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THE QUANTUM LEAP FROM 3D TO 6D BIOPRINTING AND ITS IMPACT ON THE MANAGEMENT OF DISEASES

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ABSTRACT

Bioprinting technology has emerged as a transformative frontier in medical science, enabling the creation of complex, functional biological structures for applications in tissue engineering and disease management. This review article explores the evolution of bioprinting from its conventional three-dimensional (3D) constructs to the more sophisticated and biologically dynamic six-dimensional (6D) systems. The initial 3D approaches enabled the layer-by-layer deposition of cells and biomaterials, allowing the fabrication of anatomically relevant structures. However, their static nature and limited physiological functionality stimulated the development of four-dimensional (4D) bioprinting, which integrates time as a functional variable. Subsequent progress led to the development of five-dimensional (5D) bioprinting, which incorporates additional axes of complexity by enabling gradient material deposition and improved mechanical anisotropy. Besides, the most recent advancement, 6D bioprinting, introduces real-time adaptability, biological responsiveness, and multiscale integration. The review not only elucidated the applications but also shed light on the synergistic role of nanotechnology in cellular signaling, and tissue vascularization. The major applications include tissue regeneration, skin and cartilage repair, cancer modelling, neurodegenerative disease studies, and personalized organ fabrication. Despite advancements there are critical challenges such as limited vascular integration, mechanical fragility and ethical concerns which need to be addressed. The integration of artificial intelligence, in situ printing, and smart biomaterials is expected to drive the next phase of bioprinting innovation. This quantum leap from 3D to 6D printing not only enhances the precision of tissue engineering but also offers transformative potential for disease management and the future of precision medicine.

Keywords: *Bioprinting; Biofabrication; In situ bioprinting; Smart biomaterial; Tissue regeneration; Vascularized tissue models.*

INTRODUCTION

In early 2000s, bioprinting began with the first successful demonstration of printing living cells and biomaterials in 2003 [1]. Atala's team at Wake Forest Institute for Regenerative Medicine created the first 3D-printed organ scaffold in 2006 [2]. The technology has since evolved, with advances in materials and techniques leading to more complex

tissue constructs and applications [3]. In present world, bioprinting is at the head of regenerative medicine, with ongoing research pushing the boundaries of tissue and organ engineering [4].

As a primary technology in biofabrication, bioprinting uses cells, proteins and biomaterials as building blocks for 3D-printed biological models, biological systems and therapeutic products. This emerging technique has rapidly developed with the use of functional building blocks, for example, from printing biomaterials for tissue scaffolds and implants, to printing cells or organoids for 3D biological models. It has also progressed to include the printing of micro-organ-chips for microphysiological platforms and engineered living systems, such as cellular machining and biorobots [5].

Bioprinting is a fascinating field that merges 3D printing technology with biological applications, such as tissue engineering and regenerative medicine. The dimensions of bioprinting typically refer to the spatial aspects involved in creating structures that replicate biological tissues or organs. There are three fundamental dimensions in 3D bioprinting, representing the width (X), depth (Y), and height of the printed structure (Z). The printer deposits bioink layer by layer to build up the desired 3D structure. Resolution in 3D bioprinting refers to the precision of the print, often measured in micrometers. High resolution is important for replicating fine details in tissue structures. The thickness of each deposited layer affects the overall resolution and structural integrity of the bioprinted tissue [6]. Time is fourth dimension in 4D bioprinting. Structures will change or adapt over time in response to environmental conditions, such as temperature, pH, or other stimuli in it. These dynamic structures could better mimic natural biological processes [7]. Additional dimensions can include varying material properties across the printed structure in 5D bioprinting. It may include gradient changes in stiffness, porosity, or other mechanical properties that better replicate the complex nature of biological tissues. This dimension includes incorporating complex cell behaviors and interactions into the printed structure, such as cell differentiation or migration patterns [8]. Integration of multiple functional elements into a single bioprinted construct could be seen as sixth dimension. This may include combining different types of cells, biomaterials, and growth factors in a way that simulates the dynamic and multifaceted nature of biological systems. Incorporating advanced features like vascular networks or responsive behaviors (e.g., structures that can repair themselves or respond to external stimuli) could be considered a sixth dimension, increasing the capabilities of bioprinting beyond mere structural replication [9].

Bioprinting is revolutionizing disease management and tissue regeneration by enabling the creation of complex, personalized tissue constructs and models. As the technology advances from 3D to more intricate dimensions like 4D and 6D, it allows for the development of custom implants, prosthetics, and even functional organ replacements tailored to individual needs [10, 11]. These advancements improve early disease detection, personalized medicine, and regenerative therapies by facilitating precise tissue repair, controlled release of growth factors, and dynamic, responsive structures [12, 13]. While promising, the technology faces challenges such as scalability, cost, and integration with human physiology, which must be addressed to fully realize its potential in clinical settings [14].

THE GENESIS OF BIOPRINTING: FROM 3D TO 6D

Bioprinting, a revolutionary advancement in the field of regenerative medicine, has developed significantly from its inception. Originally harnessing 3D printing technologies to create simple, three-dimensional structures, bioprinting has now progressed into the branch of 6D printing, incorporating temporal and functional dimensions into its design. This development reflects a growing sophistication in our ability to not only print tissues and organs but also integrate dynamic biological processes and real-time functional adaptations. As we move from 3D to 6D, the potential to create complex, adaptive biological systems becomes progressively tangible, promising to convert the landscape of medical treatments and tissue engineering [15].

3D BIOPRINTING: THE FOUNDATION PRINCIPLE

Three-dimensional (3D) bioprinting is a technique used to create tissues and organs by printing with biological materials. Instead of just making shapes out of plastic or metal like traditional 3D printers, bioprinting uses special "bio-inks" made from cells and materials that mimic the natural environment of tissues [16].

The process starts with designing the tissue or organ using computer software, which helps in deciding both the detailed layers and the overall shape. Then, the printer carefully deposits these bio-inks layer by layer to build up the structure. It is crucial to control factors like how thick the ink is, how fast it is printed, and the temperature to keep the cells alive and functioning well [17].

After printing, the tissue is placed in an incubator where it receives nutrients and growth factors to help it mature and develop properly. The key advantage of 3D bioprinting over traditional methods is the precise control it offers, ensuring that cells are evenly distributed and the final product closely resembles natural tissues [18].

LIMITATIONS

One major challenge in bioprinting is the lack of agreement on how to do it best because there are so many variables to consider. These include choosing the right mix of cells and materials for the bio-ink, adjusting printer settings like temperature and speed, and deciding on the right maturation process with signals and bioreactors. With so many options, it is tough to establish a universal standard for each type of tissue [19].

Another big issue is creating blood vessels within the printed tissues. For cells to survive, they need to be within approximately 400 micrometers of a blood supply. This is why it is difficult to print large living tissues thicker than 1 millimeter. While there has been progress, such as printing small blood vessel-like tubes, integrating a full network of blood vessels into printed tissues is still beyond current technology [20].

EARLY APPLICATIONS

The early development of 3D bioprinting involved several groundbreaking advancements. It all began in 1984 with Charles Hull's invention of stereolithography (SLA), a method that allowed for the creation of 3D objects from digital designs, laying the foundation for modern 3D printing. In 1988, Klebe showcased the potential of bioprinting by using a standard inkjet printer to deposit living cells, marking a significant leap towards creating living tissues [21]. By 1996, Forgacs and his team had made a crucial discovery about tissue cohesion, providing insights into how cells adhere to each other. The introduction of laser-assisted bioprinting in 1999 by Odde and Renn enabled the creation of complex tissue structures. In 2001, researchers made strides by printing a scaffold in the shape of a bladder and seeding it with human cells, demonstrating the potential for functional tissue engineering [22]. In 2002, launch of the "3D-Bioplotter" and in 2003, the development of an inkjet bioprinter by Wilson and Boland further advanced the field. Notably, in 2004, scientists succeeded in creating 3D tissue without scaffolds, and by 2006, electrohydrodynamic jetting was used for cell printing. The year 2009 saw the creation of vascular tissues, crucial for developing complex structures. By 2012, new applications such as in situ bioprinting on mouse models and the creation of cartilage and artificial liver tissues emerged [23]. The field continued to evolve with the introduction of coaxial technology in 2015 for tubular structures, rapid continuous optical 3D printing in 2016, and significant achievements like the printing of small-scale, functional hearts in 2019. These milestones highlight the rapid advancements in bioprinting, transforming it from a basic technique into a sophisticated technology with the potential to create complex tissues and organs [24].

ADVANCEMENTS TO 4D BIOPRINTING

A new era of 4D (four-dimensional) shape-morphing structures was required due to the constraints and difficulties with static 3D materials, as there are numerous applications in different scientific domains. The shape-morphing behavior of 3D constructs over time is known as 4D design. The static aspect of 3D is eliminated by 4D printing technology, which also significantly increased mechanical strength, adaptability, clinical and nonclinical usefulness of implants

under predetermined environmental circumstances including both artificial and physiological [25].

