Recent Advances in 4D Bioprinting

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Abstract

Four-dimensional (4D) bioprinting, is generally accepted as the future of biofabrication technologies. 4D bioprinting develops dynamic and 3D based biological materials which can shift their shapes or alter their behaviors when several stimulants like electricity, temperature, humidity, magnetic etc. are applied. In this review, we highlighted the important aspects of several smart materials for 4D bioprinting that have been used recently for biofabrication researches. It is believed that in immediate future, smart materials and 4D Bioprinting techniques will have an excessive importance for designing of soft robotic systems and architecture of hierarchial, compex, thick and vascularized tissue structures

4B Biyobaskı Çalışmalarında Güncel Yenilikler

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Özet

Dört boyutlu (4B) biyobaskı tekniklerinin biyofabrikasyon teknolojilerinin geleceği olduğu düşünülmektedir. Elektrik, sıcaklık, nem, manyetik vs. gibi uyarıcılar aracılığı ile şekil değiştiren akıllı malzemeler kullanılarak ortaya çıkartılmış olan 4B biyobaskı tekniği, 3B biyolojik materyallerden oluşmuş ve zamanla şekil değiştirebilen yapılar üretilebilmektir. Bu mini derlemede bu alanda son yıllarda ortaya konmuş olan pek çok akıllı malzeme ve bunların önemleri açıklanmıştır. Akıllı malzemelerin ve 4B biyoyazıcı tekniklerinin çok yakın bir gelecek içerisinde yumuşak robotik sistemlerin tasarımlarında ve hiyerarşik, kompleks, kalın ve damar dokusu eklemlenmiş doku yapılarının tasarımlanmasında aşırı derecede önem kazanacağı düşünülmektedir.

1. Introduction

Numerous useful information and applications have been obtained from the structures obtained as a result of the combination of living cells and biomaterials in many different methodologies used by scientists. However, there are significant problems in real applications of the obtained cell and non-cell biostructures in the dynamic environment of the human body. For this reason, 4D biofabrication have recently emerged, especially by taking advantage of 3D bioprinters with adding 'time' as the fourth dimension. Here, the objects obtained from the 3D bioprinting can change their morphology and functionality by external stimuli such as temperature, pH, ion or internal ones i.e. cells and their components. These biostructures, which can adapt to the dynamic environment in the human body, are expected to bring significant advantages in tissue regeneration, treatment of diseases and drug development.

Environmentally responsive biomaterials have a strong potential to be used in 4D bioprinting which can be grouped under two main headings. These are chemical stimuli that alter molecular interaction, such as pH, CO_2, salt and various solvents. For example, pH-responsive hydrogels

based on alginate can be used for this purpose. Furthermore, physical stimuli for example heat, light, magnetic with electrical field, ultrasound and mechanical strength play an important role in altering polymer chain dynamics to guide 4D biostructures. For this purpose, various studies have been conducted on biostructures that can be changed at different temperature ranges [1-2]. In addition to external stimuli, biochemical stimuli, such as cell traction forces, enzymes, glucose, antigen, amino acid, nucleic acid, polysaccharides, and etc. are important potentials to utilize the actual functions of biological components. For example, the mixtures obtained by inoculating the antigen and antibody groups interacting with each other into different polymer chains may form crosslinked structures, while the free antigen added to the medium can be displaced by breaking the existing antigen-antibody interaction yields gel swelling behavior [3].

2. Limitations of 3D Bioprinting

3D-bioprinting is an additive manufacturing method for rapid fabrication of large number of complex three-dimensional constructs with precise control in an automated manner. However, there are several drawbacks related with 3D printing. Presently printed constructs may not completely mimic the hierarchical structure of living tissues that have multiple layers [4]. For this reason, 3D-bioprinting technology needs time-dependent behavior that can respond to environmental stimuli. By the help of this, it may be possible to form multiple layers that can accurately mimic the hierarchial and complex structure of the various organ tissues [4]. In the following section of the mini-review, the type of stimuli-responsive biomaterials effectively used in 4D-printing technology is briefly summarized.

In addition, one of the other challenges of 3D-bioprinting technology is non-homogeneous cell encapsulation within the printed constructs that is due to the low-viscosity bioin [5]. Bioink term is mainly used for cell encapsulated biomaterials. Also, high-viscosity bioinks require higher pressures during printing process, and that adversely affect cell viability because of the increased shear forces during the extrusion process [5]. To overcome these bottlenecks among existing bioinks, many scientists are focusing on designing the advanced bioinks with shear-thinning (thixotropic) and rapidly self-healing characteristics. In that way, the designed bioinks will have easy printability and cell viability. This will hinder mechanically damaging effects on the cells. After the extrusion, the viscosity rises, resulting in high-precision printability [6, 7].

3D-Bioprinting technology has gained an enormous attention to fabricate synthetic tissues and organs for transplantation. Greatest current challenge of 3D-printed constructs toward the clinical translation is the absence of multifunctional vascular network for oxygen and nutrient diffusion. Without vascularization, 3D-printed constructs will have insufficient nutrient availability, growing up incomplete tissue formation or necrosis [8, 9]. This is more often due to the printing restrictions in resolution and speed [8]. More time, effort, and multidisciplinary expertise will be needed to fulfill shortcomings in clinical potentials of 3D-Bioprinting technology. All these problems are also needed to solve by 4D bioprinting techniques.

