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A REVIEW ON THE HYDROGELS USED IN 3D BIO-PRINTING

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ABSTRACT

The bioprinting process describes as computer-assisted material transfer process of biological structures such as cells and biomaterials to fabricate medical constructions for different medical fields (scaffolds engineering, tissue renewal, and etc.). Especially, 3D (Three-Dimension) bioprinting technology is used to print living organs like livers, kidneys, lungs, and any other organs in the body. Hydrogels are frequently used in 3D bioprinting process due to their high biocompatibility. In addition, hydrogels simulate the soft tissues which present in the body, swollen in water at high levels and have similar mechanical behavior as those of biological soft tissues, many researchers have been interested in hydrogels and their reputations are increasing day by day. They are generally divided into two groups as (i) natural hydrogels and (ii) synthetic hydrogels. Key documents in the literature about both naturally and synthetically derived hydrogels have been reviewed in this study. Also, 3D bioprinting process of the hydrogels has been discussed as detailly.

Keywords: 3D Printer; Bioprinting; Hydrogels

3D BİYO-YAZICILARDA KULLANILAN HİDROJELLER ÜZERİNE BİR İNCELEME

ÖZET

Biyoyazıcı işlemi, farklı tıbbi alanlarda (doku mühendisliği, rejeneratif tıp veya diğer biyolojik çalışmalar) tıbbi yapıları imal etmek için hücreler ve biyomateryaller gibi biyolojik yapıların üretilmesi için bilgisayar destekli bir transfer işlemi olarak tanımlanmaktadır. Üç boyutlu (3B) biyoyazıcı teknolojisi, özellikle karaciğer, böbrekler, akciğerler ile vücuttaki diğer canlı organlar ile diğer organların yazdırılmasında kullanılır. Hidrojeller, yüksek biyouyumlulukları nedeniyle doku mühendisliği için hücre yüklemek için 3B biyoyazıcılar da yaygın olarak kullanılmaktadır. Buna ek olarak, hidrojeller vücutta bulunan yumuşak dokuları simüle etmesi, suda yüksek oranda şişebilme kabiliyeti ve doğal yumuşak dokularınki ile benzer mekanik özelliklere sahip olmasından dolayı birçok araştırmacı hidrojel ile ilgilenmiştir ve onlara olan ilgi her geçen gün artmaktadır. Genellikle (i) doğal hidrojeller ve (ii) sentetik hidrojel olarak iki gruba ayrılırlar. Bu çalışmada hem doğal hem de sentetik hidrojeller hakkında literatürdeki önemli belgeler derlenmiş olup ek olarak, hidrojellerin 3B biyoyazıcı işlemi detaylı bir şekilde tartışılmıştır.

Anahtar Kelimeler: 3B Yazıcı; Biyoyazıcı; Hidrojeller

1. INTRODUCTION

3D printers have considerable attention in recent years because, the products which come from 3D printers give an opportunity to see the final version of products. In fact, this phenomenon, which does not seem to be very important, brings serious convenience to the manufacturer as it saves both cost and potential problems. In the coming years, 3D printer technology is expected to lead to dramatic changes in many areas. In a long-range space mission like going to Mars, astronauts will be able to produce the food they want in space, such as pizza, bread, and steak, just because of a stock of main ingredients that they take with them. Or doctors can take biological live cells from the patient's bone scaffolds and reproduce them in the laboratory environment, creating a new organ thanks to the 3D printer. With the production of organs by the cells taken from the patients, there are no need to use medicines that prevent the rejection of the organ.

2. 3D BIOPRINTING

Three-dimensional (3D) bioprinting is a new field that has revolutionized medical science. Bioprinting research has emerged as an innovative approach, with biocompatible inks replacing the plastic or metal raw materials used in convex 3D printers. Bioprinting was first shown by Klebe [1] in 1988 under the name of cytoscribing technology, as procedure of micro positioning of live-cells and formation of 2D synthetic tissues. Bioprinting can be described as a computer-assisted production process for simultaneous recording which live cells and biological materials via layered stacking organization provided to fabricate biologically engineered structures especially used in soft-tissue engineering, scaffolds engineering, tissue renewal, and etc. 3D bioprinting technologies are open a new application areas and now continues to progress as a groundbreaking technology in the sense of human health for the future with researches such as artificial organ printing experiments in the health sector, completion of missing bone parts and construction of a private prosthesis [2]. In addition, 3D bioprinters allow the use of direct copies of patient's architectural parameters, obtained with different scanning systems such as X-ray CT [3,4] and MR, and produce biomimetic 3D biological tissue at high sensitivity [5]. 3D bioprinting is at the forefront of medical engineering areas hence it offers a straight forward method for the fabrication of 3D structures that can contain both complex geometric shaped and homogeneity of live-cell types, biologic-like materials and biochemical structures that make reliable system in the development of functional tissues [6].

