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Araştırma Makalesi / Research Article

Assessing the Proliferative Impact of Alginate-Gelatin Composites on Mesenchymal Stromal Cells

Mesude BİÇER[1*]

Abstract

Traditional conducted on flat two-dimensional (2D) culture do not closely mirror the physiological and pathophysiological features of the in vivo environment including cell migration, soluble factor gradient and cell-matrix interactions. Three-dimensional (3D) cell culture overcomes these drawbacks by using 3D biomaterials, such as alginate hydrogels, to promote cell migration, vary biomaterial stiffness or permit cell-matrix interactions. In this study, it was aimed to propose a novel 3D platform including alginate-gelatin composites to address the need for tissue engineering techniques that support mesenchymal stromal cell viability and differentiation potential. In the first part, the absorbance spectra of different hydrogel-based biomaterials were examined using UV-visible light. After finding the best performing hydrogel, the work focused on cell proliferation using XTT viability assay and Live/Dead cytotoxicity assay. The cell viability of mesenchymal stromal cells in the best hydrogel biomaterial was compared to other scaffolds including cellulose, alginate-gelatin and only alginate. Alginate-gelatin hydrogel increased MSC viability, in comparison with other scaffolds such as alginate and cellulose. This increase also was significant compared to the cells grown in 2D culture. The findings of this study are consistent with the data of other studies in the literature. Thus, alginate-gelatin composites could be a promising candidate in tissue engineering to improve cell proliferation.

Keywords: Mesenchymal stromal cells, Alginate-gelatin composite, Cell viability, Tissue engineering, 3D cell culture.

Aljinat-jelatin Kompozitlerin Mezenkimal Kök Hücreleri Üzerindeki Proliferatif Etkisinin Değerlendirilmesi

Öz

İki boyutlu (2D) kültür üzerinde yapılan geleneksel çalışmalar, hücre göçü, çözünür faktör gradyanı ve hücre-matris etkileşimleri dahil olmak üzere in vivo ortamın fizyolojik ve patofizyolojik özelliklerini yakından yansıttmaz. 3-boyutlu (3D) hücre kültürü, hücre göçünü teşvik etmek, biyomateryal sertligini degiştirmek veya hücre-matris etkileşimlerine izin vermek için aljinat hidrojelleri gibi 3D biyomateryalleri kullanarak bu dezavantajların üstesinden gelmektedir. Bu çalışmada, mezenkimal kok hücre canlılığını ve farklılaşma potansiyelini destekleyen doku mühendisliği tekniklerine olan ihtiyacı karşılamak için aljinat-jelatin kompozitleri içeren yeni bir 3-boyutlu platformun önerilmesi amaçlanmıştır. İlk bölümde farklı hidrojel bazlı biyomateryallerin absorbans spektrumları görünür ışık kullanılarak incelenmiştir. En iyi performansı gösteren hidrojeli bulduktan sonra çalışma, XTT canlılık testi ve canlı/ölü sitotoksisite testi kullanılarak hücre çoğalmasına odaklanılmıştır. Hücre canlılığı aljinat-jelatin, aljinat ve selüloz gibi iskelelerle karşılaştırıldığında, aljinat-jelatin hidrojelin mezenkimal kök hücrelerinin canlılığını arttırdığı tespit edildi. Bu artış, 2D kültürde büyütülen hücrelerle karşılaştırıldığında da aynı şekilde gözlendi. Bu çalışmadan elde edilen bulgular, literatürdeki diğer çalışmaların verileriyle tutarlıdır. Bu nedenle, aljinat-jelatin kompozitleri doku mühendisliğinde hücre proliferasyonunu iyileştirmek için umut verici bir aday olabilir.

Anahtar Kelimeler: Mezenkimal kök hücreler, Aljinat-jelatin kompozit, Hücre canlılığı, Doku mühendisliği, 3D hücre kültürü.

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1. Introduction

Bone tissue engineering entails the fabrication of biomaterials tailored to foster bone regeneration within living organisms. Strategies for bone regeneration include cells, biomaterials, growth factors and a synergistic interaction between these components (Gugliandolo et al. 2021). Even though autologous bone grafting are widely regarded as the best-performing standard due to their osteoinductive properties, which are attributed to vascularization, and osteogenic potential, this technique still has some risks in terms of donor morbidity and intervention-induced inflammation (Habibovic and de Groot 2007; Kolk et al. 2012). Thus, foreseeable therapies fail to meet clinical need for bone defects. Stem cell-based treatment has been suggested as an up-and-coming avenue to tackle the constraints in bone grafting methods. On a vast scale, adult stem cells (ASCs) have been investigated in various domains for addressing various diseases, with neurological abnormalities, osteoarthritis, and musculoskeletal disorders (Chang et al. 2014; Brown et al. 2019). Among ASCs, Mesenchymal stromal cells (MSCs) are particularly pivotal because of their capability for self-rejuvenating and differentiating into specific niche, such as adipocytes, osteocytes or chondrocytes, thereby underlining their prominence in clinical cell therapy (McKee and Chaudhry 2017; Brown et al. 2019).

Stem cell-based therapy is large-scale expansion and homogenous differentiation for efficient clinical applications. Generally, there are two methods applied for cellular proliferation and differentiation potential: Traditional 2D culture and Modern 3D cell culture. Notable differences between 2D and 3D culture include the efficiency of MSC behaviour. MSCs cultivation in 2D culture leads to some difficulties including cellular senescence, lack of site-specificity and suboptimal engraftment (Zhao et al. 2019). This monolayer cell culture for the stem cell expansion gives also rise to a lack of cellular compatibility and morphological changes (Kapałczyńska et al. 2018; Chen et al. 2022). Given disadvantages of traditional culture techniques, modern 3D methods have been established to provide structural support and mimic the extracellular matrix (ECM) (Jose et al. 2020). By designing and engineering hydrogels with mechanical properties and biochemical cues, 3D culture systems have recently been improved to promote multi-layered tissue-like models and cell-matrix interactions in vivo conditions (Park et al. 2021). Therefore, hydrogels, due to their adaptability, biocompatibility, and ease of fabrication, are frequently employed as scaffolds in cell therapy and tissue engineering applications (Ho et al. 2022; Liu et al. 2022). Importantly, the regulation of mineral deposition within these composites is required to ensure their closely similarity to natural bone properties and stimulate bone formation to the greatest extent possible.

