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Progress of 3D Bioprinting for Neural Tissue

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Abstract: *3D bioprinting technology has made great progress in cell engineering and can be used to construct complex geometries with the help of computer set-up programs. This paper introduces the background and concepts of 3D bioprinting technology, describes in detail the main techniques of 3D bioprinting, the biomaterials used for 3D bioprinting technology and the advances in 3D bioprinting technology in terms of neural cells and its applications, providing information for better cell formation and modelling.*

Keywords: *3D bioprinting, nervous system, nerve injury, neural tissue engineering*

1. Introduction

AM (additive manufacturing) is a manufacturing technology that produces complex geometric structures by selectively adding materials. This technology can control the size, shape, distribution and connectivity of the scaffold pores, but it cannot place multiple cell types, biomaterials and bioactive[1]

Therefore, 3D printing technology is created on the basis of AM technology for prototype production and modeling. Three-dimensional bioprinting technology successfully combines biology with 3D printing technology and applies it to tissue engineering, providing new solutions for cell formation, tissue regeneration and organ reconstruction.

As a manufacturing technology for building personalized bionic structures, 3D bioprinting technology is based on the path planning parameters after slicing the 3D digital model, and can print layer by layer with the help of various biological materials and environmental conditions to produce three-dimensional biomedical products such as artificial implants, tissues and organs[2].

The product presentation process of cell 3D bioprinting technology needs the participation of various biological technologies, and different target products need to match different printing technologies. At present, the forming technologies adopted by cell biology printing are mainly inkjet, micro-extrusion and laser-assisted. Among them, inkjet biological 3D printing technology is the easiest to operate and widely used printing method, but the jet force of biological ink will damage the activity of cells, and the size of the nozzle will also affect the accuracy of printing.

In addition to selecting the appropriate 3D bioprinting technology, the selection of biological printing materials is also crucial. Bioprinting materials should have the characteristics of printability, biocompatibility, degradability, mechanical properties, etc[3]. At present, the materials used for 3D bioprinting are polymer materials. So as to ensure that 3D bioprinting products can normally carry out physiological and biochemical activities such as cell migration.

Nerve cells are one of the most important cells in animals, which are closely connected with the occurrence of physiological processes such as animal body sensation and movement, and usually exist in the brain and spinal cord. For example, brain glioma uses 3D bioprinting technology to build its stem cell model in vitro, so that it is easier to find anti-cancer targets; Spinal cord injury may lead to loss of motor ability or paralysis. Therefore, 3D bioprinting technology is used to construct cells in the spinal cord and design biological scaffolds that conform to the mechanical characteristics of the spinal cord.

The development and application of 3D bioprinting technology have increased steadily in recent years, making great contributions to biological and medical research. In this review article, we introduced the widely used bioprinting technology, different types of biomaterials and the application of 3D bioprinting in the neural field.

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2. Methods for 3D bioprinting

At present, 3D bioprinting mainly includes three methods: suspension printing, coaxial printing, and projection-based printing. Suspension printing technology deposits biological ink into a container filled with suspension medium, in which the suspension medium plays a supporting and limiting role to prevent ink from settling, deformation and diffusion. Coaxial cell printing is an extension and development of biological 3D printing. By combining multiple layers of coaxial needles, tissue engineering scaffolds with internal communication networks can be directly and rapidly prepared.

Since cells cannot survive in an area above 200 μm away from nutrients when cultured in vitro, the construction of vascular network is crucial for tissue and organ regeneration in the field of tissue engineering. Therefore, coaxial cell printing provides a new way for the construction of vascular-like channels in vitro. The projection-based 3D bioprinting has high resolution and high printing speed, can realize fine printing of complex structures, has significant advantages on high-precision batch manufacturing of hydrogel/cells, is suitable for batch manufacture, and has gradually become a mainstream biological 3D printing method.

3. Materials for 3D bio-Printing

The key attribute of material for biological 3D printing is good printability, biocompatibility and degradability. Since the objects of biological 3D printing and the methods used are different, the selection of appropriate biological materials is crucial for obtaining high-quality biological tissues. This section gives a brief introduction to materials used in 3D bio-printing technology.

3.1. Properties of biological 3D printing materials

3.1.1. Printability

One of the most critical features of 3D biological printing is printability. Printability refers to the performance of biological materials that can be accurately and controllably molded in three-dimensional space. That is to say, the materials must be accurately deposited in the space designated by the printer within a certain period of time, so as to ensure that 3D printing products can achieve the desired structure and size[4].

During biological printing, the state of the biological ink directly influences the printing parameter setting, and further influences the quality the product. Factors such as smooth nozzles and ambient temperature for printing also indirectly affect the structure and ultimately the function of biological products[5]. Regardless of the parameter configuration selected and the printing environment, printability is required to describe the printed results, and the printability of the material can be evaluated by known techniques such as qualitative description, quantitative analysis, and computer simulation[6].

