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3D BIOPRINTING OF TISSUES AND ORGANS FOR TRANSPLANTATION- A REVIEW

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ABSTRACT

Three-dimensional (3D) bioprinting, an additive manufacturing-based technique for fabricating biomaterials, is a novel and promising strategy in the medical and pharmaceutical fields. Because of its ability to generate regenerative tissues and organs, this technology has paved the way for the development of artificial multicellular tissues/organs. This review discusses the state of the technology as well as a wide range of biomaterials used in 3D bioprinting. It provides an update on recent bioprinting and application developments. Finally, a realistic overview of organ bioprinting technologies is presented, including recent achievements in bioprinting tissue-engineered constructs, limitations and challenges, and future research opportunities.

INTRODUCTION

A. Definition of 3D bioprinting technology

3D bioprinting is an emerging field in regenerative medicine that has gained significant attention in recent years. It is a collection of additive manufacturing technologies that aim to fabricate parts imitating real tissue and organ functionalities by combining both living and non-living materials in a specific three-dimensional (3D) spatial organization structure.[1] Bioprinting can provide patient-specific spatial geometry, control over cell placement, and the ability to create complex structures that mimic the natural architecture of tissues and organs.

The main goal of 3D bioprinting is to fabricate complex biological constructs in the field of tissue engineering and regenerative medicine.[3] It has the potential to revolutionize the field of medicine by providing a new approach for producing cell-laden, three-dimensional structures to mimic bodily tissues, which has an important role not only in tissue engineering but also in drug delivery and cancer studies.[2]

The main 3D bioprinting techniques are described in detail in research articles, and key limitations are highlighted. Successful cases, demonstrating the relevance of 3D bioprinting, are also presented. However, there are still many research challenges and future perspectives in the field of 3D bioprinting that need to be addressed.[4]

B. Potential benefits of 3D bioprinting in transplantation

3D bioprinting is a promising technology that has the potential to revolutionize the field of tissue and organ transplantation. Here are some potential benefits of 3D bioprinting in tissue and organ transplantation.[3,5,8]

1. Customization
2. Reduced waiting times
3. Improved safety
4. Reduced cost
5. Research and development

BIOPRINTING TECHNOLOGIES

3D bioprinting is a promising approach for fabricating complex biological constructs in the field of tissue engineering and regenerative medicine.[3,1] It involves the controlled deposition of bioinks, which are formulations of cells suitable for processing by an automated system.[9] Bioprinting is a collection of additive manufacturing technologies that aim to fabricate parts imitating real tissue and organ functionalities by combining both living and non-living materials in a specific three-dimensional spatial organization structure.[1] Recent advances in 3D bioprinting have led to the development of cardiac tissue, which has the potential to address cardiovascular diseases.[7]

A. Inkjet bioprinting

Inkjet bioprinting is a type of 3D bioprinting that uses inkjet technology to deposit small droplets of bioink onto a substrate to create 3D structures.[12] The bioink is a mixture of living cells and a hydrogel that provides a supportive environment for the cells to grow and differentiate.[12] Inkjet bioprinting has high resolution in the <30 µm range, making it suitable for printing complex structures such as blood vessels and capillaries.[10]

Inkjet bioprinting has been used to print a variety of biomaterials, biomacromolecules, DNAs, and cells.[12] It has been used to create in vitro tissue models for drug screening, disease modeling, and several other in vitro applications.[3] Inkjet bioprinting has also been used to create personalized medicines and to study disease mechanisms.[3]

There are several types of bioprinters, including inkjet droplet, extrusion, laser droplet, and stereolithography.[11] The selection of bioink and the approach used (scaffold-based vs. scaffold-free) is significantly influenced by the type of bioprinter that is going to be used.[11] Inkjet bioprinting is one of the most widely used 3D bioprinting methods.[10]

Inkjet bioprinting has limitations and challenges, such as the need for a suitable bioink that can maintain cell viability and function during the printing process.[12] However, inkjet bioprinting has shown promise in creating complex functional tissues and organs.[11] The field of 3D bioprinting is rapidly evolving, with researchers developing new printing modalities and improving existing ones.[3]

B. Extrusion bioprinting

Extrusion-based 3D bioprinting is a widely used method for producing 3D tissue constructs.[9] The process involves the controlled deposition of bioinks, which are formulations of cells suitable for processing by an automated system.[9] The bioinks are extruded through a nozzle in a continuous form of filaments, creating a 3D structure.[3] The extrusion-based bioprinting employs mechanical, pneumatic, or solenoid dispenser systems to deposit bioinks.[3] The bioinks can be granulated in a support bath containing yield stress hydrogels, which solidify around the extruded structure and prevent it from collapsing.[3]

Recent research has focused on developing advanced fabrication techniques for extrusion-based 3D bioprinting of hydrogel biomaterials for tissue regeneration.[14] Hydrogels are widely adopted as a bioink in cell printing technologies based on the extrusion principle.[14] The crucial parameters of extrusion-based bioprinting of hydrogel biomaterials, such as hydrogel properties, printing conditions, and tissue scaffold design, have been proposed.[14] The methods covered include multiple-dispenser, coaxial, and hybrid 3D.[14]

The selection of hardware, software, and bioinks is critical in 3D extrusion bioprinting.[9] The printability of candidate biomaterials for extrusion-based 3D printing has been studied.[9] Software for bioprinting has also been developed.[9] The researchers at Rensselaer are working with NASA to develop complex algorithms that will control the arm's movement, enabling it to accurately transport and refuel a satellite.[13]

C. Laser-based bioprinting

Laser-based bioprinting is a 3D printing technology that utilizes laser power to print structures such as in SLA by a photopolymerization principle. It can also be used for precise positioning of cells such as in laser direct-write and Laser Induced Forward Transfer (LIFT). Laser-based bioprinting has the potential to print cells with high resolution and precision, making it a promising technology for tissue engineering and regenerative medicine. Laser-based bioprinting has been used to print various types of cells, including stem cells, and has been shown to be effective in creating complex tissue structures.[3]

Research articles suggest that laser-based bioprinting is one of the four major governing approaches for 3D bioprinting technologies for engineering functional human tissues and organs that recapitulate their native prototypes. The other three approaches are droplet-based, extrusion-based, and stereolithography bioprinting.[14] Laser-based bioprinting has been used to create complex tissue structures, including blood vessels, cartilage, and bone.[9]

In conclusion, laser-based bioprinting is a promising technology for tissue engineering and regenerative medicine. It has the potential to print cells with high resolution and precision, making it a promising technology for creating complex tissue structures. Laser-based bioprinting has been used to create various types of cells, including stem cells, and has been shown to be effective in creating complex tissue structures.