Constituted by (1→4) linked chains of β-D-mannuronic acid (M-residues) and α-L-guluronic acid (G-residues), alginate is a linear polysaccharide integral in the application of bone tissue

engineering. These chains contain a combination of homopolymer G- and M-residues, alongside copolymer G-M residues (see Figure 1) (Daemi and Barikani 2012; Neves et al. 2020). The proportion of G-, M- and G-M residues, along with the G:M ratio, designate the properties of the hydrogels. Parameters affected contain mechanical strength, substrate stiffness, the molecular weight, the internal porosity, and degradation rate of the alginate solution (Kuo and Ma 2001; Draget et al. 2006). Alginate hydrogels are formalized by the dissolution of alginic acid at the desired weight per volume (w/v) to produce an alginate solution, followed by the immersed into a polymerisation buffer containing divalent ions for crosslinking of the solution (Augst et al. 2006). The impression of distinctive crosslinking agents on the viability of Schwann cells in 3D hydrogel has been reported by previous research. This study reported the positive effects of barium ions (Ba2+) on mechanical properties, and also the significant outcomes of calcium ions (Ca2+) on cellular viability (Sarker et al. 2018). Alginate hydrogels combined with gelatin have been suggested to improve cell viability, and thus displayed the homogeneous distribution within fibroblasts (Sarker et al. 2014). A recent study has reported that alginate hydrogels can ameliorate the biocompatibility and the proliferation of MSCs in 3D niches (Neves et al. 2020). In another study, Alginate hydrogels crosslinked with calcium have been notified the similarity to the ECM, which outweighs their disadvantages such as long-term stability and viscosity (Yuan et al. 2017; Dranseikiene et al. 2020).

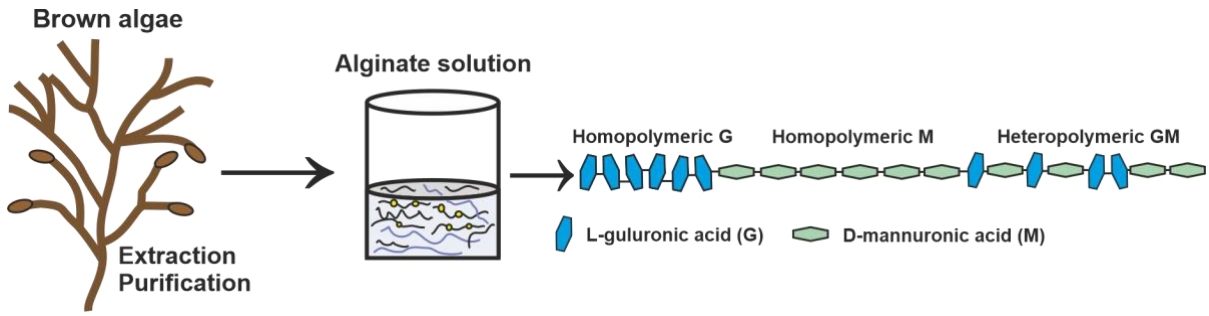


Figure 1. The texture of alginate derived from brown algae, which is comprised of β-D-mannuronic acid (M-residues) and α-L-guluronic acid (G-residues) blocks. Modifications in alginate molecular weight and monomeric composition improve by means of some factors such as species, extraction process and purification method. These distincts lead to divergent structural features, namely homopolymeric and heteropolymeric content. Those are significant in shaping the development of biomaterials and determine their properties and applications.

In this research, alginate combined with CaCl2 was utilized to fabricate 3D extracellular microenvironments for MSC therapy, aiming to enhance cellular biocompatibility. The main objective was to to propose a novel 3D platform including alginate-gelatin composites to address the need for tissue engineering techniques that support mesenchymal stromal cell viability and differentiation potential. The investigation assessed the absorbance spectra of different hydrogel-based biomaterials (including cellulose, alginate&gelatin, alginate and TrueGel3D) using UV-visible

light. After finding the best performance under alginate&gelatin, MSCs derived from different donors were embedded into alginate&gelatin, and then compared with those in conventional 2D cell culture settings. Moreover, cellular viability was analyzed using XTT Viability Assay and monitored using Live/Dead Assay under confocal microscopy. Therefore, alginate could be a promising agent to construct a 3D extracellular microenvironment for its roles in promoting cellular biocompatibility.

2. Materials and Methods

2.1. Cell Culture

Mesenchymal stromal cells (MSCs) obtained from three different non-diabetic adult donors in Lonza Inc. (Slough, United Kingdom) were purchased. All MSCs were characterized by tri-lineage differentiation assay and immunocytochemical analysis as detailed by The International Society for Cellular Therapy (Dominici et al. 2006). The MSCs were cultured in a humidified incubator at 37°C and 10% CO2. MSCs were preserved in cryovials in liquid nitrogen until used.

2.2. Test Compounds

Cellulose hydrogel was provided by UPM Biochemicals, Helsinki and 1% cellulose hydrogel was used. TrueGel3D Polymer was purchased from Sigma-Aldrich and made ready by mixing 170 μL FAST DEXTRAN (Cat. No. TRU-FDE), 175 μL water (Cat. No. TRUWA) and 34.5 μL TrueGel3D buffer (Cat. No. TRUBUF-55PH). Alginate solution was prepared by combining with alginate and gelatin. Alginic acid including sodium salt and Gelatin was purchased Sigma-Aldrich (Cat. No. 71238-250G, Cat. No. G1393 respectively) and alginic acid sodium salt was dissolved in Dulbecco's Phosphate Buffered Saline (DPBS) (Thermo-Fisher, Cat. No. 12037539) in order to prepare 1% (w/v) alginate. pH was adjusted to 7.2–7.4 and the solution was sterile filtered before further use. Gelatin was used in order to prepare 1% (w/v) gelatin beads. As a cross-linking solution, 100 mM CaCl2/10 mM HEPES buffer was prepared by mixing CaCl2 and HEPES buffer (Sigma-Aldrich, C1016 and Sigma-Aldrich, H3375) to DPBS.

