Current Progress of 3D Bioprinting of Cardiac Tissues

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Abstract. Heart disease is the leading cause of death worldwide. Heart transplants are used when treating end-stage heart disease. The main problem faced by heart transplantation is the scarcity of donors. Therefore, finding a way to replace the donor heart remains a major medical challenge. In recent years, 3D bioprinting has often been used in tissue engineering, and it has achieved results in the preparation of many types of human tissues. Therefore, it is regarded as a promising method for alleviating donor heart scarcity. In this article, we summarize the current research progress of 3D bioprinting of cardiac tissue, and after introducing several 3D bioprinting methods, bioinks, and 3D bioprinting steps for heart tissue, we proposed our perspectives on 3D bioprinting, discussed several current challenges, and analysed the future of development.

Keywords: Bioprinting, Cardiac tissues, Bioprinting methods, Bioink.

1. Introduction

Heart disease is one of the diseases with the highest mortality rate in the world, which causes one-third of the deaths in the world [1]. At present, heart transplantation is the main method for the treatment of end-stage heart disease, but due to the scarcity of donors, researchers have to find other ways to replace the donor's heart. In recent years, 3D bioprinting technology has been widely concerned by the biological community because of its application potential in the production of human tissue, and cardiac tissue 3D printing has made great progress [2].

3D bioprinting technology is a new technology developed based on traditional 3D printing technology. It can print the required tissue through bioink, and the printed tissue can reproduce part of the structure and function of the body tissue in vitro. 3D bioprinted heart tissue provides new materials for the research and treatment of heart disease.

The earliest inkjet bioprinting, the most commonly used extrusion bioprinting and laser-assisted printing are the mainstream bioprinting methods at present. With the continuous development of 3D bioprinting technology, some new bioprinting methods have also emerged in recent years, such as bioprinting based on multi-axis robots [3]. Bioink is the raw material used by bioprinters to print tissue, which contains biomaterials, cells and biological active factors. The properties of bioink, such as formability, fluidity and biocompatibility, are very important for tissue printing. The 3D bioprinting process of heart tissue is mainly divided into three parts: pretreatment, printing process and post-printing processing. Through these steps, heart tissue can be manufactured artificially. Here, we introduce the methods of 3D bioprinting, bioink and 3D bioprinting steps of heart tissue, summarize the recent research progress of 3D bioprinting on heart tissue, and analyze the development and current challenges of 3D bioprinting.

2. Methods of 3D bioprinting

2.1. Inkjet Bioprinting

Traditional inkjet printing is a printing method in which small black or color ink droplets are sprayed onto the paper surface through the nozzle. This method has the characteristics of non-contact with objects and can spray ink droplets evenly. Traditional inkjet printing first appeared in 1878, with a long history of development, and was mainly used in the printing industry in the early stage. At the end of the 20th century, researchers realized the potential value of this technology in biotechnology

and tissue engineering [4], because the development of computer technology has greatly improved the accuracy of inkjet printing, and finally reached the level of tissue refinement. Inkjet bioprinting is the first bioprinting technology [5]. In 1988, researchers transformed the bioprinter based on the traditional inkjet printer. The ink cartridge of the bioprinter is "bioink" containing cells [4]. The principle of inkjet bioprinting is similar to that of traditional inkjet printing. The bioink is divided into a series of micro-drops using a piezoelectric or thermal drive nozzle. After printing layer by layer, a three-dimensional structure containing cells can be formed. In this process, the ejected droplets need to be bonded and fused to form a whole. Inkjet bioprinting is fast and efficient. At the same time, because commercial printers can be transformed into inkjet bioprinters, inkjet bioprinting is a relatively low-cost bioprinting technology. Inkjet bioprinting has the longest development time in 3D bioprinting, so this technology has sufficient experimental basis and experience. However, this technology also has some disadvantages. First, it is difficult to ensure the directionality of the liquid droplet during injection, which ultimately leads to the failure of the shape and function of the tissue to meet expectations. Secondly, if the viscosity of the bioink is too high, the nozzle of the inkjet biological printer will be blocked and piled up, which will affect the printing process and cause the integrity of the organization. This means that inkjet bioprinting can only use bioink with low viscosity (less than 10 cells per milliliter) [6], which is also the main problem restricting the development of this technology. Therefore, researchers later made improvements to inkjet bioprinting.

