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Production of Hydrogel with Alginate and Pericardial Fluid for use in Tissue Engineering Applications

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Abstract

Hydrogels are used in the treatment of soft tissue damage, controlled drug release systems, tissue/organ production with 3D bioprinters, smart material production, and many other tissue engineering studies. Although polymers obtained from natural polymers or synthetically produced polymers are used in hydrogel production, they may have various biocompatibility problems. In this study, Pericardial fluid structure (PFS) was used to increase the biocompatibility of the alginate and was used in the production of bioink for use in 3D bioprinters. PFS is a structure isolated from pericardial fluid (PF) and consists of complex components that are very similar to natural Extracellular Matrix (ECM) both morphologically and in content. According to the results of SEM images, the collagen-elastin fiber network was clearly observed in the groups with PFS added, since PFS contains high levels of collagen and elastin proteins. It was concluded that the biocompatibility of the material was also increased thanks to the structure similar to the natural ECM in the alginate hydrogels with PFS added.

Keywords: Alginate, Pericardial fluid, Hydrogel, 3D bioprinter, Tissue engineering

Doku Mühendisliği Uygulamalarında Kullanılmak üzere Aljinat ve Perikardiyal Sıvı ile Hidrojel Üretimi

Öz

Yumuşak doku hasarının tedavisinde, kontrollü ilaç salınım sistemlerinde, 3D biyoyazıcılarla doku/organ üretiminde, akıllı malzeme üretiminde ve daha pek çok doku mühendisliği çalışmalarında hidrojeller kullanılmaktadır. Doğal polimerlerden elde edilen polimerler ya da sentetik olarak üretilen polimerler hidrojel üretiminde kullanılıyor olmasına rağmen çeşitli biyouyumluluk problemleri taşıyabilmektedir. Bu çalışmada 3D biyoyazıcılarda biyomürekkep üretiminde kullanılan aljinatın biyouyumluluğunu artırmak için Pericardial fluid structure (PFS) kullanılmıştır. PFS Perikardiyal sıvıdan (PF) izole edilen bir yapı olup

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doğal Ekstrasellüler Matrikse (ECM) hem morfolojik hem de içerik olarak çok benzeyen kompleks bileşenlerden oluşmaktadır. Dondurarak kurutulmuş olan PFS değişen oranlarda aljinat ile karıştırılarak oluşturulan grupların karşılaştırması ve analizi için SEM görüntüleme yapılmıştır. SEM görüntüleri sonuçlarına göre PFS yüksek oranda kolajen ve elastin proteini içerdiği için kolajen-elastin fiber ağı PFS eklenen gruplarda belirgin bir şekilde gözlenmiştir. PFS eklenen aljinat hidrojellerinde doğal ECM'ye benzeyen yapı daha iyi oluşturularak malzeme biyouyumluluğunun da arttırıldığı sonucuna varılmıştır.

Anahtar Kelimeler: Aljinat, Pericardiyal sıvı, Hidrojel, 3D biyoyazıcı, Doku mühendisliği

1. INTRODUCTION

3D bioprinters are one of the tissue engineering methods widely used in artificial tissue/organ production. Bioprinting requires a structure that allows cell viability and proliferation and best mimics native ECM. Hydrogels are used in the production of bio-inks for bioprinting by providing 3D and hydrophilic properties, as well as cell viability, cell proliferation, differentiation, and interaction with other cells. Hydrogels are three-dimensional polymer materials that contain high amounts of water and maintain their structural integrity through physical and chemical cross-links between polymer chains. The water retention potential of hydrogels is due to their hydrophilic structure and cross-links in their structure [1].

Hydrogels have a wide range of applications in the medical field, with properties such as swelling-shrinkage, response to stimuli, liquid-gel transitions, shape memory, and protection of biomolecules or drugs from the external environment [2]. Additionally, it is used as bioink for 3D bioprinters due to its similarities to natural ECM and its ability to create a three-dimensional environment for cells [1-3]. Natural polymers such as alginate, chitosan, collagen, fibrin, hyaluronic acid, and synthetic polymers such as PEO, PEG, polyvinyl alcohol (PVA), and polyacrylic acid (PAA) can be used in the production of hydrogels. Hydrogels are made of synthetic and natural polymers. With their biodegradation, bioabsorption, mechanical properties, and high biocompatibility, they are widely used in tissue engineering as drug release materials, wound dressing materials, bioinks, and scaffolds for bioprinters [1,4,5].