As general principles in 3D bioprinting technology, the whole printer and its hardware's controlled by a well programmed software to produce layered biological materials. A basically explanation can be made as, bioprinters uses a very high precision syringe to obtain biomaterials and live-cells in the accurate xyz coordinates with a software controlled step motors for required shape. With the releasing of material, both printing speed and feed volume can be optimized for ensure that object resolution [7]. Traditional 3D bioprinting approaches are divided into various groups such as: (i) ink-jet, (ii) micro-extrusion, and (iii) laser assisted bioprinters. In most cases, the most important factor is the selection of an appropriate material for each approach. The printability depends not only on the physico-chemical properties of pre-gel solutions (eg. viscosity) but also on the gelation process [5].

The 3D printing process field has made rapid progress in recent years for markets both tissue engineering and regenerative medicine applications [8]. The global 3D bioprinting market is estimated nearly $ 487 million in 2014, which foreseen to reach $ 1.82 billion in 2022. Given the future potential of bioprinting and reconstructive surgery, and considering their goals, it is no doubt true that 3D bioprinting applications will take place in plastic surgery [7]. For this reason, future research is intended to develop new biomaterials in the field of 3D bioprinting for usage in tissue engineering and focusing on printable biomaterials (eg. bio-ink) [8].

Lee, Kwon and Park [9], used the term hydrogel for the first time in 1894. Unlike the hydrogel word today, they meant hydrogel was a form of colloidal gel derived from inorganic salts. Hydrogels are frequently using in the medical field as a matrix for encapsulation of 3D bioprinter's cells and recently for the repair and replacement of a wide variety of soft-tissues and organs that can't functional

anymore. They are polymeric-based materials that are compatible with body tissues, can be highly water-impermeable and can carry materials with various functions [9,10]. According to hydrogel structures, there are two main groups: (i) natural and (ii) synthetic. A literature search will be made of the hydrogel types in these two main groups, which are frequently used in scientific studies such as Alginate, Chitosan, Fibrin, Poly-vinyl alcohol, Poly-hydroxyethyl methacrylate, and Poly-ethylene glycol.

2.1. Natural Hydrogels

2.1.1. Alginate Hydrogels

Alginates are mainly obtained from algae which known as brown algae (Figure 1a) and it is a type of anionic polysaccharide mostly has been present walls of brown algae. It is kind of a polysaccharide which consisting both Beta D-mannuronate and epimer Alpha-L-guluronate units [2]. With increasing number of The Beta-D-mannuronates resulted with higher gel formability (gelation degree) of alginate. Alginate hydrogel has shape-memory capability, they are also cytocompatible, promote cell proliferation, superior biocompatibility, ability of degradation, non-immunological effects, improved porosity and mechanical strength [11,12]. Generally, in tissue engineering alginates have been used in immune systems [13]. But also hydrogels obtained from alginates have been frequently used in recently in tissue engineering used scaffolds, biological structure such as protein, enzyme or cell delivery and cartilage engineering as a part of tissue engineering [14]. To extend these areas recently there are number of studies that also used as some matrix elements for in-vitro culture studies of embryos [15].

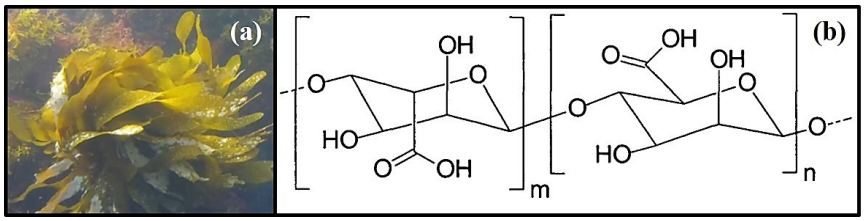

Figure 1. (a) picture of brown algae and (b) chemical structure of alginate.

The abundant hydroxyl groups of alginates can be re-changeable, connect various biological structures to these hydroxyl groups, high function capability alginate based hydrogels can be obtained (Figure 1b). Result of these hydroxyl groups, there are so many alginate hydrogels which have been developed to meet enhanced specialties for different applications [16].