D. Stereolithography bioprinting

Stereolithography bioprinting is a 3D printing technology that uses a laser to solidify a liquid photopolymer into a 3D structure. The process involves the use of a vat of liquid photopolymer, which is selectively cured by a laser beam to create a solid structure. The laser beam is directed by a computer-aided design (CAD) file, which controls the movement of the laser and the curing of the photopolymer. Stereolithography bioprinting has high resolution and can print complex structures, making it suitable for the fabrication of tissue constructs with high precision. The development of novel photo cross linkable biomaterials with enhanced physical and chemical properties has advanced the field of stereolithography bioprinting.[16,15] Stereolithography bioprinting has been used for the fabrication of human corneal stroma equivalent, hydrogel scaffolds, and tissue and organ regeneration.[15] The research on stereolithography bioprinting is progressing, and it is an emerging technology with tremendous potential to revolutionize modern medicine and healthcare.[1,16]

MATERIALS USE FOR BIOPRINTING

3D bioprinting is a technology that involves the printing of bioinks mixed with living cells to construct natural tissue-like three-dimensional structures. Bioinks are made of natural or synthetic biomaterials that can be printed layer by layer to create a 3D structure that mimics the behavior and structures of natural tissues. The materials used for 3D bioprinting are critical to the success of the process.

Hydrogels, bioinks, and decellularized extracellular matrix (dECM) are some of the most commonly used materials for 3D bioprinting.[7] Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb large amounts of water. They are commonly used as a scaffold for cell growth due to their biocompatibility and ability to mimic the extracellular matrix (ECM).[2] Bioinks are hydrogels that are specifically designed for 3D bioprinting. They are composed of a mixture of cells and biomaterials that can be printed layer by layer to create a 3D structure. dECM is created by removing the cells from natural tissues, leaving behind the ECM. This dECM can then be used as a scaffold for cell growth.[2]

3D bioprinting can be used for several biological applications in the fields of tissue engineering, bioengineering, and materials science. It has the potential to revolutionize the field of regenerative medicine by producing cell-laden, three-dimensional structures to mimic bodily tissues.[2] 3D bioprinting can provide patient-specific spatial geometry, which is important not only in tissue engineering but also in drug delivery and cancer studies.[2]

A. Hydrogels

Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb large amounts of water and biological fluids, making them ideal for use in 3D bioprinting.[17] Hydrogels have a soft structure and porosity that closely resembles living tissues, making them an excellent material for creating scaffolds for 3D cell cultures.[18] Hydrogels can be designed as an artificial extracellular matrix scaffold for providing spatial orientation and promoting cellular interactions with surroundings.[18]

Hydrogels can be composed of natural or synthetic polymers, and can be crosslinked either through covalent bonds or held together via physical intramolecular interactions.[19] The choice of hydrogel material is critical to the success of 3D bioprinting, as it can affect the biocompatibility, mechanical properties, and printability of the final product.[19]

Recent research has focused on the development of hydrogels with improved mechanical properties, such as increased stiffness and toughness, to better mimic the natural environment of living tissues.[19] Microsphere-containing hydrogel scaffolds have also been developed for tissue engineering applications, which can provide a more controlled release of growth factors and other bioactive molecules.[17]

B. Bioinks

Bioinks are the materials used to contain cells when bioprinting tissues. They provide structure for the bioprinted tissue and support and promote cell growth and differentiation.[20] Bioinks can be distinguished into two categories: bioinks and biomaterial inks. Bioinks contain living cells and are mainly composed of hydrogels, while biomaterial inks do not contain living cells and are mainly composed of synthetic or natural polymers.[21] The selection of appropriate cells for 3D bioprinting is crucial for ensuring the success of any fabricated construct.[22]

Hydrogels are the most commonly used materials for bioinks in 3D bioprinting. They are biocompatible, have high water content, and can mimic the extracellular matrix of natural tissues. Gelatin methacrylate (GelMA), collagen, and alginate are some of the most commonly used hydrogels for bioinks.[20] GelMA is a photocrosslinkable hydrogel that can be easily modified to control its mechanical properties and degradation rate. Collagen is a natural protein that is abundant in the extracellular matrix of many tissues and can promote cell adhesion and proliferation. Alginate is a natural polysaccharide that can form a gel in the presence of divalent cations such as calcium ions.[23]

Multicellular and stem cell-based bioinks are also being developed for 3D bioprinting applications. These bioinks can promote cell differentiation and tissue formation, and can be used to fabricate complex structures with multiple cell types.[22] However, further research is needed to optimize the properties of these bioinks for specific tissue engineering applications. One of the challenges in 3D bioprinting is the development of a bioink that maintains a low viscosity, which allows for the precise deposition of cells and biomaterials.[23]

C. Decellularized extracellular matrix (dECM)

Decellularized extracellular matrix (dECM) has emerged as a promising material for 3D bioprinting due to its ability to maintain both mechanical and biological properties.[24,25] dECM is produced by removing all cellular components from natural biomaterials such as collagen, gelatin, and hyaluronic acid while preserving the composition and integrity of the native ECM.[25,26] The resulting dECM retains the native extracellular matrix structure and composition, which can provide a suitable microenvironment for cell growth and differentiation.[27] Many researchers believe that 3D bioprinting decellularized ECM is a promising strategy for a regenerative scaffold.[26] dECMs have demonstrated excellent utility as bioscaffolds in recapitulating the complex biochemical microenvironment.[27] Printing three-dimensional tissue analogues with dECM is the best choice, as no natural or man-made material can recapitulate all the features.[28] However, the widespread use of dECM-based bioprinting is currently limited due to the high cost, difficulty of large-scale production, and tissue specificity of dECM from different tissues. Further research is required to make dECM a viable option for 3D bioprinting applications. The development of new decellularization methods and the optimization of dECM properties for bioprinting applications are areas of active research. The combination of dECM with other biomaterials such as hydrogels and synthetic polymers can also improve the mechanical and biological properties of the resulting constructs.[24] Overall, dECM-based bioinks have great potential for the fabrication of complex tissue constructs for regenerative medicine applications.

