasses a rich tapestry of techniques, each wielding its unique set of principles and offering a diverse array of applications. Building upon the foundations of inkjet-based and laser-assisted bioprinting, extrusion-based bioprinting reigns as one of the most widely employed methods in the bioprinting landscape. This method orchestrates the precise extrusion of bioink, enriched with living cells, to meticulously craft intricate 3D biological structures in a layer by layer fashion [5]. Extrusion-based bioprinting methods offer versatility and precision, holding immense potential for applications in tissue engineering, regenerative medicine, and drug testing. For instance, researchers have employed extrusion-based bioprinting to create intricately structured cartilage tissue [6]. By depositing hydrogel bioink containing chondrocytes and supportive factors, they mimic the complex architecture of cartilage, offering potential solutions for joint repair and regeneration. As we delve deeper into the world of bioprinting, methods such as stereolithography, microfluidics-based bioprinting, magnetic bioprinting, and electrospinning-based bioprinting expand our toolkit. The choice of method is driven by the specific needs of the tissue or organ being printed, propelling the field ever closer to the remarkable prospect of bioprinting functional,

transplantable organs [7]. Pneumatic extrusion operates on the fundamental principle of air pressure, which acts as the driving force propelling bioink through a nozzle. Hydrogels, renowned for their shear-thinning properties, are prominently featured in this technique. Hydrogels such as alginate, gelatin, and agarose, with their facile extrusion characteristics and subsequent solidification, are extensively employed [8]. Piston-driven extrusion method introduces mechanical precision into the bioprinting process by employing a mechanical piston to delicately regulate the flow of bioink. Similar to pneumatic extrusion, hydrogels find favor due to their compatibility with the extrusion mechanism. The choice of hydrogel can be tailored to the specific needs of the cells and tissues being printed. Screw-driven extrusion approach relies on a precisely controlled screw mechanism to govern the rate of bioink flow. Hydrogels continue to reign supreme in this method. The versatility of hydrogels permits diverse formulations that can be customized to meet the unique demands of the cell types and tissues targeted by the bioprinting process. Microvalve-based bioprinting take center stage in this bioprinting methodology, orchestrating the meticulous release of bioink. This precision ensures high-resolution printing. Although hydrogels are the preferred bioink, microvalve-based bioprinting also allows for the precise integration of cells and growth factors, affording exceptional spatial control [9]. The multi-material approach redefines the boundaries of possibility by harnessing multiple printheads to extrude distinct bioinks concurrently. This technique enables the creation of multifaceted, multi-material structures. Diverse hydrogels, with their unique properties, combine harmoniously, facilitating the generation of heterogeneous tissues that closely mimic natural counterparts. Inkjet-based bioprinting operates similarly to conventional inkjet printers, but instead of ink, it dispenses cell-laden droplets onto a substrate. Researchers have harnessed this method to construct in-vitro nerve tissues [10]. By printing droplets containing neurons and glial cells in precise arrangements, they aim to model neural networks and study neurological disorders. Laser-based bioprinting employs precision lasers to transfer bioink material to a substrate. This technique has shown promise in creating intricate blood vessel networks [11]. Therefore, it has been utilized to fabricate complex vascular structures, potentially revolutionizing organ transplantation by ensuring proper blood circulation within engineered organs. Stereolithography based (SLA) bioprinting relies on photopolymer based bioinks and ultraviolet light to solidify each layer. This approach is well suited for dental applications, such as creating customized dental implants [12]. It becomes possible to produce dental prosthetics that perfectly match a patient's unique oral anatomy via precisely shaping photopolymer bioinks. Magnetic bioprinting involves the use of magnetic nanoparticle-loaded bioinks [13]. This method has potential applications in cardiac tissue engineering. Researchers are exploring the incorporation of magnetic nanoparticles into bioinks to control the orientation of muscle cells [14]. This alignment can facilitate the development of functional muscle tissue for regenerative purposes. Electrospinning-based bioprinting leverages electrostatic forces to deposit nanofibers of bioink onto a substrate [15]. One notable application is in the creation of wound dressings that promote healing [16]. Wound dressings with enhanced regenerative properties can be engineered based on the electrospinning bioinks containing growth factors and antimicrobial agents. Fused Deposition Modeling (FDM) bioprinting utilizes a nozzle to extrude bioink layer by layer, much like traditional FDM 3D printing. This method is often used for its simplicity and accessibility. For instance, researchers have employed FDM bioprinting to create scaffolds for bone tissue engineering [17]. It has been aimed to generate structures that support bone regeneration by extruding a composite bioink containing biodegradable polymers and bone forming cells. Bio plotting involves the precise deposition of bioinks through a plotting system. It enables the creation of complex structures with high resolution. This method has been utilized for applications such as mimicking the architecture and function of a liver tissue by plotting bioinks enriched with hepatocytes and supporting materials [18].