3. Novel smart materials for 4D Bioprinting

Thermo-responsive materials have gained great deal of attention due to their potential application in drug delivery and regenerative medicine [10, 11]. These materials can exhibit significant changes in their physicochemical properties following the change of temperature.

In particular, thermo-responsive material should have lower critical solution temperature (LCST) close or below to physiological temperature in order to manipulate them in easiness [12]. Poly(N-isopropylacrylamide) (PNIPAM) is probably the most widely employed material because of its appropriate sol-gel behavior with its relatively low LCST (≈32–35 ºC) [13, 14]. When the external temperature is below its LCST, PNIPAM behave like a sol. However, above this temperature, polymer solution returns into gel due to sol-gel transition [12]. For instance, Liu et al. designed a dual printed gel tubes using PNIPAM and polyacrylamide. They show the printability of material as well as a number of shape changes such as uniaxial elongation, radial expansion, bending, and gripping [15]. Moreover, there are some attractive reports on printability and cellular viability of printed cell laden thermos-responsive materials to show potential usage of these materials to print 4D structures [16, 17].

Humidity responsive materials are able to absorb or release moistures with the variation in humidity [18, 19]. In particular, the shape and size of the material can change due to swelling up or shrinkage of material in a response to humidity alteration. Herein, it is vital to certainly control the swelling or shrinking rate of humidity responsive materials to maintain the printed construct. Additionally, since cells need a specific osmotic pressure to live, degree of alteration in shape can be adjusted to osmotic pressure [20]. Lv et al. humidity responsive 3D printed poly(ethylene glycol) diacrylate hydrogel using two-photon photopolymerization. They inspired from open and close the stomata of plants to achieve nano-interconnected pores of the structure. Humidity-driven swelling ability was controllable and reproducible and obtained structure was stable even after 10000 cycles [18].

Light can also be used as stimulus to induce shape and size changes of 3D printed devices. Yang et al. [21] generated photoresponsive shape memory composites by incorporation of carbon black into shape-memory polymer polyurethane and printed using fused-deposition modelling (FDM) sunflower like devices. The light illumination converted the closed sunflower shape to opened state as the blooming of the flowers. In a recent study, Cui et al. [22] incorporated photothermal graphene into thermally responsive shape memory polymer to create 4D printed near-infrared light (NIR) responsive nanocomposite brain model. Thermal energy is produced by the absorption of photons of the NIR illumination by graphene. A great advantage of this method is that NIR can efficiently penetrate tissues without biological harm. Light responsive materials and 3D printing can be also combined to release therapeutics. Gupta and

colleagues 3D printed drug capsules containing therapeutics in the core and covering the core with a poly(lactic-co-glycolic) acid (PLGA) shell containing plasmonic gold nanorods [23]. Using an irradiating laser, capsules can be ruptured for a programmable and selective release of drugs.

The functionalization of polymers with magnetic nanoparticles consisting of e.g. iron (Fe), cobalt (Co), or nickel (Ni) enables the generation of magneto-responsive polymeric systems [24]. Wei et al. [25] produced tubular 4D shape-changing structures by incorporation of magnetic iron oxide nanoparticles into poly(lactic acid) (PLA) polymer and direct write printing and ultraviolet (UV) cross-linking of PLA. Heating of iron oxide by alternating magnetic field was able to create sufficient energy to induce the transformation of the shape to the initial tubular configuration. In another study, magnetically responsive structures with a fast response time were created by incorporation of Fe into poly(dimethylsiloxane) (PDMS) and 3D printing [26].

Finally, electric fields can be applied to orientate cells to defined directions or manipulate cells to desired positions. Especially, muscle or nerve tissue engineering can be improved by the electrical stimulation. Cvetkovic et al. [27] developed stereolithographic 3D printed poly(ethylene glycol) diacrylate (PEGDa) hydrogel. The electrical stimulation triggered the contraction of skeletal muscle cell laden strip. Sayyar and colleagues generated flexible conductive composites by incorporation of graphene into methacrylated-poly(thrimethylene carbonate) and UV crosslinking [28]. Thereby, the tensile strength and the electrical conductivity of the polymer was significantly increased. The electrical stimulation further improved the osteogenesis of seeded mesenchymal stem cells.

4. Conclusion

With the exciting developments in the field of 4D bioprinting, more intensive research is needed, especially in order to predict the changes that may occur after printing in a safe and predictable manner. In this sense, it is thought that the development of mathematical models will contribute positively. On the other hand, if live cells are used, changes in the biostructure during and after bioprinting must have minimal adverse effects on the cells, like cellular stress. Particular attention should be paid to the structural design of the biomaterials, rheological properties of the bioink, crosslinkers, additives and etc. Therefore, it is expected that the use of suitable hybrid structures containing soft and hard materials which can adapt to multiple stimuli encountered in vivo with not causing any negative reaction in the immune system. As a result, inevitably total costs of production will begin to rise more rapidly in short term. 4D biostructures, which are expected to bring an advanced level to biofabrication by enabling the realization of exciting real applications in a short period of time, particularly necessitating multidisciplinary studies [29-35].

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