TISSUE AND ORGAN ENGINEERING

Tissue engineering and regenerative medicine are rapidly evolving fields that aim to solve the challenges of conventional tissue engineering methods by precise and controlled layer-by-layer assembly of biomaterials in a desired 3D pattern.[3,7] 3D bioprinting has emerged as a promising approach for fabricating complex biological constructs in the field of tissue engineering and regenerative medicine.[3] 3D bioprinting is widely used in synthesizing tissue and organs, making it highly promising for regenerative medicine. Availability of donors for organ transplantation is limited, and 3D bioprinting offers a potential solution to this problem. The 3D bioprinting of cells, tissues, and organs Collection at Scientific Reports brings together a myriad of studies portraying the capabilities of different bioprinting modalities.[3] The collection amalgamates research aimed at 3D bioprinting organs for transplantation, as well as in-vitro tissue models for drug screening, disease modeling, and several other in-vitro applications.[3] 3D bioprinting is an extended application of additive manufacturing that involves building a tissue or organ layer-by-layer.[7] Advances in 3D bioprinting of tissues/organs for regenerative medicine have been made, and the technology is evolving into an unparalleled multidisciplinary technology for engineering 3D biological tissue.[29] The applications of 3D bioprinting technology on the construction of various representative tissue and organs, including skin, cardiac, bone, and cartilage, have been demonstrated.[7] The steps involved in each of those tissues/organs printing have been highlighted, and the challenges and opportunities for future research have been discussed.[7] Overall, 3D bioprinting has great potential for the fabrication of complex tissue constructs for regenerative medicine applications, and further research is needed to optimize the technology and develop novel materials and techniques for the advancement of tissue engineering and regenerative medicine.

A. Scaffolds

Tissue engineering is a multidisciplinary research field aiming at the regeneration, restoration, or replacement of damaged tissues and organs.[30] Scaffold-based developmental tissue engineering is a classical approach that combines scaffolds, cells, and soluble factors to fabricate constructs mimicking the native tissue to be regenerated.[30] The scaffold provides the necessary support for cells to attach, grow, proliferate, and maintain their differentiated function.[31] The scaffold serves as a three-dimensional template for initial cell attachment and subsequent tissue formation both in vitro and in vivo.[31]

Different procedures for fabricating sponge-like and fibrous scaffolds using a variety of technologies have been developed. These methods include electrospinning and microfluidic-based methods, among others.[31] Researchers are also working on developing TE 3D scaffolds for teeth, hair follicles, and salivary and lacrimal glands, with particular focus on the selection of biomaterials and cell culture configurations.[30]

Regenerative medicine is a vast and highly topical theme with many important aspects to consider. Scaffold technology, tissue, and organ engineering are new horizons in surgery. The ability to regenerate a function of cells and tissues has never actually been exploited until today.[32] Revolutionary technologies such as biomaterials, biology, and nanotechnology in tissue engineering are being used to bio-fabricate virtually every human tissue or organ.[31]

Polymers are the primary materials for scaffolds in various tissue engineering applications, including bone and other tissues. The choice of polymer depends on the specific application and the desired properties of the scaffold. The development of scaffolds for tissue fabrication is an active area of research, with new materials and fabrication methods being developed to improve the performance of scaffolds in tissue engineering applications.[33]

B. Cell seeding

Cell seeding is a crucial process in tissue engineering to achieve functional tissue regeneration. Seeding three-dimensional scaffolds with suitable cells is an approved technique for rapid blood vessel ingrowth into transplanted constructs, which represents the key requirement for successful tissue engineering. Conventionally, high-concentration cell delivery is a significant process in bone tissue engineering to attain functional bone regeneration. Researchers have investigated the incorporation of cell-seeded poly-L-lactide-co-glycolide scaffolds in hypertensive and non-hypertensive mice, and the results showed that seeding approved scaffolds with organ-specific or pluripotent cells is a promising technique for tissue engineering in hypertensive organisms.[34]

The semi-dynamic seeding and dynamic perfusion seeding methods resulted in more homogeneous cell distribution than static seeding. The semi-dynamic seeding method combines the high seeding efficiency of static seeding and satisfactory distribution homogeneity of dynamic seeding while circumventing their disadvantages, which may contribute to improved outcomes of bone tissue engineering. Researchers have also investigated the influence of scaffold design on the distribution of adhering cells after perfusion cell seeding.[35]

Cells were seeded at a density of 10^5 cells/well in 200 µL growth medium, after 2 h of incubation, the medium was replaced with fresh medium, and the cells were cultured for 24 h4. The method of cell seeding onto the scaffolds is illustrated in Figure 1.[36]

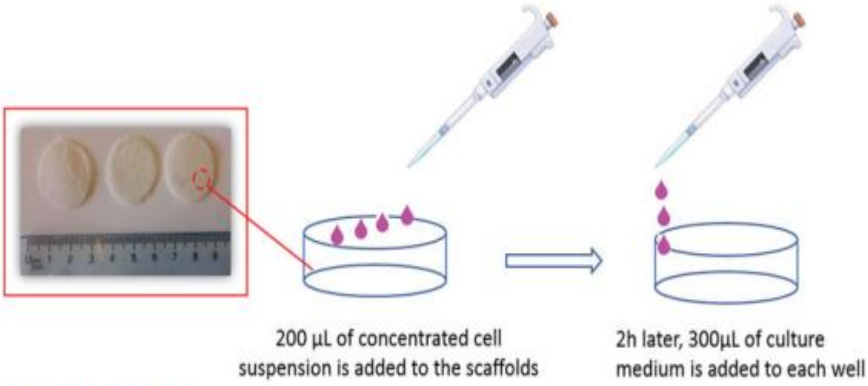

Figure 1. Method of cell seeding onto the scaffolds.