2.3. Alginate hydrogel preparation and 3D cell culturing

1% alginate solution (w/v) was prepared with 1.0% Alginate and 0.1% Gelatin bead solution. MSCs was added to the solution to make a final concentration of $1x10^5$ cells/100μL in a 1% alginate solution. The cell/alginate/gelatin solution was added into CaCl2/10mM HEPES buffer, and then pH

was adjusted to 7.3. Cells were cultivated in DMEM high glucose with 2 mM L-glutamine, 1% penicillin/streptomycin, 20% heat-inactivated fetal bovine serum and 1 ng/mL basic fibroblast growth factor. While all chemicals were purchased from Sigma-Aldrich, fibroblast growth factor was purchased from Peprotech, UK. MSCs were mixed with 1.0% Alginate solution with cell densities of $1x10^5$ cells/100μL for 3D cell culturing. After 30 min at 37°C, 50μL of MSC culture medium was added on top of the cell suspension with 3D alginate. MSCs embedded in 3D alginate were cultured in a humidified incubator at 37°C and 10% CO2. Medium was changed every two-three day. All cells were used before passage 8. MSCs obtained from three different donors were used for biological replicates.

2.4. 4'6-Diamidino-2-phenylindole dihydrochloride (DAPI) and Fluorescein Isothiocyanate Isomer I (FITC/dextran)

DAPI solution was purchased from Sigma Aldrich with Cat. No. D9542 and this solution was prepared by diluting 1:2000 in sterile DPBS. The stock solution of FITC/dextran was purchased from Sigma Aldrich and was diluted in Dimethyl sulphoxide (DMSO, BDH Chemicals 103234L) to a final concentration of 6 μg/mL. Dilutions of biomaterials were prepared and placed into a 96-well plate to be stained with FITC and DAPI. The emission values were recorded between 280 and 800 nm using the plate reader.

2.5. Cellular XTT Viability Assay

XTT dye was used to detect cell viability by incubating cells in 96-well plates for 30min, 1h, 2h, 3h, and 4h. $1x10^5$ cells/100μL of MSCs embedded into different scaffolds per well were cultivated in a 96 well plate. And then the experiments were incubated at 37°C with 10% CO2 until the end of third day. Cell proliferation kit II was purchased from Sigma-Aldrich, and then was mixed as detailed in the constructor's protocol. After placing XTT labelling reagent and electron coupling reagent into a microcentrifuge tube, XTT solution was transferred to each well. Absorbance values of MSCs in 3D were then measured spectrophotometrically at a wavelength of 490 nm at the 30th minute and at the end of the 1st hour, 2nd hour 3rd hour and 4th hour. Results were analysed by means of the reference wavelength as 650 nm.

2.6. Live/Dead Cytotoxicity Assay

$1x10^5$ cells/100μL of MSCs were cultivated in alginate hydrogel within a non-tissue culture treated 24 well plate for three days at 37°C with 10% CO2. Triplicate experiments were done for each condition and controls. The dye was prepared as detailed in the constructor's protocol. MSCs in 3D cell culture were dyed with a Live/Dead viability/cytotoxicity assay and incubated until the end of 45 minutes, followed by the fixation in 4% paraformaldehyde for 30 minutes and stained with DAPI solution for 15 minutes. Cells in 3D were visualized by means of Nikon A1-R inverted confocal microscope. While live cells stained with calcein was visualized at 494/517 nm, dead cells stained with Ethidium Homodimer-1 (EthD-1) at excitation/emission of 528/617 nm.

2.7. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA, USA). Results were analysed for significant differences between groups using one-way analysis of variance (ANOVA) followed by Bonferroni correction and Student's t-test. $P<0.05$ was considered statistically significant. Three independent experiments were made in triplicate.

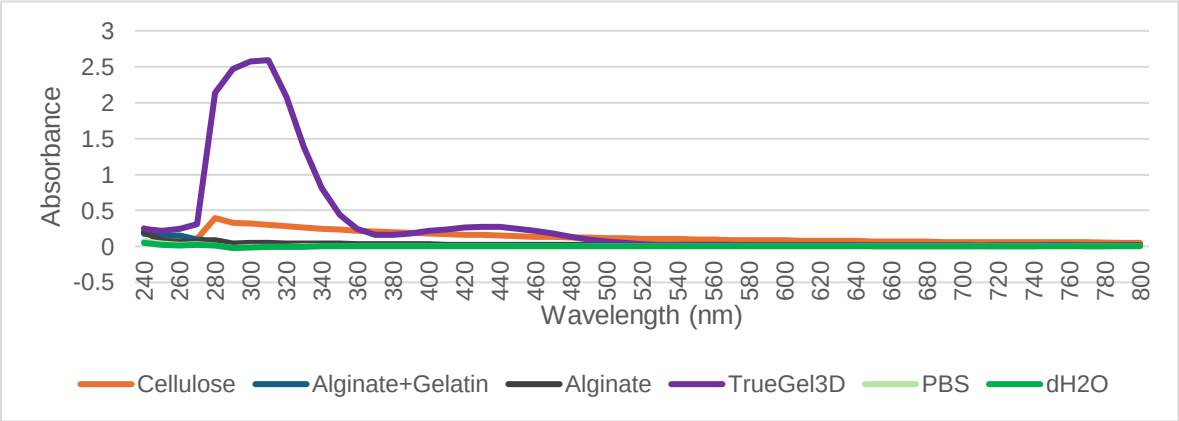
3. Findings and Discussion

3.1. Development of 3D cell culturing using Alginate-Gelatin hydrogel

The optical properties of alginate were assessed across a wavelength range of 240-800 nm, with comparison to other scaffolds including cellulose, alginate, TrueGel3D. 1% Alginate-Gelatin exhibited a lower absorption across the whole spectrum, as illustrated in Figure 2A and Figure 2B. The fluorescence emission for each substrate including DAPI and FITC-dextran was measured and evaluated the compatibility of alginate hydrogel with fluorescence-based techniques. This evaluation involved excitation wavelengths of 405 nm for DAPI, 488 nm for Fluorescein Isothiocyanate-dextran (FITC-dextran) with their emission recorded between 400 and 800 nm, as depicted in Figure 3A and Figure 3B respectively. The findings indicated that the fluorescence emission in alginate-gelatin hydrogel was not higher than in other compounds, establishing the convenience of this hydrogel with fluorescence-based techniques. That is why, the fluorescence emission in alginate-gelatin hydrogel is suitable for cell culture studies as it does not give any background. These results align with the findings of Bhattacharya et al. (Bhattacharya et al. 2012). Regarding spectral properties, alginate

depicted autofluorescent properties at various wavelengths corresponding to commonly used fluorophores including UV for DAPI and green for FITC-dextran.

