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ORIGINAL ARTICLE

Designing of Alginate-Based Tissue Scaffolds and Their Use in Mesenchymal Stem Cell Culture

Aljinat Temelli Doku İskelelerinin Tasarımı ve Mezenkimal Kök Hücre Kültüründe Kullanımları

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Abstract

Introduction: Tissue engineering is a novel approach in tissue and organ regeneration or replacement. Tissue engineering has three components: cells with the potential to differentiate into functional tissues, biomaterials that will provide the three-dimensional environment to these cells, and bioactive molecules such as growth factors or cell attachment peptides. The aim of this study is to design alginate-based cell scaffolds for use in tissue engineering and search for the effectiveness of these scaffolds in culturing mesenchymal stem cells.

Methods: Alginate-based gels were produced by using alginate, glucose, and carboxymethyl cellulose. The gels were tested for their stability, swelling, and surface characteristics. The interaction of the selected gels with mesenchymal cells and their toxicity on these cells were analyzed. All results were evaluated by variant analysis statistically.

Results: It is found that glucose and carboxymethyl cellulose increased the porosity and stability of the alginate gel. The gels had no toxic effect on cells, and the cell attachment and proliferation were improved as compared to the culture plate alone.

Discussion and Conclusion: It is concluded that the gels designed here may provide efficient scaffolds for tissue regeneration studies on which the stem cells differentiate into cartilage or other tissues.

Keywords: Alginate; Gels; Carboxymethyl cellulose; Glucose; Mesenchymal stem cell; Scaffold

Loss of tissues is a big burden for the body in certain cases. The need for the regeneration or replacement of tissues has led to the emergence of a new discipline called tissue engineering.[1,2] Tissue engineering aims to develop biomimetic tissue on a scaffold that should stimulate and allow tissue formation. The cells that are grown on scaffolds are either tissue-specific blast or stem cells.[3,4] The scaffold may be natural or synthetic, and it must certainly be nontoxic, biocompatible, and biodegradable.[5] A great number of tissue scaffolds are formed as they are useful

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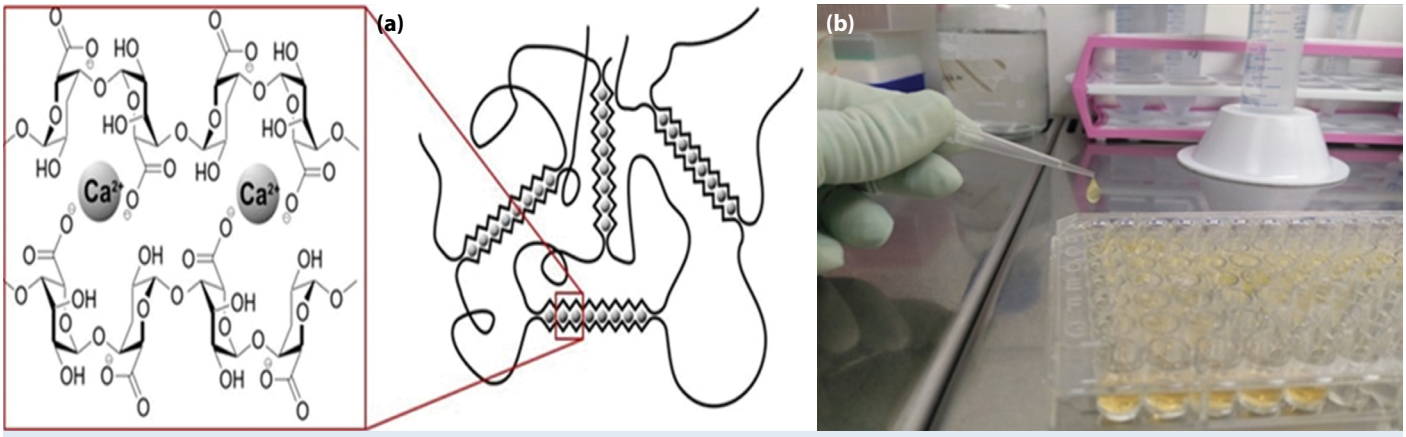

Figure 1. Alginate gel formation by divalent cations via the "egg-box" model.

