

21 BIOFORMULATION AND CHARACTERIZATION OF 3D PLATFORM FOR TISSUE REGENERATION- A FUTURE PERSPECTIVE

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ABSTRACT

Tissue engineering is a field that aims to regenerate tissues in a particular region of the body through the creation of scaffolds that can mimic the extracellular matrix to allow cell adhesion, proliferation, and differentiation. This paper aims to create a new 3D-printed sodium alginate gelatin hydrogel scaffold through an extrusion bioprinting method, CaCl_2 crosslinking, UV irradiation, and freeze-drying to obtain a biocompatible and mechanically sound scaffold. Fourier Transform Infra-Red spectroscopy results verified the interaction between hydrogen bonds and electrostatic interactions between the carboxylate groups of sodium alginate and amide groups of gelatin, indicating a polyelectrolyte complex formation with preserved secondary structures. X-ray Diffraction results verified the integration of the hydrogel matrix through broad amorphous diffraction peaks at certain 2θ values. FESEM results indicated a hierarchical porous structure with a nanofibrous surface resembling collagen to allow cell migration and vascularization. Water absorption and swelling ratios of the scaffolds were high due to the presence of hydrophilic groups. Biodegradability tests in collagenase II solution indicated a 55% mass loss after 48 h due to the presence of hydrophilic groups. Sodium alginate gelatin scaffolds are promising scaffolds for tissue regeneration due to their appropriate mechanical properties.

KEYWORDS: Tissue Engineering, Biomaterials, 3D Platform

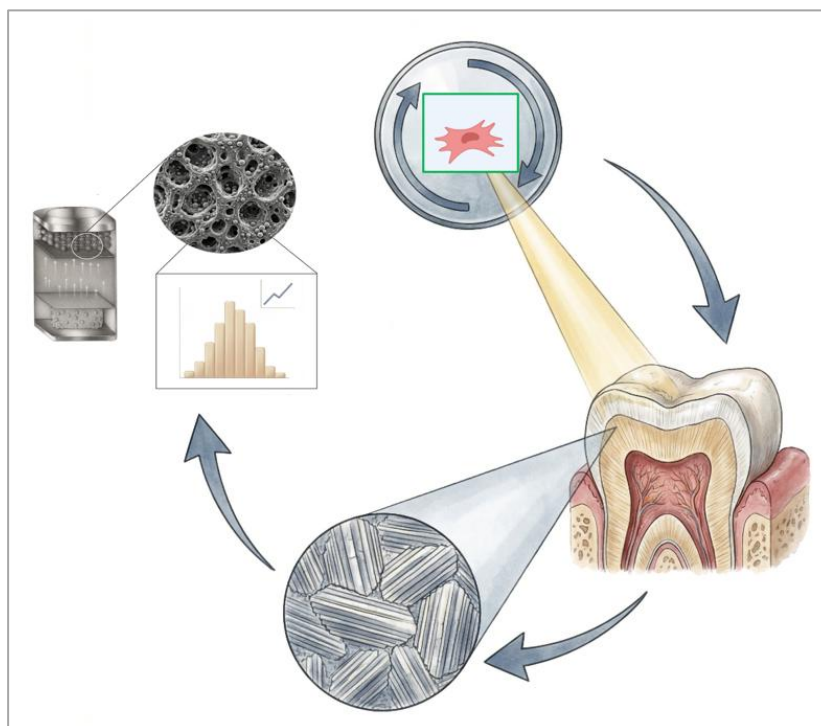


Fig.: Graphical Abstract

1. INTRODUCTION

As the world's population has grown, the number of diseases and health problems has reached an all-time high. And if any organs are hurt, tissues are the first line of defense and get hurt or change shape first. So, tissue engineering is the only modern way to fix this problem by healing the damaged area and bringing it back to normal. Tissue engineering seeks to repair or replace damaged or insufficient body tissues. To do this, it needs three main things: biomaterials, growth agents,

and a source of cells [1]. The biomaterial is very important for tissue regeneration in this method because it serves as a three-dimensional scaffold that gives cells a place to grow and change into new tissue. To promote efficient tissue regeneration and augment the manifestation of organ-specific functions and cellular differentiation, a scaffold must replicate the natural extracellular matrix (ECM) milieu of artificial tissue [1]. Scaffolds act as temporary extracellular matrices (ECMs), providing an optimal environment for cell adhesion, development, proliferation, and differentiation, thereby facilitating the formation of new tissue [2,3]. For scaffolds to work with human tissues, the materials they are made of must be very biocompatible. Hydrogel scaffolds are three-dimensional networks made of materials that dissolve in water [4]. These scaffolds can quickly soak up water and hold a lot of it without falling apart. These scaffolds are a good choice for tissue engineering because they don't need to be taken out after implantation, so patients don't need to have any more procedures.

An excellent scaffold must be biocompatible, biodegradable, have strong surface conductivity, good porosity, and acceptable mechanical qualities [5,6]. The scaffold's properties are determined by its intrinsic qualities and the interaction of the material's chemical and physical properties [7,8]. Also, the scaffold needs to break down in a controlled way and be strong enough to handle the stress of cells that are growing and changing, both in the lab and in the body. The scaffold should also be biocompatible, which is another important quality. Recent advancements in tissue engineering have enabled the creation of scaffolds with optimal properties through the utilization of composites or mixtures. Like the biopolymers sodium alginate and gelatin, these synthetic polymers will not be used in tissue engineering because they break down slowly and are not very biocompatible.

Sodium alginate (ALG) is a linear polysaccharide made up of β -D-mannuronic acid (M) and α -L-guluronic acid (G). It is inexpensive, easy to use, and has great gelation properties [4]. Because of these characteristics, it is a good place for encapsulated cells to live. This is why it is so popular in tissue engineering. The molecular chains of sodium alginate also have a lot of -COO- groups in them. These groups help metal ions and cationic polymers form networks by interacting with each other through electrostatic or complexation interactions [9,10]. Sodium alginate makes hydrogels in a safe, effective, and pollution-free way when it comes into contact with divalent cations like Ca^{2+} . Amit Kumar Singh et al. [11] utilized 3D printing and CaCl_2 crosslinking to produce sodium alginate/chitosan (SA/CH) polyelectrolyte composite scaffolds, enhancing structural stability and cellular adhesion properties, thereby promoting tissue regeneration. Clarissa Ciarlantini et al. [12] produced chitosan-alginate composite scaffolds through freeze-drying and crosslinking with CaCl_2 . This made scaffolds that worked and had biological activity and features that mimicked living things.

