Research Progress and Prospect of Bio-ink Application in 3D Printing

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Abstract. In the past few decades, bioprinting technology has gradually become an indispensable part of biomedicine, among which bio-ink is an essential printing material in 3D printing technology. Biological scaffolds printed by bio-ink have a huge role and potential in tissue repair and regenerative medicine. The right bio-inks could enable 3D printing technology to print human tissue and organ replacements. Through the explanation and explanation of bio-ink as the main material of bio-3D printing, and review as a bio-3D material, the current advantages and disadvantages of bio-ink, its application and different classifications, as well as the future development trend and prospect of bio-ink. Biological inks still have many shortcomings, the comprehensive performance needs to be improved, such as how to maintain biocompatibility while having mechanical properties, in the future, there will be more and more biological inks to be studied to meet People's Daily life and medical needs. At present, a variety of emerging bio-inks with good comprehensive properties have been successfully developed, which have not only good biocompatibility but also good mechanical properties. It can meet the needs of 3D bioprinting. By studying the shortcomings of existing bio-inks, this study summarized and concluded the advantages and disadvantages of existing bio-inks and obtained the development direction of bio-inks.

Keywords: Material, Bio-ink, 3D bioprinting, Inkjet 3D bioprinting, Extrusion 3D bioprinting, Hydrogel.

1. Introduction

3D bioprinting is an emerging technology that combines biomaterials, cells, and bioprinting technologies to achieve 3D printing of complex biological tissues and organs [1]. With the development of science and technology, 3D bio-printing technology is gradually widely used in daily life, often used in engineering tissues for therapeutic tissue repair or drug screening. Many 3D bio-printing technologies exist in the fields of medicine and biology, such as the construction of biological tissues and organs and the exploration of cell morphological changes [2]. The overall goal of this review is the selection of different bioprinting materials in different aspects of medicine, as well as the development and transformation of bioprinting materials under the continuous update and progress of materials and technologies.

Through the understanding and in-depth thinking of the relevant materials, through the reading and thinking of the relevant papers, it is found that bioprinting technology is a "technical" revolution in the medical and tissue engineering fields, the development of bioprinting technology plays a vital role in the development of medicine in the new era, and the main printing material bio-ink needs to be constantly updated and improved.

Reasons for choosing this topic: In recent years, with the development of 3D bioprinting technology, the importance of 3D bioprinting technology in medicine is increasing day by day, 3D bioprinting technology is an important part of biomedical engineering if it cannot solve the problem of selection and development of biological ink, It will lead to the 3D printed complex biological tissues and organs and biological scaffolds cannot be safely and reasonably transplanted and solve the problem of organ supply shortage. By exploring bio-inks that are more in line with today's medical and tissue engineering needs, this study provides a direction for the subsequent development of bio-inks and an important reference value for bio-3D printing of human organs.

2. Background of 3D Bioprinting Technology

2.1. The Significance of 3D Bioprinting Technology

Bio-inks are inks that can be used in 3D printers. In the past, 3D printers used "bio-ink" and hydrogels produced by collagen as raw materials, which had great obstacles in realizing complex tissue printing [3]. The research result is to produce a "bio-ink" after the tissue or organ is processed to remove the cells. In the past few decades, bioprinting technology has gradually become an indispensable part of biomedicine. Among them, bio-ink is an extremely important printing material in 3D printing technology, biological scaffolds printed by bio-ink have a great role and potential in tissue repair and regenerative medicine. Bio-ink mainly exists in the form of a hydrogel, which can simulate the natural extracellular matrix environment and has adjustable physical and chemical properties [4]. In the same way, you can take biological cells and grow them, grow them in fibrotic petri dishes so that they have hydrogel properties, and then filter out the microbial ink, as shown in Figure 1, the preparation process of bio-ink is to extract and prepare tissue cells first, and then culture them twice (culture of engineered bacteria and nanofiber culture).