C. Growth factors

Growth factors play a crucial role in tissue engineering by stimulating cell proliferation, differentiation, and division. Growth factors are soluble secreted proteins that can affect a variety of cellular processes.[37] Controlled release of growth factors is essential in many tissue engineering applications, as it leads to appropriate cell responses such as proliferation, differentiation, and angiogenesis.[39] However, the application of growth factors in clinics remains limited due to the lack of robust delivery systems and biomaterial carriers. Clinically approved therapies involving growth factors utilize some sort of a biomaterial carrier for growth factor delivery, suggesting that biomaterial delivery systems are extremely important for successful usage of growth factors in regenerative medicine. Researchers have developed delivery systems for multiple growth factors in tissue engineering, which can enhance the regeneration of various tissues.[37]

For example, local controlled release of VEGF and PDGF from a combined brushite-chitosan system enhances bone regeneration. Growth factor delivery-based tissue engineering can regulate the response of cells to growth factors by cell-cell signaling, affecting cell proliferation, differentiation, and angiogenesis.[38] Insulin-like growth factors (somatomedins) stimulate growth by mediating the secretion of growth hormone from the circulation in order to generate cells that can be harvested and used for autologous bone marrow transplant. The abnormal production and regulation of growth factors can play a role in the progression of disease, such as vascular endothelial growth factor inducing endothelial cells to penetrate a tumor nodule and begin the process of angiogenesis.

D. Comparison of tissue and organ engineering approaches

Tissue engineering and organ engineering are multidisciplinary research fields aiming at the regeneration, restoration, or replacement of damaged tissues and organs.[30] Tissue engineering approaches combine scaffolds, cells, and soluble factors to fabricate constructs mimicking the native tissue to be regenerated.[30] Organ engineering approaches use scaffolds, cells, and growth factors to regenerate or replace the entire organ. Tissue engineering has been used in skin grafts, cartilage repair, small arteries, and bladders in patients. Scaffold technology has been used to develop acceptable replacements for breast-operated patients using new materials, even in complex medical conditions.[32] The source of cells is an important choice for scaffolds, and a range of cell types can now be combined with scaffolds to regenerate tissues.[40] The advantages and disadvantages of different scaffolds have been studied to develop materials that focus on the regeneration process of living tissues.[41] Therefore, both tissue and organ engineering approaches have their advantages and disadvantages, and the choice of approach depends on the type of tissue or organ to be regenerated.[30]

BIOPRINTING APPLICATIONS

Bioprinting is a promising technology for tissue engineering, which involves the rapid printing of bio-functional materials and their supporting components in a layer-by-layer form. Bioprinting has been applied to various tissue engineering applications, including skin, bone, cartilage, liver, heart, and lung tissue engineering.[7] Bioprinting of skin tissue engineering constructs has been achieved using a combination of keratinocytes and fibroblasts.[7] Bioprinting of bone tissue engineering constructs has been achieved using a combination of osteoblasts and hydroxyapatite.[42] Bioprinting of cartilage tissue engineering constructs has been achieved using a combination of chondrocytes and hydrogels.[43] Bioprinting of liver tissue engineering constructs has been achieved using a combination of hepatocytes and endothelial cells.[44] Bioprinting of heart and lung tissue engineering constructs is still in the early stages of development.[7] Therefore, bioprinting is a promising technology for tissue engineering, and its applications in skin, bone, cartilage, liver, and other tissues are being actively researched.[7,42,43,44]

A. Skin tissue engineering

3D bioprinting is a cutting-edge technology that allows for the precise layer-by-layer construction of complex cell models and tissue types with high precision, repeatability, and reproducibility.[45] In skin tissue engineering, 3D bioprinting has shown potential for wound repair and regeneration.[47] Scaffold-based tissue engineering methods have been used to reconstruct skin tissue by seeding and cultivating cells in bioactive, printed scaffold templates.[46] However, techniques that print cell-laden hydrogels or bioinks have been developed to create more complex skin structures.[48]

Recent research has focused on the development of 3D bioprinted skin substitutes that resemble human skin and its wound healing process.[49] For example, 3D inkjet bioprinting was used to print full-thickness skin equivalents, using alternating layers of a printable and cytocompatible bioink and a dermal fibroblast cell suspension that showed high viability after printing. Keratinocytes were seeded onto the dermal cell construct and allowed to differentiate into a stratified epidermis.[46]

Another promising application of 3D bioprinting in skin tissue engineering is the development of vascularized skin substitutes. 3D bioprinting can be customized for skin shape with cells and other materials distributed precisely, achieving rapid and reliable production of bionic skin.[47] In a recent study, researchers used 3D bioprinting to create a vascularized skin substitute that was implanted onto a mouse model. The results showed that the 3D bioprinted skin substitute was able to integrate with the host tissue and form functional blood vessels.[45]

B. Bone tissue engineering

3D bioprinting technology allows for flexibility in both material choice and design paradigm, making it an excellent alternative for bone tissue engineering.[50] 3D bioprinting has become an attractive method that allows the direct deposition of small units of biomaterials, biochemicals, and living cells, which are positioned precisely with functional components to fabricate tissue-like 3D structures. Compared to the conventional use of 3D printing to form cell-free scaffolds, 3D bioprinting requires different technical approaches to construct 3D structures with mechanical and biological properties. Recent advances in 3D bioprinting for tissue engineering have covered the medical applications of biomaterials and cell sources for 3D bioprinting.[51] The use of 3D bioprinting and stem cell technology holds much promise in bone tissue engineering. Scaffold-based tissue engineering methods have been used to reconstruct bone tissue, by seeding and cultivating cells in bioactive, printed scaffold templates.[46] Therefore, 3D bioprinting is a cutting-edge technology that can provide an excellent alternative for bone tissue engineering and regeneration.

C. Cartilage tissue engineering

Recent research has focused on the development of bioinks and 3D bioprinted constructs for cartilage tissue engineering. The 3D bioprinting process allows for the production of complex porous structures, making it an excellent technique for cartilage tissue engineering.[52] The use of 3D bioprinting and stem cell technology holds much promise in cartilage tissue engineering, as it allows for the development of viable grafts that are clinically relevant. Scaffold-based tissue engineering methods have been used to reconstruct cartilage tissue, by seeding and cultivating cells in bioactive, printed scaffold templates. However, techniques that print cell-laden hydrogels have shown more promise in the development of cartilage tissue.[46] Therefore, 3D bioprinting is a cutting-edge technology that can provide an excellent alternative for cartilage tissue engineering and regeneration.