2.2. Extrusion Biopriting

Extrusion bioprinting can be called an improved version of inkjet printing. It is the most widely used bioprinting method at present, with moderate cost. The principle of extrusion printing is similar to that of inkjet technology. It uses pressure to discharge bioink in a precise direction. Because inkjet bioprinting requires low-viscosity bioink, extrusion bioprinting uses pneumatic and mechanical screw plugs to distribute bioink, and does not form droplets, but generates a continuous cylindrical line through continuous force. Therefore, when extruding the greatest advantage of bioprinting, you can use bioinks of various viscosities, especially high-viscosity bioinks [7]. The range of printable biocompatible materials is also wide, including cell clusters, cell-carrying hydrogels, microcarriers, acellular matrix components, etc., and the viscosity ranges from 30mPa/s to 6×10^7mPa/s biomaterials, especially hydrogel materials with shear thinning and rapid crosslinking characteristics [8]. This means that low-viscosity materials that can provide a suitable microenvironment for maintaining cell vitality and functionalization, and high-viscosity materials that can provide mechanical support for printing structure can be well combined with extrusion bioprinting, so that the types of tissue printed are increased and the morphology is more complete. The problem with this technology is that the printing speed is slow and the resolution is low, which means that the printed tissue fineness may not be high enough [9]. Extrusion bioprinting has become the mainstream of biological 3D printing. With the development of this technology, some of its defects will be gradually improved.

2.3. Laser-assisted Biopriting

The origin of laser-assisted bioprinting is laser direct writing and laser-induced transfer technology. Now the main method to realize this technology is photopolymerization. The crucial part of the laser-assisted bioprinting system is the donor layer that responds to laser stimulation. The donor layer includes a "ribbon" structure containing an energy absorption layer (such as titanium or gold) at the top and a layer of bioink solution suspended at the bottom. Compared with inkjet bioprinting and extrusion bioprinting, laser-assisted bioprinting can avoid direct contact between the dispenser and bioink. This non-contact printing method will not cause mechanical stress on cells, thus maintaining more than 95% of cell vitality [10]. In addition, laser-assisted bioprinting can also print high-viscosity materials. Compared with inkjet bioprinting, more types of bioinks can be used. It can also select a single cell for transfer, which can improve the accuracy very well, so as to get better tissue. The disadvantage of this technology is that compared with other nozzle-based printing methods, the laser

diode with high resolution and intensity is expensive, and the control of the laser printing system is relatively complex. The printer body is also rather bulky. The most important thing is that the side effects of laser irradiation on cells are not fully understood, which may lead to tissue mutation and functional defects [11]. These problems limit the use of laser-assisted bioprinting. But in general, this technology has a good development prospect.

2.4. Multi-axis Robot-based Bioprinting

Although the above-mentioned methods have made progress in the printing of cardiac tissue, they are all traditional Cartesian biological 3D printers, that is, 3D printers with only three directions of X axis, Y axis and Z axis. This printer has proved that it can be used to manufacture simple tissues, but there is still a certain gap in the realization of large, complex and functional tissues [12]. According to a research report published in 2022, researchers abandoned the traditional 3D biological printer and used a six-axis robot to transform a new tool that can rotate 360 degrees freely to print tissues in all directions. Compared with the traditional 3D biological printer, this robot printer can print cells from more angles. In order to follow the law of tissue development, after printing out several layers of cells, the robot will first let the cells grow, form intercellular connections and new capillaries, and then print new cells. In this way, a vascular network similar to the internal organs can be formed in the heart, thus supporting the long-term survival of tissues. On top of this, scientists have also designed an oil bath printing method, which can make the printed cells free from the influence of gravity under the hydrophobic force of mineral oil, thus making the cells tightly connected. In specific practice, the experimenter used two six-axis robots to print vascular endothelial cells and cardiac cells on the vascular stent. Finally, the printed complete endothelial layer can grow new blood vessels and capillary networks with the help of angiogenic factors; The printed myocardial cells can also form gap connections quickly and maintain regular pulsation for 6 months [3].

3. Bioink for 3D Biopriting

Bioink is generally composed of biomaterials, cells and bioactive factors. Among them, biomaterials are mainly biocompatible hydrogel materials. Cells generally select cells with biological activity, and bioactive factors are mainly used to regulate cells and maintain the activity of bioink.

3.1. Biomaterials

The purpose of bioprinting is to obtain functional tissues, so the biomaterials used for bioprinting should be non-toxic to cells, biodegradable in vivo, and have a certain mechanical strength to support the shape and function of tissues. Ideal biomaterials should also enhance cell survival and function maintenance [13]. Hydrogels are non-toxic, can be degraded in vivo, and can provide mechanical support for developing tissues. Because hydrogel has good biocompatibility, it has gradually become an ideal material to simulate extracellular matrix. The substances in hydrogels are generally divided into natural polymers and synthetic polymers according to their composition.