The best scaffold for tissue regeneration must be biocompatible, biodegradable, have the right amount of porosity, and be strong enough to withstand a lot of stress while also helping chondrocytes form extracellular matrix. The ALG/CS hydrogel scaffold has properties that help cells grow, but it doesn't have the right mechanical properties to match those of real cartilage. Using synthetic polymers that have physical, chemical, and mechanical properties similar to biological tissues for physical crosslinking is thought to be a good idea [13]. This combination can find a middle ground between mechanical properties and biocompatibility, both of which are very important for tissue engineering. The US Food and Drug Administration (FDA) has given the go-ahead for polylactic acid-hydroxyglycolic acid (PLGA) and polycaprolactone (PCL) to be used in clinical settings as biomaterials [14]. PLGA can be broken down at a controlled rate, making it a good choice for a biodegradable matrix that can be slowly replaced by new tissues. This is why it is a great choice for replacing natural tissues [14,15]. PCL is a semi-crystalline polyester that can stretch a lot before it breaks (more than 1100%) and lasts a long time. The PCL part of the composite scaffold made it tougher, and the PLGA part made it stronger [15]. One major problem with the synthetic polymers mentioned above is that they don't have places for cells to attach. Adding gelatin (GEL), which is a hydrophilic material, is one way to fix this problem. It helps cells grow and stick together. The gel matrix material can make stronger binding forces because it has a lot of functional groups, like -NH₂ and -COOH. The goal of this project is to make a new tissue regeneration scaffold by mixing sodium alginate with gelatin. This will make the scaffold stronger and more compatible with living cells.

2. MATERIALS AND METHODS:

2.1. Scaffold Preparation

The materials used in this study were gelatin (Sigma Aldrich G1890, USA) and medium molecular weight sodium alginate (Sigma Aldrich W201502, USA). 1% gelatin was dissolved by stirring distilled water at 50°C. The gelatin solution was swirled to ensure even distribution of the 2% SA that had been added. The mixture was moved into the BD syringe and fixed into the 3D bioprinter once the pH was found to be neutral (pH = 7). Prior to printing, the inks were prepared. The gelatin (1% w/v) and SA (2% w/v) were dissolved in deionized water to produce a homogenous ink. The ink was transferred using a printing barrel with a 400 μm inner diameter nozzle, which was connected to a 3D printer (BioForm Neo 3D Bioprinter, Sytolab / Kreator 3D Printers & Solutions Pvt. Ltd., India) (Fig.1). One millimeter separated the grids. The printing platform was set to 5 °C and the ink temperature was set to 25 °C in order to cause the extruded strands to solidify and acquire an initial intensity. The Gel/SA scaffold was printed layer by layer upto 4 layers with each layer height of 0.6mm using extrusion in accordance with the design. The X-, Y-, Z- axis was set as 50mm, 50mm, 2.4mm respectively. After printing, the scaffold was submerged in a 2% CaCl_2 solution, cross-linked for five minutes, then washed three times with deionized water to remove the excess calcium deposition, frozen at -80 °C for the night, and then freeze-dried for forty-eight hours [16].

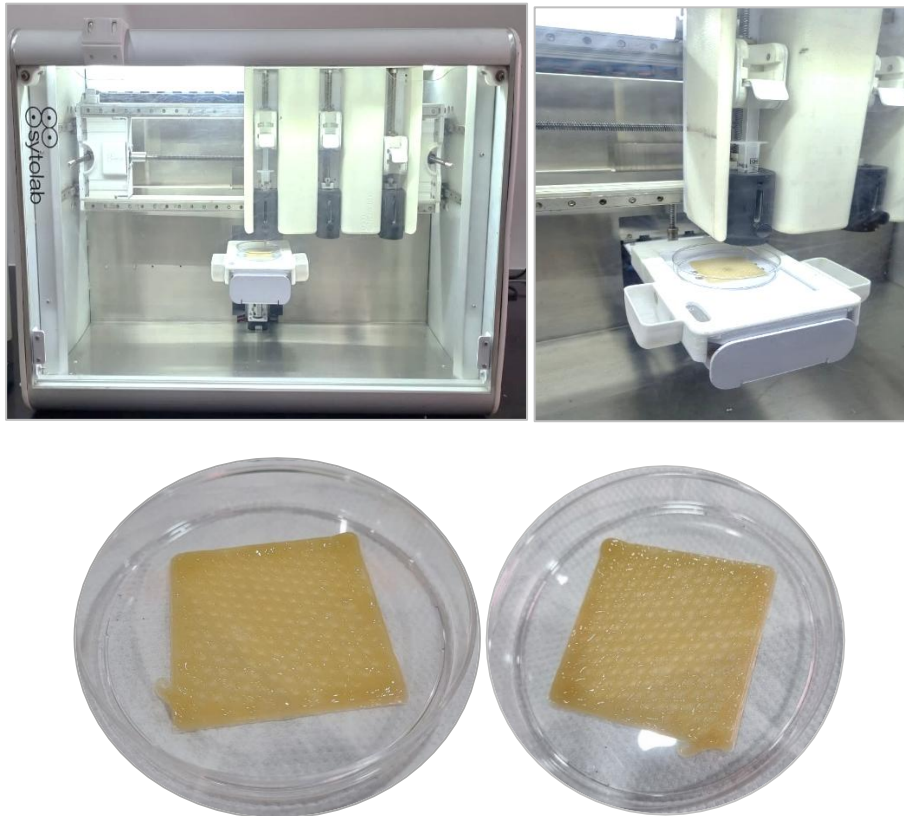


Fig.1: Image shows the scaffold during preparation and post

2.2. Scanning Electron Microscope

The scaffolds were coated with gold using a gold sputter coating machine (JEOL, IT800HL), and then their surfaces and cross-sectional shapes were looked at with a scanning electron microscope.

2.3. Swelling/ Water Uptake Assay

The swelling and water-uptake properties of the scaffolds were evaluated to determine their ability to absorb aqueous media, which is important for nutrient diffusion and cellular infiltration. Scaffolds of size 1×1×1 cm were dried, and their initial dry weight (W_d) was recorded. The scaffolds were then immersed in phosphate-buffered saline (PBS; 0.2 M KH_2PO_4 and 0.1 M NaOH, pH 7.4) at 37 °C for 24 hours. After incubation, the scaffolds were carefully removed, and excess surface water was gently blotted with filter paper before recording the swollen/wet weight (W_s or W_w).