3.1.2. Biocompatibility

The ability of biomaterials to have an appropriate host response in a given situation is called biocompatibility. For 3D printing, it means that the material is not toxic or harmful to the cells. With the advancement of biological research in cell engineering, the realization of biocompatibility has developed from the initial requirement that the implant material coexists with the host tissues and organs without causing bad local or overall immune response of the host, to the positive effect of tissue interaction between the implant material and the host. In a recent study, dental implants from the mining industry were subjected to acid etching and sand blasting to form nanostructured surfaces. Only a small number of inflammatory cells were observed after 4 and 12 weeks of implantation, demonstrating the biocompatibility of the implant. In addition, the microenvironment within the stent is expected to promote the growth of blood vessels within or around the implant in the weeks following implantation, creating an enabling environment for the movement of nutrients, oxygen, and waste. Since all by-products should be harmless, easily digested, and rapidly eliminated from the body, the by-products produced during degradation also determine the biocompatibility of the material[7].

3.1.3. Degradability

The ideal bioprinting materials should be degradable. After transplantation, it should be degraded at a similar rate as the extracellular matrix (ECM) is generated after the material is transplanted into a host and degraded[8]. At the same time, the by-products produced in the degradation process should be non-toxic, harmless, easy to metabolize, and can be quickly discharged out of the body. Harmful degradation

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by-products generally include small molecular weight proteins or other substances capable of altering the physiological environment of the host, thereby negatively affecting the structure and function of cells in the host.

3.2. Biological 3D printing polymer materials

3.2.1. Synthetic polymer materials

Synthetic polymer materials have the characteristics of high stability, good solubility and measurable degradability, and play an important role as a highly applicable component in the construction of 3D biological products. Chemical synthesis is often used to precisely tailor materials for a variety of biological donors based on specific mechanical and chemical properties.

Polyglycolic acid (PGA) has the characteristics of high chemical adaptability, simple processability, good biocompatibility, etc. Glycolic acid monomer generated from PGA degradation can be converted into carbon dioxide via in vivo catabolism pathway, thus being excluded from the body, while serine continues to enter the circulation for catabolism or synthesis[7]. Copolymers of PGA can also retain the mechanical and physical properties of PGA.

Polycaprolactone (PCL) is non-toxic and insoluble in water, with good biocompatibility, good compatibility with organic polymers and good biodegradability. Under the heating condition, PCL exhibits good elasticity and fluidity, and is often used as a scaffold material[9]. For example, SLS- PCL scaffolds have the characteristics of porous structure that promotes attachment, rough surface, and bone-like tightness that promotes bone regeneration and cell growth. However, the strong hydrophobicity of PCL results in a decrease in its biological activity, negatively affecting tissue adhesion and cell growth and development.

3.2.2. Natural polymer materials

Natural polymer materials generally exist in solution or hydrogel state and have a certain liquidity.

Gelatin is a kind of protein obtained by partial hydrolysis of collagen, which belongs to natural protein and has good degradability, biocompatibility and low antigenicity. The gelatin solution is relatively sensitive to temperature, and can be gelatinized by temperature change to maintain the shape of the printed structure [7].

Gelatin with different concentrations and its mixtures with other high-molecular materials are widely used in biological 3D printing. In a previous study, the myoblasts were wrapped with gelatin-alginate composite material, and the mechanical properties of the bioprinting of soft tissue structures using this material were also studied. The researchers observed that the mechanical strength of cell-loaded constructs decreased during culture, but that the low porosity and geometry of the structures maintained their mechanical durability, while the cells continued to proliferate as culture time increased, despite a dramatic decrease in cell activity in the first few days after printing due to the effects of low temperature[10].Meanwhile, modification products of gelatin, such as photoreactive methacrylated gelatin (Gel MA) are also often used for biological 3D printing. Gelatin was sensitive to temperature, but temperature induction was extremely unstable. However, Gel MA could crosslink with other molecules very stably, making it simple to regulate the physical and chemical properties of printed products, while maintaining high cell proliferation activity[7].

4. Bio-printing of neural tissues

The nervous system is the most important connection and control system in the human body. Moreover, it is the most functionally complex system in the human body, consisting of individual specialized cells that regulate the activities of various organs and receive and conduct signals from inside and outside. The nervous system mediates sensation, perception, movement, language and emotion. Our understanding of the structure and function of nervous system allowed us to perform 3D-BIOPRINT in vitro.

4.1. Main cell types in the nervous system

The basic functional unit of the nervous system is neurons, which have many protrusions. A large number of dendrites increases the surface area for receiving nerve signals. Neural axons are usually long and are responsible for conducting nerve impulses by means of universal electrical signals. Axons have

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terminal branches at the end, and each branch then comes into contact with the cytosol or dendrite of the neuron, and also with other controlled cells such as muscle cells. In human central nervous system, nerve fibres become wrapped in membranes formed by other cells called myelin sheaths.