degradable materials, which support cell infiltration and proliferation until the tissue is formed. The physical properties such as pore size and number are as important as the chemical properties that direct growth signals to cells and degradable composition. Carbohydrate-based scaffolds have advantages over other scaffold materials with their low costs and easy preparation methods along with desired features of biocompatibility and biodegradability. Hydrogel-forming carbohydrate gels are formed by cross-linking and they swell in water. Alginate is used in many types of biomedical applications.[6] Therefore, they allow diffusion of nutrients for the cells, and their viscosity may be controlled by the amount of cross-linking divalent cation. Alginate hydrogels are easily formed in the presence of divalent cations.[7] The gel is formed by ionic cross-linking of sodium alginate by divalent cations via the "egg-box" model as shown in Figure 1a.[8] However, the low mechanical stress-bearing texture of alginate gels should be improved by copolymerization with more resilient polymers.[9] We have aimed to form porous alginate gels to promote cell attachment by a novel procedure using glucose and analyzed the effect of adding cellulose to the cell structure to improve stability and tension-bearing capacity. These gels are characterized for their swelling capacity, stability, and porous structure and are used to culture human mesenchymal stem cells to analyze cell attachment and growth-stimulating properties.

Materials and Methods

Chemicals

Sodium alginate, carboxymethyl cellulose, DMEM, bovine serum albumin, glucose, and ethyl alcohol are obtained from Sigma, Germany. All other chemicals were obtained from Merck and Riedel, Germany.

Cells

Bone marrow-derived human mesenchymal stem cells (PoieticsTM, Lonza, Basel, Switzerland) were cultured on 25-cm cell culture plates using DMEM with glutamine, containing 10% FCS at 37°C in a humidified atmosphere containing 5% CO_2.

Preparation of Gel Scaffolds

Solutions of sodium alginate with or without cellulose are prepared. These were placed in a 1-mL syringe and injected into a 2% $CaCl_2$ solution. Cross-linking time is estimated as 4 h, and gels were washed with PBS to remove the non-cross-linked material.

Analysis of Gel Surface by Scanning Electron Microscopy

Alginate-based gels were analyzed using a scanning electron microscope. Gels were immersed in alcohol and then dried in a critical point drying apparatus. They were coated with gold–palladium and observed under 500× magnification (Zeiss EVO50 XVP 10.00 KW).[10]

Analysis of Physical Properties of Gels

Gels were dried and weighed. They were placed in saline, and weight change is determined at certain intervals to analyze the swelling capacity of alginate hydrogels. To determine the biodegradability of the gels, 50 mg/1 ml albumin was added to the alginate solutions while forming the gels. Albumin release as a measure of gel degradation is detected in PBS media using spectrophotometry with Bradford reagent at 595 nm.[11,12]

Cell Culture on Gel Scaffolds

A quantity of 20 µL of gel preparations was placed on 6 well plates. Gels were sterilized by ethylene oxide gas application. Then, 30 000 cells in 2 mL of DMEM were added to each well. Cultures were maintained by changing the me-

dia every 2 days up to 10 days. Cell growth was monitored by phase-contrast microscopy daily. Cell growth was detected using MTT (3-(4,5-dimethlthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test.

Statistics

The significance of results is determined by multidirectional analysis of variance, XLSTAT, Microsoft Excel, USA.

Results and Discussion

Alginate is a water-soluble linear polysaccharide having dimeric units of glucuronic acid (G) and mannuronic acid (M). This structure leaves areas between the dimeric block to where Ca^{2+} ions can bind and cross-link the soluble alginate to form a gel.[13,14] The alginate gels were aimed to be more stable and porous. The gels that were formed by dropwise addition of alginate solution into $CaCl_2$ solution are shown in Figure 1b. Glucose was added to the alginate solution and then the gel formed was washed to improve the pore structure, as glucose is not involved in any covalent interaction with dimers of alginate or calcium during the gelling process and leaves pores behind as it diffuses out of the gel. Cellulose is added to the gel structure to improve resilience and increase stability. As shown in Figure 2a, glucose-treated gels absorbed water more readily reaching a maximum at 2 mg glucose, which may be due to a more porous structure. One of the aims of tissue engineering is to design scaffolds with sufficient porous structure to promote attachment and spreading of cells as well as allow the growth-stimulating nutrients and growth factors to reach the cells. Therefore, salts are used to form pores in alginate gels, but here using glucose as a novel approach is shown to be effective.[15] Similarly, the water absorption of cellulose-containing gels was also significantly more than the gels containing only alginate reaching a maximum at 2.5 mg/mL cellulose. This may also result from disruption of the regular gel structure of alginate (Fig. 2b).