The swelling percentage and water uptake ratio (WUR) were calculated using the following equation:

$$\text{Swelling (\%)} \text{ or } \text{WUR (\%)} = \frac{W_w - W_d}{W_d} \times 100$$

where W_w is the wet weight of the scaffold after PBS incubation, W_d is the initial dry weight, and $W_w - W_d$ represents the amount of water absorbed by the scaffold. Three replicate samples ($n=3$) were used for each condition, and the results are presented as the mean of the replicates. This assay also provides indirect information on scaffold porosity, as a higher water uptake generally reflects greater pore volume and interconnectivity.

2.5. Degradation

The solution containing collagenase II was used for the assessment of the composite hydrogel scaffold structures degradation. The weighed frozen samples were identified by the notation W_0 . The samples were exposed to the solution containing 2.5 U/mL concentration of collagenase II solution with a temperature of 37°C. The samples were taken at regular intervals of 6, 12, 24, and 48 hours. The samples were later washed with deionized water and finally dried by the lyophilization process. The weights of the samples taken after degrading were identified by the notation W_d . The percentage of degradation was determined by the following formula: $(W_d/W_0) \times 100\%$ [16].

2.6. Fourier Transform Infrared Spectroscopy

The chemical composition of the film and any possible interaction between gelatin and sodium alginate was analyzed by FTIR spectroscopy. FT-IR spectra of gelatin, sodium alginate, and films of sodium alginate/gelatin were recorded using a Shimadzu IR Tracer-100 (Japan) Fourier IR-spectrometer. Dried samples of sodium alginate, gelatin, and their mixtures were mixed with KBr powder and pressed into discs using a manual tablet presser for two minutes at a pressure of seven kN. The analysis was done for each spectrum with a resolution of 4 cm^{-1} and scanned from 4000 to 400 cm^{-1} . Each sample was analyzed three times [19,20]. The secondary structure of the gelatin in gels after the addition of sodium alginate was analyzed by FTIR spectroscopy. To analyze the intermolecular interactions, the shift of the bands was also analyzed. The OriginPro 9.0 graphical analysis tool was used to analyze the FTIR spectra in the absorption region to obtain detailed information on the influence of alginate addition on the secondary structure of the gelatin.

2.7. X-Ray diffraction analysis

The X-ray diffraction (XRD) characterization of composites was investigated at ambient temperature; using a Bruker D8 diffractometer with Cu K α radiation (40 kV and 30 mA).

2.8. Statistical analysis

The mean $\pm$ standard deviation is used to display all data. A Student's t-test was used to compare two sets of data. Dunnett's test was used after a one-way ANOVA to compare data from different groups. Group and experimental time point differences were deemed significant if $p < 0.05$.

RESULT AND DISCUSSION:

FTIR

The FTIR spectrum of the sodium alginate sample (spectrum 1) (Fig.2) shows a broad band in the region of 3200-3400 cm^{-1} , which indicates the presence of the O-H stretching of hydroxyl groups and water molecules (21). The presence of bands at 1606-1643 cm^{-1} and 1404-1411 cm^{-1} indicates the presence of the asymmetric and symmetric stretching of the carboxylate groups of the COO^- groups of the guluronic/mannuronic units of the alginate chain (22). The bands observed in the region of 1030-1040 cm^{-1} indicate the presence of the C-O-C stretching of the pyranose ring of the alginate chain (22). The FTIR spectrum of the gelatin sample (spectrum 2) shows a band at 3400 cm^{-1} , which indicates the presence of the N-H/O-H stretching of the water molecules and the N-H stretching of the gelatin chain (22). The presence of a peak at 2926 cm^{-1} indicates the presence of the C-H stretching of the aliphatic groups of the gelatin chain (21). The presence of the bands at 1647, 1543, and 1244 cm^{-1} indicates the presence of the Amide I, Amide II, and Amide III bands, respectively, which indicate the presence of the secondary structure of the gelatin chain (23). These bands indicate the presence of the secondary structure of the gelatin chain (23). The FTIR spectrum of the sodium alginate sample (spectrum 1) shows a broad band in the region of 3200-3400 cm^{-1} , which indicates the presence of the O-H stretching of hydroxyl groups and water molecules (21). The presence of bands at 1606-1643 cm^{-1} and 1404-1411 cm^{-1} indicates the presence of the asymmetric and symmetric stretching of the carboxylate groups of the COO^- groups of the guluronic/mannuronic units of the alginate chain (22). The bands observed in the region of 1030-1040 cm^{-1} indicate the presence of the C-O-C stretching of the pyranose ring of the alginate chain (22). The FTIR spectrum of the gelatin sample (spectrum 2) shows a band at 3400 cm^{-1} , which indicates the presence of the N-H/O-H stretching of the water molecules and the N-H stretching of the gelatin chain (22). The presence of a peak at 2926 cm^{-1} indicates the presence of the C-H stretching of the aliphatic groups of the gelatin chain (21). The presence of the bands at 1647, 1543, and 1244 cm^{-1} indicates the presence of the Amide I, Amide II, and Amide III bands, respectively, which indicate the presence of the secondary structure of the gelatin chain (23). These bands indicate the presence of the secondary structure of the gelatin chain (23). The presence of C-O and C-N vibrations of the polypeptide backbone (21) near 983–1020 cm^{-1} in addition to this gelatin chain. A broad band centered around 3200-3400 cm^{-1} is present in the composite spectrum of A1 (Sodium Alginate + Gelatin). This band is present but shifted to a different position, indicating the formation of new hydrogen bonds between the -OH groups of the alginate and the -NH/-OH groups of the gelatin, as reported in the alginate-gelatin hydrogels (24). The carbonyl bands of the composite spectrum of the blend of sodium alginate + gelatin show a combined band centered between 1606 and 1643 cm^{-1} , which may be attributed to the carboxylate groups of the alginate ($\sim 1606 \text{ cm}^{-1}$) and the carbonyl groups of the gelatin ($\sim 1647 \text{ cm}^{-1}$). This indicates the formation of electrostatic complexes between the carboxylate groups of the alginate and the protonated amino groups of the gelatin, leading to the formation of a polyelectrolyte complex (24-25). The band centered around 1404-1408 cm^{-1} corresponds to the symmetric stretching of the COO^- groups of the alginate and the gelatin, which may be influenced to a certain extent due to the ionic interaction between the two polymers (26). Moreover, the presence of the bands of the amide II ($\sim 1543 \text{ cm}^{-1}$) and the bands of the amide III ($\sim 1244 \text{ cm}^{-1}$) with slight changes in position and broadening, indicates the presence of the backbone of the gelatin, which was preserved during the development of the network with the alginate (24-25). The bands indicate hydrogen bonding as well as the electrostatic interaction of the two types of polymers, which agrees with the development of a polyelectrolyte complex or an interpenetrating network.