Recent Trends in Bioprinting Methods

In the ever-evolving landscape of bioprinting, recent advancements have spurred the development of cutting-edge techniques that hold significant promise for the field of tissue engineering and regenerative medicine. These novel approaches harness innovative principles to enhance precision, functionality, and versatility in bioprinting technology. Four noteworthy trends have emerged in recent years, each offering distinct advantages and opening new avenues for research and application. These trends include FRESH bioprinting, 4D bioprinting, acoustic bioprinting, and volumetric printing. Each method addresses specific challenges in bioprinting, showcasing the dynamic nature of this field and its potential to revolutionize the way we approach tissue engineering.

The Freeform Reversible Embedding of Suspended Hydrogels (FRESH) bioprinting method is a game-changer, employing a support bath of gelatin or alginate to secure the printed structure during fabrication. This unique approach

enables the precise deposition of soft and delicate bioinks, yielding high fidelity and resolution [19]. Once printing is complete, the support bath can be easily liquefied, leaving behind a bioprinted construct with its intricate structure intact. FRESH bioprinting has demonstrated remarkable potential, particularly in the creation of complex, vascularized tissue constructs and even functional organs, marking a significant leap forward in the quest for implantable bioprinted tissues [20,21].

Going beyond conventional 3D bioprinting, 4D bioprinting introduces dynamic responsiveness. It employs materials that can adapt or transform over time or in response to external stimuli, such as changes in temperature, pH, or exposure to specific biochemical cues. These dynamic properties empower bioprinted structures to evolve, mimicking natural physiological processes, and potentially leading to self-assembly or shape-changing behaviors [22,23]. This emerging field holds immense potential for the creation of tissues that actively respond and adapt to their environment, offering exciting prospects in personalized medicine and tissue regeneration.

Leveraging the power of acoustic waves, acoustic bioprinting method enables the precise positioning and assembly of cells and bioinks into intricate tissue structures [24]. By employing ultrasonic standing waves, cells are gently guided to specific locations within a hydrogel substrate, affording precise control over cellular organization. This non-contact, high-throughput approach minimizes cell stress and damage, making it a promising method for creating large-scale tissues with high cellular viability [25]. Acoustic bioprinting shines in applications where precise cellular placement is critical, such as in the creation of functional organs or complex tissue models.

Departing from traditional layer by layer bioprinting, volumetric printing is a revolutionary technique. It utilizes advanced light patterning and photosensitive bioinks to create intricate, 3D structures within a volume of gel in a single step [26]. By precisely controlling the spatial distribution of light, researchers can generate complex, cellularly dense constructs with unprecedented speed and accuracy. Volumetric printing holds great promise for rapid prototyping and the creation of intricate, multi-cellular tissues, bringing us closer to achieving the complex architectures found in natural tissues. These recent trends exemplify the dynamic nature of bioprinting, showcasing the ongoing pursuit of innovation to overcome existing limitations. As researchers continue to refine these techniques, the potential for bioprinting to revolutionize healthcare and personalized medicine becomes increasingly tangible. This progress heralds a promising future for tissue engineering and regenerative medicine, with the potential to transform the landscape of healthcare as we know it.

Limitations of Current Methods

One of the prominent challenges is the speed of printing. Even with advancements, the pace at which these printers operate remains a bottleneck. For instance, it can take an hour to produce a relatively small 1.5-inch cube [27]. This sluggish speed hinders the scalability of 3D printing for larger and more complex tissue constructs. Additionally, the requirement for a perfusable and highly efficient vascular network poses another significant hurdle. Tissues and implants created via 3D printing demand a functional circulatory system to ensure the delivery of nutrients and removal of waste, both of which are imperative for cell viability and tissue survival [28]. Furthermore, across all the aforementioned technologies, several critical aspects such as resolution, vascularization, perfusion, automation, cost, precision, and the development of ideal bioinks demand further refinement before they can make substantial contributions to the field of bioengineering tissues [29]. These limitations underscore the ongoing efforts within the scientific community to innovate and enhance 3D printing techniques, aiming to overcome these obstacles and unlock the full potential of additive manufacturing in tissue regeneration and engineering.