The glucose-containing gels were less stable than the ones without glucose. However, this difference in albumin release as a measure of gel degradation is not found to be significant ($p>0.05$). Cellulose led to an increase in stability as detected after 10 days (Fig. 3a, b). Copolymerization of alginate and carboxymethyl cellulose has shown to yield a

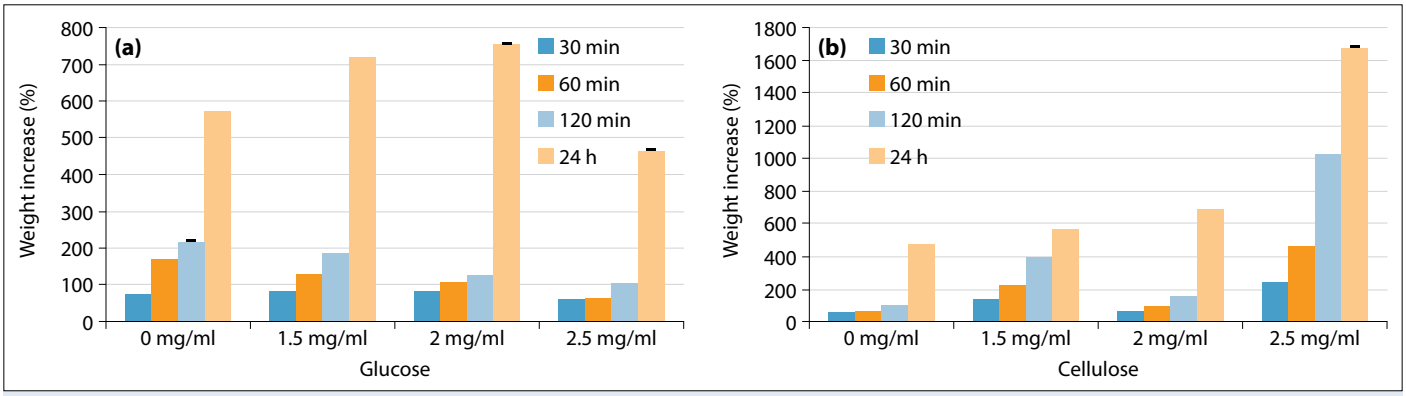

Figure 2. Effects of glucose-induced pore formation and cellulose on the water absorption capacity of alginate gels. Results represent the mean and standard deviation of five experiments.

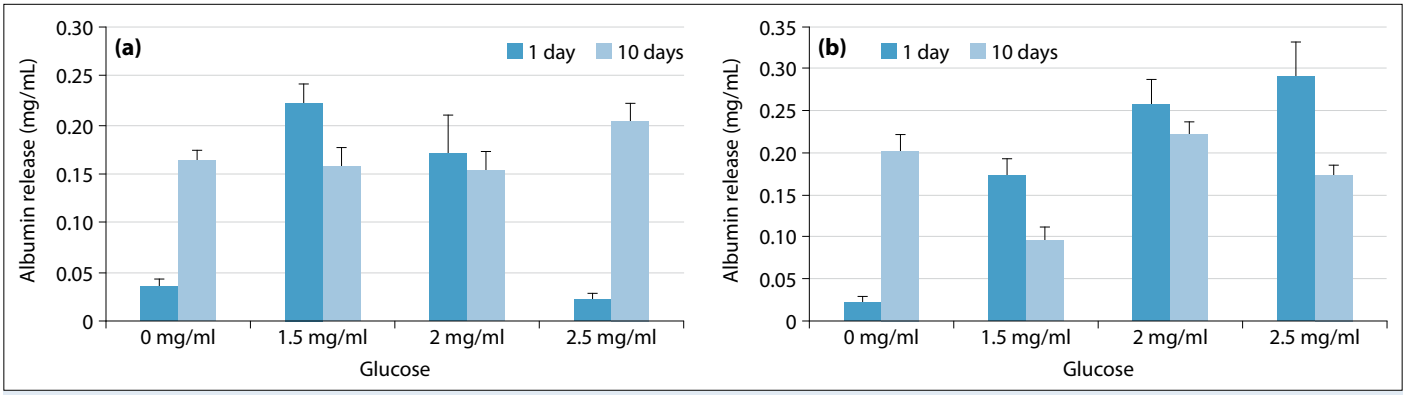

Figure 3. Effects of glucose-induced pore formation and cellulose on the stability of alginate gels. Results represent the mean and standard deviation of five experiments.

