Retraction

Retraction: 3D Bio-Printing Review (IOP Conf. Ser.: Mater. Sci. Eng. 301 012023)

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[1] Ibrahim T. Ozbolat, Monika Hospodiuk, Current advances and future perspectives in extrusion-based bioprinting, Biomaterials, Volume 76, 2016, Pages 321-343, ISSN 0142-9612, https://doi.org/10.1016/j.biomaterials.2015.10.076

[2] Murphy, S., Atala, A. 3D bioprinting of tissues and organs. Nat Biotechnol 32, 773-785 (2014). https://doi.org/10.1038/nbt.2958

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3D Bio-Printing Review

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Abstract. Ultimate goal of tissue engineering is to replace pathological or necrotic body tissue or organ by artificial tissue or organ and tissue engineering is a very promising research field. 3D bio-printing is a kind of emerging technologies and a branch of tissue engineering. It has made significant progress in the past decade, 3D bio-printing can realize tissue and organ construction in vitro and has wide application in basic research and pharmacy. This paper is to make an analysis and review on 3D bio-printing from the perspectives of bioink, printing technology and technology application.

1. Introduction
Tissue engineering is an emerging science that has been developed since 1980s and it’s related to chiastopic fusion of a series of disciplines including clinical medicine, biomaterials science, cytobiology, molecular biology and bioengineering. Its goal is mainly to generate substitutive tissue and organ inside or outside the body to recover the functions of injured tissue and organ. Huang Jiefu once published an article in The Lancet to claim that about 1.5 million people were in need of organ transplantation due to organ failure at late phase every year in China but the quantity of organs could be used in each year was less than 10,000, and the supply-demand ratio reached 1:150[1]. What’s more, the quantity of patients in need of organ transplantation is increasing by over 15% annually in China. Luckily, the progress of tissue engineering may bring hope to these patients for survival.

Although many manufacturing technologies have been introduced to tissue engineering, most of them have large limitation in biocompatibility, control precision and controllability 3D bio-printing technology is a revolutionary method that is introduced lately. It is used to manufacture artificial three-dimensional tissue and organ, and extensively applied in manufacturing stent for tissue engineering. Comparing with traditional methods of seeding after stent manufacture, 3D bio-printing technology directly deposits the mixture of cells and biological materials and mixes cells into stent model in manufacturing process of stent. The mixture of high-density living cells and bioink is printed out by bio-printer through computer control and it is a biological overlapping process of 3D layers. Bio-printing has the following advantages. It’s a completely automatic mode and can be used to manufacture renewable tissue engineering products in a large scale [2]. Besides, it allows precise layout of three-dimensional positions of different types of cells, which makes structured artificial tissue have relatively large cell density and relatively more cell types.

2. Bio-Ink Frequently Used in 3D Bio-Printing
Hydrogel is the most frequently used bio-ink in bio-printing. It is a kind of colloidal material mainly composed of moisture, and can form cross-linked structure through covalent bond. In recent years, hydrogel has become more popular in the field of tissue engineering because of its excellent biocompatibility and biodegradability. When cells are sealed in hydrogel, its stereoscopic net cross-linked structure will facilitate exchange between cells and surrounding medium. Hydrogel includes natural hydrogel and synthetic hydrogel. Up to now, the hydrogel used in 3D bio-printing includes
natural hydrogel such as collagen, alginic acid, agarose and chitosan, and synthetic hydrogel such as hyaluronic acid and PEGDA etc. A few kinds of hydrogels frequently used in bio-printing are introduced as below.

2.1. Sodium Alginate
Sodium alginate is a kind of polysaccharide mainly derived from brown algae and bacteria. It is widely used in 3D bio-printing because of its biocompatibility, low price and rapid gel. It is also used in different 3D bio-printing systems due to its property of instant gelation in calcium ion solution (such as calcium carbonate, calcium chloride and calcium sulfate). In recent studies, sodium alginate is frequently mixed with other biological materials [3] which are convenient for molding with favorable biological nature. In addition, the low-concentration sodium alginate has poor mechanical capacity but it is beneficial to promote viability and proliferation of cells.