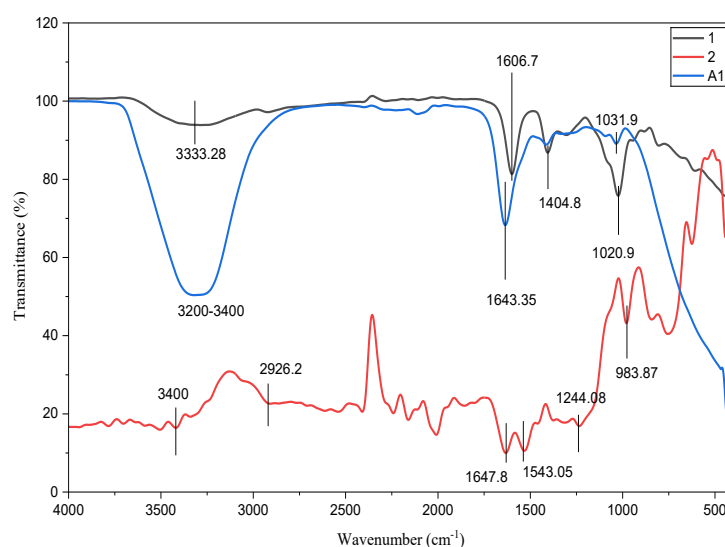


Fig. 2: FTIR spectra of sodium alginate, gelatin and cross-linked gelatin-sodium alginate. The cross-linking occurred through the formation of Hydrogen bonding and electrostatic interaction linkage between amino groups of the gelatin and carbonyl groups of sodium alginate.

3.2. XRD:

The biomaterial, which was prepared by sodium alginate and gelatin, has been shown through the results obtained from the XRD graph (Fig. 3). The broad peaks observed, which indicate the 2θ value ranging from 60° to 70° , indicate the amorphous nature of the hydrogel, which is commonly observed in the structure of the hydrogel matrix [27]. These changes indicate the integration of the molecule into the hydrogel matrix, which would improve the functionality of the hydrogel matrix. These changes observed could be as a result of the chemical change that takes place when the two compounds, which are acetic acid and the heat, which was 50°C , react. The results obtained from the experiment depicted that the scaffold developed has an amorphous structure [28].

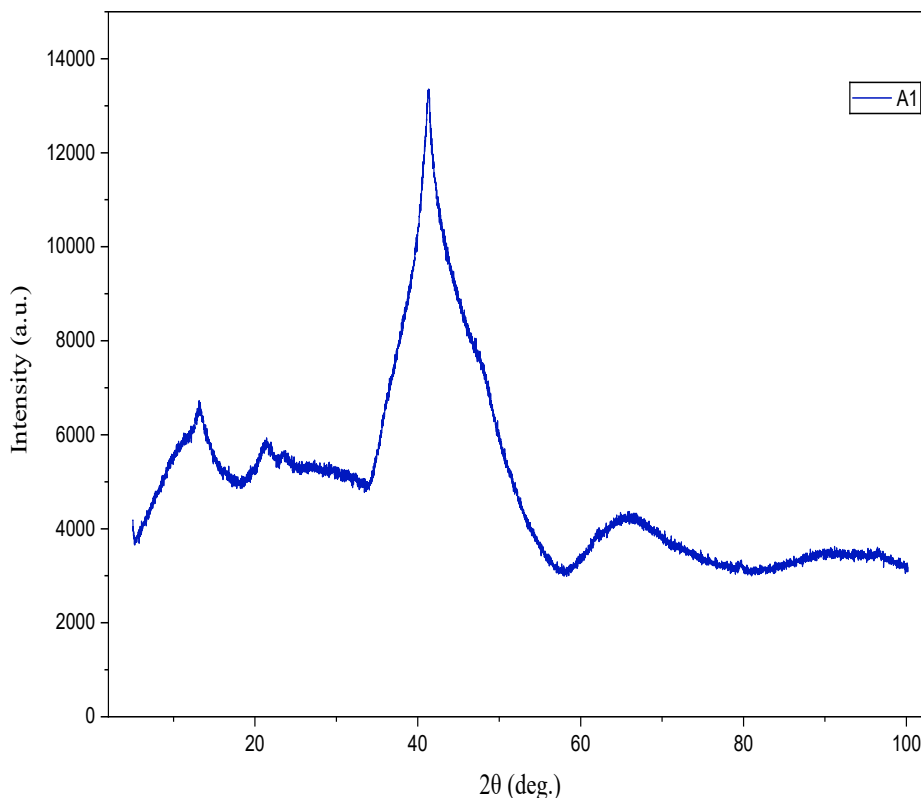


Fig. 3: XRD of sodium alginate-gelatin (A1) hydrogel

FESEM:

The microstructure of the composite scaffold made up of sodium alginate and gelatin has been found to be unique in the sense that the pores are interconnected and the surface is not flat, which is quite suitable for the application in tissue engineering. The microstructure has been found to be characterized by the development of pores that are hierarchical in nature, irregular in shape and size, ranging from sub-micron to several microns in dimension, which may be attributed to the freeze-drying process or the phase separation mechanism used in the development of the scaffolds. Furthermore, the surface of the pores is not flat, which is attributed to the nanofibrous nature of the surface of the pores. Moreover, the surface is free from any cracks and delamination, which is indicative of the integrity of the composite scaffolds developed using the lyophilization process. The surface of the composite scaffolds has been found to be unique (Fig.4) in the sense that the surface is undulating in nature, attributed to the presence of nanofibers on the surface, which are $1\ \mu\text{m}$ in dimension and attributed to the presence of collagen mimicry in the gelatin-alginate composite material. Furthermore, isolated pores and cavities ranging from 2 to $5\ \mu\text{m}$ have open interconnectivity, which is suitable for cell migration and vascularization. At lower magnification levels, pores have an elongated and sheet-like structure, which transforms into dense nanofibrous clusters at higher magnification levels. There is interconnectivity at all levels, with the pore throats providing interconnectivity and facilitating the permeation of fluids, and the average pore sizes (estimated to be in the range of 1 to $10\ \mu\text{m}$) approaching the optimum range (50 to $200\ \mu\text{m}$) to achieve 80 to 95% porosity, which is characteristic of such blends. Bright spots in the image at high magnification levels may indicate the presence of salts, which may have formed due to the crosslinking reaction, and the lack of nanoparticle clusters is evident in the image. This morphology is suitable for the development of effective alginate and gelatin scaffolds for the delivery of stem cells, which have substantial porosity ($>90\%$ likely), mechanical compliance, and bio-adhesiveness through hydrogen bonding, which has been validated through the FTIR analysis done previously, and the nanofibrous roughness of the surface is suitable to mimic the extracellular matrix and has significant potential to increase stem cell adhesion and proliferation.