CONCEPT AND MECHANISM

4D bioprinting is a latest extension of 3D printing technology that involves the dimension of time into the creation of dynamic and adaptive structures. It was introduced by Tibbits Skylar in 2013, the concept of 4D bioprinting includes the use of stimuli-responsive materials that can change shape, properties, or functionality over time in response to external factors [26]. These materials, such as hydrogels and shape-memory polymers (SMPs), are created to react to physical stimuli (like temperature and light), chemical stimuli (such as pH changes), and biological stimuli (e.g. biomolecules). The mechanism of 4D bioprinting includes the preparation of these dynamic materials, printing them into 3D structures, and then exposing them to specific stimuli that trigger time-dependent transformations [27]. This capacity allows printed objects to develop and adapt, offering advantages over static 3D-printed items. For example, 4D bioprinting can allow the creation of structures that self-repair, change shape, or perform complex functions over time, which has significant imputations for fields like tissue engineering, drug delivery, and personalized medicine [28].

ENHANCED FUNCTIONAL CAPABILITIES

The advanced functional capabilities of 4D bioprinting represent a significant leap beyond traditional 3D printing by introducing dynamic and adaptive features to bioengineered constructs. Unlike static 3D-printed structures, 4D bioprinting allows for the creation of constructs that can undergo pre-determined morphological changes over time in response to external stimuli such as temperature, pH, or light [29]. This is achieved through the use of stimuli-responsive biomaterials and the incorporation of natural cell forces, which enable the printed constructs to actively interact with their environment and modify their shape or properties. Advanced mathematical modeling further enhances this technology by predicting how these constructs will transit between different states and what their final forms will be [30]. These capabilities open up a range of applications beyond tissue engineering, including bioactuation (creating responsive structures), biorobotics (developing biologically integrated robotic systems), and biosensing (designing sensors that react to environmental changes). Looking ahead, 4D bioprinting holds great promises for advancing biomedicine and related fields by enabling the development of more complex and functional biological systems [31].

The advanced functional capabilities of 4D bioprinting include several innovative and dynamic features that significantly enhance the utility of bioengineered constructs. Firstly, 4D bioprinting introduces the ability to incorporate time as a fourth dimension, enabling printed structures to undergo controlled, dynamic transformations over time. This capability addresses a major limitation of traditional 3D bioprinting, which only considers static, initial conditions of printed objects. By utilizing stimuli-responsive materials, 4D bioprinting allows for the creation of biologically active architectures that can adapt and change in response to various stimuli, such as temperature, pH, or light [7]. Additionally, 4D bioprinting enables the integration of natural cell forces into the constructs, allowing for the maturation and functionalization of cells or tissues over time, which is crucial for replicating the dynamic nature of natural tissues. This technology also advances the field of bone tissue engineering by providing constructs that can adapt to irregular bone defects and support personalized bone regeneration through shape-recovery and mechanical property modulation [32]. Moreover, 4D bioprinting supports the development of self-folding micro-tubes for vascularization, and it facilitates the spatiotemporal release of bioactive cues and cells, promoting complex tissue regeneration that includes bone, vascular, and nerve tissues. These capabilities collectively enhance the adaptability, functionality, and long-term viability of bioprinted constructs, offering new solutions for tissue engineering and regenerative medicine [33].

EVOLUTION TO 5D BIOPRINTING

3D bioprinting is widely successful yet debated technology utilized across numerous sectors,

including aerospace, automotive, fashion, marine applications, orthodontics, prosthetics, and various types of tooling and design. Despite its many uses, 3D printing has several drawbacks. These limitations are addressed by 4D printing, which incorporates shape memory effects (SMEs) such as one-way, two-way, and three-way SMEs. Building on 3D and 4D printing, 5D printing has been developed to produce more streamlined and advanced products [34].

INTEGRATION OF TEMPORAL DYNAMICS

The integration of temporal dynamics in 5D bioprinting represents a groundbreaking evolution in additive manufacturing, particularly in the area of biological and tissue engineering. Unlike traditional 3D printing, which builds static structures, 5D bioprinting integrates time as a dynamic element, allowing for the creation of materials and tissues that can adapt and develop over time. This advancement involves the use of shape memory and self-healing materials, which can change their form or properties according to environmental stimuli such as temperature and pH [35]. Moreover, temporal dynamics enable the manipulation of cellular behaviors, allowing cells to differentiate and grow in a manner that imitates natural biological processes [36]. This capability to develop adaptive and responsive tissues is crucial for applications in regenerative medicine, drug testing, and personalized treatments, as it ensures that bioprinted constructs can maintain their functionality and integrate effectively over time [37]. The incorporation of these time-dependent factors make 5D bioprinting a significant advancement over previous technologies, offering enhanced performance and longevity in practical applications [38].

CASE STUDIES AND INNOVATIONS

The innovations and case studies in 5D bioprinting point out its transformative potential in personalized medicine. By combining temporal dynamics with 3D printing technology, 5D bioprinting advances beyond static models to form adaptive, responsive structures tailored to individual patient needs [8]. For example, 5D bioprinting influences dynamic materials that can change according to physiological conditions, boosting the functionality of medical devices and scaffolds. This approach involves the development of advanced bio-inks and composite materials that control chemical and physical properties accordingly, confirming that the bioprinted constructs meet specific biological requirements. Particularly, in the context of peripheral arterial disease (PAD), 5D bioprinting highlights the limitations in existing treatments by allowing the creation of personalized vascular models and drug-eluting devices that release nanoparticles effectively [39]. One case study demonstrated the use of rapid freeze prototyping to construct nano-laden aerogels for quick nanoparticle release, which was tested for in vivo applications, showcasing the potential for improved drug delivery and treatment personalization. These innovations underscore how 5D bioprinting is composed to revolutionize medical treatments by combining detailed patient-specific data with dynamic material properties, contributing to more effective and customized therapeutic solutions [40].

EMERGING 6D BIOPRINTING

Emerging 6D bioprinting builds on the advancements of 5D bioprinting by incorporating an additional dimension that integrates dynamic physiological interactions over time, involving cellular and molecular processes. This technology improves the creation of complex tissue models by allowing real-time adaptation to biological changes and environmental conditions, potentially contributing to more accurate and functional implants and regenerative tissues. The integration of these multi-scale interactions offers a significant leap forward in personalized medicine, allowing the development of medical solutions that more closely duplicate and interact with natural biological systems [41].

MULTISCALE AND MULTIMATERIAL APPROACHES

Multiscale and multimaterial approaches in 6D bioprinting represent advanced strategies that integrate various spatial and temporal scales, as well as diverse materials, to enhance the complexity and functionality of biofabricated tissues. Multiscale techniques involve designing

structures that can mimic natural biological hierarchies, from nanoscale extracellular matrix components to macroscale tissue architecture, thereby promoting better cell behavior and tissue integration [42]. Concurrently, multimaterial printing enables the use of different biomaterials within a single construct, facilitating the creation of gradients in mechanical properties and biological signals that can support heterogeneous cell types and functional gradients [43]. Together, these approaches aim to replicate the intricate organization of native tissues more accurately, potentially improving the performance of engineered constructs in regenerative medicine and transplantation [44].

THEORETICAL AND PRACTICAL IMPLICATIONS

6D bioprinting, which incorporates three spatial dimensions along with three additional dimensions of time, material properties, and biological signals, offers significant theoretical implications for tissue engineering and regenerative medicine. Theoretically, this approach allows researchers to model and predict complex biological processes more accurately, considering not just the structural elements of tissues but also their dynamic behaviors and interactions over time [45]. By integrating temporal factors into bioprinting, researchers can simulate how tissues respond to various stimuli, such as mechanical loading or biochemical signals, enhancing the design of functional biomaterials that promote desired cellular behaviors and tissue maturation. This comprehensive modeling can lead to better understanding and predictions of how engineered tissues will perform in vivo [46].

On the practical side, 6D bioprinting holds the potential to revolutionize the way complex tissues and organs are fabricated, leading to advancements in personalized medicine. By utilizing multimaterial printing capabilities, practitioners can create constructs that closely mimic the heterogeneous nature of native tissues, accommodating different cell types and material properties within a single print [47]. This versatility enables the production of customized grafts tailored to individual patient needs, potentially improving the outcomes of tissue repair and regeneration. Furthermore, the integration of dynamic biological signals during the printing process can facilitate real-time tissue development, making it possible to optimize the mechanical and biochemical environments for specific applications, such as wound healing or organ regeneration [48]. Thus, the practical applications of 6D bioprinting could significantly enhance therapeutic strategies and tissue engineering methodologies. Figure 1 shows the summary of evolution of bioprinting from 3D to 6D. The evolution of bioprinting has progressed from simple 3D tissue models to advanced 6D systems, enabling dynamic, responsive, and functional biological structures [49].

