Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/22/20230972

Techniques and applications of 3D bioprinting

Jingru Wang

SDU-ANU Joint Science College, Shandong University, Weihai, 264200, China

jingru_wang@mail.sdu.edu.cn

Abstract. 3D bioprinting is an emerging technology that uses computer printing technology and unique bio-ink components to create artificial organs and biomedical objects. It is an interdisciplinary development technology integrating biology, chemistry, materials science, life science and other disciplines, and the technology behind biological 3D printing has improved in its development over the past few decades. Precisely printed biomaterials could benefit both tissue engineering and regenerative medicine. 3D bioprinting technology makes it possible to print tissues and organs, providing favorable conditions for organ transplantation and medical experiments. This paper mainly reviews the development of bioprinting technology and related concepts. According to the morphological change and chemical composition, the commonly used bioprinting materials (bio-inks) and their characteristics are described. The main methods of bioprinting are summarized, including traditional inkjet 3D bioprinting, extrusion 3D bioprinting, laser-assisted 3D bioprinting, photocuring 3D bioprinting and emerging 4D bioprinting and in situ bioprinting technologies. Finally, the paper introduces the development trend of bioprinting in the transplantation of skin, blood vessels and complex organs, as well as the precision research of tumors. In addition, current research challenges and prospects for future bioprinted 3D printing materials are discussed.

Keywords: 3D bioprinting, bio-ink, tissue engineering, bioprinting techniques, applications.

1. Introduction

According to the literature, organ demand and organ donations by the world population are in short supply every year. In order to create a healthier and better society, 3D organ printing can well solve the problem of organ shortage. In addition, 3D printing provides a good objective condition for the research of medical experiments. Through printing technology, the medical community can obtain more accurate organs, further study the etiological mechanism, and provide basic material conditions for the solution of difficult and intractable diseases [1].

The assembling of living and non-living biomaterials into perfect complex layouts is made possible by the new technology known as "bioprinting," which helps to accelerate tissue development. Bioprinting strives to automate, coordinate, and optimize the production of modified tissues or organs. Printing biological structures in a variety of forms, scales, and resolutions has been done using a wide range of biomaterials and methods.

Bioprinting has four levels in terms of classification. The first is to print non-biocompatible structures through traditional printing technology; the second is to print biocompatible but non-degradable structures; the third is to print biocompatible and degradable structures; the fourth is also called narrow bioprinting, also known as cell printing. It is the process of building biomimetic tissues and organs using

DOI: 10.54254/2753-8818/22/20230972

special bio-ink and bio-printing technology [2]. This paper mainly discusses the narrow sense of bioprinting. At present, 3D bioprinting technologies based on scaffold materials are mainly divided into extrusion type, droplet type and laser-assisted technology, and have been developed into in situ bioprinting and 4D bioprinting technology, each of which has its own advantages and disadvantages. According to the different printing methods, the choice of biological ink will also change accordingly, often according to the rheological properties of ink, viscosity, crosslinking chemistry and biocompatibility to choose.

The first time that living cells were printed was in 2003, according to scientists at Clemson University in the United States. The researchers adjusted an inkjet printer, loaded the cell-containing ink substance, and created straightforward two-dimensional cell designs [3]. The patent for multi-nozzle extrusion cell printing was submitted that same year by Professor Sun Wei of Drexel University in the US. In the same year, Bio Fabrication, the first SCI journal in the field of biological 3D printing and bio fabrication, was published by the IOP and edited by Professor Sun Wei. The impact factor of Bio fabrication in 2017 was 6.838, ranking second in the field of biomaterials.