A)



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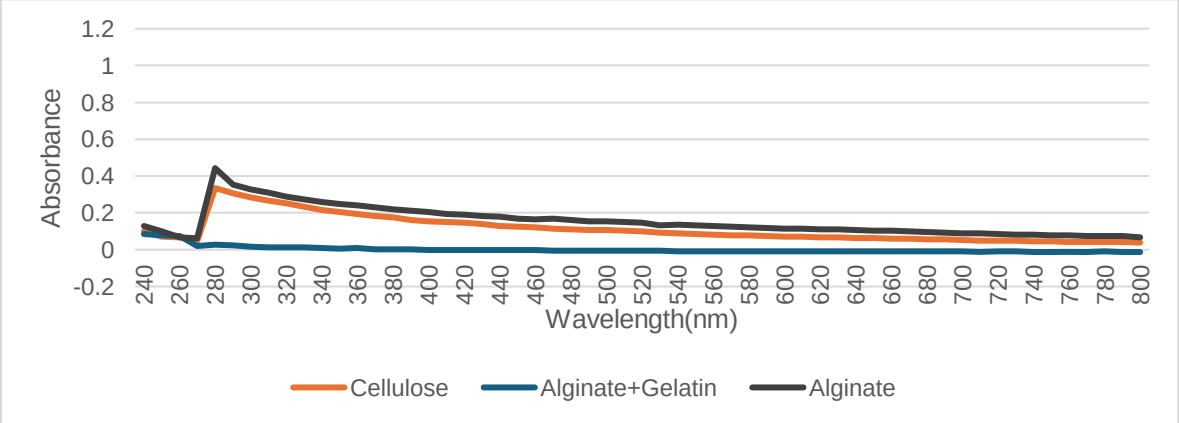
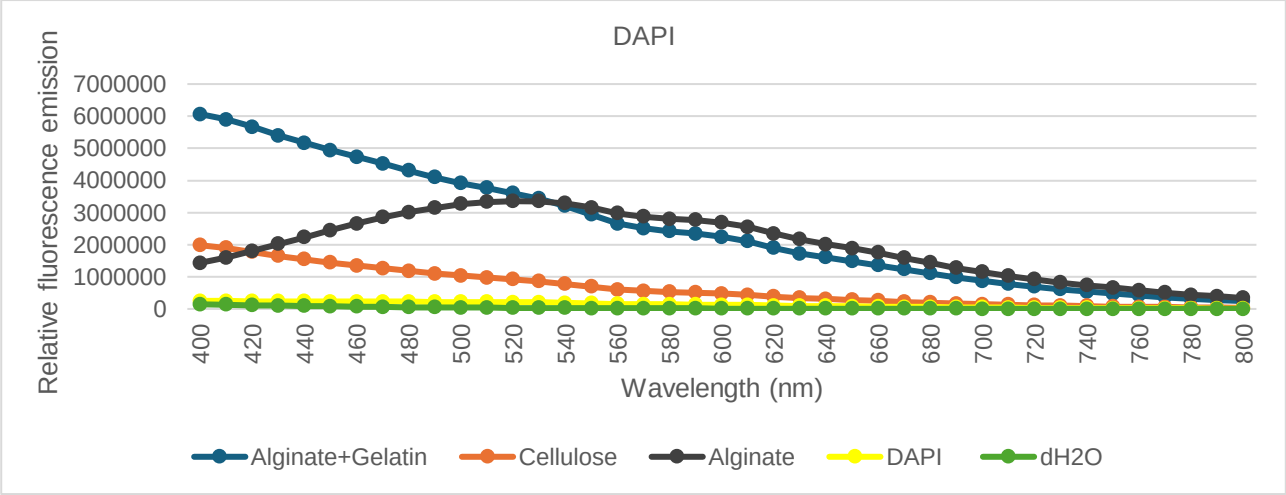


Figure 2. Alginate-based hydrogel depicted favorable optical properties in terms of its light absorbance spectrum. (A) The composite of alginate and gelatin demonstrated a consistently lower absorbance across the entire light spectrum. When comparing the light absorbance spectrum of alginate-gelatin hydrogel with other scaffolds including cellulose, alginate, TrueGel3D, it was found that the alginate-gelatin composite exhibited the lowest absorbance among the tested biomaterials. (B) When comparing the light absorbance spectrum of alginate-gelatin hydrogel with cellulose and only alginate, it was found that the alginate-gelatin composite exhibited the lowest absorbance among the tested biomaterials.

A)



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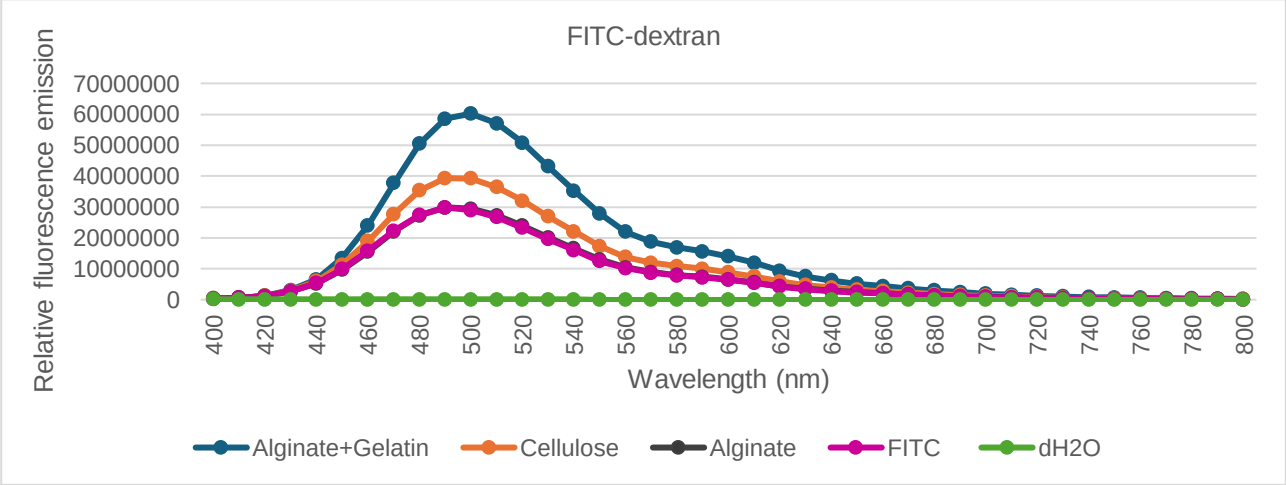