In addition to neurons, glial cell is also an important part of the nervous tissue. Glial cells do not conduct nerve impulses, but they support, nourish and protect neurons. Therefore, healthy glia cells are very important for the normal function of neurons. In the central nervous system, glial cells include oligodendrocytes, astrocytes and microglia; in the peripheral nervous system, glial cells include Schwann cells and satellite cells.

4.2. 3D bioprinting for the central nervous system

The central nervous system mainly includes brain and spinal cord. Due to the complexity of the central nervous system, there is a huge challenge for 3D printing in vitro. At present, only some central nervous function units can be reproduced and simulate some structures and functions. Brain is the most complex organ in the human body. The ability to produce brain organoid from human pluripotent stem cells in vitro has been demonstrated. Scientists have shown that brains cultured in vitro with Induced pluripotent stem cells can survive and function normally when transplanted into the mouse cortex. Using a modified porous gelatin/alginate/fibrinogen hydrogel that mimics the extracellular matrix to build a glioma stem cell model, the cells will proliferate and retain characteristics in vitro without difficulty. Scientists can use this model to better study the nature of glioma stem cells and find anti-cancer targets[11].

Spinal cord injury is a serious and disabling injury to the central nervous system, and bio-3D printing offers a promising treatment for SCI. Three-dimensional bioprinted scaffolds are a promising method for spinal cord injury (SCI) repair. They can be customized in size, structure, mechanical properties, degradation, and bioactivity. These patient-specific scaffolds are designed to create a suitable microenvironment for the injured spinal cord, promoting nerve regeneration and improving functional recovery. Researchers believe that choosing the right biological material can help damaged axons in the spinal cord to recover. For example, collagen-chitosan combinations have shown good compatibility and improved mechanical properties, resulting in enhanced regeneration of nerve fibres, functional recovery and reduced scar formation. 3D printed conductive hydrogels are also being investigated for their potential use in SCI repair. There have now been many successful animal experiments[12].

4.3. 3D bioprinting for Peripheral nervous system

The peripheral nervous system is responsible for the afferent and efferent signals that allow the central nervous system to control all parts of the body, and many organs cannot function autonomously without the peripheral nervous system. Peripheral nerves can get damaged easily by trauma or neurodegenerative diseases. It has been reported that iPSC-Derived neural crest stem cells and Schwann cells can all be used as supporting cells in bioprinting. For the printing of peripheral nerves, synthetic and natural polymers are the most popular materials. Studies have demonstrated that the right polymer can modulate cell growth signals and direct axonal growth. Using both natural and synthetic polymers allow the native physiological environment of healthy nerve tissue to be mimicked[13]. The choice of printing method was also crucial, and after trying various methods extrusion and electro-hydraulic jet printing seemed the most suitable for printing peripheral nerve tissue with stem cells. Two years ago, there were surprising advances in 3D printing of the peripheral nervous system, as Ning et al. created a composite hydrogel scaffold encapsulated in Schwann cells, which supports axon growth and survival[14].

Peripheral nerve injury is generally treated with autologous nerve transplantation or nerve guidance conduit. 3D bioprinting provided the ability to create complex, customizable nerve tissues that mimic the original shape of the nerve. Owens et al. used various biomaterials, Schwann cells and mouse bone marrow-derived mesenchymal stem cells (BM-MSCs) to create multi-lumen linear nerve guidance conduits to repair peripheral nerve injuries. This design helps to better mimic the natural arrangement of nerve fibers, thus facilitating nerve injury repair and functional recovery. In a sciatic nerve injury rat model, motor and sensory functions were restored after three weeks of conduit implantation. Unfortunately, although 3D-printed nerve guides can bridge nerve gap defects, they hardly outperform nerve autografts. The perfect nerve repair material needs to be further explored, and the transformation from quantitative to qualitative change requires long-term efforts[15].

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5. Challenges and ideal standards for 3D printing of neural tissue

It goes without saying that printing neural tissue still faces many insurmountable challenges. Firstly, only a very small number of specific nerve cells have been studied. Secondly, key sites may lose function after successful transplantation, and there is no way to accurately control the differentiation and growth of stem cells by humans. Thirdly, the optimal combination of biological cells and cellular materials is still being worked out. Due to various limitations, the practical application of 3D printed neural cells in the clinic remains rare. Careful consideration should be made for the choice and design of biomaterials, based on the following criteria: i) mechanical properties of biomaterials that attempt to mimic neural tissues; ii) printability—printing resolutions of less than 50 μm are desired to match with native tissue networks structurally; iii) integrity, low toxicity, and suitability for multilayered channel construction; iv) biodegradation properties; and v) cell compatibility or containing biological components necessary for neural cell proliferation and adhesion. The ideal nerve tissue should be able to perform its physiological work smoothly and precisely, and be individually designed to suit the needs without side effects such as exclusion reaction[11].

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