Natural polymers are widely used in bioprinting research. Typical natural polymers used as biomaterials include gelatin, fibrin, collagen and alginate [14]. Gelatin is a water-soluble protein that is partially hydrolyzed from collagen. It can be dissolved at high temperatures and treated at low temperatures to obtain colloid, which has good hydrophilicity. Gelatin has good biodegradability and biocompatibility, and is widely used in the preparation of hydrogels because of its easy preparation and low cost. Collagen is the most abundant protein component in human tissues with good adhesion, contraction and biocompatibility. Therefore, collagen is widely used in tissue engineering. Alginate is a kind of natural polysaccharide carbohydrate, mostly from algae. Alginate can maintain cell activity very well. The complex of alginate and gelatin-containing myocardial cells have been used for inkjet bioprinting to produce heart tissue.

Synthetic polymers include calcium phosphate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polycaprolactone [15]. Unlike natural polymers, synthetic polymers are

made by chemical synthesis, and their chemical and mechanical properties are generally more controllable.

However, researchers have not systematically studied their interactions with cells and their effects on cells. One of the main advantages of synthetic polymers is that they have relatively consistent chemical and mechanical properties. In addition, the mechanical strength of synthetic polymers usually exceeds that of natural polymers. However, unlike natural polymers, synthetic polymers can hardly promote cell function. Therefore, some researchers have developed and used a combination of natural and synthetic polymers. The survival rate and growth effect of cells are better than that of synthetic polymers alone.

3.2. Cells

The cell is the most important functional component in bioink. Cells are generally wrapped by hydrogel and survive in it, so cells with strong activity must be selected. In addition, cells should be able to maintain their normal functions. Different cells have different preferences for hydrogels, so it is necessary to constantly screen the hydrogels suitable for the target cells in the experiment. At the same time, the density of cells in the hydrosol is also important [16]. For the printing of some complex tissues, it is also necessary to mix several different cells at the same time, and precisely inoculate the cells in the correct position, so as to maximize the simulation of tissue structure.

3.3. Bioactive Factor

Bioactive factors are a class of proteins that can regulate cell growth or function maintenance, and play a very important role in cell culture in vitro [17]. There are many kinds of bioactive factors, and they have certain specificity for different kinds of cells. Different cells need different bioactive factors. Although bioactive factors mainly play an auxiliary role in bioink, they are crucial for the maintenance of cell viability, especially tissue function after printing.

4. The Steps in 3D Bioprinting of the Cardiac Tissues

The printing process of cardiac tissue is roughly divided into three parts, namely pretreatment, printing process and post-printing processing. Cardiac tissue needs to be preprocessed before printing. First, the target tissue is scanned by MRI or CT to obtain the corresponding data, and then the 3D model is created through the CAD modeling interface. A more accurate model may also be obtained using PET and other advanced clinical imaging technology in the field of nuclear medicine [18]. After pretreatment, the biological 3D printer can start the printing process according to the modeling on the computer. The printing process is to print the bioink containing heart cells into heart tissue through various printing methods such as inkjet biological printing. In order to ensure the stability of bioink and printing structure, the printing process may need to continuously adjust the formula and printing parameters of bioink to make the printing complete normally. Post-printing processing is mainly used to cultivate and maintain printed cardiac tissue. The media formula of cardiac tissue must be carefully selected after printing to maximize the integrity of tissue morphology and function. The normal function of adult heart tissue is the result of the harmonious interaction between morphology, mechanics, electrophysiology and gene expression [19]. Although the heart tissue obtained by biological 3D printing has not fully reached the level of natural heart tissue, the current research has made outstanding breakthroughs in the engineering of heart tissue.

5. Perspectives

3D bioprinting originated in the 1980s, and the development level of this technology is still in its infancy. From the perspective of the market, the enterprises developing 3D bioprinting are mainly in the R&D stage, and there is still huge room for the future development of the industry. 3D bioprinted heart tissue can not only be used for development and disease research, but also be one of the main

sources of organ transplantation in the future [20]. However, this technology is faced with the challenge of difficult research and development technology, in which it is the most difficult to arrange multiple cells in order, such as the multi-scale network of the vascular system, which is also a critical breakthrough problem for researchers now. This technology also faces problems such as high technical cost, fewer categories of critical materials, imperfect standard systems, and difficult transformation of scientific research achievements. Therefore, at present, the development of 3D bioprinting is in a situation where opportunities and challenges coexist. Future development needs to grasp opportunities and breakthrough challenges to promote the continuous development of this technology.

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