2.2. Chitosan
Chitosan has the capacity of biodegradation, antibacterial and sterilization, and is used in wound dressing etc. Chitosan hydrogel is widely used in bone, skin and cartilage tissue engineering. Chitosan has slow gelation speed (10min after injection) with low mechanical capacity; therefore, only stent with high viscosity can maintain the shape for hours. Chitosan can be dissolved in acid solution and cross-linked through ion and covalent bond. It will be gelatinized rapidly when its PH value is increased. Under condition of neutral PH value, the chitosan dissolved in water will be gelatinized when temperature is 40°C. In 3D bio-printing, chitosan is used to produce all kinds of stents and micro-flow channels.

2.3. Gelatin
Gelatin has excellent biocompatibility, hydrophilic property and non-immunogenicity. It is 100% biodegradable in vivo [4]. Gelatin is a kind of thermo-reversible gel; it is solid under low temperature and its mechanical property is unstable under physiological conditions. In order to make gelatin structure stable fewer than 37°C, its chemical property will be changed. Cross-linking will happen to the gelatin modified with methacrylamide with existence of photoinitiator. Methacrylate complex hydrogel (GelMA) can be easily extruded for molding through ultraviolet irradiation, which is widely applied in 3D bio-printing. The printing property of GelMA is closely related to concentration of gel, duration of ultraviolet exposure and cell density. The duration and intensity of ultraviolet irradiation will affect viability of cells, density and intensity of hydrogel.

2.4. Hyaluronic Acid
Hyaluronic acid is also called as sodium hyaluronate. It has been widely applied in clinics as skin filler and joint lubricant [5]. It plays a crucial role in adjustment of cell behavior and function, such as cell spread, proliferation and angiogenesis etc. In the 3D printed hyaluronic acid hydrogel sealed in cartilage tissue, the viability of cells is higher than those in collagen hydrogel. However, hyaluronic acid has poor mechanical property due to its feature of rapid degradation, which can be improved through chemical modification to control degradation rate. Due to such reason, hyaluronic acid is not suitable for 3D bio-printing. Nevertheless, cross-linking of hyaluronic acid can be realized through functional treatment by photocuring methyl acrylate (MA) to control duration of photopolymerization.

2.5. Agarose
Agarose hydrogel is a kind of linear polymer with heat reactive and thermo reversible characteristics. During printing of low-melting-point agarose, the extruded agarose filaments will be rapidly solidified on refrigeration platform. Campos et al [6] sealed mesenchymal stem cells in agarose gel for 3D bio-printing. The whole structure was supported by fluorocarbon. Tubular structure was formed after cell deposition, and nearly 100% cells survived 21 days later. Agarose is also frequently applied in 3D cell culture platform, supporting cell clusters through its natural cell attachment inertia.
3. Mainstream Technologies of 3D bio-printing

3.1. Extruded Biological 3D Printing Technology

Extruded printing used material crosslinking mechanism is generally divided into three types: (1) chemical crosslinking, sodium alga acid and chitosan for example; (2) photo-crosslinking, GelMA for example; (3) physical crosslinking, agarose for example. Different printing methods are used for crosslinking means.