D. Liver tissue engineering

3D bioprinting technology allows for flexibility in both material choice and design paradigm, which is useful in the context of tissue engineering.[50] The aim of tissue engineering with 3D bioprinting technology is to construct fully functional and viable tissue and organ replacements for various clinical applications, including liver tissue engineering. 3D bioprinting allows for the precise layer-by-layer construction of different complex cell models and tissue types with high precision, repeatability, and reproducibility. The incorporation of developmental biology, cell biology, regenerative medicine, and bioengineering, specifically tissue engineering, provides a platform for the development of liver tissue engineering using 3D bioprinting.[45]

E. Heart and lung tissue engineering

Recent research has focused on the development of bioinks and 3D bioprinted constructs for cardiac tissue engineering. 3D bioprinting has attracted much attention due to its ability to integrate multiple cells within printed scaffolds with complex 3D structures, and many advancements in utilizing composite bioinks have been made.[53] 3D bioprinting can be used to create 3D structures that are key to bridging the gap between current cell culture and natural tissues. 3D bioprinting is a technology that could facilitate the construction of in vitro tissue to meet both goals. The introduction of the third dimension and the possibility of depositing cells in a controlled manner on engineered supports are advantages of 3D bioprinting.[54]

Bioprinting Challenges

Despite the potential benefits of 3D bioprinting, there are several challenges that need to be addressed. One of the main challenges is the development of bioinks that can support cell growth and differentiation while maintaining their structural integrity.[55] Another challenge is the need for vascularization, which is essential for the survival of large tissue constructs.[50] The mechanical properties of the printed constructs are also a challenge, as they need to be strong enough to withstand the forces of the surrounding environment. The lack of standardization in 3D bioprinting techniques and the need for optimization of printing parameters are also challenges that need to be addressed.[50] Additionally, the cost of 3D bioprinting technology and the availability of suitable biomaterials are also challenges that need to be overcome.[46] Therefore, while 3D bioprinting holds great promise for tissue engineering and regenerative medicine, there are several challenges that need to be addressed to fully realize its potential.

A. Biocompatibility

One of the most important challenges in 3D bioprinting is to find suitable printing materials with excellent printability, biocompatibility, and mechanical properties.[56] The biocompatibility of the materials used in 3D bioprinting is critical to ensure that the printed constructs are not rejected by the body's immune system.[55] The biomaterials used in 3D bioprinting must provide crucial physical and chemical signals and can have significant impacts on cell activities, such as adhesion, metabolism, proliferation, and differentiation. The choice of biomaterials must be carefully made based on the tissue of interest.[50] The mechanical properties of the printed constructs are also a challenge, as they need to be strong enough to withstand the forces of the surrounding environment.[56] Therefore, the development of biocompatible materials with suitable mechanical properties is a critical challenge in 3D bioprinting. While significant progress has been made in this area, further research is needed to fully address this challenge and realize the full potential of 3D bioprinting in tissue engineering and regenerative medicine.[57]

B. Scalability

Scalability is a significant challenge in 3D bioprinting, as the technology needs to be able to produce large quantities of tissue constructs to meet the demands of clinical applications. The current limitations of 3D bioprinting technology include the inability to produce large, complex structures with high resolution and accuracy.[50] The scalability of 3D bioprinting is limited by the size of the printing bed, the speed of the printing process, and the availability of suitable biomaterials. The development of new printing technologies and the optimization of printing parameters are needed to improve the scalability of 3D bioprinting. Additionally, the cost of 3D bioprinting technology and the availability of suitable biomaterials are also challenges that need to be overcome to improve scalability.[55] Therefore, while 3D bioprinting holds great promise for tissue engineering and regenerative medicine, there are several challenges that need to be addressed to fully realize its potential, including scalability.

C. Ethical considerations

The development of 3D bioprinting technology raises several ethical considerations that need to be addressed. One of the main ethical concerns is the potential for the commercialization of 3D bioprinted tissues and organs, which could lead to unequal access to healthcare and exacerbate existing health disparities.[58] Another ethical concern is the use of 3D bioprinting for non-medical purposes, such as cosmetic enhancements or performance enhancement in sports.[59] The use of 3D bioprinting for research purposes also raises ethical concerns, such as the use of human cells and tissues in research and the potential for the creation of chimeras.[50] Additionally, the use of 3D bioprinting for personalized medicine raises ethical concerns related to privacy and informed consent. Therefore, while 3D bioprinting holds great promise for tissue engineering and regenerative medicine, there are several ethical considerations that need to be addressed to ensure that the technology is used in an ethical and responsible manner.

Applications Of 3d Bioprinting In Tissue And Organ Transplantation

3D bioprinting is a revolutionary technology that has the potential to transform the field of tissue and organ transplantation. Here are some applications of 3D bioprinting in tissue and organ transplantation:

1. Tissue Engineering: 3D bioprinting has been used to create living tissues such as cartilage, skin, and heart valves. The technology enables the creation of tissue constructs with heterogeneous compositions and complex architectures.[5] This has contributed to many breakthroughs in the preparation of tissue engineering scaffolds.[6]

2. Organ Transplantation: With a global shortage of organs suitable for transplant into critically ill patients, some researchers are looking at 3D printing of living tissue as a potential solution. 3D bioprinting can be used to create functional organs such as liver,

kidney, and heart. This technology can also be used to create personalized organs that are tailored to the specific needs of individual patients.

3. Drug Testing: 3D bioprinting can be used to create tissue models that mimic the structure and function of human organs. These models can be used to test the efficacy and toxicity of drugs before they are tested on humans.[7] This can help to reduce the number of animals used in drug testing and improve the accuracy of drug testing.

