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Application of 3D Bioprinting in Cartilage Tissue

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Abstract. 3d bioprinting is a branch of additive manufacturing rapid prototyping technology that precisely controls the spatial assembly of cells, biomaterials and biological factors, and personalizes macro and micro structures on demand. In addition to personalizing the repair of tissues and organs, the emergence of this technology provides a new way for transplant technology to solve the source and rejection. This article not only introduces the principle technology of 3d bio-printing and bio-ink, but also uses the experiment of cartilage tissue to further discuss.

Key words: 3d Bioprinting, Bio-Ink, Cartilage Tissue.

BACKGROUND AND PRINCIPLE

With the development of the economy and the improvement of material living standards, the movement has become more and more popular, the joints of the limbs have been damaged more and more, and the incidence of articular cartilage wear has also increased. The demand for various cartilage tissue loss and deformity repair is greatly increased. However, traditional autologous cartilage transplantation has limited sources, so people began to repair cartilage tissue through 3D bioprinting technology. 3d bioprinting technology has great potential in tissue engineering. 3d bioprinting technology is a branch of 3d printing technology. The material used in this technology is biomaterial or living cell, which requires biological material or living cells as its bio-ink. . The most prominent advantage of 3d bio-printing is that it can achieve co-printing of living cells, biological materials and growth factors, and create personalized macroscopic and microscopic structures. At the same time, it can construct a biologically active tissue or organ. By using autologous cells to construct three-dimensionally printed tissues or organs, immune rejection can be greatly avoided, and ethical issues and questions are greatly reduced. At present, the widely used 3D bio-printing technology mainly includes inkjet technology (thermal inkjet and piezoelectric inkjet), pressure-assisted printing technology, and laser-assisted printing technology. The composition of bio-inks, in addition to the original living cells, also includes natural polymers and synthetic polymers, each with advantages and disadvantages.

TECHNOLOGY AND METHODS

3D bio-printing technology and its characteristics currently available 3D bio-printing technology mainly has the following types: ink-jet bio-printing technology, pressure-assisted bio-printing technology, laser-assisted bio-printing technology. Each method has a wide range of research, and each has its own advantages and limitations (Table 1).

TABLE 1. Comparison of common bioprinting technologies

Printing method	Fast printing	Cell viability (%)	Resolution (μm)	cost
inkjet printing	speed	~90	20~100	low
Squeeze printing	slow	40~80	200	Medium
Laser bioprinting	Medium	>95	>20	high

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Inkjet Technology

Inkjet technology is a commonly used 3D tissue printing technology. Under the control of a computer, bio-ink droplets are formed by heat or electricity, and are non-contact-exited from the nozzle of the inkjet head in microliters, and accurately printed. To a specific location in the hydrogel matrix or culture dish. Among them, thermal inkjet technology uses the power of foam expansion to eject ink from a narrow nozzle by locally heating the inside of the print head to a range of 200-300 ° C. This technique is widely used because of low cost, but bio-ink often appears. Some problems related to thermal energy: resulting in uneven droplet size and disordered species; frequent nozzle clogging of nozzles is difficult to meet the smoothness of printed tissue; shear and thermal stress also affect cell and protein activity. Piezoelectric inkjet technology uses a pulse generated by a piezoelectric crystal pulse actuator to periodically eject small water droplets, because the process does not use thermal energy, does not cause nozzle clogging and ensures uniform droplet size and directional ejection; however, too frequent The use of piezoelectric inkjet technology destroys the cell membrane and produces cell lysis.

Pressure-Assisted Technology

Pressure-assisted technology is a method of creating the desired 3D structure based on the principle of physically squeezing the biological ink droplet directional deposition. The biological material used for printing is usually a liquid or dispersion, by pneumatic pressure or a plunger. The form of the screw pressure coordinates the squeezing motion to form continuous filaments in the micro-scale nozzle holes or micro-pinholes fixed to the substrate. Among them, the air pressure assisting technology uses a set of electromechanical microvalves to generate droplets by opening the microvalve at a constant pressure. This technology has the advantage of using various types of liquid biomaterials to directly distribute the cells evenly, and the viscosity can be as high as possible. 200 Pa•s and can be handled at room temperature.