The viscosity, porosity and cross-linking time of alginate based hydrogel determined by concentration of alginate. The mechanical stiffness of alginate based hydrogels is major challenge for 3D bioprinting process in tissue engineering. Used alginates, that in tissue engineering, have lower compressive strength than native articular tissues [12]. On the other hand, depending on the purpose of the 3D bioprinting process, the bio printed constructs can be strengthening in terms of mechanical properties with other functional materials and by other addition of the cross-linkers both types and amounts [17], bifurcated vascular tissue constructed with fluorocarbon 3% weight percent at volume, low melting agarose and 3% addition non-viscous alginate and they found that with the addition of fluorocarbon solution was able to support tissue construct. As a conclusion, for successful 3D bioprinting process with alginate based hydrogels, concentration and viscosity, surface tension, gelation times, wettability and addition of functional materials are important parameters [18].

2.1.2. Chitosan Hydrogels

Another type of polysaccharide which linearly and random dispersed β-(1→4)-linked D-glucosamine (de-acetylated unit) and N-acetyl-D-glucosamine (acetylated unit) glucosamine structures known as

Chitosan. Chitosan generally derived by treating the chitin shells of seafood such as shrimp and other crustaceans (Figure 2a) with the help of alkaline substance, like sodium hydroxide.

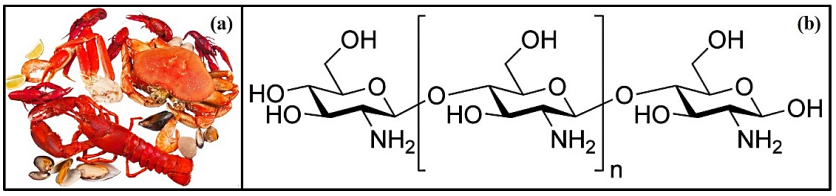

Figure 2. (a) Picture of seafood and (b) chemical structure of chitosan.

Its glucosamine groups (Figure 2b) responsible for high possibility for chemical differences and also can be obtain high bioactivity, bio-functionalities and etc. For instance, the cell adhesion properties can be controlled by N-acetylation groups [19]. Chitosan is one of the most significant materials which generally used as biomaterials due to, its many advantages such as high biocompatibility, biodegradability, non-toxic response, gives antibacterial ability and non-immunologically [20]. Chitosan, has a wide range usage in biological engineering applications like cartilage, productions of scaffolds and recovery of wounds [21].

Because of chitosan's weak mechanical strength and restricted 3D bio printability, its optimal material for cell support as a hydrogel, not suitable for large-scale scaffold synthesizing in 3D process [22]. The shortcoming of chitosan is generally, low mechanical properties and responsible for its gelation time; only viscous samples can hold themselves their shapes for limited times. These mechanical restrictions can be solved by mixing chitosan with other hydrogels or addition of different materials for obtain high strengthen structures. Also chitosan's mechanical strength can be increased with dissolving in acidic solutions followed by cross-linking mechanism via ionic or covalent agents [23]. On the other hand, Huang et al. [24] was examine addition of halloysite nanotubes (HNT) to strength and store modulus of chitosan hydrogels and showed that addition of HNT or regeneration in ethanol solution leads to extraordinary increase in mechanical stiffness of chitosan hydrogels.

2.1.3. Fibrin Hydrogels

Fibrin is a biologic structure that has fibrous shape and non-globular protein involved in the wrapping of blood cells which represented in Figure 3b. Fibrin also can be used as hydrogel by numerous enzymatic reactions between thrombin and fibrinogen structures by different steps, the key proteins which also stopping blood clotting is Factor VIII Ca^{2+} showed in Figure 3a [23]. The polymerized fibrins form hemostatic clot with other compounds such as platelets over an injured area.