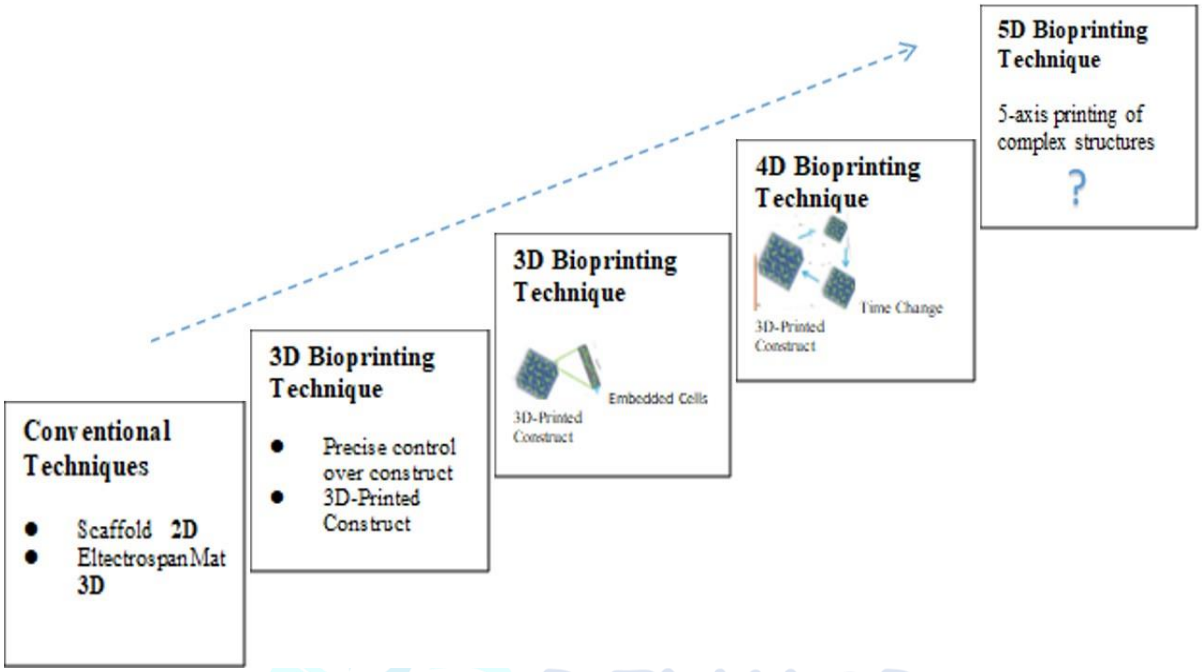

Fig 1. Bioprinting has advanced from 3D to 6D, evolving from static tissue models to dynamic, responsive structures. Early 3D bioprinting focused on layering cells and biomaterials, while 4D added time, allowing self-assembly and adaptability. 5D introduced mechanical property manipulation, mimicking natural tissue heterogeneity. Today, 6D bioprinting enables tissues that grow, respond, and interact with their environment in real-time, moving closer to functional organ printing.

TECHNOLOGICAL INNOVATIONS DRIVING THE EVOLUTION

Recent advancements in bioprinting technologies, such as enhanced bio-inks and precision printing techniques, have significantly improved tissue engineering outcomes [50]. These innovations enable the creation of more complex structures that better mimic natural tissues [51]. Moreover, the integration of 3D printing with stem cell technology is paving the way for personalized medicine [52].

ADVANCEMENTS IN PRINTING MATERIALS

Advancements in printing materials for bioprinting have dramatically enhanced the ability to create complex and functional tissue constructs. Recent innovations focus on smart hydrogels, which can respond to environmental stimuli, allowing changes in properties such as stiffness and porosity that mimic natural tissue behavior [44]. These hydrogels can be engineered to support cell adhesion, growth, and controlled release of bioactive factors, significantly improving tissue regeneration outcomes. Furthermore, the development of bio-inks from natural polymers like alginate and gelatin has gained traction due to their biocompatibility and ability to facilitate cellular interactions, making them ideal for bioprinting applications [42]. Composite materials that combine multiple biopolymers or incorporate nanoparticles have also emerged, enhancing both mechanical strength and biological functionality. These advancements allow for greater customization of tissue constructs, enabling the design of structures that closely resemble the native architecture of tissues. This flexibility in material design is crucial for creating scaffolds tailored to specific tissue types, such as cartilage, bone, or skin. Additionally, the integration of

nanomaterials has led to improved mechanical properties and bioactivity, further broadening the scope of potential applications. As researchers continue to explore new material combinations and formulations, the ability to produce more sophisticated and effective tissue-engineered solutions is becoming increasingly feasible. Overall, these developments in printing materials are paving the way for significant advancements in regenerative medicine, providing new opportunities for personalized treatment strategies [53].

SOFTWARE AND COMPUTATIONAL MODELS

Recent advancements in software and computational models have significantly transformed the field of bioprinting, enhancing the design, simulation, and optimization of biofabrication processes. Sophisticated software tools now enable researchers to create intricate 3D models of biological structures, facilitating precise control over the arrangement of cells and materials within the print [45]. These tools not only streamline the design process but also allow for the incorporation of complex geometries that replicate the architecture of native tissues. Furthermore, computational modeling plays a critical role in predicting how printed constructs will behave in vivo, taking into account factors such as mechanical stress, fluid dynamics, and cellular interactions [46].

By simulating these conditions before physical printing, researchers can optimize designs to improve cell viability and tissue functionality. Additionally, machine learning algorithms are increasingly being integrated into bioprinting workflows, enabling real-time adjustments during the printing process based on feedback from sensors and imaging technologies. This integration of computational intelligence enhances the adaptability and precision of bioprinting, paving the way for personalized medicine and customized tissue engineering solutions. As these software and modeling techniques continue to evolve, they will play a crucial role in pushing the boundaries of what is achievable in bioprinting, ultimately leading to more effective therapeutic applications [54].

DISEASE MANAGEMENT AND TISSUE REGENERATION: IMPACT OF BIOPRINTING

Bioprinting is revolutionizing disease management and tissue regeneration by enabling the precise fabrication of complex tissue structures tailored to individual patient needs. This technology allows for the creation of scaffolds that promote cellular growth and integration, significantly enhancing the healing process [50].

BIOPRINTING FOR TISSUE ENGINEERING

Three-dimensional (3D) printing, or additive manufacturing (AM), is increasingly utilized across various fields, including manufacturing, food production, art, and architecture. Recently, regenerative medicine has embraced this technology to create computer-designed cellular structures incorporating cells, biomaterials, and signaling molecules for the regeneration of artificial tissues and organs, known as 3D bioprinting [55]. By layering bio-inks composed of living cells, hydrogels, and bioactive molecules, bioprinting allows for the creation of scaffolds that promote cellular growth and differentiation. This method addresses key challenges in traditional tissue engineering, such as the uniform distribution of cells and the replication of the intricate architectures found in native tissues. As a result, bioprinting holds immense potential for applications in regenerative medicine, including the development of artificial organs, repair of damaged tissues, and personalized implants tailored to individual patient needs. Moreover, ongoing advancements in bioprinting techniques continue to enhance the functionality and viability of engineered tissues, paving the way for innovative therapeutic solutions [56].

SKIN AND CARTILAGE REPAIR

Because bioprinting can produce intricate, biomimetic structures that promote regeneration, it has become a potential method for regenerating skin and cartilage. Bioprinting enables the exact layering of extracellular matrix components, growth factors, and cells in skin restoration, which can improve wound healing and restore skin integrity [57]. Recent studies have demonstrated the successful fabrication of 3D skin constructs

that closely mimic natural tissue, providing a platform for both therapeutic applications and drug testing [58]. Similarly, bioprinting facilitates the creation of scaffolds for cartilage repair that mimic the mechanical characteristics and anatomical structure of natural cartilage, encouraging cell adhesion and growth [6]. By utilizing bio-inks that include chondrocytes and suitable hydrogels, researchers have achieved significant advancements in developing functional cartilage tissues [59]. The integration of bioprinting with stem cell technology also shows promise for enhancing tissue regeneration, offering personalized solutions for patients suffering from skin and cartilage defects. As bioprinting technologies continue to evolve, their application in regenerative medicine is likely to expand, providing innovative strategies for tissue repair and regeneration [60].

ORGAN TRANSPLANTATION AND REGENERATION

Organ transplantation has a rich history, with early successes in kidney transplants during the 1950s marking significant milestones [61]. However, challenges such as organ rejection and donor shortages led scientists to explore alternative solutions [62]. By the late 20th century, advancements in stem cell technology and tissue engineering began to transform the field of regenerative medicine [63]. A major breakthrough came in the 2000s when pioneers like Dr. Anthony Atala and Dr. Jennifer Lewis developed 3D bioprinting technique to create living tissues [64, 65]. These innovations opened new possibilities for printing more complex biological structures, offering hope for addressing organ shortages [50]. Today, 3D bioprinting holds immense potential for transforming organ transplantation and regenerative medicine, bringing us closer to personalized, life-saving solutions [66].