Bioinks

The cornerstone of the entire bioprinting process is the bioink, a specialized material meticulously designed to serve as the foundation for the 3D structures housing living cells [9]. Bioinks predominantly comprise hydrogels, a class of biomaterials that possess the remarkable ability to emulate the physical and mechanical attributes of natural tissues. Typically, hydrogels are constituted by a blend of water and a polymer, with options such as alginate, collagen, and gelatin frequently employed [30]. The magic of these materials lies in their capacity to be precisely tailored, creating a conducive physical and chemical microenvironment indispensable for the sustenance and proliferation of encapsulated cells. In the preparatory phase of bioprinting, bioink takes on the pivotal role of harboring living cells, including but not limited to stem cells, ready for the intricate printing process [31]. This critical step involves the extrusion of bioink, laden with cells, layer by layer, from a specialized printhead to gradually materialize the envisioned 3D structure. However, the journey doesn't culminate with printing; instead, the nascent structure is transferred to an incubator, where optimal conditions prevail to stimulate growth and development [32]. The significance

of bioink in bioprinting cannot be overstated. It serves as the linchpin that enables the survival and flourishing of the enclosed cells while simultaneously upholding the structural integrity of the construct. Devoid of an aptly designed bioink, the cells would succumb to the rigors of the printing process, rendering the resulting structure nonviable, and thus, incapable of fulfilling its intended purpose [33].

Polymers for Bioinks

In the realm of 3D bioprinting, the choice of bioink is of paramount importance, as it must exhibit specific critical properties and characteristics to facilitate successful tissue engineering. Notably, bioinks necessitate attributes such as printability and mechanical integrity to ensure accurate deposition and structural integrity during the bioprinting process. Additionally, they should possess the capability for functional modifications, enabling tailored adjustments to meet the specific requirements of desired tissues and organs. Controlled biodegradability and non-toxicity to cells are also imperative features, allowing cells to receive essential nutrients for growth and metabolic activity during tissue regeneration [2]. To this end, a diverse array of biomaterials, including both natural and synthetic polymers, has been identified as viable bioinks. Natural biomaterials derived from biological sources, offer distinct advantages in terms of biomimicry, self-assembly, biocompatibility, and biodegradability, aligning closely with the composition and structure of the Extracellular Matrix (ECM) [34]. Conversely, synthetic polymers bring their own set of merits, such as precise control over mechanical stability, photo-crosslinking capabilities, and responsiveness to pH and temperature variations [29]. The subsequent section will delve into the intricate landscape of natural biomaterials, elucidating their unique attributes and applications as bioinks in 3D printing.

Collagen, a fibrous protein abundant in the ECM, plays a pivotal role in providing structural integrity and support to tissues. It is considered a gold standard biomaterial in tissue engineering and regenerative medicine due to its excellent biocompatibility and bioactivity [35]. Collagen based bioinks offer an ideal substrate for cell adhesion, proliferation, and differentiation, closely mimicking the natural microenvironment of cells in vivo. Moreover, collagen possesses inherent signaling motifs that can influence cellular behavior, making it an invaluable component in bioprinting applications.

Gelatin, derived from collagen through denaturation, inherits many of collagen's beneficial properties while offering additional advantages. As a natural polymer, gelatin maintains high biocompatibility, allowing for favorable interactions with cells. It provides a supportive matrix for cell growth, making it an excellent choice for bioprinting applications [36]. Gelatin-based bioinks can be easily manipulated and processed, offering researchers a versatile material for creating intricate tissue constructs. Furthermore, gelatin's responsiveness to temperature changes allows for precise control over the gelation process during printing, ensuring the structural integrity of the final construct.

Alginate, a polysaccharide extracted from brown seaweed, is renowned for its ability to form gentle and stable gels under mild conditions. This characteristic makes it an exceptional choice for encapsulating cells in 3D bioprinting applications [37]. Alginate-based bioinks offer structural stability to the printed construct, providing a supportive environment for cell growth and tissue development. Additionally, alginate's unique gelation process does not compromise cell viability, making it a reliable option for bioprinting living tissues. Its biocompatibility and ease of gelation render alginate a valuable biomaterial in tissue engineering, particularly for applications where cell encapsulation is crucial for successful tissue regeneration.

Hyaluronic Acid (HA), a naturally occurring glycosaminoglycan, is known for its role in maintaining tissue hydration and promoting cell migration. HA-based bioinks are employed for their ability to support cell viability and proliferation [38]. These bioinks are often used in applications related to wound healing and tissue regeneration.