Future Directions

A. Hybrid bioprinting approaches

The future of 3D bioprinting lies in hybrid bioprinting approaches that combine different printing techniques to create complex structures. This approach involves combining different types of bioinks, cells, and materials to create functional tissues and organs. Hybrid bioprinting can also be used to create structures with multiple materials, such as soft and hard tissues, which is not possible with traditional bioprinting techniques.[60]

B. Advancements in material science

The development of new biomaterials is crucial for the advancement of 3D bioprinting. The use of advanced materials such as hydrogels, nanomaterials, and biodegradable polymers can improve the mechanical properties of printed tissues and organs. The use of these materials can also improve the biocompatibility of printed structures, which is essential for their integration into the human body.[61]

C. Use of artificial intelligence

The use of artificial intelligence (AI) in 3D bioprinting can improve the accuracy and precision of printed structures. AI can be used to optimize the printing process, predict the behavior of cells and tissues, and design complex structures. AI can also be used to analyze large amounts of data generated during the printing process, which can help researchers to identify new biomaterials and optimize the printing process.[42]

CONCLUSION

3D bioprinting has emerged as a promising approach for engineering functional tissues and organs by layer-by-layer precise positioning. The current state of the art in 3D bioprinting involves the use of advanced biomaterials, such as hydrogels, nanomaterials, and biodegradable polymers, to improve the mechanical properties of printed tissues and organs. However, there are still several challenges that need to be addressed, such as the need for more advanced printing techniques, the development of new biomaterials, and the need for more accurate cell and tissue modeling. The future of 3D bioprinting lies in hybrid bioprinting approaches, advancements in material science, and the use of artificial intelligence to improve the accuracy and precision of printed structures.

3D bioprinting has the potential to revolutionize tissue and organ transplantation by enabling the creation of functional tissues and organs that can be transplanted into patients without the risk of rejection. 3D bioprinting can also be used to create personalized implants and prosthetics that are tailored to the specific needs of individual patients. Additionally, 3D bioprinting can be used to create in vitro models of human organs, which can be used for drug testing and disease modeling. However, there are still several challenges that need to be addressed before 3D bioprinting can be used for clinical applications, such as the need for more advanced printing techniques, the development of new biomaterials, and the need for more accurate cell and tissue modeling.

REFERENCES

1. Santoni, S., Gugliandolo, S.G., Sponchioni, M. *et al.* 3D bioprinting: current status and trends—a guide to the literature and industrial practice. *Bio-des. Manuf*, 2022; **5**: 14–42.
2. Kačarević ŽP, Rider PM, Alkildani S, Retnasingh S, Smeets R, Jung O, Ivanišević Z, Barbeck M. An Introduction to 3D Bioprinting: Possibilities, Challenges and Future Aspects. Materials (Basel), 2018 Nov 6; 11(11): 2199. PMID: 30404222; PMCID: PMC6266989.
3. Dey, M., Ozbolat, I.T. 3D bioprinting of cells, tissues and organs. *Sci Rep* 10: 14023, 2020.
4. Paulo Bartolo, Ajay Malshe, Eleonora Ferraris, Bahattin Koc.3D bioprinting: Materials, processes, and applications. CIRP Annals, 2022; 71(2): 577-597,ISSN 0007-8506.
5. Xia Z, Jin S, Ye K. Tissue and Organ 3D Bioprinting. SLAS Technol., 2018 Aug; 23(4): 301-314. Epub 2018 Feb 23. PMID: 29474789.
6. Jiang W, Mei H, Zhao S. Applications of 3D Bio-Printing in Tissue Engineering and Biomedicine. J Biomed Nanotechnol, 2021 Jun 1; 17(6): 989-1006. PMID: 34167615.
7. Agarwal Swarnima, Saha Shreya, Balla Vamsi Krishna, Pal Aniruddha, Barui Ananya, Bodhak Subhadip. Current Developments in 3D Bioprinting for Tissue and Organ Regeneration–A Review. Frontiers in Mechanical Engineering, 2020.

8. Matai I, Kaur G, Seyedsalehi A, McClinton A, Laurencin CT. Progress in 3D bioprinting technology for tissue/organ regenerative engineering. Biomaterials, 2020 Jan; 226: 119536. Epub 2019 Oct 11. PMID: 31648135.
9. Zhang, Y.S., Haghiashtiani, G., Hübscher, T. *et al.* 3D extrusion bioprinting. *Nat Rev Methods Primers*, 2021; 1: 75.
10. Yi, HG., Kim, H., Kwon, J. *et al.* Application of 3D bioprinting in the prevention and the therapy for human diseases. *Sig Transduct Target Ther,* 2021; 6: 177.
11. Jamieson Colin, Keenan Patrick, Kirkwood D'Arcy, Oji Saba, Webster Caroline, Russell Keith A., Koch Thomas G. A Review of Recent Advances in 3D Bioprinting With an Eye on Future Regenerative Therapies in Veterinary Medicine. Frontiers in Veterinary Science, 2021; 7.
12. Li X, Liu B, Pei B, Chen J, Zhou D, Peng J, Zhang X, Jia W, Xu T. Inkjet Bioprinting of Biomaterials. Chem Rev., 2020 Oct 14; 120(19): 10793-10833. doi: 10.1021/acs.chemrev.0c00008. Epub 2020 Sep 9. PMID: 32902959.
13. Cui, X., Li, J., Hartanto, Y., Durham, M., Tang, J., Zhang, H., Hooper, G., Lim, K., Woodfield, T., Advances in Extrusion 3D Bioprinting: A Focus on Multicomponent Hydrogel-Based Bioinks. *Adv. Healthcare Mater,* 2020; 9: 1901648.
14. M. Askari, M. Afzali Naniz, M. Kouhi, A. Saberi, A. Zolfagharian and M. Bodaghi, *Biomater. Sci.*, 2021; 9: 535 —573.
15. Kumar H, Kim K. Stereolithography 3D Bioprinting. Methods Mol Biol., 2020; 2140: 93-108. PMID: 32207107.
16. Ritu Raman, Rashid Bashir. Chapter 6 - Stereolithographic 3D Bioprinting for Biomedical Applications. Essentials of 3D Biofabrication and Translation. Academic Press, 2015; 89-121.
17. S. Zhang, A. Lin, Z. Tao, Y. Fu, L. Xiao, G. Ruan, Y. Li, *Chem. Asian J.*, 2022; *17*: e202200630.
18. Fahima Akther, Peter Littled, Zhiyong Lie, Nam-Trung Nguyen and Hang T. Ta. Hydrogels as artificial matrices for cell seeding in microfluidic devices, 2020; 10: 43682-43703.
19. El-Sherbiny IM, Yacoub MH. Hydrogel scaffolds for tissue engineering: Progress and challenges. Glob Cardiol Sci Pract, 2013 Nov 1; 2013(3): 316-42. PMID: 24689032; PMCID: PMC3963751.