Laser Assisted Technology

Laser assisted technology typically consists of three parts: a pulsed laser source, a ribbon deposited on a metal film coated with a liquid biomaterial, and a receiving substrate layer. The laboratory mainly uses nanosecond lasers and ultraviolet or near-ultraviolet wavelengths as energy sources to print biomaterials such as hydrogels, cells, and proteins. The laser illuminating the ribbon causes the liquid biomaterial to evaporate, reaching the receiving layer in the form of droplets containing a biopolymer or cell culture medium to support cell adhesion and subsequent growth. The thickness, rheological properties of the biomaterial, the energy of the laser pulse, and the wettability of the substrate, the printing speed, and the type of tissue structure all affect the resolution of the laser printed tissue. In addition, because this technology can only use low-viscosity or liquid biomaterials to make tissue/organ analogs, there is often not enough mechanical strength to maintain its shape, and the cost of using laser sources is high, and the control of laser pulses is also very good complexity.

BIO-INK MATERIAL

Biological Ink

3d bioprinted materials are usually fluids containing nutrients, mechanism components, and cells. There is currently no ideal "bio-ink" specifically for cell printing. Various hydrogel materials are known to be used to construct 3D tissue structures. However, many factors related to the material must be considered, such as printability and cross-linking ability, rheological properties and mechanical properties, biocompatibility and polymerization mechanism, cytotoxicity and degradation controllability.

Biomaterials

Biomaterials usually give cell strength and protection. They must meet the following conditions: (1) printable; (2) suitable physical and chemical properties; (3) good biocompatibility and biological activity, (4) Good mechanical properties; (5) clinical feasibility.

The most widely used natural biomaterials are organogel materials such as alginate, gelatin, hyaluronic acid, collagen and the like. Currently the most widely used in bioprinting are hydrogels, which have good biocompatibility and biodegradability, and their hydrophilic nature makes them contain a large amount of water in a three-dimensional structure. At the same time, it is very similar to the tissue extracellular mechanism components.

Collagen is a structural protein of the extracellular matrix. It is mainly found in the connective tissue of animals. It plays a role in supporting, protecting, binding and forming compartments of the body and organs. Collagen contains a large amount of glycine, proline and hydroxyl. Proline residues, and participate in cell-cell, cell-matrix signal transduction, growth factor and cytokine transport, and collagen can promote cross-linking by thermogel action under physiological conditions. Therefore, most of the substrates in 3D bioprinting consist of this material.

Alginate is a hydrogel formed by complexing natural anionic polysaccharide alginic acid derived from brown algae with multivalent cations including Ca2+, Ba2+ and Fe3+. Alginic acid can be easily modified to provide bioactive molecules for a variety of tissue engineering applications; cross-linked alginic acid also has a structure similar to that of human cell matrices, has good biocompatibility, and has a short gelation time. It is more popular than alginic acid in the field of tissue engineering.

The polylactic acid-glycolic acid copolymer is a functional high molecular organic compound obtained by randomly polymerizing lactic acid and glycolic acid. The ratio of lactic acid and glycolic acid determines the biocompatibility and controllable degradability of the polylactic acid-glycolic acid copolymer (the ratio of the two is 50:50, the degradation rate is faster), and the degradation products have no toxic and side effects. It has been used to make artificial catheters, drug delivery vehicles, and tissue engineering scaffold materials. Polylactic acid-hydroxyacetic acid copolymers are thermoplastic materials and are therefore popular in 3D biolaser printing technology.

APPLICATION

3D bioprinting of tissue analogs containing living cells has shown great potential in the field of tissue engineering. Despite extensive research, current printing techniques can only accomplish relatively simple tissue structures and biological functions, and 3D tissue bioprinting with living cells is still in a very early stage of exploration.