Chitosan, derived from the exoskeleton of crustaceans like shrimp and crab, has found utility as a bioink in 3D printing of tissue engineering scaffolds [39]. Chitosan offers biocompatibility and biodegradability, making it an attractive choice for creating bioactive constructs. Its positive charge facilitates interaction with negatively charged cells and molecules, promoting cell adhesion and proliferation. Additionally, chitosan based bioinks can be tailored to incorporate bioactive agents for enhanced tissue regeneration.

Cellulose, sourced from plant fibers, presents a unique option as a bioink in extrusion-based bioprinting [40]. This natural polymer is biocompatible, biodegradable, and highly modifiable, allowing researchers to tailor its mechanical properties to match specific tissue requirements. Cellulose-based bioinks have demonstrated success in creating 3D structures for tissue engineering and regenerative medicine, highlighting their versatility and potential in a variety of applications.

Fibrin, a natural ECM protein involved in blood clotting

and wound healing, is another noteworthy bioink [41]. Fibrin bioinks provide an ideal microenvironment for cell survival, proliferation, and tissue regeneration. Fibrin bioinks also replicate the mechanical properties of native tissues, enhancing their utility in creating physiologically relevant constructs.

Gelatin Methacryloyl (GelMA), derived from gelatin through methacryloyl group crosslinking, offers versatility as a bioink [42]. GelMA bioinks exhibit good biocompatibility and can be easily crosslinked, ensuring the stability of printed structures. Researchers have harnessed GelMA for 3D printing of various soft tissues, including cardiac and skeletal muscle, blood vessels, and nerve tissue.

Silk, a natural protein from silkworms, has emerged as a promising bioink for 3D printing [43]. Silk bioinks support cell survival and proliferation while faithfully replicating the mechanical properties of natural tissues. They have found applications in the creation of blood vessels and nerve tissue constructs.

Matrigel is a specialized bioink primarily composed of extracellular matrix proteins derived from mouse sarcoma cells. It closely mimics the natural microenvironment and is often employed in research settings for studying cellular behavior, though its clinical applications are limited due to its non-human origin [44].

Polycaprolactone (PCL), a synthetic biodegradable polymer, has gained prominence as a bioink in extrusion-based bioprinting [45]. PCL's thermoplastic nature makes it amenable to melt processing, enabling precise deposition during printing. Its good mechanical properties and biodegradability render it suitable for scaffold fabrication, particularly for load-bearing tissues in musculoskeletal applications. PCL's ability to maintain structural integrity while supporting cell growth makes it an essential component in bioprinted constructs.

Polyethylene Glycol (PEG) and its derivatives are used to create bioinks that offer excellent control over mechanical properties and printability [46]. These bioinks are customizable and can be fine-tuned to match the requirements of specific tissues. Each of these common bioinks possesses distinct advantages and limitations. For instance, alginate provides excellent printability and biocompatibility but may lack the ability to replicate the intricacies of the native ECM.

Polyethylene Glycol Diacrylate (PEGDA), a synthetic hydrogel, is widely used in extrusion-based bioprinting due to its numerous advantages [47]. PEGDA is highly hydrophilic, making it easy to process and manipulate during printing. Its robust mechanical properties and biocompatibility make it a versatile choice for various tissue engineering applications. Moreover, PEGDA can be readily crosslinked, allowing the formation of stable 3D structures that retain their shape and integrity.

Thermoresponsive hydrogels such as poly(N-isopropylacrylamide) (PNIPAAm) and PEO-PPO-PEO copolymers introduce a unique dimension to extrusion-based bioprinting [48]. These hydrogels exhibit changes in mechanical properties and solubility in response to temperature fluctuations, enabling precise control over the bioink's state during printing. This feature streamlines the printing process, ensuring accurate deposition while maintaining cell viability.

Selection of an appropriate bioink is a critical step in 3D bioprinting for successful tissue engineering. Bioinks must possess key attributes like printability, mechanical integrity, and the ability for functional modifications to ensure accurate deposition and structural integrity. Moreover, they should be biodegradable and non-toxic to cells, enabling proper nutrient delivery for tissue regeneration. A wide range of biomaterials, including natural and synthetic polymers, have been identified as viable bioinks, each offering unique advantages. Natural biomaterials closely mimic the ECM's composition and structure, providing biomimicry, self-assembly, biocompatibility, and biodegradability. On the other hand, synthetic polymers offer precise control over mechanical stability, photo-crosslinking capabilities, and responsiveness to environmental factors. These considerations underscore the intricate landscape of bioink choices in 3D printing, highlighting the importance of tailoring selections to specific tissue engineering needs.