Human organ transplantation involves implanting healthy organs from donors into patients with failing organs to extend their lives and improve their health. Despite significant advancements in surgical techniques and immunosuppressive therapies, the demand for organs far exceeds supply, with over 90% of patients still waiting for transplants. In response to this critical shortage, researchers are exploring innovative solutions, including bioprinting technology. Three-dimensional (3D) printing offers a promising avenue for creating patient-specific organ structures, utilizing data from imaging techniques like computed tomography (CT) and magnetic resonance imaging (MRI) [67]. This additive manufacturing process allows for the production of complex, custom organ designs that traditional methods cannot achieve. As the technology matures, it opens up new possibilities for alleviating donor shortages and improving outcomes for patients requiring transplants. Moreover, the decreasing costs of 3D printers and the emergence of open-source designs are making this technology more accessible, potentially revolutionizing the field of organ transplantation and enhancing personalized medicine [68].

BIOPRINTING IN DISEASE MODELING AND DRUG TESTING

Bioprinting is increasingly recognized as a pivotal technology in disease modeling and drug testing, offering innovative approaches to create more accurate and reliable models of human tissues. Bioprinting allows for the careful stacking of biomaterials and living cells to create three-dimensional tissue constructs that closely resemble the structure and functions of natural tissues. This capability allows researchers to study disease progression in a controlled environment, providing insights into cellular interactions and disease mechanisms that traditional two-dimensional models cannot offer [50]. Furthermore, bioprinted tissues can be used for high-throughput drug screening, allowing for the evaluation of therapeutic compounds on human-like tissues, which enhances the predictability of drug responses and toxicity [52]. This not only accelerates the drug discovery process but also reduces reliance on animal models, leading to more ethical and efficient research practices. As bioprinting technology advances, its applications in disease modeling and drug testing are expected to expand, paving the way for more personalized and effective therapeutic strategies [69].

PERSONALIZED MEDICINE

Personalized medicine benefits greatly from bioprinting since it makes possible to create customized tissue structures that precisely match the demands of each patient. By using bioprinting, patient-specific models that take into account the distinct cellular and molecular properties of each individual's tissues can be created, in contrast to conventional 2D and even some 3D cell culture systems that frequently generalize the intricate interconnections within human tissues [70]. By utilizing computer-aided design (CAD) and advanced imaging techniques, bioprinting can produce highly customized scaffolds that are designed to replicate the architecture and functional organization of human tissues, promoting more relevant cellular interactions [71]. This customization is particularly beneficial in drug testing, where bioprinted tissues can provide more accurate predictions of how a patient might respond to specific therapies, thereby reducing the reliance on less relevant animal models. Additionally, the ability to incorporate bioactive materials and growth factors into the bioprinted constructs further enhances their functionality, allowing for the potential regeneration of tissues that closely mimic the patient's original tissues. As a result, bioprinting not only advances the field of tissue engineering but also contributes significantly to the development of personalized therapeutic strategies that are more effective and aligned with the biological intricacies of individual patients [72]. Figure 2 shows different applications of bioprinting across various fields.

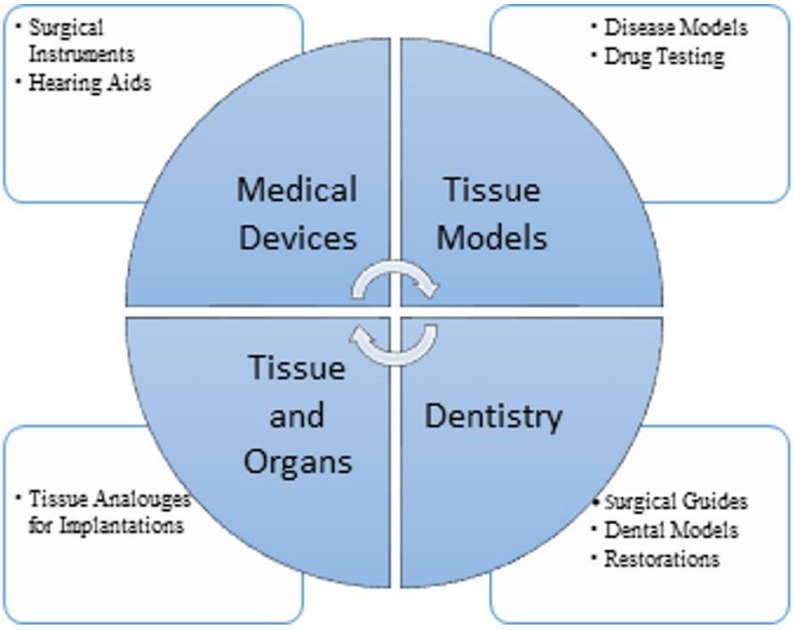

Fig 2. 3D printing has found applications across various fields, including dentistry, the creation of printed tissues or organs for tissue restoration and transplantation, and the development of printed tissues or cells for pathological modeling. It is also utilized in producing medical devices, anatomical models for surgical planning, education and training, drug testing, and high-throughput drug screening [73].

HIGH-THROUGHPUT SCREENING AND VALIDATION

Bioprinting plays a significant role in enhancing high-throughput screening (HTS) and validation processes in drug development. Traditionally, HTS relies on two-dimensional (2D) cell cultures, which often fail to replicate the complex in vivo environment, leading to inaccuracies in drug response and toxicity assessments. Bioprinting, on the other hand, allows for the creation of three-dimensional (3D) cell cultures that better mimic the physiological characteristics of human tissues

[74]. By enabling the construction of multiple 3D cell cultures in a controlled and reproducible manner, bioprinting enhances the ability to study drug interactions and tissue responses more effectively. Various bioprinting techniques such as extrusion, inkjet, and laser printing facilitate the incorporation of different cell types, extracellular matrix components, and biomaterials. This capability allows researchers to create complex tissue models that can provide more relevant data during drug testing [75]. Furthermore, bioprinting supports automation and high-throughput capabilities, enabling the rapid fabrication of 3D structures. This speed and precision are essential for processing large datasets and performing simultaneous experiments, which are crucial for identifying potential drug candidates quickly. As bioprinting technology advances, it is increasingly being integrated into HTS workflows, paving the way for more physiologically relevant and accurate drug screening and validation processes [76]. Despite these advantages, there are challenges to fully integrating bioprinting into HTS, such as ensuring that the 3D models maintain the appropriate microenvironment and optimizing assays for quantitative analysis. Ongoing research is focused on addressing these hurdles, aiming to improve the overall efficacy of drug development pipelines by leveraging bioprinting for better tissue and disease modeling [77].

ADDRESSING CHRONIC AND COMPLEX DISEASES

Bioprinting plays a crucial role in addressing chronic and complex diseases by enabling the creation of sophisticated in vitro models that closely replicate human tissue architecture and function. This technology allows researchers to use patient-derived cells to develop personalized models that provide more accurate insights into disease mechanisms and treatment responses. By mimicking the intricate cellular environments found in vivo, bioprinting enhances the understanding of various diseases and aids in the identification of new therapeutic targets. Moreover, bioprinting facilitates the rapid prototyping of complex tissue structures, making it easier to test new drugs and treatment approaches [78]. Its ability to customize models with specific cell types and biomaterials allows for the development of advanced therapeutic products, such as 3D-printed drugs that can be tailored to individual patient needs. This customization is particularly valuable in the context of chronic diseases, where patient-specific responses to therapies can vary widely [79].

Figure 3 shows the conventional vaccine and therapeutic discovery pipeline model which typically involves stages such as target identification, preclinical testing, and clinical trials. 3D bioprinting technologies have the potential to enhance these processes by enabling more accurate disease models and personalized drug testing, improving the efficiency of vaccine and therapeutic development.