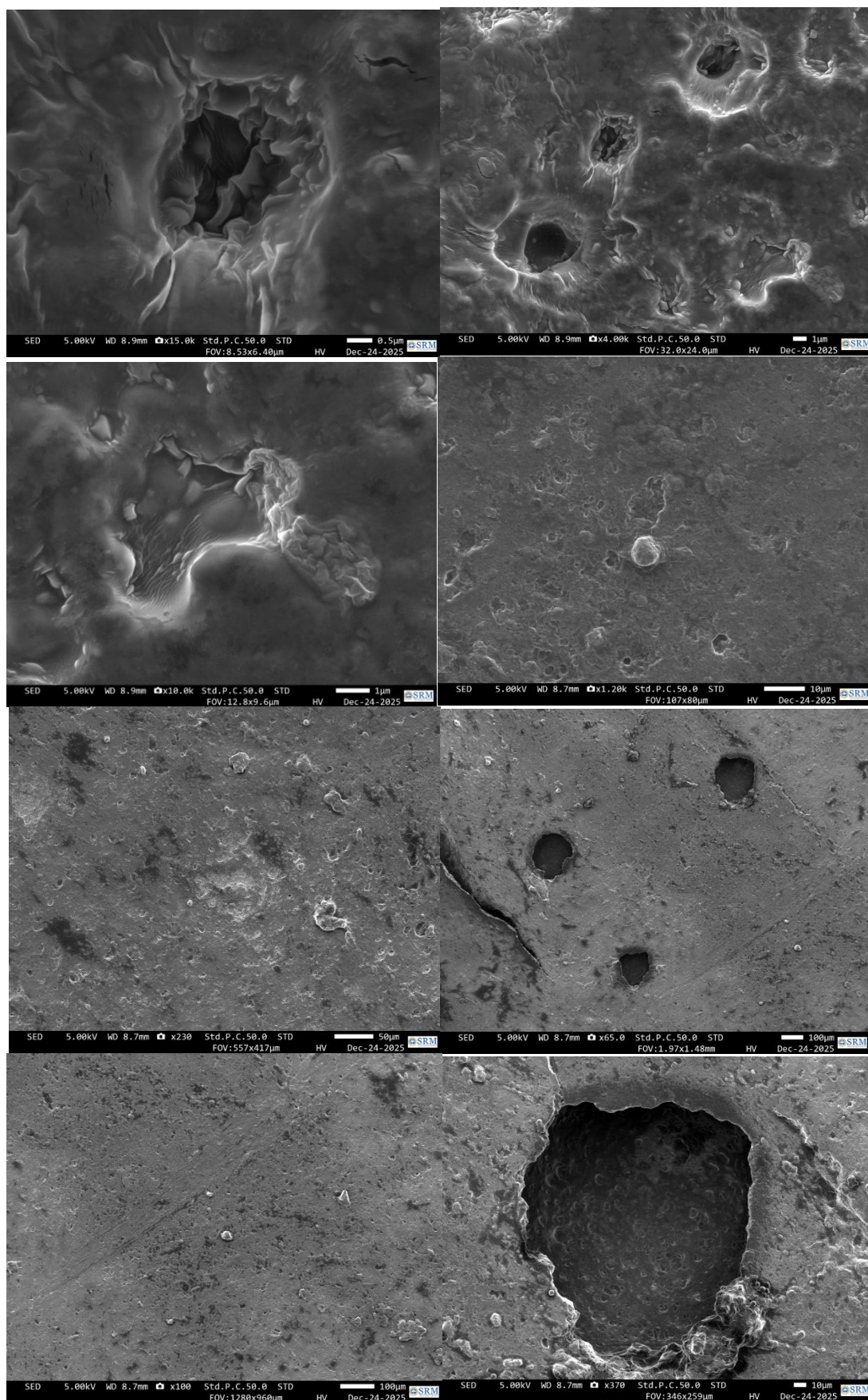


Fig. 4: SEM micrographs on the surface of sodium alginate-gelatin material

Water Uptake Assay/ Swelling Assay:

Water Uptake Assay: -

The water uptake ratio (WUR) of the scaffold, consisting of the mixture of sodium alginate and gelatin, has also demonstrated an increase with time, with high WUR values being recorded after 72 hours of immersion (Fig.5). This shows the high hydrophilic, porous, and swelling capabilities of the scaffold, which are highly beneficial in the field of tissue engineering. This high WUR has shown to improve the nutrient, oxygen, waste removal, and growth factor retention

capabilities, which in turn promotes cell migration, proliferation, and distribution in a three-dimensional environment, simulating the hydrated environment of the extracellular matrix to sustain the viability of the cells in the alginate-gelatin scaffold, specifically designed for chondrogenesis. The swelling behavior of the scaffold, from a materials perspective, shows the presence of nanoporous structures, as well as the synergistic effect of the hydrophilic groups of the carboxylic groups of the alginate and the amide groups of the gelatin, which have shown a synergistic effect. Generally, a nanoporous structure, as well as a low content of inorganic fillers, shows a high-water uptake ratio (WUR). The WUR value of more than 200% is optimal, particularly in the case of bone and cartilage tissue engineering scaffolds, whereas a WUR value of 300-400% or above may be an indicative of potential mechanical instability or accelerated degradation.