Bioink Requirements

There are several critical requirements that a bioink must fulfill to be suitable for use in 3D bioprinting [49-57]. Biocompatibility is paramount, as the bioink must be non-toxic to cells and should not trigger an immune response in the host [58]. Rheological properties are crucial for printability, including appropriate viscosity and shear-thinning behavior [59]. Cell viability must be maintained throughout printing and culture, requiring the absence of toxic materials and a conducive environment for cell growth and proliferation [60]. Furthermore, the bioink must exhibit suitable gelation properties to maintain construct shape post-printing without harming cells [61]. Matching the mechanical properties of the native tissue, including stiffness and strength, is essential for functional tissue constructs [62]. Biodegradability or harmlessness to the host is necessary for integration with the host's body [63]. The ability to release growth factors or molecules that promote cell growth and differentiation is advantageous [64]. Scalability is vital for the creation

of large-scale tissue constructs [65]. Meeting these requirements is challenging, and a bioink that fulfills all of them comprehensively remains elusive [66]. Researchers continually strive to enhance bioink properties and develop novel formulations to address these challenges. Additional considerations include the capacity to incorporate various cell types into the bioink to create multifaceted tissue constructs [67], as well as the ability to control cell behavior and differentiation through the release of signaling molecules or growth factors [68]. Cost-effectiveness and availability are also vital factors determining the practical feasibility of bioprinting technology [69]. The development of a bioink that satisfies the diverse and often conflicting requirements of 3D bioprinting is a complex and ongoing endeavor. Researchers are dedicated to advancing bioink technology to pave the way for the creation of functional organs for transplantation and other innovative applications in the field of regenerative medicine.

RESULT AND DISCUSSION

Table 1 presented below offers a comprehensive view of a wide array of bioinks commonly employed in bioprinting, shedding light on their diverse functionalities, inherent properties, advantages, and disadvantages. These bioinks encompass a spectrum of both natural and synthetic materials, each carefully tailored to address specific needs within the realms of tissue engineering and regenerative medicine. A critical examination of the table reveals intriguing insights into the intricate balance between a bioink's merits and limitations, aligning each choice with its intended applications. Among the notable entries in the table, hydrogels emerge as a prevalent choice. Gelatin-based bioinks, in particular, exhibit a remarkable versatility by providing the advantages of supporting cell adhesion, encapsulation, and differentiation, while their biodegradability is a notable benefit for applications in wound healing and tissue engineering [70-75]. Yet, they may require crosslinking to enhance their stability and counter potential mechanical limitations. Alginate-based hydrogels, on the other hand, offer remarkable printability and biocompatibility [76,77], but can be limited by their mechanical strength and may require modifications to fine-tune their properties [78]. Furthermore, silk-based bioinks stand out for their natural origin and ability to mimic the mechanical properties of native tissues. Silk bioinks have been successfully employed in creating blood vessels and nerve tissues, providing support for cell survival and proliferation [79-81]. Nevertheless, they may necessitate additional crosslinking to ensure stability. Chitosan, derived from crustaceans, boasts good biocompatibility and biodegradability, making it an attractive bioink option for tissue engineering scaffolds [82-83]. However, potential limitations may arise concerning mechanical strength and the possibility of cytotoxicity [84]. In the realm of synthetic polymers, Polycaprolactone (PCL) stands as a biodegradable and easily processed bioink with commendable mechanical properties [85-87]. Its printability and adaptability have found utility in creating tissue engineering scaffolds. Meanwhile, Polyethylene glycol Diacrylate (PEGDA), a synthetic hydrogel, offers high hydrophilicity and robust mechanical properties, making it an ideal choice for extrusion-based bioprinting [88]. Its biocompatibility and ease of crosslinking further enhance its appeal. Beyond the conventional bioinks, thermoresponsive hydrogels like poly(N-isopropylacrylamide) (PNIPAAm) and block copolymers like poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) (PEO-PPO-PEO) introduce an element of responsiveness to temperature changes. This characteristic facilitates the ease of processing before printing and maintains the desired structure post-printing. Fibrin, a natural extracellular matrix protein, has been instrumental in the 3D printing of blood vessels and soft tissues. It not only supports cell survival and proliferation but also closely mimics the mechanical properties of native tissue [89-91]. However, its application may require careful consideration of the blood clotting and wound healing functions associated with fibrin. Additionally, bioinks like Gelatin Methacryloyl (GelMA) find widespread use due to their versatility in 3D printing various types of soft tissues, including cardiac and skeletal muscle, blood vessels, and nerve tissue [92-94]. Their biocompatibility and ease of crosslinking offer substantial benefits. Still, achieving the desired mechanical properties may necessitate optimization. The choice of bioink hinges on the specific tissue engineering or regenerative medicine application, as each bioink type exhibits its unique set of properties and capabilities. While natural polymers like hydrogels and silk are biocompatible and biodegradable, they may require additional measures to enhance mechanical stability. Synthetic polymers like PCL and PEGDA provide mechanical strength and ease of processing but must align with specific application needs. Thermoresponsive hydrogels enable dynamic manipulation during printing, and fibrin offers biomimicry with potential considerations regarding its biological functions. GelMA, with its adaptability, and chitosan, with its natural origin, illustrate the diversity within the bioink landscape. This complexity underscores the dynamic nature of bioprinting and the need for ongoing research to refine and expand the array of bioinks available, thereby advancing the field's potential to create functional, transplantable organs and