Cartilage is a banded tissue structure with no nerves and no blood vessels. 3D bioprinting technology generates precise spatial patterns and creates different grades of cartilage tissue by controlling the different biomaterial properties and cell types in each print layer. For example, articular cartilage tissue components have different cell densities, morphologies, mucopolysaccharide compositions, and mechanical properties in different regions: precise deposition of chondrocytes and polyethylene glycol diacrylate in 3D printed settings, mimicking different anatomy the region produces cartilage structures, which can produce different porosity and elastic modulus by changing fiber spacing or fiber deposition. Human chondrocytes and osteoblasts can also be added to different anatomical regions to simulate osteochondral tissue structure. In addition to physically distinguishing different regions, biochemical gradients can also be achieved by combining printing and packaging of human mesenchymal stem cells, bone morphogenetic protein 2, and transforming growth factor β1.

Cartilage defect repair is a clinical problem in orthopedics. The development of this technology has brought hope to the repair of cartilage defects, in which the mechanical and biological properties of the scaffold material are the key factors for cartilage regeneration. The design of cartilage tissue engineering scaffolds must take into account the growth of cells and tissues, as well as the mechanical properties and degradation rate of the scaffolds. In recent years, with the aid of computer-aided design and 3D printing technology, 3D printed porous scaffolds constructed using medical polymer materials have controllable appearance and internal structure and high mechanical strength, which is an ideal scaffold material for repairing cartilage damage.

The type II collagen and the silk fibroin were uniformly mixed at a mass ratio of 3:7, filtered through a mesh filter to remove the uneven bulk compound, and then placed in a syringe, centrifuged to remove bubbles and sealed. The stent model was designed using Solidworks software in Fig.1: the shape of the model and the internal pore shape were set to square, the hole spacing was 0.8 mm, and the layer thickness was 0.3 mm, for a total of 8 layers. A silk fibroin-type II collagen complex having a lattice structure was prepared by using a low-temperature 3D printer to adjust the printing speed and the extrusion speed, and then the silk fibroin-type II collagen composite scaffold was obtained by freeze-drying.

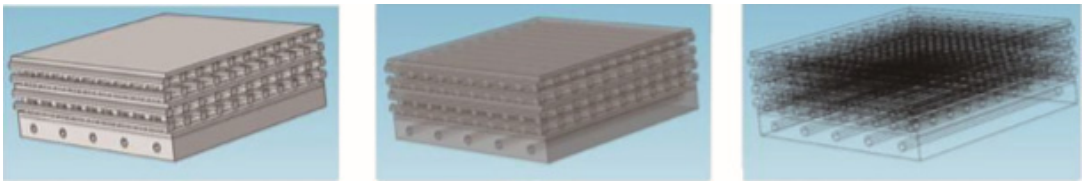

FIGURE 1. A proposed hybrid scaffold design for vascularization.

CONCLUSION

The proposed cartilage tissue engineering provides a more effective treatment for cartilage repair. Choosing materials suitable for the development of artificial cartilage with mechanical properties and biological functions has become a top priority. Artificial cartilage scaffolds should also have suitable porosity and pore size to facilitate cell growth and organization. The main materials currently used for cartilage repair are natural materials, artificial materials and composite materials, among which natural materials are mainly collagen, hyaluronic acid and chitosan. The pore structure of the cartilage scaffold has an important influence on the mechanical properties of the scaffold. At present, a method for preparing a cartilage scaffold with high porosity and good permeability is particle leaching. Filtration, freeze drying, electrospinning, and 3D printing. Low temperature 3D printing technology acquires the physical structure of an object by tomography or magnetic resonance imaging. Data, using computer 3D design software to reconstruct the image model, according to various parameters, the appropriate materials are printed and stacked layer by layer at a low temperature in a low temperature state.

Form a three-dimensional scaffold. In recent years, with the advancement of 3D bio-printing technology, people have further refined the repair of cartilage defects, and now they are coming. The more people begin to realize that articular cartilage and subchondral bone are a complete functional unit.

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