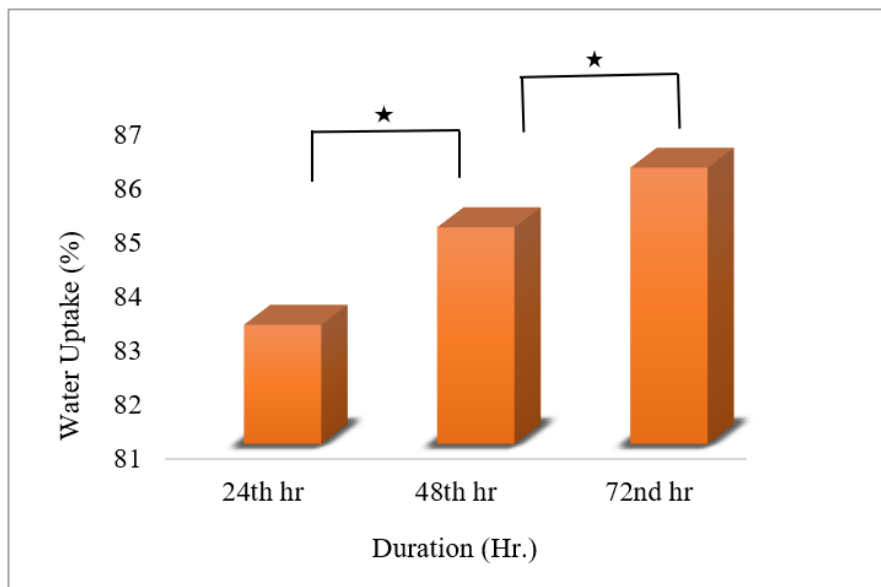


Fig. 5: Water uptake ratio (WUR) (mean value): 24th hr- 83.2%; 48th hr- 85%; 72nd hr- 86.1%

Swelling Assay:

Swelling capacity is another parameter of the scaffold used in tissue engineering. It means the pore size, conditions of interconnectivity, and volume of the scaffold. The capacity of the scaffold to swell is very important in the absorption of fluids of the human body, which in turn enables the absorption of nutrients and metabolites of the cells [29]. During the culture of cells, the swelling of the scaffold enables the infusion of the cells into the scaffold in three dimensions. It also enables the increase of the pore size of the scaffold, which in turn maximizes the surface area of the scaffold used in the culture of the cells. The sample that has a higher degree of swelling has a higher surface area-volume ratio, which maximizes the probability of infusing the cells into the scaffold in three dimensions, which in turn maximizes the proliferation of the cells through the adhesion of the cells to the surface of the scaffold. The formula mentioned above was used to determine the degree of swelling of the scaffold, which increased after 24 hours, showing that the scaffold made of sodium alginate and gelatin was conducive to the adhesion of the cells.

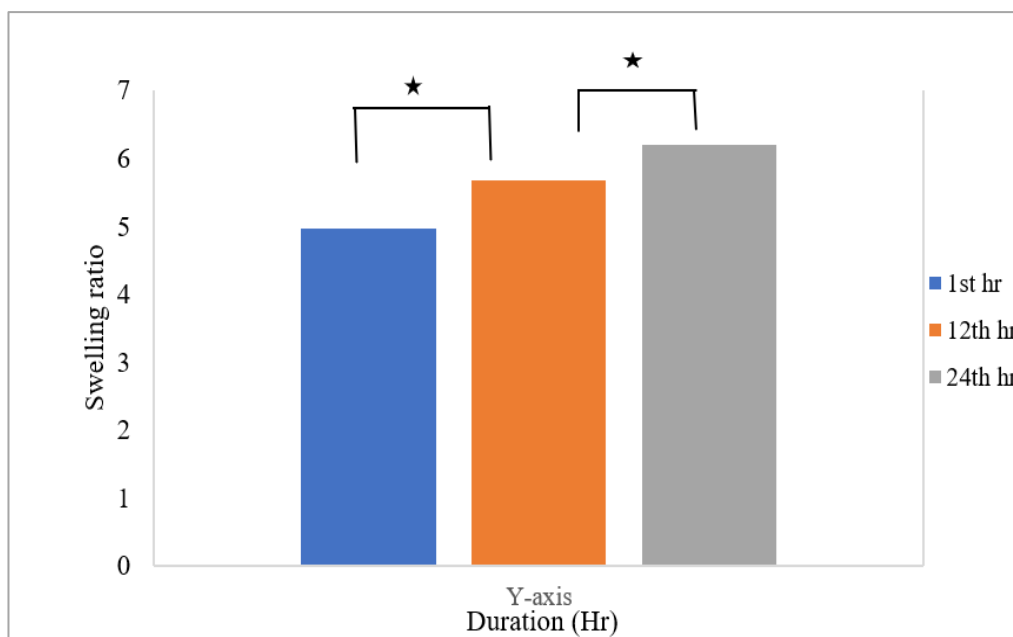


Fig. 6: Swelling ratio of scaffold at time intervals of 1st hr- 4.959; 12th hr- 5.682; 24th hr- 6.196

Degradation assay

Scaffolds showed a rapid degradation rate in the type II collagenase solution. After soaking for 48 h, the degradation of the composite SA/Gel scaffold was faster, and the mass remaining in the scaffold was almost 44.57% at the end which signifies the degradability nature and thus is a better and desirable for biodegradable scaffold to be characterize as a feature.

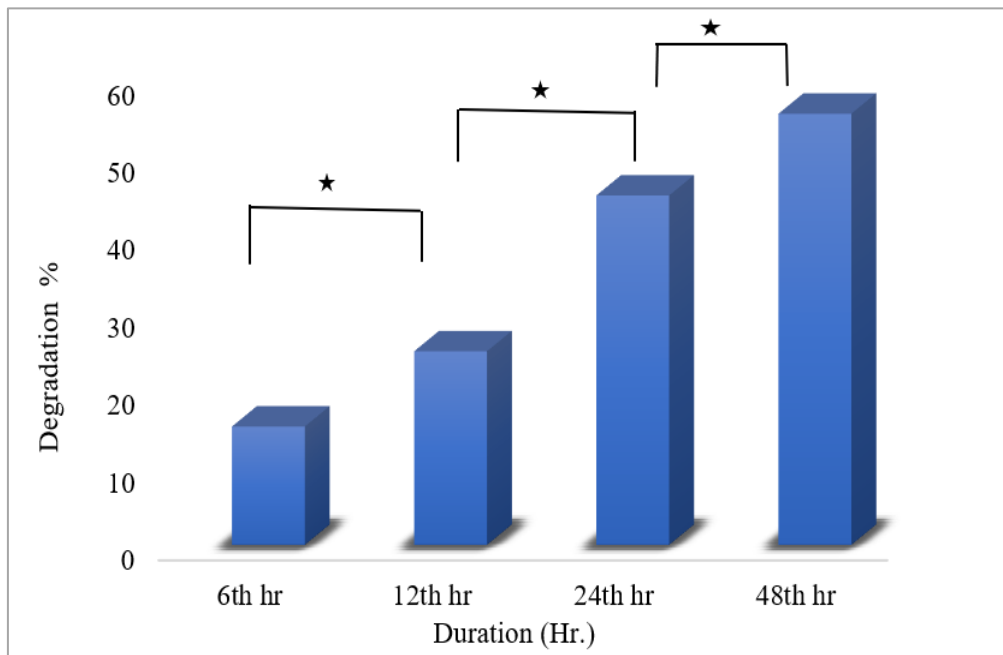


Fig.7: Degradation percentage of scaffold at different time intervals from 6th hr – 15.22%; 12th hr- 24.89%; 24th hr- 44.92%; to 48th hr- 55.43%