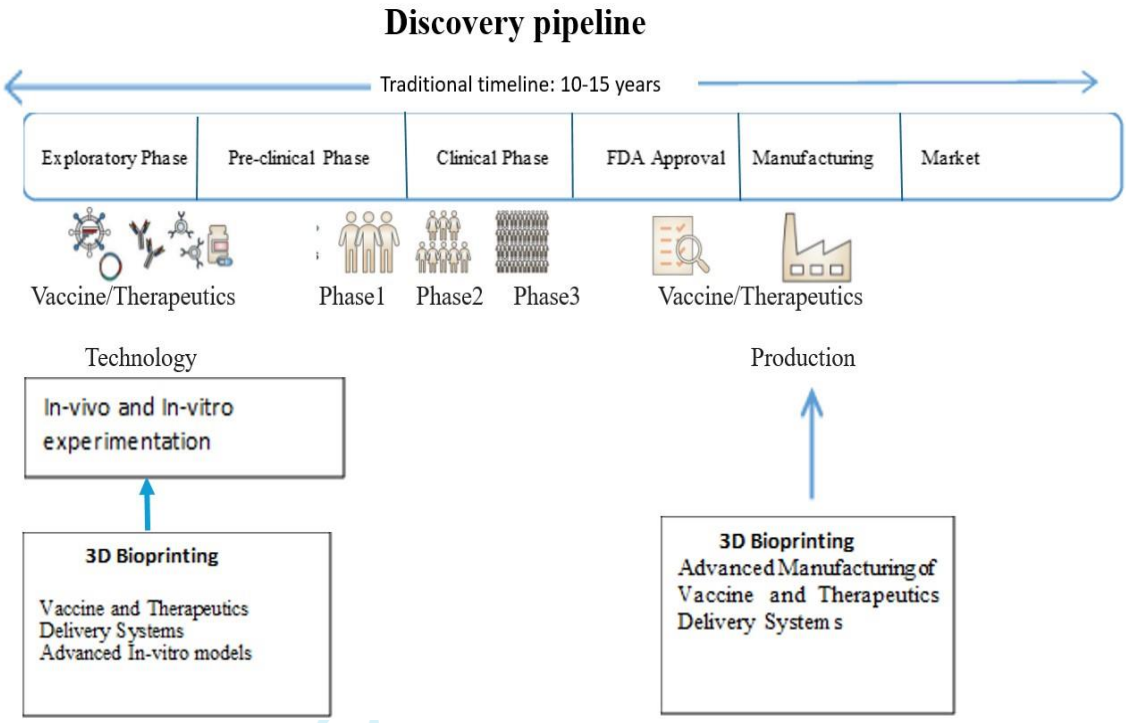

Fig 3. Schematic representation of the conventional vaccine and therapeutic discovery pipeline, along with potential enhancements through 3D bioprinting technologies (indicated by gray boxes) [80].

CANCER

In 2012, there were 8.2 million mortalities and nearly 14 million new cases of cancer recorded globally. It is predicted that the number of cases would rise to 22 million over the next 20 years, making cancer a major source of illness and mortality [81]. The majority of cancers have characteristics in common, such as a complex microenvironment with dense tissue and a hypoxic core, which is seen in stomach, liver, lung, breast, and colon cancers, among others [82]. Beginning as healthy epithelial cells, cancer cells undergo genetic mutations that confer upon them characteristics such as invasive behavior, resistance to cell death, uncontrollably growing, and the capacity to induce aberrant blood vessel formation via the action of factors such as vascular endothelial growth factor (VEGF) [83,56]. Since only cells that are close to blood vessels obtain enough oxygen, these skills are critical for the survival of tumors. Interactions with the surrounding stroma, which consists of a variety of cell types and the extracellular matrix, impact the formation of tumors. Cancer cells can change their activity and migrate by interacting with stromal and inflammatory cells [84].

State of the art in vitro cancer models have evolved from traditional two-dimensional (2D) monolayers to more advanced three-dimensional (3D) cultures utilizing various tissue engineering techniques. While 2D models have been used since the 1950s, they often fail to accurately replicate drug responses observed in vivo due to their inability to mimic the complex 3D tumor microenvironment, resulting in inadequate cell–cell and cell-extracellular matrix (ECM) interactions [85]. To address these limitations, researchers have developed 3D cancer models, primarily based on multicellular tumor spheroids and 3D matrices. Multicellular spheroids can be created by seeding tumor cells on non-adherent surfaces, allowing them to self-aggregate, which leads to the formation of a necrotic core due to nutrient and oxygen deprivation, mirroring in vivo conditions [86]. Although these spheroids have advanced cancer research and drug testing, they still lack essential ECM components. To enhance their biomimetic properties, 3D matrices made

from hydrogels or porous scaffolds, derived from natural and synthetic polymers, have been employed. These matrices can be engineered for specific stiffness and other properties, providing a more accurate representation of the tumor microenvironment and improving cell behavior, including proliferation and secretion of growth factors [87]. Overall, 3D cancer models using these innovative materials show significantly enhanced performance compared to traditional 2D cultures and even 3D tissues embedded in matrices like Matrigel [88].

Current 3D in vitro cancer models face significant challenges, particularly due to their simplified structures and limited vascularization capabilities, which restrict their ability to replicate later stages of tumor development. Multicellular tumor spheroids and 3D scaffold-based tumors can simulate early tumor genesis but often lack the spatial organization and ECM composition found in actual tumors [89]. To address these issues, there has been growing interest in 3D bioprinting technologies to create more biomimetic cancer models. Bioprinting encompasses various techniques, such as inkjet, extrusion-based, laser-assisted, and stereolithography, all of which enable the precise deposition of bio-inks containing cells and biomaterials. Inkjet bioprinting is cost-effective and quick, while extrusion-based methods provide moderate speed and affordability [57]. Laser-assisted bioprinting allows for high-resolution patterns but can be expensive. Stereolithography, traditionally used for materials fabrication, offers excellent precision and rapid production. The bio-inks used in these processes, typically hydrogels, must possess favorable characteristics like printability and biocompatibility, with cross-linking strategies that stabilize the structures post-printing [90]. Recent advancements include using decellularized ECMs as bio-inks, which may enhance the structural complexity and functionality of bioprinted tissues by closely mimicking native tissue properties. Additionally, bioprinting can facilitate the creation of vascular structures through sacrificial templates, enabling the incorporation of perfusable channels into engineered tissues. Overall, these innovations in bioprinting enhance the ability to create complex 3D cancer models that better reflect the tumor microenvironment and support advanced research in cancer biology [91].

Current 3D cancer models face significant challenges, including batch-to-batch variability, limited control over cell arrangement, and low throughput. In response, 3D bioprinting offers a versatile solution by allowing for the precise deposition of complex structures with high fidelity. However, applications of bioprinting in creating biomimetic cancer models have only recently emerged. As previously mentioned, vascularization is crucial for tumor growth, providing essential nutrients and oxygen [92]. For instance, Yoo *et al.*, developed a glioblastoma vascularization model that integrated sacrificial bioprinting with multicellular spheroids [93]. In this approach, a microchannel seeded with human umbilical vein endothelial cells (HUVECs) was bioprinted into a collagen matrix, and a spheroid made from patient-derived glioma stem cells was placed adjacent to the vascular channel to mimic in vivo conditions [94]. Over a period of seven days, the glioma spheroid exhibited remodeling, with indications of sprouting and potential vascularization. This model is versatile and can be adapted to study various cancer types within the context of the tumor-vascular niche [95].

In addition to vascularization, research has increasingly focused on the tumor microenvironment, which plays a vital role in tumor development. For instance, Sun *et al.*, created a 3D cervical cancer model by encapsulating HeLa cells in a composite bioink made of gelatin, alginate, and fibrinogen, demonstrating that this bioprinted model better replicated cancer cell proliferation, viability, and response to chemotherapy compared to traditional 2D cultures. The microfibrous matrix used in this model can be tailored to various shapes and architectures to accommodate different cancer cell types [5]. Similarly, Xu *et al.*, developed a co-culture model using OVCAR-5 ovarian cancer cells and normal fibroblasts in Matrigel with a droplet bioprinter, allowing for efficient co-culture and a perfused system. This droplet-based approach improved spatial control over cell distribution and density, leading to more

reproducible cell patterning and a controlled tumor microenvironment [96]. Furthermore, immune cells are critical components of the tumor microenvironment, actively participating in tissue remodeling [77]. Coextrusion bioprinting technique was utilized to examine interactions between MDA-MB 231 breast cancer cells and macrophages, which can create a paracrine loop that enhances cell motility and may facilitate tumor cell extravasation into the bloodstream. In their model, the two cell types were separately loaded into cartridges and coextruded with precise spatial control. The macrophages, initially positioned in a core microchannel, were found to migrate outward and interact with surrounding breast cancer cells, with the extent of interaction influenced by the geometry of the bioprinted macrophage channels. This innovative model enables detailed studies of tumor-stromal cell interactions, cancer metastasis, and migration within a designed niche [97].

NEURODEGENERATIVE DISORDERS

The human nervous system's complexity makes studying its development particularly challenging, as traditional reliance on animal models fails to capture unique human aspects and can be both costly and time-consuming [98]. The breakthrough of reprogramming human somatic cells into induced pluripotent stem cells (hiPSCs) has revolutionized this field, enabling the differentiation of hiPSCs into various nervous system cell types and facilitating the study of neurodevelopmental and neurodegenerative disorders. However, much of the research has been limited to two-dimensional cultures, which do not accurately replicate the 3D architecture of neural tissues [49]. To address this, 3D bioprinting technology offers a promising solution, allowing for the precise arrangement of cells and extracellular matrix components to create complex tissue structures that better model human neural environments [99]. This method not only enhances our understanding of nervous system development but also holds potential for developing more effective therapeutics, particularly by using biomaterial scaffolds that support cell survival and integration in regenerative applications [100].