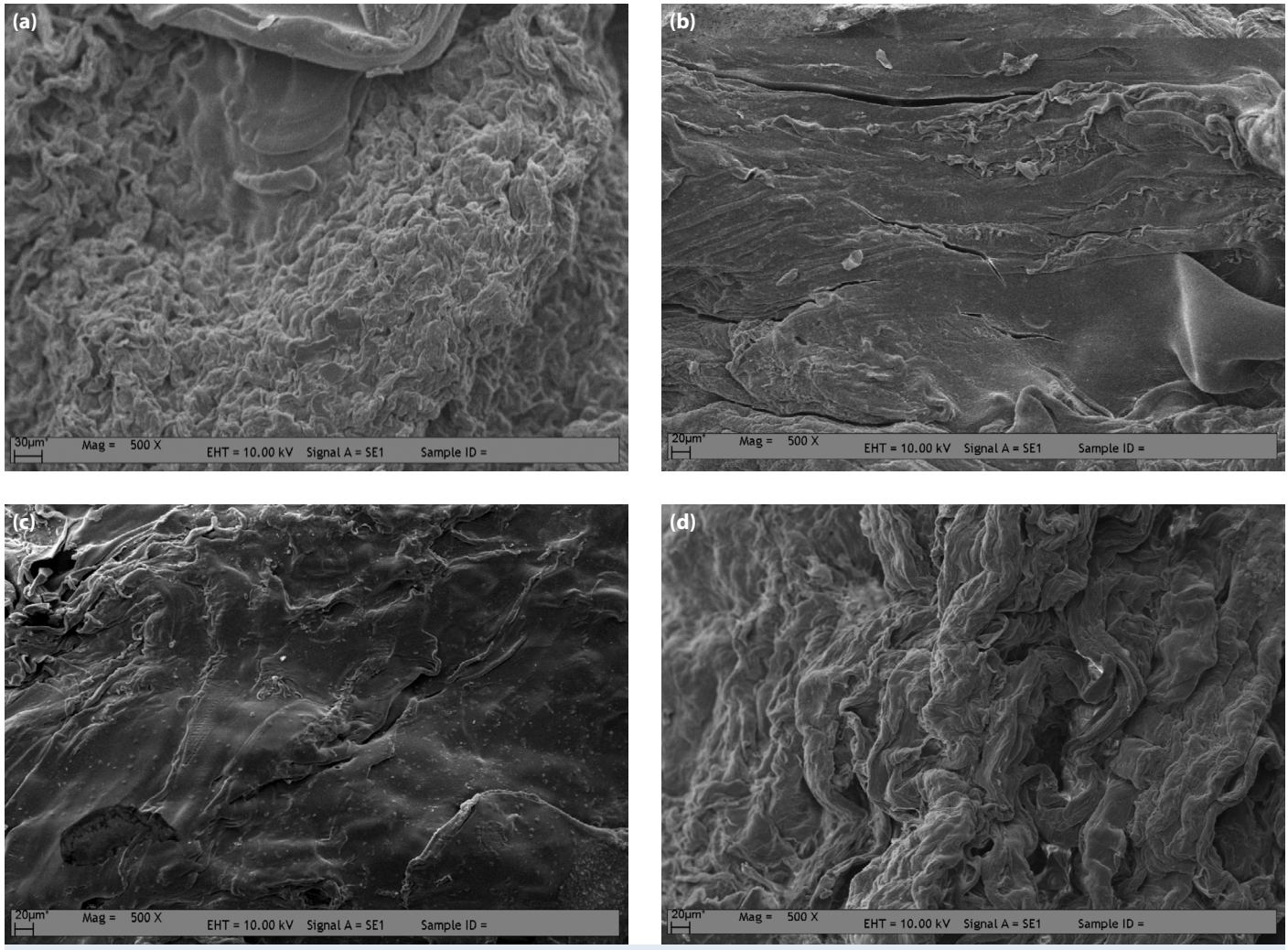

Figure 4. Scanning electron micrographs of alginate gel **(a)** and modified by 1.5 mg glucose **(b)**, 2.5 mg glucose **(c)**, and 2.5 mg cellulose at 500× magnification (Zeiss EVO50 XVP 10.00 KW).

more stable gel formation with increased tensile strength.[9,16] The scanning electron micrographs of alginate and modified forms of gels have further revealed the porosity introduced by glucose treatment (Fig. 4a–c) and the curved surface introduced by the cellulose (Fig. 4d).