CONCLUSION:

The 3D bioprinter was used to fabricate a novel combination of scaffold for tissue regeneration. After the fabrication it has underwent various characterization both physical and biological and in all the analysis it has been observed that the composite was blend perfectly and with biodegradable property. Thus, it possesses all the required feature and convincing for giving an artificial yet biological 3D platform for regeneration of tissues on the surface.

DECLARATION

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

1. Farshi Azhar, F., Olad, A., & Salehi, R. (2014). Fabrication and characterization of chitosan–gelatin/nanohydroxyapatite–polyaniline composite with potential application in tissue engineering scaffolds. *Designed Monomers and Polymers*, 17(7), 654–667. <https://doi.org/10.1080/15685551.2014.907621>
2. Deng W, Tang Y, Mao J, Zhou Y, Chen T, Zhu X. Cellulose nanofibril as a crosslinker to reinforce the sodium alginate/chitosan hydrogels. *Int J Biol Macromol*. 2021 Oct 31;189:890-899. doi: 10.1016/j.ijbiomac.2021.08.172. Epub 2021 Aug 26. PMID: 34455006.
3. Zielińska A, Karczewski J, Eder P, Kolanowski T, Szalata M, Wielgus K, Szalata M, Kim D, Shin SR, Słomski R, Souto EB. Scaffolds for drug delivery and tissue engineering: The role of genetics. *J Control Release*. 2023 Jul;359:207-223. doi: 10.1016/j.jconrel.2023.05.042. Epub 2023 Jun 8. PMID: 37286137.
4. Li C, Shang W, Huang Y, Ge J, Ye J, Qu X, Guo Q, Wang C, Hu P, Liu Y. Sodium alginate/chitosan composite scaffold reinforced with biodegradable polyesters/gelatin nanofibers for cartilage tissue engineering. *Int J Biol Macromol*. 2025 Jan;285:138054. doi: 10.1016/j.ijbiomac.2024.138054. Epub 2024 Nov 27. PMID: 39613057.
5. Rianti, D., Fanny, G., Nathania, R. V., Purnamasari, A. E., Putri, R. R., Soekartono, H., Soebagio, Yuliati, A., & Syahrom, A. (2022). The Characteristics, Swelling Ratio and Water Content Percentage of Chitosan-gelatin/limestone-based Carbonate Hydroxyapatite Composite Scaffold. *International Journal of Integrated Engineering*, 14(2), 13-23. <https://doi.org/10.30880/ijie.2022.14.02.003>