For chemical crosslinking materials such as sodium alga acid, there are generally the following printing methods: (1) bioplotting: as shown in Fig 1A, hydrogel with encapsulated cells is squeezed into cross-linking agent pool, extrusion shall be done in the pool, printed stent shall be left in the pool until printing is completed. The density of extruded bioink shall be larger than that of crosslinking pool. The process of extrusion and deposition can be controlled through changing the temperature and viscosity of crosslinking pool. (2) Secondary nozzle assisted printing: as shown in Fig 1B, secondary nozzle is used to spray cross-linking agent to main nozzle to make bioink extruded from main nozzle be crosslinked rapidly, secondary nozzle can rotate around main nozzle. (3) Coaxial nozzle system printing: as shown in Fig 1C, a coaxial nozzle device is used to extrude bioink from inner tube, and cross-linking agent is extruded from the interlayer between inner tube and outer tube. (4) pre-crosslinked printing: as shown in Fig 1D, make bio-printing of pre-crosslinked alginic acid and the alginic acid with pre-crosslinking ability (relatively low crosslinking degree) provides enough material strength. After printing is completed, bio-printed stent is immersed in crosslinking solution to realize complete crosslinking. This method of printing guarantee better structural mechanical property but discontinuous filaments may appear in spinning process if bioink is not balanced. (5) Atomized crosslinking printing: as shown in Fig 1E, alginic acid is printed on a platform and ultrasonic humidifier is used to atomize the crosslinking solution to make the entire space full of liquid drops. Tiny crosslinking solution particles are generated in atomizing process and can be symmetrically distributed on the entire printing structure, which realizes simultaneous crosslinking among layers to form a complete structural body.

Figure 1. Schematic diagram of printing principle for ion crosslinking materials

Printing method for light-cured materials such as methacrylate composite hydrogel ((GelMA) is shown in Fig 2A. Ultraviolet irradiation is utilized when material is extruded. Printing performance of GelMA is closely related with concentration of hydrogel, ultraviolet exposure time and quantity of cells. Duration and intensity of ultraviolet irradiation will influence the viability of cells and mechanical strength of hydrogel.
There is thermo sensitive and thermal reversible hydrogel. For example, agarose can be printed by the device shown in Fig 2B. The agarose of a low melting point is in liquid state when heated in charging barrel, extruded agarose rapidly solidifies on the refrigeration platform but structural collapse may easily occur when printed structure is higher and high but low temperature of the platform can not be conveyed rapidly to agarose filament.

Figure 2. 3D bio-printing schematic diagram

3.2. Inkjet 3d Bio-Printing Technology
The first technology used for organ printing is inkjet bio-printing technology and it belongs to contactless printing. Inkjet bio-printing has two printing types, i.e., piezoelectric type and hot-bubble type. Hot-bubble printing technology utilizes heating element in operation. Heating element acts on the ink near nozzle to make rise of material temperature and gasification to generate bubbles and force liquid drops to overcome surface tension and extrude successfully. However, high temperature and pressure in the process will cause irreparable damage and influence the activity of cells after printing. As shown in Fig 2C, thermal inkjet printer utilizes electric heating print head to generate steam bubbles so as to make nozzle spray liquid drops while piezoelectric inkjet printer utilizes piezoelectric effect to generate pressure pulse.

3.3. 3D Laser Bio-Printing Technology
Laser bio-printing (BioLP) device is mainly constituted of laser source, target boss and receiving layer. Laser source mainly adopts single wavelength and pulse laser device, laser boss is constituted of transparent substrate, light absorbing layer and biological solution coating layer. Receiving layer serves as buffer layer and generally it refers to ordinary glass slide. Transparent substrate in target boss usually adopts quartz slide that almost absorbs no laser. Laser absorbing layers are mostly made through film coating of metal or metallic oxide and only a few of them adopt thin film of high-molecular polymer. Biomaterial coating layer generally refers to mixing layer of cells and biological materials [7]. As shown in Fig 2D, high-energy laser pulse is illuminated on energy absorbing layer. Instantaneous high temperature generates steam bubbles to push the mixture of cells and materials to the basal plate.
4. Application of 3D bio-printing technology

4.1. Artificial Blood Vessel
Doctor Gunter Tovar in Germany has already utilized 3D printing technology to create artificial blood vessels [8]. Although artificial blood vessels had been developed successfully early in the 1950s but it was limited to large artery blood vessels and no breakthrough progress has been made in research on vein blood vessels or blood capillaries whose diameter is less than 6mm. The primary cause is that artificial capillaries shall not only be enough tiny but also have elasticity and biocompatibility comparing favorably with real capillaries. The blood capillary created by a German scientist by utilizing 3D printing two-photon polymerization and biological functional modification has favorable elasticity and human body compatibility and it is not only able to replace necrotic blood vessels but also combine with artificial organ. It’s possible to realize revascularization of structured tissue or organ.