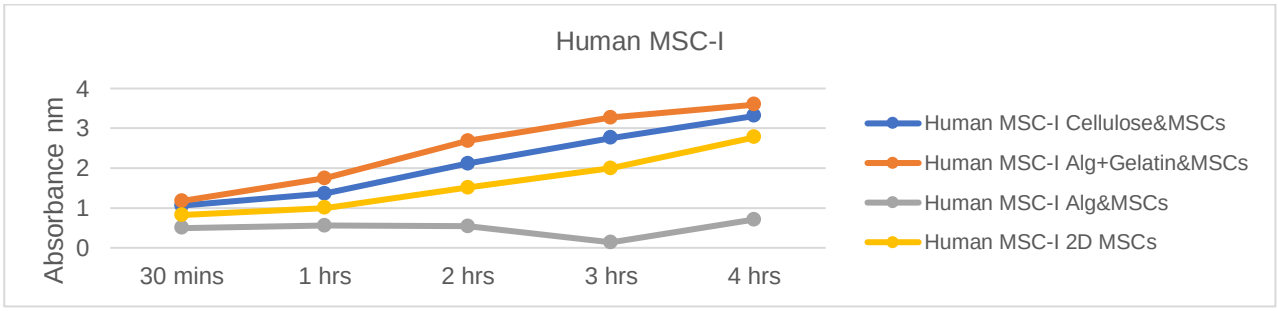
Figure 3. Alginate-based hydrogels illustrated a high level of fluorescence emission when analyzed using fluorescence spectroscopy. The fluorescence spectra from different substrates including cellulose, alginate and alginate-gelatin were recorded using a spectral scanning plate reader. (A) In order to reflect typical conditions used in biological imaging, the excitation wavelength was selected for DAPI at 405 nm. (B) In order to reflect typical conditions used in biological imaging, the excitation wavelength was selected for FITC-dextran at 488 nm. (A, B) The alginate-gelatin composite exhibited higher fluorescence emission at these excitation wavelengths, commonly used by molecular probes with 405 nm and 488 nm.

3.2. Viable MSCs could be supported in 3D Alginate hydrogels

The viability of MSCs cultivated within different biomaterials (including cellulose, alginate-gelatin and only alginate) and traditional 2D culture was evaluated utilizing an XTT viability assay. The findings indicated that MSCs derived from different three donors (MSC-I, MSC-II and MSC-III) embedded in alginate-gelatin hydrogel exhibited significantly higher viability compared to those grown in a traditional 2D culture, with normalization to the 2D control group, as depicted in Figure 4A, Figure 4B and Figure 4C. The values were stated as means ± standard errors, followed by their analysis using GraphPad Prism software. Statistical analyses were compared using a one-way

ANOVA followed by Bonferroni correction (Cl 95%). Three different tests were conducted, and the results were performed as mean ± SEM.

A)



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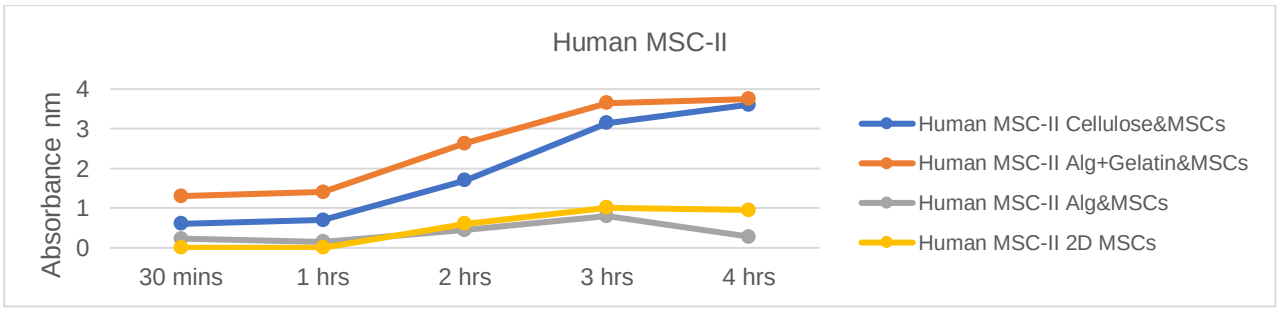

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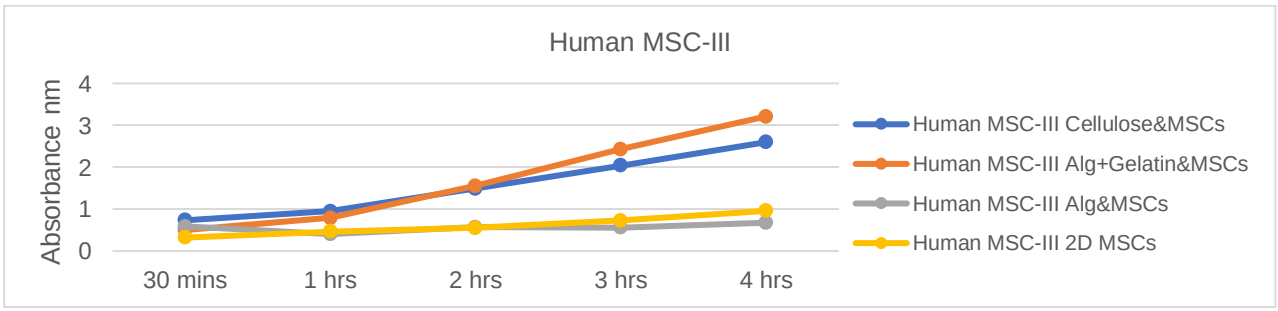