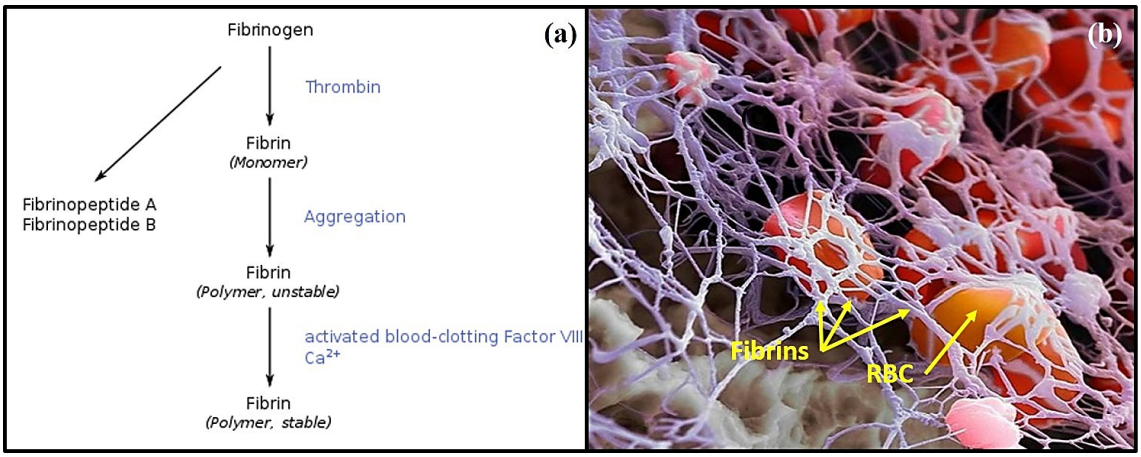

Figure 3. (a) From fibrinogen to fibrin transformation steps and (b) illustrations of both RBC (Red blood cells) and fibrins.

Fibrins are supports enhanced cell proliferation [25], plays a significant role in injure healing, inherent cell-adhesion capabilities, high cell seeding and has been used in modifications and fabrications of skin grafts [26,27]. For these capabilities fibrin hydrogels and its different forms are generally used in many areas such including cardiovascular treatments, different types of muscles, liver proliferations, skin replacements, cartilage engineering and bone tissues regenerates [28].

Both mechanical stability and integrity of fibrin hydrogels are commonly depended on the fibrinogen amount and pH of environments [29]. The major disadvantages of them are both weak mechanical strength and fast degradability [12]. Also, because of the degradation time of fibrin hydrogels are very fast, they are not suitable for bulk cell laden materials for encapsulation of the cells in a long time period. On the other hand, their nature of the weak shear-thinning abilities, fibrin structure is occasionally used in 3D bioprinting process (especially in extruding process) [30]. However, there are some studies were conducted to optimize these drawbacks which fibrins have [31]. The mechanical weakness of fibrins in extruding process can be overcome with other techniques such as photo-crosslinking of fibrinogen.

2.2. Synthetic Hydrogels

2.2.1. PHMEA (Poly (hydroxyethyl methacrylate)) Hydrogels

Poly (hydroxyethyl methacrylate: HEMA) also known as PHEMA or pHEMA, is kind of a polymeric compound which formability to hydrogel in aqueous solutions like water and chemical structure of PHME represented in Figure 4. PHEMA hydrogel for contact lenses materials was synthesized by solution polymerization using different chemicals including raw material (HEMA), ammonium persulfate and sodium pyro sulfite as catalyst chemicals and as cross-linking agents tri-ethylene glycol di-methacrylate can be used as additive [32]. It was described by two scientists who names are Lim and Wichterle, and later patented in 1953.

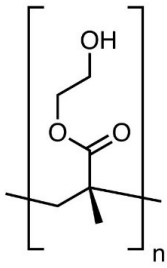

Figure 4. PHEMA chemical structure.

However, this synthetic polymer was firstly prepared by DuPont scientists in 1936 [32], but they did not start polymerization process of the monomers in aqueous solvent media by adding crosslinking agents as did Lim and Wichterle. In addition of using areas differ from contact lenses, PHEMA can be seen in lots of medical fields such as artificial skin manufacturing, dressings, especially burn wounds, responsible for its ensures good healing conditions [33].

The synthesis of PHEMA can be occurred with simultaneous cross-linking by UV-radiation. Also, the properties of PHEMA depend on many factors including, synthesis methods, polymer content, cross-linking degree, temperature and environment which applied for polymerization [33]. The improvement at the stiffness of these hydrogels could extend its used fields for higher mechanical properties. According to some research in the literature, such a development can be achieved by production of the amphiphilic (possessed as both hydrophilic and lipophilic character) material into its structure [34].

2.2.2. PVA (Poly (vinyl alcohol)) Hydrogels

PVA is a kind of soluble in water type synthetic polymer. Idealized formula of it $[CH_2CH(OH)]_n$ or otherwise $(C_2H_4O)_x$ can be seen in Figure 5. It is frequently used in industries that both papermaking

and textile and various coating sectors. It is specific properties are colorless and odorless. It is sometimes supplied as solid beads or generally solute from which present in water.