Hydrogels are generally produced by using natural ECM components, which are collagen, hyaluronic acid, elastin, and fibrin, or by using Matrigel as a commercial product. Matrigel is extracted from mouse tumor cells, which include a protein source. In addition, hydrogels can be produced by using decellularized tissues that are enzymatically broken down and gelling at appropriate pH or temperature values [6,7].

Collagen, gelatin, fibrin, elastin, chitosan, and alginate, which are natural polymers, are widely used in tissue engineering studies due to their biocompatibility. Natural polymers are used together with synthetic polymers because their structures can be damaged during the production phase, their degradation is rapid, and they are mechanically weak polymers. Synthetic polymers are preferred due to their high mechanical strength, ease of manipulation, and slow degradation. Polyurethane (PU), PEG, PGA, PLGA, polyvinyl alcohol (PVA), PLA, and PCL are synthetic polymer types used in tissue engineering studies [8,9].

Due to their similarity to natural ECM, gelatin-containing hydrogel structures such as chitosan-gelatin, fibrin-gelatin, alginate-gelatin, and dextran-gelatin are also used in tissue engineering studies [10-12]. Alginate, a hydrophilic, anionic, and linear polymer, is a polysaccharide obtained from brown seaweed. Alginate consists of subunits of mannuronic acid (M) and glucuronic acid (G) [13-15]. G monomer blocks in the structure of alginate react with divalent cations (Ca2+, Mg2+, Ba2+, or Sr2+), forming cross-links, and gelation occurs [1,16-21].

Alginate is used in many biomedical applications such as cell encapsulation, tissue engineering, and drug delivery due to its gel-forming ability, hydrophilic structure, low toxicity, lack of

immunological effects, and lack of resources [22,23]. Because alginate allows cell growth and has high biocompatibility, it is also used to create 3D scaffolds in bioprinting studies [15,24]. In order to increase cell growth and proliferation, alginate can be used by mixing with other biocompatible materials such as collagen, chitosan, and fibrin [25-29]. The definition of PFS, a PF component, was first introduced in a study conducted in 2023. In that study, it was shown that PFS has similarities to native ECM according to morphological and contained materials [30]. In this study, hydrogel groups produced by mixing alginate and PFS in varying proportions. Groups of hydrogels for the production of bioinks for use in 3D bioprinting were compared and analyzed.

2. MATERIAL AND METHOD

The harvesting of PF from bovine hearts and isolation PFS from the PF was described in our previous studies [31-34]. PFS samples were frozen at −80°C before being freeze-dried in a freeze drier (Alpha 1–2 LD Plus, Martin. Christ, Germany).

2.1. Preperation of Hydrogels Samples

Freeze-dried PFS was dissolved in varying amounts (50, 100, and 150mg) in Dulbecco's PBS (DPBS) purchased from Thermo Fisher Scientific (USA) and mixed with 3% Alginate (A) (Alginic Acid Sodium Salt, Sigma, Germany) solution. Thus, four different hydrogel groups were formed: A, A-50mg PFS, A-100mg PFS, and A-150mg PFS. The Alginate (A) hydrogel was used as a control for comparison with the PFS-added hydrogel groups. Alginate and alginate-PFS hydrogels were synthesized by cross-linking reactions. After preparing the hydrogel solution, the hydrogels were cross-linked in $CaCl_2$ (2%; v/v) for 15 min [35,36].

Figure 1 shows the flow images showing the formation of the hydrogel structure obtained from A-PFS solutions. Firstly, A-PFS hydrogel solutions were prepared, and then the gelation processes were done for all hydrogel groups.

Figure 1. Images of the production of A-PFS hydrogel structures.

2.2. SEM Imaging of Hydrogels

Scanning electron microscopy (SEM) was used for imaging and examining the microarchitecture of the hydrogels. Hydrogel samples were frozen at −80°C before being freeze-dried in a freeze drier (Alpha 1–2 LD Plus, Martin. Christ, Germany). After frezee drying to hydrogel samples were mounted onto microscope stubs and coated with gold (3–6 nm). SEM images (SEM; Evo ls-10 Life Science Zeıss, Germany) were taken at 500x and 2500x magnification with a resolution of 1024x768 in 8-bit grayscale.