4.2. Artificial Skeleton
Human skeleton has extremely irregular forms and there is a large difference between individuals. Thus, it makes little sense to make batch manufacturing of artificial bones but personalized artificial bones are extensively required in clinical application. A research group led by Christian Weinand from Bernsel Hospital in Switzerland successfully makes a biological copy of thumb bone [9]. Joint Research Center of the First Hospital Affiliated to AMU (Southwest Hospital) has already had a three-dimensional print for skeleton printing. Doctor Wang Fuyou in this section utilizes the printer to print teaching entity organs and also explains operation plans to patients with printed “artificial organs”. It’s possible to use printed organs in human experimentation.

4.3. Stomatology
Forms and structure of human skeletons and teeth are both very complicated. Organizational structures are diverse. In order to adapt to alveolar structure, development and change in teeth show totally different trends. Thus, there are a lot of problems difficult to be solved by using traditional problems too difficult to solve in tooth regeneration. However, 3D bio-printing technology can make computer-aided prototyping design to meet the requirement on individualized production. Thus, 3D printing technology also has extensive application in stomatology. Xue Shihua, et al from Peking University School of Stomatology has successfully made 3D bio-printing of human dental pulp cell blends. It is found through evaluation that the survival rate of printed cells can reach 87±2%. The research indicates that bio-printing technology has feasible application in human tooth tissue engineering and can be possible used in tooth regeneration engineering in the future.

4.4. Artificial Skin
Researchers including Lee from Harvard Medical School adopt inkjet printer to print skin texture and method of layer accumulation is adopted. At first, a layer of fibroblasts are sprayed after two layers of collagen are printed, and then 6 layer of collagen are printed and 2 layers of keratinocytes are printed on the surface. The cross-linking agent used in printing process is sodium bicarbonate [10]. Due to relatively large thickness of 6 layers of collagen in the middle, in order to strengthen intercellular communication, a channel is printed among 6 layers of collagen and gelatin is used as sacrificial material. The mixture of collagen and gelatin is printed in low temperature, temperature temperature after printing is 37°C and gelatin will turn into liquid state and flow out from the tube when cultivated in the environment with 5% of CO₂, and it contributes to the formation of skin texture and perfusion channel similar to blood vessel may be generated. Experimental results are compared to find that the survivability of skin texture cells with pores is larger than those without pores.

5. Conclusion
3D bio-printing technology has high precision and fast construction speed. Printing as required meets the requirement on individualized medical treatment and has advantages such as low rejection reaction. But it is also confronted with relatively large challenges in biomechanics, selection of stent material, guarantee of bacteria-free environment, molding of printed structure, blood supply for printed structure
and long-term survival of printed structure at the same time. Thus, 3D bio-printing technology is not yet a completely mature technology and it is still in need of researchers’ unceasing efforts and breakthroughs. At present, the technology has not been extensively applied in clinic. Such as it is, not a few of domestic and foreign research centers and labs have extensively made clinical experiments of 3D bio-printing technology that is mostly applied in bone and cartilage tissue engineering. Besides, blossom and yield fruit are also achieved in various clinical areas including oral medicine and aesthetic medicine. In general, 3D bio-printing technology is one of 3D stereocheimical structure construction technologies in stereocheimical structure. It has a wide application prospect and is a research focus in many current disciplines including life science, materials science, engineering science and medicine science. If “bio-printing” technology is mature, human organ may be replaced at any time in the future decades so as to extend human life cycle. However, the road ahead is still long and it’s in need of joint efforts, integration and breakthroughs of various disciplines to solve the problems encountered in its development.

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7. References
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