Human mesenchymal cells proliferated on the gels of alginate and its modified forms, namely glucose-treated and cellulose-containing alginate gels (Fig. 5). The growth of human mesenchymal cells on these gels after 10 days of culture was much greater than the control cells cultured on tissue culture flasks in a two-dimensional form. This difference was found to be significant, whereas the difference between gel forms was not found to be significant. Cells attached to the alginate and modified alginate gels are shown in Figure 6. This is in accordance with the studies carried out to grow cells in hydrogels.[17] For example, insulinoma cells grown on alginate gels have grown and produced more insulin as compared to the controls.[18] Hydrogels mimic the extracellular matrix, and further modifications to these three-dimensional scaffold systems such as adding growth factors or integrin recognition peptides

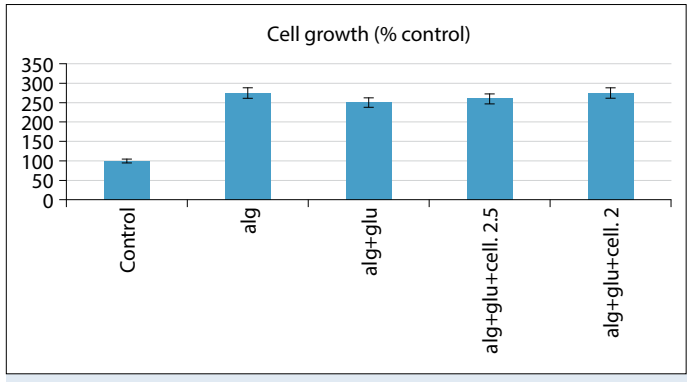

Figure 5. Proliferation of mesenchymal stem cells on alginate and modified alginate gels. Data represent percent growth as compared with control cells in 10 days as the mean and standard deviation of five experiments. Cultured on uncoated flasks.

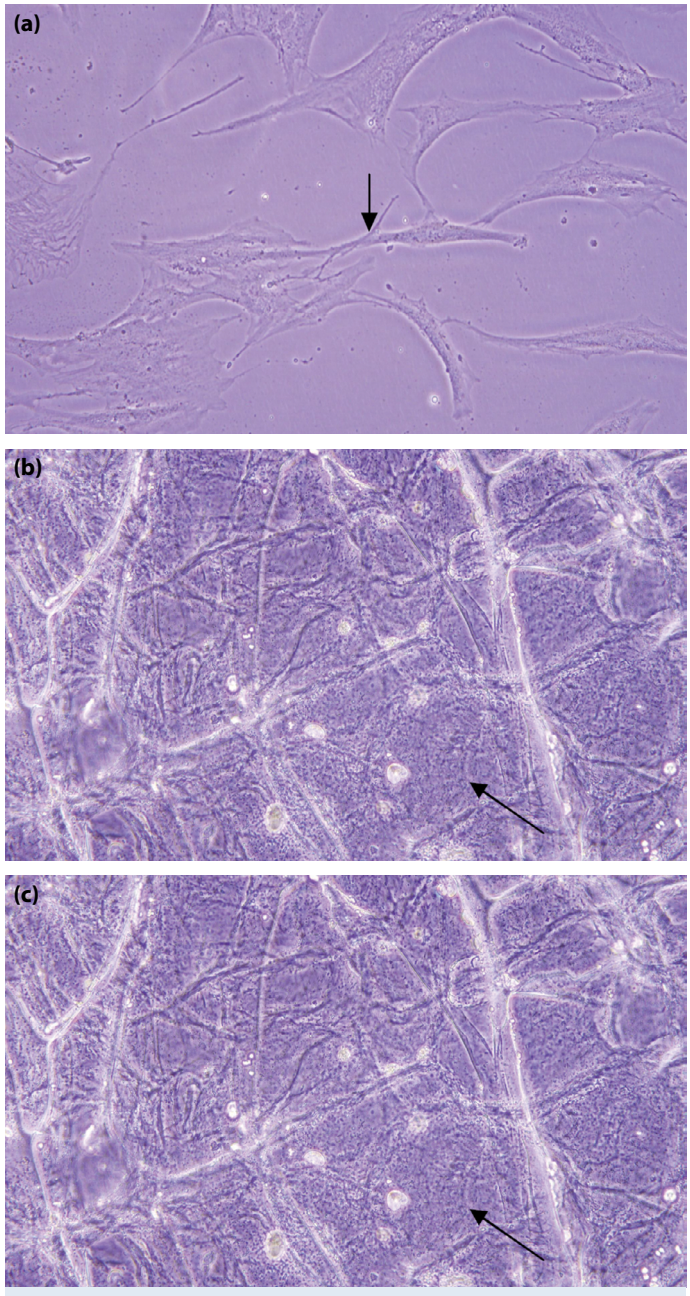

Figure 6. Mesenchymal cell attachment on alginate gel **(a)** and modified by 2.5 mg glucose **(b)**, and 2.5 mg glucose and 2.5 mg cellulose **(c)**.

are shown to improve cell binding and cell proliferation.[19] Alginate gels are also used in forming 3D models to analyze cancer cell behavior and drug targeting research.[16] Therefore, it is essential to develop alginate features to develop efficient biomedical tools.