3. RESULTS AND DISCUSSION

In the present study, the morphology of hydrogels was observed by SEM. SEM images of hydrogel groups are shown in Figure 2.

Figure 2.a-b shows the SEM image of the honeycomb-like porous structure of the hydrogel prepared using alginate. The alginate hydrogel structure consists of a porous and very smooth morphology. Figure 2.c-d shows that although PFS is mixed homogeneously with alginate in the A-50mg PFS hydrogel, its amount is low. In Figure 2.e-f, although PFS is seen more densely in the A-100mg PFS hydrogel compared to the previous group, PFS fiber networks are not seen much. Figure 2.g-h shows that PFS is more dense and homogeneously distributed in a porous structure in

the A-150mg PFS hydrogel. Additionally, the PFS fiber network appears to be very clearly dispersed within the hydrogel. It is seen that the PFS distribution in the A-50mg PFS and A-100mg PFS hydrogel groups is less due to the amount. SEM images show that the PFS distribution is better in the A-150mg PFS hydrogel. The PFS fiber networks are composed of collagen, elastin, fibrinogen, glycosaminoglycans (GAGS), and proteoglycans [30]. These collagen fiber networks can be seen in detail in our previous study, in which PFS was characterized and analyzed.

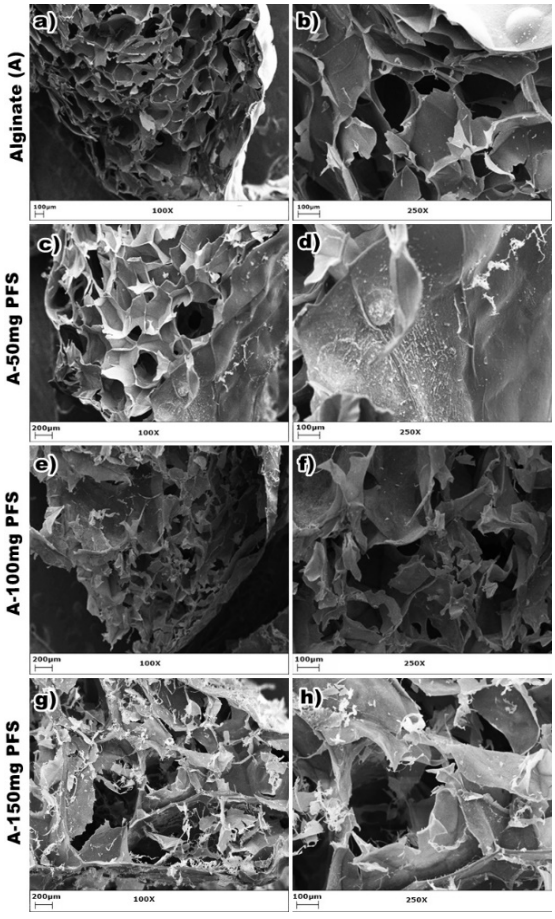

Figure 2. SEM images of hydrogel groups. a, b; SEM images of Alginate (A) hydrogel. c, d; SEM images of A-50mg PFS; e, f; SEM images of A-100mg PFS; g, h; SEM images of A-150mg PFS hydrogels with magnification at 100x and 250x.

4. CONCLUSION

In our previous study, pericardial fluid was characterized and analyzed, which has similarities to native ECM. That study demonstrated that PF includes collagen types I, III, and IV, elastin, fibrin, fibrinogen, GAGs, proteoglycans, and albumin. In addition to similarities in content, PF also has morphological similarities to native ECM [30].

In this study, we pretended to use PF in hydrogel production and also show that it can be used as a natural biomaterial. PFS was used for the production of hydrogel to increase the biocompatibility of alginate, which is widely used in bioink production in 3D bioprinters. When the SEM images of the hydrogel groups produced by adding varying amounts of PFS were examined, the hydrogels produced using alginate had a smooth and porous structure, while the hydrogel groups with PFS added had a rough structure consisting of porous and fiber networks. Additionally, it was observed that as the amount of PFS increased, the density of the PFS fiber network in the hydrogel structure also increased. Thus, by increasing cell adhesion in the cell-laden bioink produced using PFS, it will be possible to create the desired tissue with 3D bioprinters, allowing cell proliferation, differentiation, and migration. PFS, the PF component, has the potential to be used not only in 3D bioprinters but also as a natural biomaterial in various tissue engineering studies and as a scaffold to mimic natural ECM.

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