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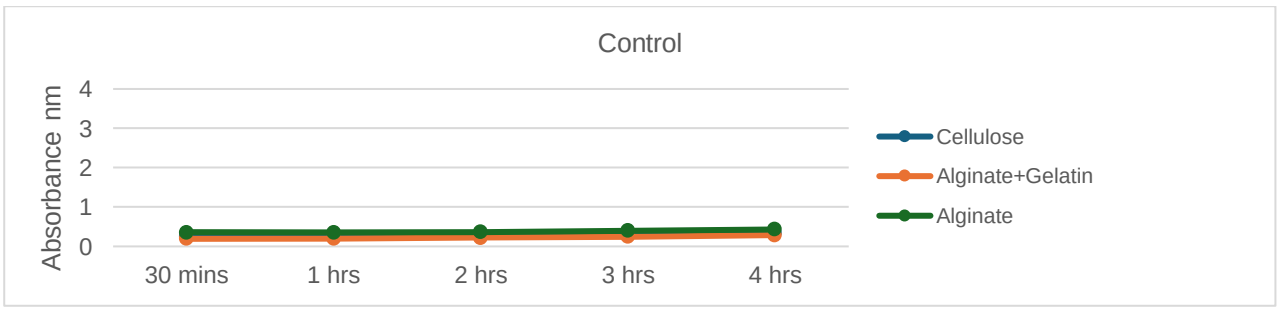

Figure 4. Human MSCs from different three donors were viable in alginate-gelatin hydrogel. The viability of MSCs derived from different three donors within diverse hydrogels was assessed using an XTT viability assay. (A, B and C) The XTT assay measured cell viability for MSC-I, MSC-II and MSC-III embedded in alginate, cellulose and alginate-gelatin composite, normalised to a traditional 2D culture. Cell viability for MSC-I, MSC-II, MSC-III exhibited significantly higher in alginate-gelatin hydrogel, compared to others in cellulose and only alginate. (D) Additionally, hydrogels without cells were evaluated as a control, demonstrating no viability within the scaffold. MSCs from three different donors cultured in 3D alginate-gelatin hydrogel exhibited significantly higher viability compared to those grown in other hydrogels and in a traditional 2D culture.

3.3. 3D Alginate hydrogel is compatible with confocal microscopy-based assays

In order to validate the proxy viability data obtained from the XTT assay, confocal imaging of MSCs embedded in alginate-gelatin hydrogel was conducted using a Live/Dead Assay. This assay was performed with calcein with green colour for alive cells, ethidium homodimer-1 with magenta colour for dead cells and stained with DAPI. The dye was prepared as detailed in the constructor's protocol. MSCs in 3D cell culture were dyed with a Live/Dead viability/cytotoxicity assay and incubated until the end of 45 minutes, followed by the fixation in 4% paraformaldehyde for 30 minutes and stained with DAPI solution for 15 minutes. The analysis revealed that MSCs seeded into the alginate-gelatin composite have predominantly viable cells, with a minimal presence of non-viable cells. MSCs embedded in different hydrogels were cultivated for 72 hours, and subsequent XTT analysis illustrated that MSCs in 3D alginate-gelatin composite are compatible with higher viability than MSCs grown in a conventional 2D culture, with normalization to the 2D control group (Figure 5A and Figure 5B).

A) B)

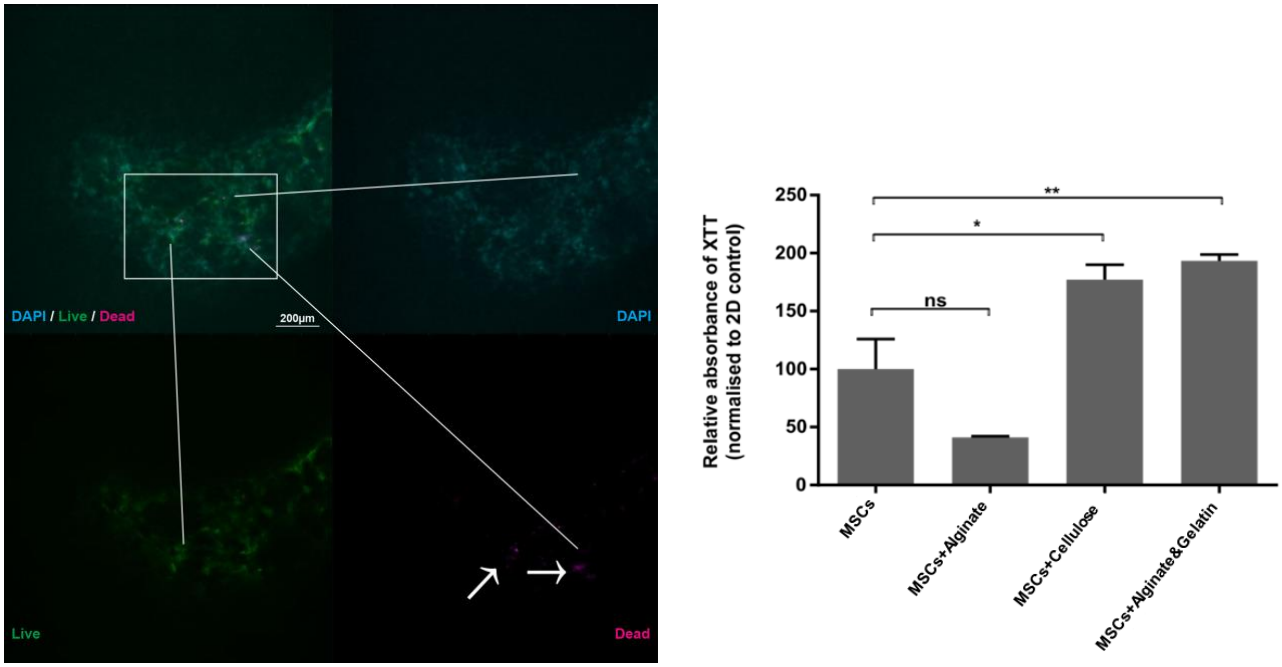

Figure 5. Imaging of viable and a few dead cells in alginate-gelatin composite. (A) Live/Dead Assay was conducted using confocal microscopy in order to visualise living and dead cells exposed to alginate and gelatin composite scaffolds. Confocal laser-scanning microscopy indicated that MSC viability did not decrease in the 3D platform. (B) MSCs embedded in various hydrogels were cultivated for 72 hours and followed by XTT viability analysis. The data analysis revealed that MSCs in the alginate-gelatin composite demonstrated higher relative cell viability in the 3D platform compared to other conditions.