Table 1: A comprehensive view of the bioinks commonly employed in bioprinting.

Bioink	Chemical Structures	Composition	Function	Properties	Advantages	Disadvantages	Application	Ref.
Collagen-based Bioink	(Glycine - Proline - Hydroxyproline)n	Collagen, Gelatin, Hyaluronic Acid	Provides cell adhesion and ECM-like environment	Soft, tissue-like, biodegradable	Excellent cell compatibility, biomimetic ECM, supports tissue regeneration	May require additional crosslinking, low mechanical strength	Skin, cartilage, wound healing	[95-97]
Alginate-based Bioink	β-D-mannuronic acid and α-L-guluronic acid	Alginate, Calcium ions	Cell encapsulation, supports chondrogenesis	Gel-like, rapid gelation	Good cell viability, ease of use, ideal for cell encapsulation	Limited mechanical strength, potential cytotoxicity due to residual calcium ions	Cartilage, bone, drug delivery	[76-78]
GelMA Bioink	Gelatin backbone and Methacryloyl groups	Gelatin Methacryloyl, Photoinitiator	Photopolymerization, supports cell adhesion	Tunable mechanical properties, biodegradable	Excellent cell compatibility, fine-tunable properties, versatile for various tissues	Requires UV exposure for crosslinking, potential phototoxicity	Cardiac, muscle, nerve tissue, vascular constructs	[92-94]
PCL-based Bioink	[-CH_2-CH_2-CH_2-C(O)O-]n ("n" number of repeating units)	Polycaprolactone (PCL)	Structural support, tissue engineering scaffolds	Rigid, durable	Excellent mechanical strength, slow degradation, precise control over structure	Limited cell adhesion properties, lacks natural bioactivity	Bone, cartilage, scaffold fabrication	[85-87]
Fibrin-based Bioink	Two polypeptide chains: Aα chain and Bβ chain	Fibrinogen, Thrombin	Blood vessel formation, soft tissue regeneration	Soft, tissue-like, biodegradable	Excellent cell compatibility, mimics natural ECM, supports angiogenesis	Limited mechanical strength, rapid degradation	Blood vessels, wound healing, soft tissue	[89-91]
Chitosan Bioink	(β-D-Glucosamine)n ("n" number of repeating units)	Chitosan, Crosslinker	Encapsulation, cartilage regeneration, drug delivery	Tunable properties, biodegradable	Good biocompatibility, versatility, potential for sustained drug release	Potential immunogenicity, may require modification for enhanced properties	Cartilage, skin, drug delivery, scaffold	[82-84]
Silk-based Bioink	(Glycine-Serine-Glycine-Alanine-Glycine-Alanine)n	Silk Fibroin	Nerve regeneration, tissue scaffolds	Soft, durable	Good biocompatibility, tunable properties, supports nerve growth	Slow degradation, limited control over mechanical properties	Nerve regeneration, blood vessels, tissue scaffolds	[95-100]
PEG-based Bioink	H-(O-CH_2-CH_2)n-OH ("n" indicates the degree of polymerization or the length of the PEG chain)	Polyethylene Glycol (PEG)	Cell encapsulation, hydrogel scaffolds	Soft, hydrophilic	Excellent cell viability, minimal immunogenicity, easily modified for bioactivity	Limited mechanical strength, potential leaching of unreacted PEG	Cell encapsulation, hydrogel scaffolds	[88, 101, 102]
Hyaluronic Acid (HA) Bioink	[-D-glucuronic acid - N-acetyl-D-glucosamine-]n ("n" indicates the number of repeating disaccharide units)	Hyaluronic Acid	Supports cell proliferation, tissue hydration	Soft, viscoelastic	Good biocompatibility, natural component of ECM, retains moisture	Low mechanical strength, rapid degradation	Skin, cartilage, wound healing, ophthalmology	[103-105]
Decellularized ECM Bioink	Various proteins like collagen, fibronectin, laminin, and others, as well as glycosaminoglycans such as chondroitin sulfate, heparan sulfate, and hyaluronic acid.	Extracellular Matrix (ECM) components	Replicates tissue-specific microenvironments	Tissue-specific properties, biocompatible	Biomimetic, retains tissue-specific cues, supports cell differentiation	Limited scalability, variability between sources, complex preparation	Various tissue engineering applications	[106-108]