When creating functional tissues through 3D bioprinting, it is essential to carefully characterize and adjust both the biophysical and biochemical properties of the selected hydrogel, as these factors significantly affect tissue behavior and functionality [101]. The characteristics of the printed constructs largely depend on the chosen bioink. Hydrogel-based bio-inks serve not only as structural scaffolds but also as microenvironment for the encapsulated cells, influencing their activities [102]. Therefore, it is important to select bio-inks that are biocompatible, bioactive, and biomimetic to produce constructs that closely resemble native tissue. This section will explore key considerations for bioink selection in nervous system applications, with Table 1 summarizing the various bioprinting modalities and bio-inks used for specific neural tissue applications.

Table 1. Bioprinting modalities and bio-inks used for specific neural tissue applications.

Applications	Bioprinting method	Bioink material	Cell type	Cell density	Cell viability	Crosslinking method	References
Modeling stem cell response	Extrusion bioprinting	Alginate, carboxymethylchitosan, agarose	Human Neural stem cells	1 x 107 cell	> 85%	2% w/v Calcium Chloride for 10 min.	[103]
	Stereolithography	Gelatin methacrylate modified with dopamine	Mouse derived neural stem cells	3 x 104 cell scaffold	-	1 wt% Irgacure, UV crosslinked	[104]
Cerebral Cortex Modeling	Hand-held bioprinter	RGD modified gellan gum	Mouse primary cortical neurons	1 x 106 cell	73% DMEM and 73% Calcium Chloride 5 days post printing	5x DMEM or 1 M Calcium Chloride	[105]
	Piezo-electric driver droplet printing into oil bath	Matrigel into undecane and silicone oil bath with DPhPC	iPSC-derived human Neural Stem cells,	3.5 x 107 cell	96% on day 1 post print	Gelation at room temperature	[106]
Cancer Modeling	Drop-on-demand bioprinting	Agarose and Collagen type I	Human primary umbilical cord derived mesenchymal stromal cells,	MSCs- 1 x 106 cell HUVECs - 3 x 106 cells/mL Neuroblastoma cells - 1 x 106 cell	-	-	
Traumatic Brain Injury Repair	Fused deposition manufacturing	Polyurethane	Murine neural stem cells	1 x 107 cell	> 100% 72 hrs post printing in PU2 scaffold	No crosslinking	
Spinal Cord Modeling	Lab-on-a-printer	Fibrinogen, alginate, and genipin with the addition of: CHIR, SB, LDN,	hiPSC-derived neural progenitor cells	-	> 81% on day 7 post print	Calcium chloride, chitosan, and thrombin	

		purmorphamine, or retinoic acid					
	Continuous printing and point dispensing printing	Scaffold: Alginate with methylcellulose Cell-matrix: Gelatin Methacrylate, gelatin and fibrinogen, or Matrigel	Primary human neonatal dermal fibroblasts.	Fibroblasts: 1 x 107 cell sNPCs: 1 x 107 cell OPCs: 1 x 107 cell	Fibroblasts: > 90% on day 4 post print sNPCs (in Matrigel): > 70% on day 4 post print	Scaffold: Calcium chloride Cell-matrix: UV crosslinking (GelMA)	[33]
Peripheral Nerve Modeling	Extrusion bioprinting	Sodium alginate and gelatin	Rat Schwann Cells (RSC96)	2 x 106 cell	> 92% on day 14 post print	Calcium chloride	[94]
Peripheral Nerve Repair	Extrusion bioprinting	Gelatin and sodium alginate	Rat Schwann Cells	2 x 106 cell	~ 93% on day 7 post print	Calcium chloride	[107]
Spinal Cord Repair	Extrusion bioprinting	Collagen type I, heparin sulfate, and basic fibroblast growth factor	Embryonic neural stem cells for *in vitro* analysis	-	- (*in vivo* analysis)	UV crosslinking	[108]
	Extrusion bioprinting	Collagen and silk fibroin	Rat neural stem cells	1 x 107 cell	-	Solidification	[57]
Drug Screening	Customized extrusion bioprinting	Gelatin methacrylate and gelatin	Mouse macrophages (RAW264.7) and Mouse glioblastoma cells (GL261)	Macrophages: 3 x 106 cell Glioblastoma cells: 2 x 106 cell	> 75% on day 10 post print	UV crosslinking	[109]
	Digital light processing bioprinting	Gelatin methacrylate and	Glioblastoma stem cells (GSC)	GSC: 2.5 x 107 cell	-	UV crosslinking	[110]

In bioprinting tissues, creating an environment conducive to cellular activities such as viability, migration, and proliferation is crucial. While various natural and synthetic polymers are commonly used as bio-inks for their biocompatibility and low toxicity, they can sometimes hinder essential cell functions like adhesion and proliferation [74]. To address this, natural materials with inherent binding sites, like gelatin and fibrin, are frequently employed [50]. Additionally, bio-inks lacking these sites can be enhanced by incorporating cell-binding peptides,

as demonstrated by Lozano *et al.*, who used gellan gum modified with the RGD sequence to promote neuronal tissue development, improving neuron viability and network formation. The composition of the native extracellular matrix (ECM) is also vital, as it directly influences cellular responses and organization within the printed construct [105]. The brain's ECM, comprising various proteins and polysaccharides, plays a significant role in cellular interactions and is essential for effective bioprinting. Mechanical properties such as stiffness and shear stress need to be carefully optimized to match those of native tissues, as deviations can impact cell behavior and viability. Moreover, the rheological properties of bio-inks are critical for ensuring printability, with non-Newtonian behaviors like shear thinning being desirable for extrusion processes [111]. Biochemical properties, including the incorporation of signaling molecules and growth factors, further enhance tissue functionality by supporting cell growth and differentiation. Finally, electrical conductivity is crucial for facilitating neural network formation; adding conductive materials like PEDOT or polypyrrole to bio-inks can significantly improve their electrochemical properties, thus aiding in neuronal differentiation and regeneration. Overall, these factors must be harmonized to develop effective and functional bioprinted tissues [56].

By precisely manipulating various cell and tissue types, 3D bioprinting makes it easier to create complex tissue structures. This is especially useful when integrating vascularization into neural tissue constructs, which is necessary to maintain long-term cellular functions by facilitating waste removal and nutrient transport. The blood-brain barrier (BBB), which is created by the complex interactions of endothelial cells, pericytes, and astrocytic end foot, functions as a crucial interface for selective permeability between the brain and systemic circulation in brain tissue [112]. The surrounding extracellular matrix (ECM), consisting of collagen IV, laminin, proteoglycans, and fibronectin, plays a significant role in regulating molecule permeability. The establishment of the BBB occurs over several weeks during fetal development and relies on specific signaling cues from endothelial cells and the neuroepithelium to orchestrate the assembly of BBB components [113]. Various 3D culture techniques and microfluidic devices have been employed to develop BBB models. For instance, researchers created a micro-engineered physiological system featuring dual compartmental microfluidic channels: the upper layer contained an endothelial cell monolayer atop a porous membrane with pericytes below, while the lower channel housed astrocytes within a 3D Matrigel matrix. This model demonstrated low permeability and replicated key structural features of the native BBB, although it faces limitations in throughput and scalability [114]. Replicating developmental signaling cues and the intricate architecture of the BBB which consists of an endothelial lumen encircled by pericytes and astrocytic end feet are two of the challenges involved in accurately reconstructing the BBB. In order to progress this field, numerous researchers co-cultured endothelium-like bEnd.3 and U87 glioma cells with microtubules made using two-photon lithography to mimic blood capillaries [43]. This allowed the researchers to successfully maintain a stable endothelial monolayer with mature tight connections. Like previous approaches, this one can be improved, especially in adding more cellular elements, obtaining trans-endothelial electrical resistance similar to in vivo circumstances, and creating models that are affordable [11]. Additionally, while 3D bioprinting has been used to create perfusable vascular tissues, these efforts are still in the early stages of optimization. Enhancing these techniques to more accurately mimic brain blood vessels would significantly advance neural tissue engineering, potentially creating a more reliable neurovascular model that could serve as a platform for screening drug candidates for their ability to cross the BBB [115].

Brain organoids are three-dimensional aggregates derived from human induced pluripotent stem cells (hiPSCs) that can be organized to represent specific brain regions, such as the cerebellum, cortex, hypothalamus, and midbrain. These organoids have been valuable for modeling normal developmental processes and various neurodevelopmental disorders, including

microcephaly, prenatal Zika virus infection, Autism Spectrum Disorder, and Schizophrenia. Consequently, they hold significant promise as platforms for drug screening and as human model systems for studying neurological development and function [116]. However, brain organoids face several limitations, including the absence of vascularization, limited diversity of cell types, and lack of spatially controlled morphogen gradients necessary for regional patterning. 3D bioprinting presents an opportunity to overcome these challenges by enabling precise placement of cells and growth factors, potentially leading to more sophisticated and reproducible organoid structures [117]. This approach could enhance the modeling of regional diversity, improve our understanding of molecular mechanisms in brain development, and facilitate investigations into nervous system disorders [118].