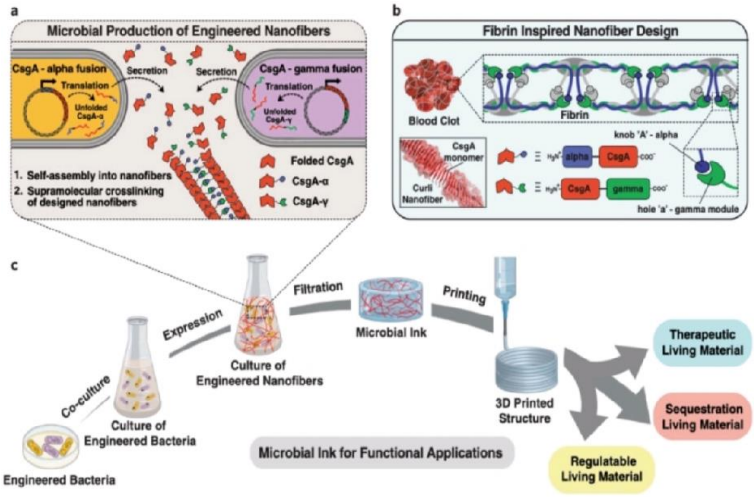

Figure 1. Preparation of functional microbial ink. (a) Microbial production of engineered nanofibers; (b) Fibrin-inspired nanofiber design; (c) Microbial ink for functional applications [4]

2.2. Current Status and Related Situation of 3D Bioprinting

At present, 3D printing technology has been widely used in the biomedical field by depositing the target tissue structure of bio-ink containing biological cells layer by layer. For example, Jiang et al. studied the application of acellular matrix bio-ink in the cardiovascular field, and reasonably used bio-ink to print human blood vessels in line with human cardiovascular mechanical properties and biocompatibility [5]. The preparation process is shown in Figure 2 [5].

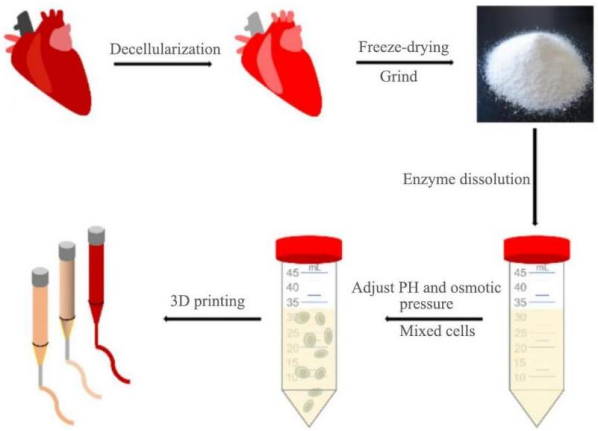

Figure 2. Preparation process of acellular matrix bio-ink [5]

2.3. The Research Content and Research Method of Bioprinting Technology

Through the use of a single or composite material biological ink, researchers have made breakthroughs in the construction of blood vessels, organoid manufacturing, skin damage repair, etc., through research, according to the forming principle and the types of biological ink, the existing 3D printing technology is divided into extrusion, inkjet, light curing. Among the three printing technologies, extrusion bioprinting is mainly divided into three ways: pneumatic, piston drive, and screw drive, with these three ways to extrude the material, but because the pneumatic extrusion bioprinting is generally compressed air to provide pressure extrusion bio-ink, although the nozzle structure is simple, the pressure during extrusion is difficult to control; Similarly, even though the other two kinds of extrusion 3D bioprinting are volume-driven, although the problem of unstable flow rate is improved, there is still a problem of large pressure fluctuation [6]. Although extrusion 3D bioprinting is the most widely used bioprinting technology at present, the printing accuracy needs to be further improved. Inkjet bio-3D printing inkjet printing generates micro bubbles at the end of the nozzle through the piezoelectric effect or thermal effect and then generates thrust to spray out the microdroplet. Combined with a CAD model and motion control, the 3D structure of the microdroplet can be manufactured [7]. Compared with extrusion printing, inkjet printing has small droplets and high resolution. However, the applicability of inkjet printing materials is limited, such to the poor fluidity of high-viscosity ink solutions, which is easy to cause jet difficulties, while low-viscosity ink solutions are easy to flow and affect the print fidelity [6]. Photocuring 3D printing technology is based on the photopolymerization reaction and uses digital signals to control UV light to selectively cure the photosensitive resin and pile up the resin layer by layer [8]. Compared with the first two kinds of bio-3D printing technology, the printing resolution and printing speed have been significantly improved [9]. Compared with other printing technologies, there is no problem of nozzle blockage or shear stress affecting cell vitality, and it is gradually widely used in daily 3D printing activities the light-curing 3D printing technology has higher printing resolution and faster printing speed than the previous two, to achieve a wider range of applications. Among them, according to the different directions of optical printing, it can be divided into SLA and digital light processing (DPL). SLA photocurable printing technology is mainly used in printing scaffolds and is rarely used in cell-bearing printing, while DPL photocurable printing technology has fast printing speed, high precision, and good uniformity, and has been widely used at present [10]. The principle of photocurable printing is shown in Figure 3.