Agarose Bioink	-3,6-anhydro-L-galactopyranose-(1→4)-D-galactose	Agarose	Supports cell encapsulation, soft tissue regeneration	Soft, gel-like	Good cell viability, ideal for cell encapsulation, ease of use	Limited mechanical strength, potential cytotoxicity	Cartilage, neural tissue, drug delivery	[109-111]
Methacrylated HA (MAHA) Bioink	[CH_2=C(CH_3)COO] n - (HA backbone) ("n" represents the number of repeating units in the HA backbone, and the methacrylate group is attached to the HA molecule)	Methacrylated Hyaluronic Acid (MAHA)	Photopolymerization, supports cell adhesion	Tunable mechanical properties, biodegradable	Enhanced cell adhesion, fine-tunable properties, natural ECM component	Requires UV exposure for crosslinking, potential phototoxicity	Cartilage, cornea, vascular constructs	[112-114]
Matrigel Bioink	Complex mixture of proteins and other molecules derived from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells.	Matrigel	Supports cell survival, angiogenesis, and differentiation	Soft, gel-like, biodegradable	Good cell compatibility, contains growth factors, promotes angiogenesis	Variable composition, batch-to-batch variability	Angiogenesis assays, neural tissue, stem cell culture	[115-117]
Pectin-based Bioink	-(Galacturonic Acid)-[Rhamnose units -(Galacturonic Acid)- Arabinose units]n	Pectin	Supports cell encapsulation, drug delivery	Soft, gel-like, biodegradable	Good cell viability, biodegradable, abundant source	Limited mechanical strength, may require crosslinking, potential cytotoxicity	Cartilage, drug delivery, wound healing	[118-120]
Dextran-based Bioink	[α-D-Glucopyranose]n	Dextran	Supports cell encapsulation, drug delivery	Soft, hydrophilic	Biocompatible, tunable properties, versatile for cell encapsulation	Limited mechanical strength, potential cytotoxicity	Drug delivery, cartilage, vascular constructs	[121-123]
Polyurethane (PU) Bioink	H-O-C-R-NH-O-R' ("H" hydrogen atom."O" oxygen atom, "C" carbon atom, "R" and "R'" different organic groups)	Polyurethane (PU)	Cartilage tissue engineering, soft tissue scaffolds	Tunable mechanical properties, biodegradable	Good mechanical strength, versatile for different tissues, customizable properties	Potential cytotoxicity, complex synthesis process	Cartilage, tissue scaffolds, wound healing	[124-126]
Pluronic F127 Bioink	HO-PEO-b-PPO-b-PEO-OH ("HO" hydroxyl (OH) group, "PEO" polyethylene oxide, "PPO" polypropylene oxide, "OH" hydroxyl group.)	Pluronic F127 (PF127)	Supports cell encapsulation, drug delivery	Soft, hydrophilic	Good cell viability, thermoresponsive, minimal cytotoxicity	Limited mechanical strength, potential phase separation at higher concentrations	Drug delivery, cell encapsulation, thermoresponsive scaffolds	[127, 128]
HA-Gelatin Bioink	Repeating units of HA along with the amino acid sequences found in Gelatin	Hyaluronic Acid, Gelatin	Supports cell adhesion, encapsulation, and differentiation	Soft, biodegradable, tunable properties	Synergistic properties of HA and gelatin, promotes tissue regeneration	May require crosslinking for stability, potential mechanical limitations	Skin, cartilage, wound healing, tissue engineering	[70-72]
Silk Fibroin-HA Bioink	Interact through a variety of physical and chemical interactions, such as hydrogen bonding and electrostatic forces.	Silk Fibroin, Hyaluronic Acid	Supports nerve regeneration, tissue scaffolds	Soft, biodegradable	Synergistic properties of silk and HA, supports nerve growth and tissue regeneration	May require additional crosslinking, potential limitations in mechanical properties	Nerve regeneration, cartilage, tissue scaffolds	[79-81]

