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# Regenerative Material for the Treatment of Articular Cartilage Damage: Synthesis and Characterization of Hyaluronic Acid and Poly(glycolic acid) Composite Matrices

# Eklem Kıkırdağı Hasarının Tedavisinde Rejeneratif Materyal: Hyaluronik Asit ve Poli(glikolik asit) Kompozit Matrislerin Sentezi ve Karakterizasyonu

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## ABSTRACT

Currently, analgesics, anti-inflammatory drugs, and hyaluronic acid viscosupplementation are used to alleviate pain associated with joint cartilage disorders. Hyaluronic acid injections are known not only for their pain-reducing effects but also for stimulating cartilage regeneration. In this study, a regenerative biomaterial platform comprising poly (glycolic acid) mesh and cross-linked hyaluronic acid was developed for the repair of degenerated joint cartilage following microfracture and subchondral bone stimulation. For this purpose, in the first stage, hyaluronic acid gels cross-linked with butanediol diglycidyl ether, containing a concentration of 23 mg/mL, were prepared. The residual butanediol diglycidyl ether cross-linker in the obtained gels was below 1 ppb. The pH value was determined to be 6.95 ± 0.2, and the osmolality was 361.3 ± 2.9 mOsm/kg. The injection force and related rheological properties were investigated. In the second stage, the cross-linked hyaluronic acid gels were impregnated into poly (glycolic acid) meshes, evaluated using scanning electron microscopy and characterized chemically. Finally, the composite matrices were recellularized with chondrocytes, and cell viability analysis was conducted using Alamar Blue. The cell viability results and scanning electron microscopy images of the composite structure consisting of poly(glycolic acid) mesh and cross-linked hyaluronic acid indicated that the structure supports chondrocyte viability.

**Key Words**

Biomaterial, cartilage reconstruction, hyaluronic acid, poly(glycolic acid).

## ÖZ

Günümüzde eklem kıkırdağı bozukluklarıyla ilişkili ağrıyı hafifletmek için analjezikler, anti-inflamatuar ilaçlar ve hyaluronik asit viskosuplementasyonu kullanılmaktadır. Hyaluronik asit enjeksiyonları sadece ağrıyı azaltıcı etkileriyle değil aynı zamanda kıkırdak rejenerasyonunu uyarmasıyla da bilinmektedir. Bu çalışmada, mikro kırık ve subkondral kemik stimülasyonu sonrası dejenerasyona uğramış eklem kıkırdağının onarımı için poli(glikolik asit) ağ ve çapraz bağlı hyaluronik asitten oluşan rejeneratif bir biyomalzeme platformu geliştirilmiştir. Bu amaçla, ilk aşamada 23 mg/mL konsantrasyon içeren bütandiol diglisidil eter ile çapraz bağlı hyaluronik asit jelleri hazırlanmıştır. Elde edilen jellerdeki kalıntı bütandiol diglisidil eter çapraz bağlayıcısı 1 ppb'nin altında elde edilmiştir. pH değeri 6,95 ± 0,2 ve ozmolalite 361,3 ± 2,9 mOsm/kg olarak belirlenmiştir. Enjeksiyon kuvveti ve ilgili reolojik özellikler incelenmiştir. İkinci aşamada, çapraz bağlı hyaluronik asit jelleri poli(glikolik asit) ağlara emdirilmiş, taramalı elektron mikroskobu kullanılarak incelenmiş ve kimyasal olarak karakterize edilmiştir. Son olarak, kompozit matrisler kondrositlerle reselülerize edilmiş ve hücre canlılığı analizi Alamar Blue kullanılarak gerçekleştirilmiştir. Poli(glikolik asit) ağ ve çapraz bağlı hyaluronik asitten oluşan kompozit yapının hücre canlılığı sonuçları ve taramalı elektron mikroskobu görüntüleri kompozit yapının kondrosit canlılığını desteklediğini göstermiştir."