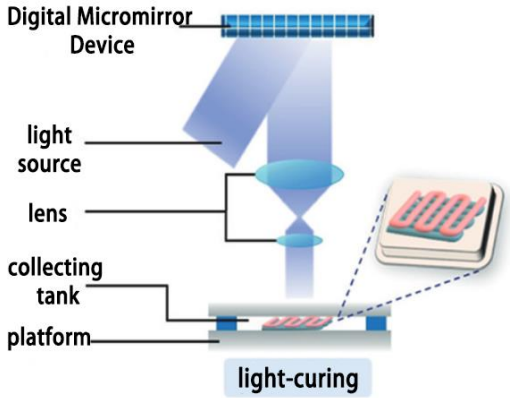

Figure 3. Light curing 3D printing [10]

3. Bio-ink in 3D bioprinting

3.1. Introduction to bio-inks and its Classifications

At present, bio-inks come from a variety of biological materials and synthetic materials, such as collagen, chitosan, polyethylene glycol, and polycaprolactone, etc., providing good biological activity, and the application range is gradually wide, such as the construction of biological scaffolds, and has

a wide range of application prospects in biomedicine, tissue engineering, drug delivery, and other fields. Future bio-inks will overcome the shortcomings of existing bio-inks, such as limited mechanical properties, and have good mechanical properties while having good biocompatibility, mainly to improve the overall performance of bio-inks.

3.2. The Role and Advantages of Bio-inks

The emergence of bio-inks and the integration with 3D printing technology, due to the emergence of a variety of bio-inks, different kinds of bio-inks have good printability, biocompatibility, and certain physical and chemical properties [4], therefore, the reasonable selection of bio-inks with different advantages and disadvantages can be applied to printing different tissues and organs and biological scaffolds.

4. Research Status of Bio-ink

4.1. Advantages and Disadvantages Different Kinds of Bio-inks

Because the bio-ink needs to have good biocompatibility and certain mechanical properties to ensure that the cell structure in the bio-ink will not be damaged due to external interference during the printing process, resulting in defects in the printed finished product, the traditional bio-ink uses hydrogel as the carrier. The physical and chemical properties of bio-inks with hydrogel as the carrier are stabilized by hydrogel as the dominant, and hydrogel also provides a good and stable environment for cells from development to maturity [11]. Therefore, in the field of bio-3D printing, bio-inks based on hydrogels account for the majority, which is mainly divided into eight categories: polyethylene glycol, collagen, alginate, gelatin, hyaluronic acid, chitosan, silk/fibroin protein, and polycaprolactone [12].

4.1.1 Advantages and Disadvantages of Traditional Hydrogel Bio-inks

Bio-ink with polyethylene glycol as the main component. Because polyethylene glycol itself is hydrophilic and absorbent and is not toxic, therefore, polyethylene glycol after a series of processing and fusion with biological cells, polyethylene glycol can mainly be used to repair human tissues and organs, but also as a printing scaffold material for bone plasticity; However, due to the poor oxidation of polyethylene glycol itself, it needs to be used in conjunction with other materials when printing as a bio-ink. Collagen, gelatin, hyaluronic acid, and chitosan, these four types of bioinks that are known for their good biocompatibility. Among them, collagen is widely used because of its strong practicality and simple way of obtaining raw materials, and it has good biodegradability and low toxicity with gelatin and chitosan. However, the mechanical properties of collagen, gelatin, and hyaluronic acid are poor, and the three biological inks are mainly liquid at low temperatures, and the gelation rate of hyaluronic acid is slow. When the three biological inks are used alone, they cannot meet the strength of the printed structure, so they need to be used in combination with other materials. Alginate is an early natural copolymer, which can gelate and cross-link based on its special polymeric chain network. In tissue engineering applications, alginate can be combined with peptides for cell adhesion, thus promoting cell proliferation and the production of extracellular matrix. Li et al [12]. studied the influence of different sterilization methods on alginate properties, and concluded that the printability of alginate could be maintained under high-pressure steam sterilization, but the disadvantage was that alginate itself had poor cell adhesion and was inert. Therefore, through the study on gelatin-based bio-ink, it was found that adding sodium alginate to gelatin seed could improve the quality of alginate printing [13]. Combining the temperature-programmable properties of gelatin with the viscosity and crosslinking ability. As for life inks with the same high mechanical properties as alginate, silk/fibroin protein and polycaprolactone can still maintain good biodegradability under the condition that they have good mechanical properties and can print finished products with certain strength. However, the effect of the three inks is also poor when used alone. It only works when it is mixed with the other materials mentioned above.