In order to support the engineering of 10 physiological systems, including the circulatory, endocrine, gastrointestinal, immune, epidermal, musculoskeletal, nervous, reproductive, respiratory, and urinary systems, the Defense Advanced Research Projects Agency (DARPA) of the United States approved an in vitro platform in 2011 and intends to use it in vivo. The cost of 3D-printed organ models for auxiliary medical treatment and surgery will be covered by standard medical insurance starting in 2016, according to the Medical Council of the Central Social Insurance under the Ministry of Health, Labor, and Welfare of Japan. This will encourage the use of 3D printing technology in the medical industry. The publication of Bioprinting and other professional publications on biological 3D printing in succession in 2016 suggests the possibility of an ongoing study in this area. This paper will clarify the meaning of bio-3D printing technology from the aspects of bio-ink, bioprinting technology, and the application of this technology.

2. Bio-ink

Bio-ink plays a very important role in bio-printing. It is a kind of 3D printer ink with biological activity and good formability, which can be grown into stable structures through printing technology.

2.1. Key characteristics of ideal materials for 3D bioprinting

The choice of bio-ink is very important for 3D printing, and the ideal bio-ink should have appropriate relevant characteristics including printability, biocompatibility, appropriate mechanical properties, biodegradability and sterilization stability [4]. Among these, the first three factors are most important. The application of bio-ink will also vary depending on the printing technology method based on its own characteristics.

2.2. Classification according to the mode of morphological change

2.2.1. Temperature-sensitive ink. By heating or cooling method, the ink material from the sol state to the gel state is the working principle of temperature-sensitive ink. Such as gelatin ink material, it is necessary to heat the nozzle to melt the gelatin during printing, so that the bottom of the printing platform is cooled and the gelatin is shaped. A smart biocompatible scaffold based on soybean oil epoxy acrylate, a temperature-sensitive ink, was also created by Miao et al. By fastening and chilling at -18 °C, the temporary shape can be created, and by heating at 37 °C, the original shape may be melted. Multipotent human bone marrow mesenchymal stem cells (hMSCs) exhibited strong adherence and proliferation on printed scaffolds, suggesting their significant potential in biological tissue engineering [4].

2.2.2. Shear thinning ink. The apparent viscosity of the material will decrease with the increase of shear stress. The shear thinning ink is gel state when it is not subjected to shear force. The shear thinning ink

DOI: 10.54254/2753-8818/22/20230972

will become a sol state after it is subjected to shear force, such as the bio-ink composed of collagen and the bio-ink mixed with carbomer gum and GelMA [2].

2.2.3. Ion crosslinked ink. The forming and curing process of hydrogels is realized through the reaction of ion crosslinking. For example, the ink series of alginate, sodium ions in sodium alginate and calcium ions are replaced when used to obtain calcium alginate hydrogels [5].

2.2.4. Photosensitive ink. By activating the photosensitive primer in the ink, the ink can be changed from the sol state to the gel state, which is the working principle of photosensitive ink. Such as photosensitive ink methacrylic anhydride gelatin material. Arakawa et al. developed a biomimetic layered blood vessel network using photodegradable bioinks. The research focused on making a fully cell-compatible bioink, including precise molecular photolysis, to produce complex three-dimensional blood vessel networks in a hydrogel biomaterial. In this work, a multifunctional synthetic peptide polymer-based material was discovered, which supported cell encapsulation, enzyme-mediated matrix remodeling and biochemical customization, with the ability to form light-programmable blood vessels. Vessels were created in a photosensitive gel by multiphoton lithography-assisted photosplitting. The project shows that bioinks with photodegrading capabilities have great potential for creating complex tissues, such as networks of blood vessels. In addition, the inclusion of biocompatible photothermal particles such as graphene, carbon nanotubes and polydopamine nanoparticles in the polymer matrix facilitates the fabrication of photoresponsive materials and 4D bioprinted structures [4].