**Anahtar Kelimeler**

Biyomateryal, kıkırdak rekonstrüksiyonu, hyaluronik asit, poli(glikolik asit).

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## INTRODUCTION

Articular cartilage damage in the knee joint can lead to functional loss in joint movement and severe pain, affecting daily life and reducing the quality of life. These damages can arise due to the decrease in the viscoelastic properties of synovial fluid associated with aging, as well as from trauma, pathological sources, or degenerative diseases that harm the cartilage structure. Since cartilage tissue lacks blood vessels, it does not heal spontaneously within the body. Currently, therapeutic and operational approaches are used in cartilage treatment. However, none of these therapies result in the formation of a stable hyaline-like tissue capable of withstanding normal joint loads. The treatment methods, depending on the type and degree of the damage, are surgical techniques aimed at forming cartilage repair tissue by cleaning/covering the damaged areas (stimulating/targeting tissue regeneration).

Although the critical defect varies according to different schools of thought, various approaches such as mosaicplasty, cellular therapies, and biomaterials are commonly applied in the clinic for large area defects. Hyaluronic acid, also known as hyaluronan, is a high molecular weight glycosaminoglycan composed of repeating sequences of glucuronic acid and N-acetylglucosamine. It serves functions such as providing lubrication to the joint, shock absorption, forming a backbone for extracellular matrix proteoglycans, and providing a hydrated pathway for cell migration[1, 2]. Recent studies highlight the use of hyaluronic acid (HA) as a tissue scaffold in tissue engineering methods [3-5]. A study has shown that viscosupplementation with HA improves the bioadhesive properties of synovial fluid and is suitable for protein and phospholipid stabilization as well as the formation of a uniform film[6].

Hydrogels are important due to their similarity to the extracellular matrix, support for cell proliferation, and ability to release drugs and growth factors[7, 8]. Injectable hydrogels hold a significant place in tissue engineering. HA-based injectable hydrogels can be crosslinked through various chemical methods[8]. With these cross-linking methods, mechanical properties, viscosity, degradation, and biological response can be easily adjusted[9, 10]. Therefore, HA and its derivatives are extensively studied as tissue engineering materials for cartilage repair. It is known that hyaluronic acid cannot remain in the body for long periods and is enzymatically degraded by hyaluronidases. For this reason, the development of cross-linked hyaluronic acid formulations is necessary. HA can be cross-linked at high pH values using agents like divinyl sulfone[11-13]. Butanediol diglycidyl ether (BDDE) is another widely used cross-linking agent[14]. Cross-linked hyaluronic acid can be produced by opening epoxy rings and forming ether bonds via hydroxyl groups on HA[15, 16].

It is known that coating tissue scaffolds with hyaluronic acid provides significant benefits for regeneration. This study aims to synthesize a three-dimensional regenerative structure for the treatment of articular cartilage damage that provides treatment without the need for preoperative cell seeding, is biocompatible and biodegradable. The synthesized structure, designed to meet tissue engineering principles, should degrade after implantation, with its degradation products and itself being compatible with the body. Therefore, hyaluronic acid and poly (glycolic acid) (PGA) matrix, known for their use in tissue engineering and cartilage treatments, were combined. Thus, it is aimed to form new and healthy cartilage tissue in the damaged area at the end of the degradation process, without causing any toxic or immunogenic effects. For this purpose, poly (glycolic acid) networks were combined with cross-linked HA hydrogels within the study scope. The rheological properties, chemical structure, microscopic structure, and physical properties of the obtained cross-linked HA were determined and impregnated into the PGA network. The PGA/cross-linked HA scaffolds were chemically and microscopically analyzed, and the composite structures obtained were reseeded with chondrocytes to examine their cell metabolic and viability activities.