4.1.2 Emerging Types of Bio-inks

In addition to the above common types of hydrogel-based bio-inks, with the continuous progress of science and technology, emerging bio-inks have also been developed, such as pure silk protein bio-inks developed by Zhang, which can not only achieve rapid printing at low concentration but also achieve rapid printing [14]. It has the characteristics of high strength and good cytocompatibility. Lifeink Collagen-only bio-ink is also a new type of bio-ink, which is known for its ability to overcome viscosity, temperature, and volatility problems. Fig.4 is the SEM image of the 3D-printed recombinant collagen hydrogel scaffold after freeze-drying. The scaffold has a good forming effect, obvious three-dimensional pores, and increased internal surface area of the scaffold, which is conducive to cell adhesion and growth into the scaffold [14].

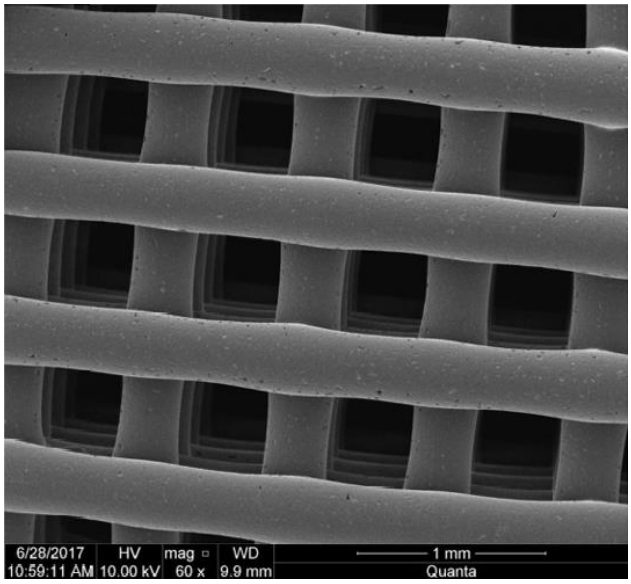

Figure 4. Collagen hydrogel 3D printed scaffold [14]

4.2. Disadvantages of Traditional Bio-inks

Therefore, based on the above summary of various biological inks, the shortcomings of traditional biological inks are mainly unable to have good mechanical properties and good biocompatibility at the same time, so a single traditional biological ink cannot be directly used to print a complete human organ and tissue structure, the main reasons for the existence of biological inks are:

(1) The cell source is limited

(2) The cell preparation cycle is long

(3) The mechanical properties are insufficient.

4.3. Problem cause analysis and Research trends

Therefore, to sum up, the traditional bio-inks that are currently being widely used still have the problem of limited scope of use, so in the future, whether it is in the fields of medicine, biology, or tissue engineering, the bio-inks used in the field of hydrogel concentration is gradually increased (mechanical properties are enhanced) based on ensuring that the hydrogel concentration is gradually increased. At the same time, it is also necessary to maintain the deposition of extracellular matrix (good biocompatibility), so that the comprehensive performance of bio-ink has been improved.

5. Conclusion

This paper mainly explains the 3D printing technology in daily life and analyzes its advantages and disadvantages, as well as the material bio-ink required in the bio-3D printing technology. Through the introduction and explanation of the bio-ink, it becomes evident that traditional bio-inks,

which utilize hydrogel as the primary carrier, exhibit certain shortcomings. How to select bio-ink and the development prospect of bio-ink are discussed and studied.

The research results show that to make the bio-ink have good biocompatibility and strong mechanical properties at the same time, so that the printed imitation human organs have the strength and corresponding activity of human organs, in addition to mixing a variety of traditional hydrogel bio-inks for use, such as adding alginate to gelatin bio-inks, It can make the mixed biological inks have good comprehensive performance, and there are a variety of single categories of emerging biological inks also have good comprehensive performance. These bio inks with good comprehensive performance can not only provide a material basis for bioprinting human organs and bone scaffolds but also promote the development of medicine and tissue engineering so that the replacement of human organs is no longer a difficult thing.

However, this study is limited to understanding the categories and improvement programs of biological inks and lacks actual data on the actual printing process, which cannot be used as a reference basis for biological 3D printing organ transplantation. Meanwhile, it is also hoped that the subsequent emerging biological inks can combine the advantages of existing biological inks, to achieve the feat of 3D printing human organs in vivo experiment success.

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