2.3. Classification according to chemical composition

2.3.1. Extracellular matrix (ECM) -based materials. ECM is the natural environment in which cells live. ECM is composed of macromolecular proteins and polysaccharides with different structures and functions, which plays an important role in the formation and function maintenance of tissues and organs, as well as the interaction between cells and tissues [1]. ECMs are composed of a variety of molecules, including the collagen family, elastic fibers, fibritin, glycosaminoglycans (GAGs) and proteoglycans, as well as various adhesive glycoproteins [4]. The interaction between cells and the extracellular matrix results in the delivery of biochemical signals and structural support for a number of critical cellular functions. In addition, the physical and biological characteristics of each tissue and organ's ECM are distinct, enabling cells to carry out certain tasks including diffusion, migration, proliferation, and differentiation. Therefore, for 3D bioprinting of tissues and organs, the researchers' understanding of the composition, structural organization, and biomechanics of natural ECMs is crucial.

2.3.2. Multi-materials. The physical and chemical characteristics of the printed product are frequently only slightly altered when using bioprinting based on a single material, which can also cause significant issues for medical applications. To solve this issue, there has been a sharp rise in interest in modifying and diversifying printing materials, which has resulted in the creation of bioprinting functional composites [4]. The composite materials are divided into chemical composite materials and physical composite materials, and the synthetic methods are divided into chemical syntheses and physical syntheses. After the synthetic process, the properties of the composite materials will change accordingly, making them suitable for bioengineering applications. The synthetic material has strong mechanical properties without cytotoxicity and immunogenicity. Therefore, composite materials can be matched with individual materials according to demand, and play a role. Because the synthetic material is cell-inert, which makes it difficult for cells to attach, it needs to bind to other bioactive hydrogels in order to function. Lai et al. used cationic cellulose nanocrystals and zwitterionic hydrogels to design effluent gels under a series of physical syntheses such as photopolymerization. This bio-ink had good mechanical strength, high transparency and 3D printability, which could be applied to wearable electronic devices and the preparation of artificial muscles. Some researchers developed hydrogels that belong to chemical composites, It was composed of methacrylate group-derived poly (N-(2-hydroxypropyl)

DOI: 10.54254/2753-8818/22/20230972

methylacrylamide lactate) A- block and hydrophilic poly (ethylene glycol) B- block with a molecular weight of 10 kD. The gel was thermotreated and formed by photopolymerization and chemical crosslinking. It had strong mechanical properties and could be used in the culture of chondrocytes. Synthetic materials have great potential in the field of human organ repair and tissue regeneration [6].

2.3.3. Stimuli-responsive materials. Stimulus-responsive polymer materials are polymer materials that can respond to subtle physical or chemical changes in the external environment. Changes in the external environment mainly refer to changes in temperature, solvent, pH, magnetic field, electric field, ionic strength, light, etc. [6]. These changes lead to changes in the interactions between molecules and various energies, as well as changes in the structure of the polymer chain at the molecular level, or in the interactions between the polymer chain and the solvent. According to the different external environmental conditions, they can be divided into temperature-sensitive type, gas sensitive type, pH response type and other polymer materials. By placing hydrogels with different stimulus responses, structures with the ability to deform can be generated. Numerous groundbreaking studies have now shown how stimulus-response materials may be successfully included in the printing process. For instance, alginate's electrical conductivity may be increased to create an electrically reactive water gel by including nanowires and carbon nanotubes [5]. It is first important to create stimulation-responsive materials with qualities including good printability, good biocompatibility, mild response, and strong mechanical support in order to satisfy the demands of 4D bioprinting.

3. Printing techniques

Biological 3D printing methods have evolved many times, from simple gel casting methods (droplet - extrusion - laser assisted printing, etc.) to multi-material bioprinting to full-range bioprinting, and emerging bioprinting methods including 4D bioprinting and in situ bioprinting.

3.1. Simple gel casting methods

3.1.1. Inkjet 3D bioprinting. Inkjet bioprinting uses heat or a magnetic field to generate energy. The bio-inks to be printed are transformed into droplets, similar to inkjet forms [2]. In this way, sound waves or heat energy can be tightly controlled, thus controlling the amount of biological material extruded from the nozzle, effectively avoiding nozzle clogging.