20. Benwood C, Chrenek J, Kirsch RL, Masri NZ, Richards H, Teetzen K, Willerth SM. Natural Biomaterials and Their Use as Bioinks for Printing Tissues. Bioengineering (Basel), 2021 Feb 20; 8(2): 27. PMID: 33672626; PMCID: PMC7924193.
21. Andrea Schwab, Riccardo Levato, Matteo D'Este, Susanna Piluso, David Eglin, and Jos Malda. Printability and Shape Fidelity of Bioinks in 3D Bioprinting. *Chemical Reviews,* 2020; *120*(19): 11028-11055.
22. N. Ashammakhi, S. Ahadian, C. Xu, H. Montazerian, H. Ko, R. Nasiri, N. Barros, A. Khademhosseini. Bioinks and bioprinting technologies to make heterogeneous and biomimetic tissue constructs. Materials Today Bio, 2019. 100008, ISSN 2590-0064.
23. Ramiah Previn, du Toit Lisa C., Choonara Yahya E., Kondiah Pierre P. D., Pillay Viness. Hydrogel-Based Bioinks for 3D Bioprinting in Tissue Regeneration. Frontiers in Materials, 2020; 7. ISSN=2296-8016.
24. Yu Jung Shin, Ryan T. Shafranek, Jonathan H. Tsui, Jelisha Walcott, Alshakim Nelson, Deok-Ho Kim.3D bioprinting of mechanically tuned bioinks derived from cardiac decellularized extracellular matrix.Acta Biomaterialia, 2021; 119: 75-88. ISSN 1742-7061.
25. Wang Haonan, Yu Huaqing, Zhou Xia, Zhang Jilong, Zhou Hongrui, Hao Haitong, Ding Lina, Li Huiying, Gu Yanru, Ma Junchi, Qiu Jianfeng, Ma Depeng. An Overview of Extracellular Matrix-Based Bioinks for 3D Bioprinting. Frontiers in Bioengineering and Biotechnology, 2022; 10.
26. Dzobo K, Motaung KSCM, Adesida A. Recent Trends in Decellularized Extracellular Matrix Bioinks for 3D Printing: An Updated Review. Int J Mol Sci., 2019 Sep 18; 20(18): 4628. PMID: 31540457; PMCID: PMC6788195.
27. Claire Yu, Xuanyi Ma, Wei Zhu, Pengrui Wang, Kathleen L. Miller, Jacob Stupin, Anna Koroleva-Maharajh, Alexandria Hairabedian, Shaochen Chen. Scanningless and continuous 3D bioprinting of human tissues with decellularized extracellular matrix. Biomaterials, 2019; 194: 1-13.
28. Pati, F., Jang, J., Ha, DH. *et al.* Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat Commun* **5**, 3935, 2014.
29. Pooja Jain, Himanshu Kathuria, Nileshkumar Dubey. Advances in 3D bioprinting of tissues/organs for regenerative medicine and in-vitro models. Biomaterials, 2022; 287: 121639. ISSN 0142-9612.

30. Contessi Negrini N, Angelova Volponi A, Higgins CA, Sharpe PT, Celiz AD. Scaffold-based developmental tissue engineering strategies for ectodermal organ regeneration. Mater Today Bio., 2021 Mar 6; 10: 100107. PMID: 33889838; PMCID: PMC8050778.
31. Mohammadreza Chimerad, Alireza Barazesh, Mojgan Zandi, Ibrahim Zarkesh, Armaghan Moghaddam, Pouya Borjian, Rojan Chimehrad, Alimohamad Asghari, Zeinab Akbarnejad, Hossein Ali Khonakdar & Zohreh Bagher Tissue engineered scaffold fabrication methods for medical applications. International Journal of Polymeric Materials and Polymeric Biomaterials, 2022.
32. Cobianchi Lorenzo, Peloso Andrea.Editorial: Scaffold Technology, Tissue and Organ Engineering: New Horizons in Surgery. Frontiers in Surgery, 2022.
33. Peter X Ma. Scaffolds for tissue fabrication. Materials Today, 2004; 7(5): 30-40.
34. Wagner MEH, Kampmann A, Schumann-Moor K, Gellrich NC, Tavassol F, Schmeltekop F, Rücker M, Lanzer M, Gander T, Essig H, Schumann P. Cell seeding accelerates the vascularization of tissue engineering constructs in hypertensive mice. Hypertens Res., 2021 Jan; 44(1): 23-35. Epub 2020 Aug 11. PMID: 32778779.
35. Shi, F., Duan, K., Yang, Z. *et al.* Improved cell seeding efficiency and cell distribution in porous hydroxyapatite scaffolds by semi-dynamic method. *Cell Tissue Bank*, 2022; 23: 313–324.
36. Emile Jubeli, Afif Khzam & Najet Yagoubi Cells integration onto scaffolds prepared from polyester based polymers – importance of polymer thermal properties in addition to hydrophilicity, International Journal of Polymeric Materials and Polymeric Biomaterials, 2018.
37. Koria, P. Delivery of Growth Factors for Tissue Regeneration and Wound Healing. *BioDrugs*, 2012; 26: 163–175.
38. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J R Soc Interface, 2011 Feb 6; 8(55): 153-70. Epub 2010 Aug 18. PMID: 20719768; PMCID: PMC3033020.
39. Whitaker, Martin & Quirk, Robin & Howdle, Steven & Shakesheff, Kevin Growth factor release from tissue engineering scaffolds. The Journal of pharmacy and pharmacology, 2001; 53: 1427-37. 10.1211/0022357011777963.
40. Howard D, Buttery LD, Shakesheff KM, Roberts SJ. Tissue engineering: strategies, stem cells and scaffolds. J Anat., 2008 Jul; 213(1): 66-72. Epub 2008 Apr 15. PMID: 18422523; PMCID: PMC2475566.