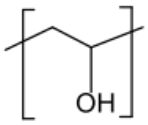

Figure 5. PVA chemical structure.

Cross-linked forms of PVA hydrogels have found a number of uses in the biomedical field such as contact lenses, cartilage regeneration applications, synthetic organs and wounds that providing beneficial for injuries [32]. Apart from this knowledge, cells or proteins are not adhering to non-modified PVA hydrogels, which makes it novel material in biomedical fields in terms of new tissue engineering purposes. Different from advantages of typical hydrogels (water absorbable ability, permeability, tissue imitation, soft behavior (high flexibility or elastic strain) and high biocompatibility). PVA hydrogels are arise from due to by their improved mechanical stiffness and ability to reshape their original form in water, which provides enhanced bulk properties at all environments [33]. Hydrogel forms of PVA polymers can be obtained via various methods like freezing or thawing. PVA hydrogels which obtained by these methods have superior mechanical stiffness than achieved by others like using UV light sources for cross-linking process [35].

2.2.3. PEG (Poly (ethylene glycol)) Hydrogels

The chemical formula of PEG is "$HO(CH_2CH_2O)_nH$" represented in Figure 6. It is a type of water soluble polyether alcohol and can be mixed with water at a certain ratio. With the solubility in alcohol and methylene chloride, they can be polymerized with UV light also with solution forms.

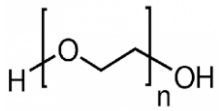

Figure 6. PEG chemical structure.

PEGs are commonly used fields are; mineral oils, emulsifiers, humectants, alcohol components, chromatic and salicylic pigments, abrasives, hydraulic fluids, organic synthesizers, office products, solvents and more. But other important usage field is medical fields such as, polymer medical equipments and regenerative medicine including cell culture studies and wound healing etc.

The production of PEG was first reported in 1859 by two independent scientist names are, Laurence and Wurtz are independently produced material that now known as polyethylene glycols. Basically, PEG is synthesized by the reaction between ethylene oxide and different solvent types such as water, ethylene glycol or ethylene glycol oligomers. Due to hydrophilic property of PEG, it has adsorption resistance for proteins but on the other hand it has high affinity to cells as attachment specialties in physiologic environments. Research for evaluate this mechanism showed that even if there is no physiological environment, cell capsulated PEG hydrogels can have survived [36]. One of the biggest drawback as in many other kinds of hydrogels is the low mechanical strength. To solve this limitation, addition of another compounds like other photo initiator or reinforcement materials is highly beneficial. However, with the addition of UV photo cross-linkable photo initiator may damage to the cells viability because of the excessive exposure to UV lights [2]. But for now, PEG is also known as the higher mechanical strength hydrogel compared with other natural hydrogels.

3. SUMMARY AND OUTLOOK

3D bioprinting emerges as an innovative method promising a solution to some challenging obstacles against tissue engineering applications, high precision shaped of tissue structures, building of complex

shaped biological architectures and cell proliferation/differentiation. In recent years, extraordinary developments have been made in the field of functional biomaterials, such as hydrogels and others. Hydrogels are commonly used in everyday products but we are still have limited knowledge about them. As we explained before actually hydrogels have lots advantages and they are well-established role in especially contact lenses but commercial hydrogels products in medical grade tissue engineering and systems that capability of drug delivery are still inadequate because of some limitations (Table 1).

Table 1. Some important parameters for hydrogels as both advantages and disadvantages used in medical tissue engineering

Advantages	Disadvantages
• Can be modified by cells, ceramic compounds or other biologic compounds	• They usually have poor mechanical properties
• They can protect cells and fragile biologic structures from environments	• Can be hard to synthesize
• Good transport capabilities from both directions: nutrient to live-cells and products derived from them	• Sometimes it's hard to load cells and fragile biologic compounds as well as then crosslink in-vitro
• They are usually biocompatible compounds with biologic tissues	• Can be difficult to sterilize them

As we discuss about 3D bioprinting process, many 3D bio printable hydrogels are researched by scientist even some of them have been patented, but not many have achieved to the global market for humankind recently. We think that there is much to do in these areas both 3D bioprinting and hydrogels. Although the findings from current laboratory studies are exciting for natural cartilage repair, produced from natural hydrogel-based structures, the results obtained from clinical trials are still limited. Scientists are questioning how to improve both mechanical strength and bio-functionality, and studies are continuing on how to improve these two very important properties for usage of hydrogels. Another important thing to keep in mind when doing these researches is that the market prices of the final products should be cheap.

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