## MATERIALS and METHODS

### Synthesis of Cross-linked Hyaluronic Acid

10% (w/v) sodium hyaluronate powders (Bloomage Freda, EP1.8, China) with low molecular weight-HA (LMW-HA) were dissolved in 0.25 M NaOH containing 1% (v/v) BDDE. The crosslinking reaction was carried out at 40 °C for 6 hours at hotplate. After the gel formation, the hydrogel was dialyzed against phosphate buffer (PBS) for 24 hours, followed by dialyzed with deionized water another 24 hours to completely remove unreacted BDDE[14]. The HA samples of 1cc were subsequently transferred to the syringes (1.5cc COP Syringe Assembly, Plas-Tech Engineering, USA) (Steriject, 27 G needle diameter, TSK Laboratory, The Netherlands). For steriliza-

tion, the syringe samples were autoclaved at 121℃ for 20 minutes.

**Poly-glycolic acid (PGA) Meshes**

Fibrous polyglycolic acid (PGA) mesh sheets were purchased from BMT Group Company (Biofelt, Ankara, Türkiye). The sheets had a thickness of 1 mm ± 0.1 mm, dimensions of 20 cm x 30 cm, and a bulk density of 300 mg/cc ± 30 mg/cc. The sheets were sterilized using 25 kGy gamma irradiation and stored at room temperature.

**Preparation of Composite Mesh**

The package of sterile PGA mesh was opened under sterile conditions in a laminar flow cabin and transferred to a culture dish using sterile forceps. 0.75 cc of the crosslinked HA gel was dispensed at specific points onto the mesh. Using a sterile spatula, the HA gel was evenly spread across the surface of the PGA mesh. The mesh, coated with HA gel, was left to dry overnight to allow the gel to be absorbed.

**Characterization of Cross-linked Hyaluronic Acid (HA)**

**Identification of Hyaluronic Acid Gel**

The chemical composition of sodium hyaluronate and PGA/crosslinked HA mesh was characterized using Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) (Agilent, USA). A 10 mg sample of sodium hyaluronate was directly placed on the ZnSe ATR crystal of the FTIR machine, and the lid was secured by turning the apparatus head. Additionally, to determine the concentration of the HA filler, a calibration curve was obtained using the Quant Algorithm of FTIR. Sodium hyaluronate solutions with concentrations of 100 mg/mL, 75 mg/mL, 50 mg/mL, 25 mg/mL, 10 mg/mL, and 1 mg/mL were prepared by dissolving the samples in PBS with a magnetic stirrer. A 50 µL sample of each concentration was directly placed on the ZnSe ATR crystal of the FTIR machine. The transmittance of the samples was measured in the range of 650-4000 $cm^{-1}$. All concentrations of sodium hyaluronate were analyzed using the Quant Algorithm, which employs Simple Beer's Law in the ATR-FTIR application.

**1,4-Butanediol diglycidyl ether (BDDE) Analysis**

The method developed for measuring BDDE concentration, based on a literature study[17], was analyzed using Liquid Chromatography-Mass Spectrometry (LC-MS/MS) (Thermo Scientific, USA). To determine the BDDE concentration in cross-linked HA, a calibration curve was obtained with BDDE concentrations of 1 ppb, 10 ppb, 25 ppb, 50 ppb, and 100 ppb. A five-point calibration curve was obtained. 1 g sample was dissolved in 10 N ACN. The liquid chromatography (LC) parameters were determined as a flow rate of 0.4 mL/min, 87% ACN-13% water (0.001% FA doped), and a 5 µL injection volume, and Mass Spectrometry (MS) determined as 1900 V positive ion voltage, sheat gas 2 arb, aux gas 2arb, ion transfer temperature 250 °C, energy 203 m/z-129 m/z 25 collision.

pH and Osmolarity of Cross-linked Hyaluronic Acid Gel

The pH of the HA samples was measured using a pH meter (HANNA, USA). Approximately 1 cc of the sample was added to a 15 mL centrifuge tube (n=3). The pH electrode was submerged approximately 3 cm into the tube and gently stirred. The pH value was recorded from the LCD display. The osmolality of the HA samples was measured using a freezing point osmometer (Astori, Italy). The sample tube was gently cleaned, and 200 µL of the HA (n=3) was dispensed into the sample tube using a micropipette. The sample tube was then inserted into the chamber, and the results were recorded.