3.1.2. Extrusion 3D bioprinting. In extrusion bioprinting, hot melt materials are melted by a heater, and then extracted into filament-like materials, which are fed into the hot melt nozzle with the help of a wire feeder. The nozzle is melted by a heating device in the nozzle, and the nozzle moves along the section outline and filling track of the component [7]. At the same time, the materials of the semi-fluid turntable can be stratified according to the Computer Aided Design data control path, and then the extruded materials are deposited in the specified position for solidification treatment, and the surrounding materials can be bonded to achieve layer upon layer accumulation. The advantages of extrusion printing are that it can provide continuous power, is not limited by the concentration of biological ink, and has a wide selection of biological materials. It can print stronger structures. But the printing cost is higher and the cell activity is lower than the droplet type [7]. Another disadvantage is that the survival rate of the cells produced by micro extrusion bioprinting is very low, which limits the development of this technology in the field of biomedicine.

3.1.3. Laser-assisted 3D bioprinting. The laser-assisted bio-printer uses the laser to focus an absorbing layer on a glass plate so that a high-pressure vacuole appears on the layer, and then pushes the biomaterial-containing cells onto the receiving substrate [7]. The advantage of this printing method is that the nozzle is open, there will be no nozzle blockage trouble, and the damage to biological cells is

DOI: 10.54254/2753-8818/22/20230972

small, cell survival rate can reach more than 95%, but the disadvantage is that the price is too high, which greatly limits the clinical application.

3.1.4. Photocuring 3D bioprinting. Photocurable printing uses light to select the interlocking bio-ink. The micro-mirror device is solidified layer by layer to form a three-dimensional structure. The special device structure of photocuring determines the high efficiency and high precision of the printing method. Because the printing is vertical, the whole plane can be cured at the same time, which is simpler and more efficient than other methods [2]. But the emitted ultraviolet light could damage the structure of the bio-ink.

3.2. Emerging methods of bioprinting

3.2.1. 4D bioprinting. The term "4D printing" describes the use of "programmable matter" and 3D printing technology to produce 3D objects with the ability to change their physical characteristics, such as shape, density, color, elasticity, conductivity, optical properties, electromagnetic properties, etc., in response to predetermined stimuli, such as submerging them in water or heating, pressurizing, electrifying, or lighting them, among others [3]. A substance that can alter its structure, density, conductivity, color, optical characteristics, electromagnetic properties, and other qualities by programming is referred to as "programmable matter" among them. The fourth dimension of 4D printing refers to the ability of an item to modify its own shape or qualities after it has been manufactured. This ability to change its structure over time or driven by an external field is very consistent with biomedical materials.

3.2.2. In situ bioprinting. In situ bioprinting system integrates surgical robot and bioprinting technology. According to the shape of the patient's tissue defect, repair materials and cells can be directly printed on the acute wound surface with appropriate biological inks to achieve efficient and accurate regenerative repair [3]. At present, this printing technology is still in the research stage. He Yong et al. developed a new type of "biological concrete" ink. In this study, pre-functionalized cell-carrying microspheres were used as "stones" and high-concentration GelMA hydrogel prepolymers were used as "cement". In addition, a robotic in-situ 3D bioprinting system was developed to deposit "bio-concrete" directly on the patient's tissue defects according to the defect morphology to achieve tissue regeneration and repair.

4. Application

4.1. Tissue and organ transplantation

There are two methods of bioprinting in the direction of biological manufacturing of tissue and organ structure, namely in vitro bioprinting of tissue and organ structure and in situ bioprinting of tissue structure [8]. Among them, the research achievements in skin printing, organ printing and tumor printing are outstanding.