It is concluded that alginate as a natural material with low toxicity and biodegradability is a preferred scaffold material.[20] Gaining porosity and stability by glucose treatment and cellulose may increase the feasibility of using in the regeneration of different types of tissues as a supporting scaffold.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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References

1. Langer R, Vacanti JP. Tissue engineering. Science 1993;260 (5110):920–6. [CrossRef]
2. Tabata Y. Recent progress in tissue engineering. Drug Discov Today 2001;6(9):483–7. [CrossRef]
3. Sokolsky-Papkov M, Agashi K, Olaye A, Shakesheff K, Domb AJ. Polymer carriers for drug delivery in tissue engineering. Adv Drug Deliv Rev 2007;59(4-5):187–206. [CrossRef]
4. Sakai S, Kawakami K. Synthesis and characterization of both ionically and enzymatically cross-linkable alginate. Acta Biomater 2007;3:495–501. [CrossRef]
5. Wei G, Ma PX. Partially nanofibrous architecture of 3D tissue engineering scaffolds. Biomaterials 2009;30:6426–34. [CrossRef]
6. Iglesias-Mejuto A, García-González CA. 3D-printed alginate-hydroxyapatite aerogel scaffolds for bone tissue engineering. Mater Sci Eng C Mater Biol Appl 2021;131:112525.
7. Yuan NY, Lin YI, Ho MH, Wang DM, Lai JY, Hsieh HJ. Effects of the cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze-gelation method. Carbohydrate Polymers 2009;78(2):349–56. [CrossRef]
8. Grant GT, Morris ER, Rees DA, Smith PJ, Thom D. Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Letters 1973;32. [CrossRef]
9. Yuan N, Lin Y, Ho M, Wang D, Lai J, Hsieh H. Effects of the cooling mode on the structure and strength of porous scaffolds made of chitosan, alginate, and carboxymethyl cellulose by the freeze-gelation method. Carbohydrate Polymers 2009;78:349–56. [CrossRef]
10. Vernon-Parry K. Scanning electron microscopy: an introduction. Analysis 2000:13(4):40–4. [CrossRef]
11. Nochos A, Douroumis D, Bouropoulos N, *in vitro* release of bovine serum albumin from alginat/HPMC hydrogel beads. Carbohydrate Polymers 2008;74:451–7. [CrossRef]
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54. [CrossRef]
13. George M, Abraham TE. Polyionic hydrocolloids for the intestinal delivery of protein drugs: alginate and chitosan--a review. J Control Release 2006;114(1):1–14. [CrossRef]
14. Zhao Y, Li F, Carvajal MT, Harris MT. Interactions between bovine serum albumin and alginate: an evaluation of alginate as protein carrier. J Colloid Interface Sci 2009;332(2):345–53.
15. Wulf A, Mendgaziev RI, Fakhrullin R, Vinokurov V, Volodkin D, Vikulina AS. Porous alginate scaffolds designed by calcium carbonate leaching technique. Adv Funct Mater 2021;2109824.
16. Mollah M, Hasan Z, Mahal Z, Faruque MR, Khandake MU. The usages and potential uses of alginate for healthcare applications. Front Mol Biosci 2021;8. [CrossRef]

17. Tanaka Y, Yamaoka H, Nishizawa S, Nagata S, Ogasawara T, Asawa Y, et al. The optimization of porous polymeric scaffolds for chondrocyte/atelocollagen based tissue-engineered cartilage. Biomaterials 2010;31(16):4506–16. [CrossRef]
18. Li Z, Ramay HR, Hauch KD, Xiao D, Zhang M. Chitosan-alginate hybrid scaffolds for bone tissue engineering. Biomaterials 2005;26(18):3919–28. [CrossRef]
19. Tai BC, Du C, Gao S, Wan AC, Ying JY. The use of a polyelectrolyte fibrous scaffold to deliver differentiated hMSCs to the liver. Biomaterials 2010;31(1):48–57. [CrossRef]
20. Sahoo DR, Biswal T. Alginate and its application to tissue engineering. SN Appl Sci 2021;3:30. [CrossRef]