Gelatin-Pectin Bioink	Interact through a variety of physical and chemical interactions, such as hydrogen bonding, electrostatic forces, and potential cross-linking.	Gelatin, Pectin	Supports cell encapsulation, drug delivery	Soft, biodegradable	Synergistic properties of gelatin and pectin, biodegradable, potential for controlled drug release	Limited mechanical strength, potential cytotoxicity	Drug delivery, cartilage, cell encapsulation	[73-75]

tissues. As the bioink field continues to evolve, researchers are working to address current limitations, enhance bioink properties, and develop novel materials to drive the field toward its ultimate goal of revolutionizing disease treatment, particularly in cases of organ failure. This dynamic interplay between the pros and cons of each bioink type illustrates the ongoing quest for the ideal bioink, one that balances biocompatibility, mechanical strength, and printability to create tissues and organs that seamlessly integrate with the human body.

In the rapidly advancing field of 3D bioprinting, the selection of an appropriate bioink stands as a critical determinant of success. As discussed, various bioinks, each with its unique advantages and drawbacks, are at the forefront of this innovative technology. Hydrogels, encompassing collagen, gelatin, and alginate, offer promising biocompatibility and mechanical properties but may require modifications to faithfully replicate intricate native tissue structures. Cellulose-based bioinks provide an intriguing natural alternative, allowing for tailored mechanical properties to meet specific tissue requirements. PCL impresses with its thermoplastic nature, facilitating precise deposition during printing, albeit potentially falling short in fully replicating biochemical cues. Chitosan, boasting biocompatibility and biodegradability, fosters cell adhesion and proliferation, and its customization potential is promising. PEGDA shines with hydrophilicity, robust mechanical properties, and biocompatibility. Thermoresponsive hydrogels introduce an intriguing dimension, simplifying the printing process while maintaining cell viability. Fibrin, an ECM protein, creates an ideal microenvironment, faithfully replicating native tissue mechanics. GelMA offers versatility and stability, while silk mimics natural tissue properties. However, the path to developing a bioink that comprehensively fulfills all requirements, from biocompatibility to scalability, remains intricate. Researchers diligently work to refine bioink formulations and explore novel materials, emphasizing the need for continuous research and development. As this field progresses, the ultimate goal of creating functional, implantable tissues and organs for regenerative medicine and transplantation draws ever nearer.

CONCLUSION

Bioinks are indispensable for 3D printing in tissue engineering and regenerative medicine, offering a range of unique advantages and disadvantages. Hydrogels, including cellulose, PCL, chitosan, PEGDA, fibrin, GelMA, silk, and thermo responsive hydrogels, stand out as commonly used bioinks due to their tailored properties. Hydrogels, for instance, mimic natural tissue mechanics and possess high biocompatibility and biodegradability. Cellulose, derived from plant fibers, exhibits biocompatibility and biodegradability. PCL, a synthetic biodegradable polymer, combines biocompatibility with excellent mechanical properties. Chitosan, a natural polymer from crustacean exoskeletons, offers biocompatibility and biodegradability. PEGDA, a synthetic hydrogel, is highly hydrophilic and mechanically robust. Fibrin, a natural protein, supports cell growth, though it may be challenging to handle. GelMA is a versatile synthetic hydrogel promoting biocompatibility and cell growth. Silk, a natural protein, supports cell growth but may require careful processing. Thermo responsive hydrogels, which respond to temperature changes, facilitate convenient bioink handling before printing. The choice of bioink hinges on the specific application, desired structure properties, and printing technique. Researchers continuously advance bioink development, aiming for enhanced functionality and broader applications. Cost considerations and the need to supplement bioinks with growth factors, extracellular matrix components, or cells for certain applications are crucial factors. The choice of bioink is also influenced by the 3D printing technique employed. This dynamic field of bioinks promises to play a pivotal role in shaping the future of tissue engineering and regenerative medicine.

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