Neurodegenerative diseases encompass a wide range of conditions marked by the gradual loss of neurons, posing significant challenges for the aging population. Human induced pluripotent stem cells (hiPSCs) have emerged as a valuable tool for modeling neurodegenerative diseases like Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis, as well as for exploring treatments for traumatic brain injury. Furthermore, hiPSC-derived organoids are being investigated as three-dimensional models to study neurodegeneration [93]. However, the application of 3D bioprinting in modeling and treating these conditions is still in its early stages. A pivotal study by Hsieh *et al.*, demonstrated the potential of bioprinting in repairing traumatic brain injuries by developing two thermoresponsive biodegradable polyurethane (PU) bio-inks containing neural stem cells. The PU2 bioink displayed rheological properties akin to those of brain tissue (ranging from 680 to 2400 Pa) and supported cell proliferation and differentiation. Additionally, injecting the PU2 hydrogel into an adult zebrafish model of traumatic brain injury showed promise in restoring nervous system function [119]. Combining tissue bioprinting with technologies like iPSCs may enhance our ability to replicate neurodegenerative conditions, providing insight into the underlying molecular mechanisms and serving as a platform for high-throughput drug screening [120]. Figure 4 shows the role of bioprinting in disease management and control.

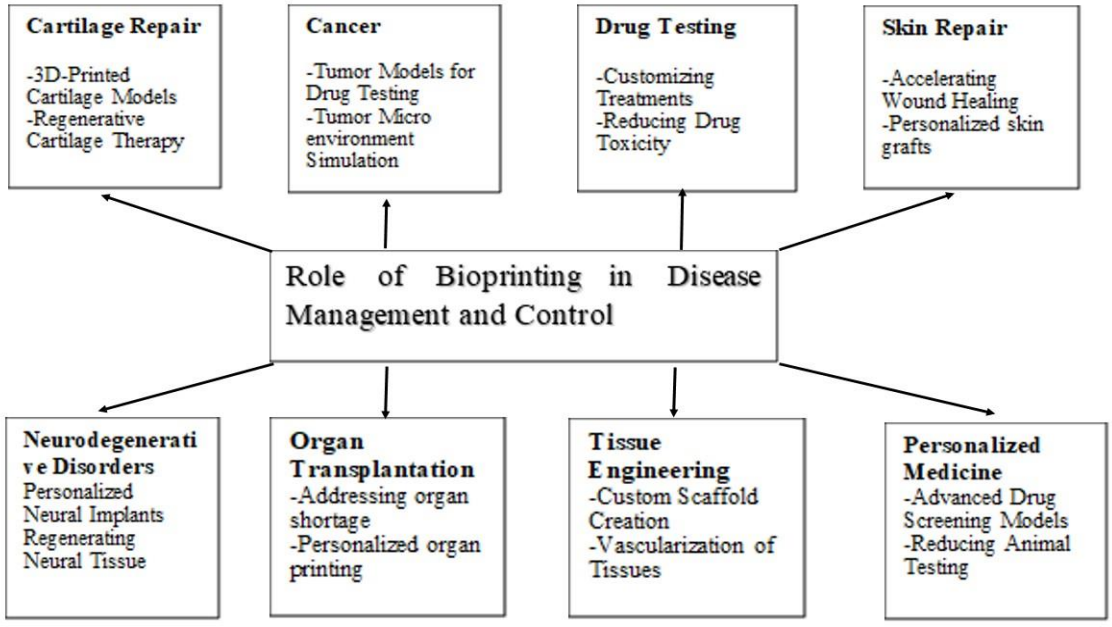

Fig 4. Bioprinting plays a crucial role in disease management and control by enabling the creation of personalized tissue models for disease modeling, drug testing, and regenerative therapies, ultimately improving the precision and efficacy of treatments while reducing the reliance on animal testing [121].

IMPACT OF NANOTECHNOLOGY ON 3D BIOPRINTING

In recent years, nanotechnology has significantly advanced the engineering of biomaterials through the development of 3D bioprinted scaffolds for tissue engineering and regenerative medicine. These nanomaterial-based scaffolds are crucial for supporting cell accommodation or guiding their differentiation into specific tissues during the regenerative process. Cellular behaviors such as adhesion, proliferation, migration, and differentiation are influenced by the structural and functional characteristics of the cellular microenvironment in which the cells are cultured. By leveraging nanotechnology, researchers can modify these properties within 3D bioprinted scaffolds to promote better cellular growth and function [122]. For instance, Nowicki *et al.*, demonstrated that the combination of 3D bioprinting and nanocrystalline hydroxyapatite enhanced the adhesion, proliferation, and osteochondral differentiation of human bone marrow mesenchymal stem cells (hBMSCs). Their study highlighted that software-assisted scaffold design could improve mechanical performance without compromising cellular activity. Moreover, incorporating nanoparticles along with biochemical factors into the scaffolds led to favorable hBMSCs differentiation into osteogenic and chondrogenic lineages [123].

Ensuring adequate vascularization in bioprinted scaffolds is vital for their long-term viability. Several studies have shown the potential to create complex vascular networks within these scaffolds. For instance, Holmes *et al.*, integrated nanomaterials with 3D printing to develop advanced scaffolds featuring both nano and micro-scale structures. They produced novel 3D bone scaffolds that not only supported bone formation but also included interconnected microvascular channels, promoting efficient osteogenic regeneration and vascular cell growth. Their polylactic acid (PLA)-based scaffolds, enhanced with nanohydroxyapatite, demonstrated mechanical properties akin to bone and fluid flow profiles resembling vascular systems [124]. In vitro studies confirmed improved hBMSCs adhesion, proliferation, and osteogenic differentiation, as well as enhanced growth and organization of human umbilical vein endothelial cells on the scaffolds. Various biomaterials are currently utilized for 3D bioprinting tissue-engineered scaffolds, including natural polymers like alginate, gelatin, and collagen, as well as synthetic polymers such as polyethylene glycol (PEG) and their composites. Recently, researchers introduced peptide bio-inks composed of lysine-containing hexapeptides that self-assemble into stable, nanofibrous 3D hydrogels with remarkable stiffness of up to 40 kPa. These biocompatible bio-inks facilitate the 3D culture of human stem cells and the differentiation of primary cells into organotypic structures for applications in high-throughput screening, diagnostics, and tissue engineering [125].

Overall, the examples provided and numerous other studies underscore the significant impact of nanotechnology on the efficiency and effectiveness of 3D bioprinted tissue engineering products. 3D bioprinted nanomaterials have a wide range of applications in tissue engineering and regenerative medicine due to their ability to create scaffolds that closely mimic the native extracellular matrix. These scaffolds support the culture and growth of cell lines or human stem cells, facilitating the regeneration of specific tissues for transplantation. They can be engineered to meet the mechanical and physiological requirements of the host tissue, enhancing cell attachment and promoting vital interactions between cells and the scaffold material. Applications include the development of bone and cartilage tissue, vascularized structures for improved nutrient and oxygen delivery, and organotypic models for drug testing and disease modeling. Overall, the integration of nanomaterials in bioprinted scaffolds enhances the effectiveness of tissue regeneration, paving the way for innovative solutions in biomedical applications [126, 127, 128].

CHALLENGES AND FUTURE DIRECTIONS

The challenges in 3D bioprinting include the need for standardization and integration of the entire biofabrication process, from model design to post-printing tissue processing. Additionally, there are obstacles that must be addressed during the preprinting, printing, and post-printing tissue maturation phases. These challenges hinder the

widespread adoption of bioprinting technology. To facilitate the transition of bioprinted tissues into clinical applications, advancements in areas such as novel bio-inks, high-resolution and high-speed imaging, improved printing techniques, and dynamic culture systems for tissue maturation are essential [129].

Technical and Ethical Challenges

3D bioprinting technology faces a range of technical and ethical challenges that need to be addressed for successful clinical application. Technically, an ideal bioprinter must possess several critical specifications, including high throughput, high resolution, and the ability to dispense multiple materials simultaneously. It must also ensure sterility and maintain cell viability after printing [130]. The biomaterials used in bioprinting must be biocompatible, biodegradable at appropriate rates, and exhibit non-toxicity while having the necessary mechanical properties and porosity to facilitate nutrient and gas exchange. Additionally, cell selection is crucial; the chosen cells should closely mimic the in vivo counterparts and maintain functionality throughout the patient's lifetime. Advanced imaging and modeling systems are also required to accurately capture the complex geometries of biological structures for effective 3D modeling [131]. Ethically, the technology grapples with regulatory issues, as existing guidelines often evaluate individual components rather than the technology as a whole, creating a gap in comprehensive oversight. Public acceptance is another significant hurdle, as societal perceptions can greatly influence the adoption of new technologies; historical precedents, such as reproductive cloning, illustrate how negative public sentiment can impede progress. Furthermore, complexities surrounding patenting and the regulatory understanding of bioprinting present additional obstacles. Addressing these intertwined technical and ethical challenges is essential for advancing 3D bioprinting into practical, clinical use [130].