4.1.1. Skin. Skin tissue is vital for wound healing, and the absence of skin larger than 4cm in diameter on the body's surface can prevent wounds from healing on their own. The skin loss caused by large area trauma or burn in battlefield first aid is a typical representation, which has been a big problem plaguing medical workers, but also a major factor causing infection and even death. Therefore, how to print skin through 3D bioprinting technology is very important. Full-thickness skin contains epidermis and dermis, and micro extruded can print full-thickness skin, allowing cell and cytoplasmic matrix distribution similar to the human dermis, with some barrier function. Lee et al. made skin with a multi-layer structure through 3D bioprinting technology, and printed the fiber layer and the cuticle layer of the skin alternately through multi-nozzle printing [2]. The latest in situ skin printing technology is to print the skin directly at the skin wound site to help heal the skin wound. Today's printed skin is still an oversimplification of the complexities of natural skin. However, natural skin also includes appendages, such as hair follicles

DOI: 10.54254/2753-8818/22/20230972

and sweat glands, and has a high degree of innervation, and these refined functions have yet to be explored. In addition, how to keep the whole skin alive is also a key problem to be solved. These issues need to be addressed in the future [2].

4.1.2. Blood vessels. 3D bioprinting printed tissues, cells, organs and other organs need oxygen and nutrients to maintain normal life activities and physiological functions. Therefore, the use of artificial blood vessels as a good transport tissue has been put on the 3D printing agenda. Through artificial blood vessels, various complex organs of bioprinted organs can be injected with physiological activities related to material transport. However, the structure of blood vessels is complicated and intricate, so how to construct the blood vessel network with normal function has become the focus of research. Zhang et al. created a stable biodegradable scaffold with a built-in network of branching microchannels. To allow for efficient molecular exchange and cell migration, nanopores and micropores are added to the blood vessel walls. A stable, permeable network of blood vessels is established in the scaffold, irrespective of material limitations, allowing various ECMs to play a role in parenchymal space and tissue remodeling, and to mimic the anisotropy of natural tissues such as myocardium. After the vascular structure was successfully fabricated by Zhang et al., the blood vessels were connected to the femoral vessels of the hind limbs of adult rats through surgery and surgical intubation. Blood vessels were reported to be free of blood clots for a week after surgery [9]. Currently, there are many laboratories that can simulate blood vessels, but 3D-printed blood vessels are still very different from natural blood vessels. The current biological material library cannot meet the requirements of mechanical and physical properties of natural blood vessels [9]. In addition, printing submicron capillaries with the current bioprinting techniques is very challenging. It is worth mentioning that the vascular network is operated under the control of the nervous system, so it is difficult to achieve independent operation. In order to construct the vascular channel network more effectively and meet the needs of nutrient transport, it is necessary to further deepen the printing technology to construct the multi-scale nutrient transport network more effectively.

4.1.3. Complex organ. Different organs work together to perform normal human physiological functions. The demand for organs is huge, so the study of how to print organs is called a research hotspot. For example, the current cardiac repair mainly focuses on myocardial function repair and cardiac valve function repair [10]. The extrusion printing method combined with bio-inks such as sodium alginate and gelatin can print heart patches to improve heart function. Regenerative medical repair of the kidney can be achieved through endogenous repair and remission of kidney disease or by providing transplantable organs or functional organ units.

4.2. Tumor research with precision

Traditional surgery mainly relies on Computer Tomography (CT) and Magnetic Resonance Imaging (MRI) images of patients, but there are limitations in planarization, so doctors need to have three-dimensional imagination to lay the foundation for follow-up surgery. Due to the complex tissue structure surrounding some tumors, it is difficult for even doctors with extensive clinical experience to achieve 100% accuracy during surgery. The operation boundary is fuzzy, and the operation is easy to leave dead corners. However, 3D printing technology solves this problem. The tumor cells produced by 3D printing are closer to the real characteristics of tumors in vivo [10]. Tumor model printing technology provides a favorable tool for tumor research and drug screening. Zhao et al. printed cervical cancer cells into three-dimensional structures by 3D bioprinting-based methods, and the research results showed that three-dimensional cells grew in a spherical structure, had stronger proliferation ability and higher protein expression than two-dimensional cells, and these characteristics were closer to the real characteristics of tumors in vivo [11]. It can be seen that tumor model printing technology provides a favorable tool for tumor research and drug screening.