3D biomaterials have been extensively familiarized in various grounds of stem cell research (Bhattacharya et al. 2012). In comparison with traditional 2D methods, 3D cell culturing represents

more similarity with the ECM of the biological environment and closely mimics in vivo tissue (Dutta and Dutta 2009; Bowers et al. 2010). Bone-graft replacements, which include natural and synthetic biomaterials facilitate cellular proliferation and osteogenic differentiation potential (Finkemeier 2002). Additionally, cylindrical titanium scaffolds have also been utilized to attach autologous bone to ameliorate injured cells (Dimitriou et al. 2011). In this study, different hydrogels were evaluated for their fluorescence emission and absorbance properties and alginate-gelatin composite was identified as the superior hydrogel because of its optical properties (Figure 2A and Figure 2B). Bhattacharya and his teams previously examined the fluorescence emission and the absorbance spectrum of nanofibrillar cellulose, finding that 0.5% cellulose displayed a lack of UV-visible light and no autofluorescence in the range 420-700 nm to absorb (Bhattacharya et al. 2012). Bhattacharya and his coworkers also recorded UV-visible absorbance between 300 and 550 nm, and then the fluorescence emission of cellulose hydrogel at diverse excitation wavelengths of 405, 488 and 560 nm (Bhattacharya et al. 2012). Their findings align with the present study, which examined higher level of fluorescence emission in the alginate-gelatin hydrogel when excited at wavelengths of 405 and 488 nm (Figure 3A and Figure 3B). The alginate-gelatin composite exhibited compatibility with fluorescence-based practices, providing a higher signal of fluorescence emission for DAPI and FITC compared with other materials (Figure 3A and Figure 3B). Research has indicated that MSCs in hydrogel demonstrated an enhanced proliferation when covered with PEG hydrogels and cohesive ligands RGDSP such as Arg-Gly-Asp-Ser-Pro (Aggarwal and Pittenger 2005). More recently, Widera lab reported that nanofibrillar cellulose have no cytotoxic effect on cellular viability for bone-marrow and palatal MSCs, however a higher concentration showed a decrease for cellular viability compared to 2D cell culture (Azoidis et al. 2017). Despite the advantages of nanofibrillar cellulose on cell proliferation, Sheard and his friends depicted that an anionic version of nanofibrillar cellulose remarkably enhanced MSCs viability (Sheard et al. 2019). Moreover, a study indicated that 3D cultivation of MSCs in polysaccharide hydrogel increases cell viability (Yin et al. 2020). In this research, the findings showed that MSCs embedded in alginate&gelatin composite exhibited greater viability than those grown in cellulose, alginate, and a traditional 2D culture (Figure 4A, Figure 4B and Figure 4C).

4. Conclusions and Recommendations

It is evident that 3D cell culturing holds significant promise for tissue regeneration in various ailments. Among all the biomaterials, natural hydrogels have acknowledged particular consideration in the development of biomaterials in terms of their biocompatibility and structural similarities to

inherent ECM. By exploring different biomaterials focusing on the optical assets of the hydrogels, the alginate-gelatin composite has been identified as the best hydrogel. Moreover, cell viability and proliferation have been examined to provide valuable insights for future experiments in determining the most effective hydrogel. These hydrogels derived from brown algae, have indicated exceptional circumstances for utilizing in physiologic milieus, by virtue of their bioactive features to enhance cellular biocompatibility. Despite these promising results, extensive in vitro and also in vivo research is required to understand the approaches of alginate hydrogels in healing various disorders. Understanding the interactions between MSCs and alginate-gelatine hydrogels is anticipated to be promising for optimizing therapeutic applications to promote bone regeneration. The promising findings of these study suggest that alginate-gelatine composite could play a significant role in advancing therapeutic approaches.

Author's Contributions

The author agreed to publish this manuscript in "The Black Sea Journal of Science". M. Bicer mainly contributed to write this manuscript and analyze the data. The author reviewed the manuscript draft and approved the final version for submission.

Statement of Conflict of Interest

The author declares no conflict of interest.

Statement of Research and Publication Ethics

The author declares that all the rules required to be followed within the scope of "Higher Education Institutions Scientific Research and Publication Ethics Directive" have been complied with in all processes of the article, that The Black Sea Journal of Science and the editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than The Black Sea Journal of Science.

Ethical approval is not required for this research article.

References

Aggarwal, S., and Pittenger, M. F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*, 105, 1815-1822.

Augst, A. D., Kong, H. J., and Mooney, D. J. (2006). Alginate hydrogels as biomaterials. *Macromol Biosci*, 6, 623-633.

Azoidis, I., Metcalfe, J., Reynolds, J., Keeton, S., Hakki, S. S., Sheard, J., and Widera, D. (2017). Three-dimensional cell culture of human mesenchymal stem cells in nanofibrillar cellulose hydrogels. *MRS Communications,* 7, 458-465.

Bhattacharya, M., Malinen, M. M., Lauren, P., Lou, Y-R., Kuisma, S. W., Kanninen, L., Lille, M., Corlu, A., GuGuen-Guillouzo, C., Ikkala, O., Laukkanen, A., Urtti, A., and Yliperttula, M. (2012). Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. J*ournal of Controlled Release*, 164, 291-298.

Bowers, S. L., Banerjee, I., and Baudino, T. A. (2010). The extracellular matrix: at the center of it all. *J Mol Cell Cardiol*, 48, 474-482.

Brown, C., McKee, C., Bakshi, S., Walker, K., Hakman, E., Halassy, S., Svinarich, D., Dodds, R., Govind, C. K., and Chaudhry, G. R. (2019). Mesenchymal stem cells: Cell therapy and regeneration potential. *J Tissue Eng Regen Med,* 13, 1738-1755.

Chang, K. A., Lee, J. H., and Suh, Y. H. (2014). Therapeutic potential of human adipose-derived stem cells in neurological disorders. *J Pharmacol Sci*, 126, 293-301.