Regulatory and Commercialization Hurdles

The 3D printing industry has significantly advanced in the last decade, reshaping the health technology landscape. 3D printing, or additive manufacturing, involves creating physical objects from digital models by adding material layer by layer. This rapid growth has led to regulatory challenges, often referred to as "regulatory lag," where existing systems struggle to keep pace with technological innovations [132]. It includes the specific regulatory hurdles of 3D bioprinting, targeting bioengineers, scientists, and clinicians involved in the commercialization of these technologies. We first outline our approach, then delve into the regulatory landscape, summarizing how regulations have been applied to 3D printed devices and cellular therapies. The challenges unique to 3D bioprinting include classification, quality control, software integration, point-of-care manufacturing, material consideration, and standardization [133].

Current regulatory frameworks are often ill-equipped to handle the nuances of 3D bioprinting, particularly in relation to three categories of medical therapies: conventional devices, custom-made 3D printed devices, and cell therapies. The ambiguity surrounding 3D bioprinting regulation poses risks for clinical translation in this sector [134]. Regulations are defined as authoritative rules or directives that establish legal requirements, primarily to protect patients by ensuring quality in device design, manufacture, and preclinical testing. The failure of medical devices can lead to widespread implications, eroding public trust in regulatory systems and the medical device industry. Thus, while it is essential to mitigate risks associated with new technologies, regulators must also foster an environment conducive to innovation, which is especially challenging in emerging areas like 3D printing [118].

Global regulatory approaches vary; in the U.S., the FDA governs medical devices, while Australia relies on the Therapeutic Goods Administration (TGA), and Europe utilizes multiple accredited European Notified Bodies under the European Medicines Agency (EMA). Although there are movements towards harmonization, significant differences remain [135].

In the context of 3D printing, existing regulatory frameworks have primarily addressed mass-manufactured devices, which are fundamentally

different from the personalized nature of 3D printed products. This divergence creates challenges for regulators in adapting processes to account for the unique aspects of bioprinting, which involves living cells and can lead to complexities not encountered with traditional 3D printed devices. The emergence of the first human clinical trial for a 3D bioprinted product in 2022 underscores the urgent need to reform regulatory systems, which have historically lagged behind technological advancements [136]. The regulatory landscape for bioprinting faces several significant challenges that must be addressed to ensure the safe and effective use of these technologies in healthcare. One major issue is classification, as determining how to categorize bioprinted products within existing regulatory frameworks is complex and often unclear. Quality control is another critical concern, as maintaining consistent safety and quality in bioprinted constructs is essential, particularly due to their unique biological components [70]. Additionally, the implications of software used in the design and manufacturing processes necessitate careful consideration, given its integral role in bioprinting. Regulations around point-of-care manufacturing further complicate oversight, as devices created at or near the site of care introduce new compliance challenges. The diverse biomaterials utilized in bioprinting also require established standards to ensure safety and efficacy. Finally, there are unresolved liability issues concerning the legal responsibilities in case of defective products, a topic that remains uncertain in the context of emerging technologies. Together, these challenges underscore the urgent need for regulatory frameworks that can evolve in response to the innovations brought by 3D bioprinting, all while ensuring patient safety and fostering technological advancement [136].

FUTURE PROSPECTS AND EMERGING TRENDS

Three-dimensional printing, also known as additive manufacturing or rapid prototyping, is a computer-assisted technique that enables the creation of physical objects by depositing layers of material. This transformative manufacturing process has made significant inroads in various industries, particularly in biomedical therapies and regenerative medicine, which are experiencing rapid advancements fueled by innovative material science. By integrating modern design approaches with biomaterials, researchers have developed new materials with specific, critical properties that facilitate enhanced interactions within biological system [137].

Natural materials offer a rich variety of structures that have evolved to interact effectively within living organisms, leveraging specific biochemical mechanisms. These natural-based systems are particularly advantageous due to their ability to respond to mild environmental stimuli that mirror biological conditions. To further refine the design of natural-based devices and mitigate the inherent variability of these materials, a deeper understanding of the mechanisms governing their responsive behavior and the relationship between their structure and properties is essential. The development of multi-responsive materials is a key aspect of this research. For instance, photo-crosslinkable methacrylated gelatin serves as a promising bio-ink due to its tunable properties and biocompatibility, making it suitable for 3D bioprinting applications [69]. Various classes of biomaterials, including craniofacial tissue analogs, cell aggregate composites, and polymer hydrogels, are currently being explored for use in scaffolds that promote cell migration and proliferation. The printing of living cells alongside these matrix materials is still in its early stages [138].

One of the most promising techniques for creating patient-specific implants is stereolithography. Researchers have developed innovative resin formulations based on monomers with alkylene or thiol groups, which can produce biocompatible polymers with specialized surfaces for bioprinting. Many researchers have also investigated a range of materials for developing autologous corneal tissue, examining both natural polymers like gelatin and synthetic options such as gelatin methacrylate [139]. While natural polymers tend to offer better biodegradability and biocompatibility, synthetic polymers provide more control over material properties, allowing for tailored applications in additive manufacturing, including the precision production of keratoprosthesis and personalized implants [140].

Additionally, the manufacture of 3D medical devices can be facilitated by using thermoplastics like polycarbonate-ISO (PC-ISO), which can be sterilized through methods such as ethylene oxide or gamma radiation, ensuring biocompatibility. Emerging trends in this field include the integration of synthetic biology with 3D bioprinting, leading to advancements in "bioadditive production," which allows for the creation of biological models that closely mimic human organs [141]. The long-term vision for bioprinting is to produce human tissues and organs suitable for surgical applications and transplants. Through techniques like embedded extrusion bioprinting using hydrogel baths, complex structures can be fabricated, enabling the creation of intricate volumetric designs made from various bio-inks. These advancements point to a future where bioprinting could revolutionize medical treatments and personalized healthcare solutions [142].

The 3D printing technique has garnered considerable interest, particularly in head and neck surgical specialties such as maxillofacial, otorhinolaryngology, and plastic surgery, due to its remarkable ability to produce intricate constructs with high precision, including small parts and even entire organs. This technology enhances surgical planning by enabling pre-operative simulations that provide surgeons with accurate representations of complex anatomical structures through physical models. In orthopedic surgery, 3D printing is utilized during the planning phase for revision and reconstructive procedures, allowing for the creation of personalized implants and specialized instruments for bone tissue engineering [120].

By leveraging 3D printing technology, surgical outcomes can be significantly improved. During the planning stage for implants and prosthetics, the technology offers precise anatomical customization, addressing the shortcomings of traditional methods that often struggle with visual discrepancies and limited anatomical representation. Additionally, a variety of 3D printing systems support the creation of anatomical models and devices in medical environments, facilitating advanced applications in spinal surgery. While there is limited research on the production of spinal implants through 3D printing, the technology is primarily employed in personalized surgical planning, aiding in the construction of anatomical models and intraoperative guides that enhance the precision of pedicle screw placement [143]. Looking ahead, emerging trends in bioprinting may focus on the development of more sophisticated materials and techniques that can better replicate the complexity of human tissues and organs. Innovations in biocompatible inks, multi-material printing, and the integration of living cells into constructs are expected to advance the capabilities of bioprinting. These advancements could lead to improved personalization of surgical implants, enhanced regenerative therapies, and greater potential for complex tissue engineering applications, ultimately transforming the landscape of surgical practices and patient outcomes [144, 145].

CONCLUSION

In summary, the evolution of bioprinting technology from 3D to 6D encapsulates significant advancements that have reshaped our approach to tissue engineering and disease management. The foundational 3D techniques have provided a solid base, paving the way for innovations such as 4D bioprinting, which incorporates dynamic properties, and 5D bioprinting, which introduces the critical element of temporal dynamics. The emerging 6D bioprinting, characterized by its multiscale and multimaterial capabilities, holds the promise of creating highly complex biological structures that mimic natural tissues more accurately than ever before. The implications for healthcare are profound, ranging from enhanced capabilities in tissue regeneration and organ transplantation to more effective models for personalized medicine and drug testing. However, these advancements come with significant challenges, including technical limitations, ethical concerns, regulatory hurdles, and commercialization issues. Addressing these challenges will be essential to harness the full potential of bioprinting technology. As we look to the future, continued innovation and interdisciplinary collaboration will be critical in overcoming these barriers and ensuring that

bioprinting can deliver its promised benefits in disease management and regenerative medicine. The trajectory of bioprinting holds immense potential, marking a pivotal moment in the intersection of technology and healthcare that could redefine patient care and treatment paradigms.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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AUTHORS CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: SZ, IK, data collection: MR; analysis and interpretation: SZ, MR, NR, ZZ; draft manuscript: MR, NR, ZZ; review and finalization of manuscript: SZ, IK. All authors reviewed the results and approved the final version of the manuscript.

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