DOI: 10.54254/2753-8818/22/20230972

5. Conclusion

In the context of bionics and healthcare, bioprinting creates a new technological platform for us to better enjoy the emerging scientific results of interdisciplinary research. This paper starts with the development background of bioprinting, classifies the ink materials and printing technology related to bioprinting, and finally introduces the related applications of bioprinting technology. Since bioprinting cells, relatively good results have been achieved. In particular, biomaterials and printing technologies have been effectively applied in tissue engineering and regenerative medicine, and eventually become substitutes for tissues and organs. 3D bioprinting technology has gradually penetrated the field of clinical medicine. The technology can produce personalized tissue cells or organs, solving the problem of donor shortage and immune rejection. The technology could also create models for a wide variety of medical research, leading to the development of drugs and treatments. The development prospect of 3D bioprinting technology is bright, but it also faces many problems and challenges. Currently, the number of bio-inks that can be applied to bioprinting and accurately reflect the tissue structure required for organ function recovery after printing is limited. In addition, models and criteria need to be established to evaluate the performance of different bio-ink materials. Due to the limited current technology, the accuracy of bioprinting is lower than that of natural tissues/organs. Therefore, it is considered to develop new bioprinting methods to solve the problem of low accuracy. Going forward, 3D bioprinting can focus on developing better bio-inks and more efficient and viable printing technologies. And can be combined with medical research, more convenient application in clinical trials. In general, 3D printing technology has shown many new possibilities. Although it has some shortcomings and deficiencies, it is believed that with the development of science and technology and the efforts of researchers, 3D bioprinting technology will continue to integrate with multiple disciplines, achieve functional breakthroughs as soon as possible, and have broader application prospects in the future.

References

[1] Mao, H., Gu, Z. “Development Status and Trend of Bio-3D Printing Polymer Materials.” Progress of materials in China 37(12), 949-969 (2018)

[2] He, Y., Gao, Q., Liu, A., et al. “Biological 3D Printing-From Similar Shape to Similar Manner.” Journal of Zhejiang University (Engineering), 53(3),407-419 (2019).

[3] Shen, J., Murat, G., “Complex 3D bioprinting methods.” APL Bioeng 5 (1), 011508 (2021).

[4] Mao, H., Yang, L., Zhu, H., et al. “Recent Advances and Challenges in Materials For 3D Bioprinting.” Progress in Natural Science International Materials (English) 30(5), 618-634 (2020).

[5] Lee, J., Yeong, W. “Design and Printing Strategies in 3D Bioprinting of Cell-Hydrogels: A Review.” Advanced Healthcare Materials, 5(22), 2856-2865 (2016).

[6] Yang, J., Hu, H., Li, S. et al. “Application Of 3D-printed Bio-ink In Tissue Repair and Regenerative Medicine.” Tissue engineering Research in China, 1-8 (2023).

[7] Chen, S., Wang, X., Gan, M., et al. “A Review of 3D Bioprinting.” Rubber and plastic technology and equipment 49(04), 9-12 (2023).

[8] Ozbolat., I. T. “Bioprinting Scale-up Tissue and Organ Constructs for Transplantation.” Trends in Biotechnology 33(7), 395-40 (2015).

[9] Ng, H., Lee, K., Kuo C., et al. “Bioprinting of Artificial Blood Vessels.” International Journal of Bioprinting 4(2), 43-58 (2018).

[10] Liu, C., Hu, X. “3D bioprinting technology and its application.” Biology Teaching 48(2), 4 (2023).

[11] Zhao, Y., Yao, R., Ouyang, L., et al. “Three- dimensional printing of Hela cells for cervical tumor model in vitro.” Biofabrication 6(3), 035001 (2014).