6. Ghassemi T, Shahroodi A, Ebrahimzadeh MH, Mousavian A, Movaffagh J, Moradi A. Current Concepts in Scaffolding for Bone Tissue Engineering. *Arch Bone Jt Surg*. 2018 Mar;6(2):90-99. PMID: 29600260; PMCID: PMC5867363.
7. Rianti, D., Fanny, G., Nathania, R. V., Purnamasari, A. E., Putri, R. R. ., Soekartono, H., Soebagio, Yulianti, A., & Syahrom, A. . (2022). The Characteristics, Swelling Ratio and Water Content Percentage of Chitosan-gelatin/limestone-based Carbonate Hydroxyapatite Composite Scaffold. *International Journal of Integrated Engineering*, 14(2), 13-23.
8. Willerth SM, Sakiyama-Elbert SE. Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery. 2008 Jul 9. In: *StemBook* [Internet]. Cambridge (MA): Harvard Stem Cell Institute; 2008-. PMID: 20614610.
9. Baysal K, Aroguz AZ, Adiguzel Z, Baysal BM. Chitosan/alginate crosslinked hydrogels: preparation, characterization and application for cell growth purposes. *Int J Biol Macromol*. 2013 Aug;59:342-8. doi: 10.1016/j.ijbiomac.2013.04.073. Epub 2013 May 7. PMID: 23664939.
10. Siddiqui N, Asawa S, Birru B, Baadhe R, Rao S. PCL-Based Composite Scaffold Matrices for Tissue Engineering Applications. *Mol Biotechnol*. 2018 Jul;60(7):506-532. doi: 10.1007/s12033-018-0084-5. PMID: 29761314.
11. Singh, Amit & Pramanik, Krishna. (2023). Fabrication and investigation of physicochemical and biological properties of 3D printed sodium alginate-chitosan blend polyelectrolyte complex scaffold for bone tissue engineering application. *Journal of Applied Polymer Science*. 140. 10.1002/app.53642.
12. Ciarlantini C, Francolini I, Silvestro I, Mariano A, d'Abusco AS, Piozzi A. Design of bioactive and biomimetic scaffolds based on chitosan-alginate polyelectrolyte complexes for tissue engineering. *Carbohydr Polym*. 2024 Mar 1;327:121684. doi: 10.1016/j.carbpol.2023.121684. Epub 2023 Dec 11. PMID: 38171693.
13. Seifi S, Shamloo A, Barzoki AK, Bakhtiari MA, Zare S, Cheraghi F, Peyrovan A. Engineering biomimetic scaffolds for bone regeneration: Chitosan/alginate/polyvinyl alcohol-based double-network hydrogels with carbon nanomaterials. *Carbohydr Polym*. 2024 Sep 1;339:122232. doi: 10.1016/j.carbpol.2024.122232. Epub 2024 May 3. PMID: 38823905.
14. González-González AM, Cruz R, Rosales-Ibáñez R, Hernández-Sánchez F, Carrillo-Escalante HJ, Rodríguez-Martínez JJ, Velasquillo C, Talamás-Lara D, Ludert JE. In Vitro and In Vivo Evaluation of a Polycaprolactone (PCL)/Polylactic-Co-Glycolic Acid (PLGA) (80:20) Scaffold for Improved Treatment of Chondral (Cartilage) Injuries. *Polymers (Basel)*. 2023 May 16;15(10):2324. doi: 10.3390/polym15102324. PMID: 37242899; PMCID: PMC10221788.
15. Abdouss H, Pourmadadi M, Zahedi P, Abdouss M, Yazdian F, Rahdar A, Díez-Pascual AM. Green synthesis of chitosan/polyacrylic acid/graphitic carbon nitride nanocarrier as a potential pH-sensitive system for curcumin delivery to MCF-7 breast cancer cells. *Int J Biol Macromol*. 2023 Jul 1;242(Pt 3):125134. doi: 10.1016/j.ijbiomac.2023.125134. Epub 2023 May 29. PMID: 37257532.
16. Miao, G., Liang, L., Li, W., Ma, C., Pan, Y., Zhao, H., Zhang, Q., Xiao, Y., & Yang, X. (2023). 3D Bioprinting of a Bioactive Composite Scaffold for Cell Delivery in Periodontal Tissue Regeneration. *Biomolecules*, 13(7), 1062. <https://doi.org/10.3390/biom13071062>
17. Sangkert, S., Kamolmatyakul, S., Gelinsky, M., & Meesane, J. (2021). 3D printed scaffolds of alginate/polyvinylalcohol with silk fibroin based on mimicked extracellular matrix for bone tissue engineering in maxillofacial surgery. *Materials Today Communications*, 26, 102140.
18. Rahman M, Peng XL, Zhao XH, Gong HL, Sun XD, Wu Q, Wei DX. 3D bioactive cell-free-scaffolds for in-vitro/in-vivo capture and directed osteoinduction of stem cells for bone tissue regeneration. *Bioact Mater*. 2021 Apr 21;6(11):4083-4095. doi: 10.1016/j.bioactmat.2021.01.013. PMID: 33997495; PMCID: PMC8091180.
19. Derkach, S. R., Voron'ko, N. G., Sokolan, N. I., Kolotova, D. S., & Kuchina, Y. A. (2020). Interactions between gelatin and sodium alginate: UV and FTIR studies. *Journal of Dispersion Science and Technology*, 41(5), 690–698. <https://doi.org/10.1080/01932691.2019.1611437>
20. Abbasiyasi S, Shun TJ, Tengku Ibrahim TA, Ismail N, Ariff AB, Mokhtar NK, Mustafa S. Use of sodium alginate in the preparation of gelatin-based hard capsule shells and their evaluation in vitro. *RSC Adv*. 2019 May 23;9(28):16147-16157. doi: 10.1039/c9ra01791g. PMID: 35521410; PMCID: PMC9064353.
21. Hassan, N., Ahmad, T., Zain, N. M., & Awang, S. R. (2021). Identification of bovine, porcine and fish gelatin signatures using chemometrics fuzzy graph method. *Scientific reports*, 11(1), 9793. <https://doi.org/10.1038/s41598-021-89358-2>
22. A Physically Cross-Linked Sodium Alginate–Gelatin Hydrogel with High Mechanical Strength. Lini Wang, Hui Jie Zhang, Xueqian Liu, Yun Liu, Xing Zhu, Xinhua Liu, and Xiangyu You, *ACS Applied Polymer Materials* 2021 3 (6), 3197-3205.DOI: 10.1021/acsapm.1c00404
23. Skopinska-Wisniewska, J., Tuszyńska, M., Kaźmierski, Ł., Bartniak, M., & Bajek, A. (2024). Gelatin-Sodium Alginate Hydrogels Cross-Linked by Squaric Acid and Dialdehyde Starch as a Potential Bio-Ink. *Polymers*, 16(18), 2560. <https://doi.org/10.3390/polym16182560>
24. Sharma, R., Malviya, R., Singh, S., & Prajapati, B. (2023). A Critical Review on Classified Excipient Sodium-Alginate-Based Hydrogels: Modification, Characterization, and Application in Soft Tissue Engineering. *Gels (Basel, Switzerland)*, 9(5), 430. <https://doi.org/10.3390/gels9050430>
25. Irfanita, N., Lestari, W., Mirghani, M. E. S., Jaswir, I., Octavianti, F., & Haris, M. S. (2022). Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy Coupled with Principal Component Analysis and Polymerase Chain Reaction (PCR) Assay for the Detection of Porcine and Bovine Gelatins in Dental Materials. *Tropical life sciences research*, 33(2), 133–153. <https://doi.org/10.21315/tlsr2022.33.2.7>
26. Łabowska, M. B., Cierluk, K., Jankowska, A. M., Kulbacka, J., Detyna, J., & Michalak, I. (2021). A Review on the Adaption of Alginate-Gelatin Hydrogels for 3D Cultures and Bioprinting. *Materials (Basel, Switzerland)*, 14(4), 858. <https://doi.org/10.3390/ma14040858>

27. Ketabat, F., Maris, T., Duan, X., Yazdanpanah, Z., Kelly, M. E., Badea, I., & Chen, X. (2023). Optimization of 3D printing and in vitro characterization of alginate/gelatin lattice and angular scaffolds for potential cardiac tissue engineering. *Frontiers in bioengineering and biotechnology*, 11, 1161804. <https://doi.org/10.3389/fbioe.2023.1161804>
28. Ghezzi, B., Foresti, R., Scialoia, L. P., Botti, M., Mersanne, A., Ratto, F., Rossi, F., Martini, C., Perini, P., Favari, E., & Freyrie, A. (2025). Preliminary Evaluation of 3D-Printed Alginate/Gelatin Scaffolds for Protein Fast Release as Suitable Devices for Personalized Medicine. *Biomedicines*, 13(6), 1365. <https://doi.org/10.3390/biomedicines13061365>
29. Venkatachalam, K., Charoenphun, N., Nitikornwarakul, C., & Lekjing, S. (2025). Effect of Sodium Alginate Concentration on the Physicochemical, Structural, Functional Attributes, and Consumer Acceptability of Gel Beads Encapsulating Tangerine Peel (*Citrus reticulata* Blanco 'Cho Khun') Extract. *Gels* (Basel, Switzerland), 11(10), 808. <https://doi.org/10.3390/gels11100808>
30. Hossainpour H, Zare S, Alvandi H, Abiri R, Aghaz F, Arkan E, Alvandi A. Gelatin-sodium alginate hydrogel infused with *Onosma dichroanthum* Boiss. root extract: Preparation, characterization, and application in wound dressing. *Biochem Biophys Rep.* 2025 Sep 9;44:102236. doi: 10.1016/j.bbrep.2025.102236. PMID: 40989717; PMCID: PMC12452783.