41. Chaudhari, Atul & Vig, Komal & Baganizi, Dieudonné [Radé] & Sahu, Rajnish & Dixit, Saurabh & Dennis, Vida & Singh, Shree & Pillai, Shreekumar. Future Prospects for Scaffolding Methods and Biomaterials in Skin Tissue Engineering: A Review. International Journal of Molecular Sciences, 2016; 17: 1974. 10.3390/ijms17121974.
42. Tripathi, S, Mandal, SS, Bauri, S, Maiti, P. 3D bioprinting and its innovative approach for biomedical applications. *MedComm*, 2023; 4: e194.
43. Mani, Mohan Prasath, Sadia, Madeeha, Jaganathan, Saravana Kumar, Khudzari, Ahmad Zahran, Supriyanto, Eko, Saidin, Syafiqah, Ramakrishna, Seeram, Ismail, Ahmad Fauzi and Faudzi, Ahmad Athif Mohd. "A review on 3D printing in tissue engineering applications" *Journal of Polymer Engineering*, 2022; 42(3): 243-265.
44. Li, J., Chen, M., Fan, X. *et al.* Recent advances in bioprinting techniques: approaches, applications and future prospects. *J Transl Med*, 2016; 14: 271.
45. Chen Earnest P., Toksoy Zeren, Davis Bruce A., Geibel John P.3D Bioprinting of Vascularized Tissues for in vitro and in vivo Applications.Frontiers in Bioengineering and Biotechnology, 2021; 9.
46. Ong, C., Yesantharao, P., Huang, C. *et al.* 3D bioprinting using stem cells. *Pediatr Res*, 2018; 83: 223–231.
47. Weng T, Zhang W, Xia Y, Wu P, Yang M, Jin R, Xia S, Wang J, You C, Han C, Wang X. 3D bioprinting for skin tissue engineering: Current status and perspectives. J Tissue Eng., 2021 Jul 13; 12: 20417314211028574. PMID: 34345398; PMCID: PMC8283073.
48. Mengde Zhang, Chao Zhang, Zhao Li, Xiaobing Fu, Sha Huang, Advances in 3D skin bioprinting for wound healing and disease modeling, *Regenerative Biomaterials*, 2023; 10: rbac105.
49. Anna Olejnik, Julia Anna Semba, Adam Kulpa, Aleksandra Dańczak-Pazdrowska, Jakub Dalibor Rybka, and Justyna Gornowicz-Porowska. 3D Bioprinting in Skin Related Research: Recent Achievements and Application Perspectives. ACS Synthetic Biology, 2022; 11(1): 26-38.
50. Xiang, Y., Miller, K., Guan, J. *et al.* 3D bioprinting of complex tissues in vitro: state-of-the-art and future perspectives. *Arch Toxicol*, 2022; 96: 691–710.
51. Xie Z, Gao M, Lobo AO, Webster TJ. 3D Bioprinting in Tissue Engineering for Medical Applications: The Classic and the Hybrid. Polymers (Basel), 2020 Jul 31; 12(8): 1717. PMID: 32751797; PMCID: PMC7464247.

52. McGivern S, Boutouil H, Al-Kharusi G, Little S, Dunne NJ, Levingstone TJ. Translational Application of 3D Bioprinting for Cartilage Tissue Engineering. Bioengineering (Basel), 2021 Oct 18; 8(10): 144. PMID: 34677217; PMCID: PMC8533558.
53. Wang Z, Wang L, Li T, Liu S, Guo B, Huang W, Wu Y. 3D bioprinting in cardiac tissue engineering. Theranostics, 2021 Jul 6; 11(16): 7948-7969. PMID: 34335973; PMCID: PMC8315053.
54. Barreiro Carpio Mabel, Dabaghi Mohammadhossein, Ungureanu Julia, Kolb Martin R., Hirota Jeremy A., Moran-Mirabal Jose Manuel.3D Bioprinting Strategies, Challenges, and Opportunities to Model the Lung Tissue Microenvironment and Its Function.Frontiers in Bioengineering and Biotechnology, 2021; 9.
55. Donderwinkel, Ilze & Hest, Jan & Cameron, Neil. Bio-inks for 3D Bioprinting: Recent Advances and Future Prospects. Polym. Chem, 2017; 8. 10.1039/C7PY00826K.
56. Hongli Mao, Li Yang, Haofang Zhu, Lihuang Wu, Peihong Ji, Jiquan Yang, Zhongwei Gu. Recent advances and challenges in materials for 3D bioprinting. Progress in Natural Science: Materials International, 2020; 30(5): 618-634.
57. Nanbo Liu, Xing Ye, Bin Yao, Mingyi Zhao, Peng Wu, Guihuan Liu, Donglin Zhuang, Haodong Jiang, Xiaowei Chen, Yinru He, Sha Huang, Ping Zhu. Advances in 3D bioprinting technology for cardiac tissue engineering and regeneration. Bioactive Materials, 2021; 6(5): 1388-1401.
58. Pallab Datta, Laura Y. Cabrera, Ibrahim T. Ozbolat. Ethical challenges with 3D bioprinted tissues and organs. Trends in Biotechnology, 2023; 41(1): 6-9.
59. S. Vijayavenkataraman, W.F. Lu, J.Y.H. Fuh. 3D bioprinting – An Ethical, Legal and Social Aspects (ELSA) framework. Bioprinting, 2016; 1–2: 11-21.
60. Yongcong Fang, Yuzhi Guo, Tiankun Liu, Runze Xu, Shuangshuang Mao, Xingwu Mo, Ting Zhang, Liliang Ouyang, Zhuo Xiong, Wei Sun. Advances in 3D Bioprinting. Chinese Journal of Mechanical Engineering: Additive Manufacturing Frontiers, 2022; 1(1): 100011.
61. Yu J, Park SA, Kim WD, Ha T, Xin Y-Z, Lee J, Lee D. Current Advances in 3D Bioprinting Technology and Its Applications for Tissue Engineering. *Polymers*, 2020; 12(12): 2958.