Chen, W., Pan, W., Wang, J., Cheng, L., Wang, J., Song, L., Hu, Y., and Ma, X. (2022). Emerging two-dimensional monoelemental materials (Xenes): Fabrication, modification, and applications thereof in the field of bioimaging as nanocarriers. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*, 14, e1750.

Daemi, H., and Barikani, M. (2012). Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. *Scientia Iranica*, 19, 2023-2028.

Dimitriou, R., Jones, E., McGonagle, D., and Giannoudis, P. V. (2011). Bone regeneration: current concepts and future directions. *BMC Med*, 9, 66.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Krause, D. S., Deans, R. J., Keating, A., Prockop, D. J., and Horwitz, E. M. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy,* 8, 315-317.

Draget, K. I., Skjåk-Bræk, G., and Stokke, B. T. (2006). Similarities and differences between alginic acid gels and ionically crosslinked alginate gels. *Food Hydrocolloids,* 20, 170-175.

Dranseikiene, D., Schrüfer, S., Schubert, D. W., Reakasame, S., and Boccaccini, A. R. (2020). Cell-laden alginate dialdehyde-gelatin hydrogels formed in 3D printed sacrificial gel. *J Mater Sci Mater Med*, 31, 31.

Dutta, R. C., and Dutta, A. K. (2009). Cell-interactive 3D-scaffold; advances and applications. *Biotechnol Adv*, 27, 334-339.

Finkemeier, C. G. (2002). Bone-grafting and bone-graft substitutes. *The Journal of bone and joint surgery American volume,* 84-a, 454-464.

Gugliandolo, A., Fonticoli, L., Trubiani, O., Rajan, T. S., Marconi G. D., Bramanti, P., Mazzon, E., Pizzicannella, J., and Diomede, F. (2021). Oral Bone Tissue Regeneration: Mesenchymal Stem Cells, Secretome, and Biomaterials. *International journal of molecular sciences,* 22, 5236.

Habibovic, P., and de Groot, K. (2007). Osteoinductive biomaterials--properties and relevance in bone repair. *J Tissue Eng Regen Med*, 1, 25-32.

Ho, T-C., Chang, C-C., Chan,, H-P., Chung, T-W., Shu, C-W., Chuang, K-P., Duh, T-H., Yang, M-H., and Tyan, Y-C. (2022). Hydrogels: Properties and Applications in Biomedicine. *Molecules*, 27, 2902.

Jose, G., Shalumon, K. T., and Chen, J-P. (2020). Natural Polymers Based Hydrogels for Cell Culture Applications. *Current Medicinal Chemistry*, 27, 2734-2776.

Kapałczyńska, M., Kolenda, T., Przybyła, W., Zajączkowska, M., Teresiak, A., Filas, V., Ibbs, M., Bliźniak, R., Łuczewski, Ł., and Lamperska, K. (2018). 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. *Arch Med Sci,* 14, 910-919.

Kolk, A., Handschel, J., Drescher, W., Rothamel, D., Kloss, F., Blessmann, M., Heiland, M., Wolff, K-D., and Smeets, R. (2012). Current trends and future perspectives of bone substitute materials – From space holders to innovative biomaterials. *Journal of Cranio-Maxillofacial Surgery,* 40, 706-718.

Kuo, C. K., and Ma, P. X. (2001). Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. *Biomaterials*, 22, 511-521.

Liu, K., Wiendels, M., Yuan, H., Ruan, C., and Kouwer, P. H. J. (2022). Cell-matrix reciprocity in 3D culture models with nonlinear elasticity. *Bioact Mater,* 9, 316-331.

McKee, C., and Chaudhry, G. R. (2017). Advances and challenges in stem cell culture. *Colloids Surf B Biointerfaces,* 159, 62-77.

Neves, M. I., Moroni, L., and Barrias, C. C. (2020). Modulating Alginate Hydrogels for Improved Biological Performance as Cellular 3D Microenvironments. *Front Bioeng Biotechnol*, 8, 665.

Park, Y., Huh, K. M., and Kang, S. W. (2021). Applications of Biomaterials in 3D Cell Culture and Contributions of 3D Cell Culture to Drug Development and Basic Biomedical Research. *International journal of molecular sciences*, 22:

Sarker, B., Singh, R., Silva, R., Roether, J. A., Kaschta, J., Detsch, R., Schubert, D. W., Cicha, I., and Boccaccini, A. R. (2014). Evaluation of fibroblasts adhesion and proliferation on alginate-gelatin crosslinked hydrogel. *PLoS One,* 9, e107952.

Sarker, M., Izadifar, M., Schreyer, D., and Chen, X. (2018). Influence of ionic crosslinkers (Ca(2+)/Ba(2+)/Zn(2+)) on the mechanical and biological properties of 3D Bioplotted Hydrogel Scaffolds. *J Biomater Sci Polym Ed*, 29, 1126-1154.

Sheard, J. J., Bicer, M., Meng, Y., Frigo, A., Aguilar, R. M., Vallance, T. M., Iandolo, D., and Widera, D. (2019). Optically Transparent Anionic Nanofibrillar Cellulose Is Cytocompatible with Human Adipose Tissue-Derived Stem Cells and Allows Simple Imaging in 3D. *Stem Cells Int*, 2019, 3106929.

Yin, Q., Xu, N., Xu, D., Dong, M., Shi, X., Wang, Y., Hao, Z., Zhu, S., Zhao, D., Jin, H., and Liu, W. (2020). Comparison of senescence-related changes between three- and two-dimensional cultured adipose-derived mesenchymal stem cells. *Stem Cell Res Ther*, 11, 226.

Yuan, L., Wu, Y., Fang, J., Wei, X., Gu, Q., El-Hamshary, H., Al-Deyab, S. S., Morsi, Y., and Mo, X. (2017). Modified alginate and gelatin cross-linked hydrogels for soft tissue adhesive. *Artif Cells Nanomed Biotechnol*, 45, 76-83.

Zhao, L., Hu, C., Zhang, P., Jiang, H., and Chen, J. (2019). Preconditioning strategies for improving the survival rate and paracrine ability of mesenchymal stem cells in acute kidney injury. *J Cell Mol Med*, 23, 720-730.