**Injection Force Analysis**

To determine the injection force of the syringes, the force applied to the syringe plunger was measured by Biomaterial Tester (CellScale, Canada) with a compression test. Each syringe that was filled with crosslinked HA was placed in a custom apparatus under the load cell of the machine (Figure 1A). The syringe barrel was loaded with 50 N and compressed at a crosshead speed of 50 mm/min. Data in the form of plunger experiment (mm) vs applied force was collected (n=3). The maximum applied was taken as the maximum injection force required during the injection.

**Rheology Measurements**

To assess the rheological properties of the material, a rheometer (Malvern, UK) was utilized in a parallel plate configuration following the manufacturer's manual. The parallel plate was attached to the rheometer, and the zero-gap position was established. A volume of 1.32 mL of each sample was loaded onto the plate, and the temperature lid was closed over the sample. An amplitude sweep test was conducted at 25 °C to investigate the Storage Modulus (G'), Loss Modulus (G"), Complex Modulus (G*), Phase Angle (°), and Complex Viscosity values of the HA samples. These properties of the HA

(n=3) were determined by conducting the test within a strain range of 0.01–1000% and at a frequency of 5 Hz.

### Characterization of Poly (glycolic acid) / Hyaluronic Acid Meshes

### Cell Culture Studies

The cell culture studies of the study were conducted using mature chondrocyte cells. Chondrocyte cells were cultured in DMEM growth medium containing 1% Antibiotic-Antimycotic (Capricorn), 1% L-glutamine, and 10% FBS. The incubation process of the cells was carried out in an incubator (Memmert, Germany) maintained at 37 ℃ temperature, 95% humidity, and 5% CO2. For the expansion of chondrocyte cells, cell culture flasks with a surface area of 75 $cm^2$ were utilized, and when the cell density reached approximately 90% confluence, passaging was performed. Then, they were detached from the surface using trypsinization process and trypsin activity was stopped with the growth medium. The cell suspension was centrifuged at 2500 rpm for 3 minutes to pellet the cells. After discarding the supernatant, a fresh growth medium was added to the cell pellet according to the cell density and homogenized.

### Determination of Metabolic Activity

To assess the metabolic activity of chondrocyte cells seeded onto PGA/cross-linked HA scaffolds, the Alamar Blue analysis method was employed. For this purpose, scaffolds divided into 6 equal parts were transferred to 48-well plates, and cell seeding was performed at a density of $4x10^4$ cells/scaffold. Each scaffold was supplemented with 2 mL of growth medium, and the cells were incubated for 14 days. On days 1, 4, 7, and 14 of the study, the culture medium was removed from the scaffolds (n=3), and 2 mL of 10% (v/v) Alamar Blue solution-containing medium was added. The samples were then incubated for 4 hours, and the absorbance values of samples taken from each well were determined using a microplate reader at 570/600 nm. A cell-free scaffold incubated with the test solution for the same duration served as a control, and the resulting solution was designated as the reference (blank) solution.

### Scanning Electron Microscopy (SEM) Imaging

Scanning Electron Microscopy (SEM) was utilized to examine the fiber and gel morphologies of the material, confirm the integration of HA gel into the PGA mesh structure, and investigate cell behavior and morphology on the material surface. On days 1, 4, 7, and 14 of the cell culture, scaffolds (n=3) had their culture medium removed and were washed with PBS to remove culture residues. The scaffolds were then fixed with a 4% (w/v) paraformaldehyde solution (Sigma, USA) for 30 minutes. Following fixation, they were washed twice with PBS, dehydrated through a series of graded alcohols, and left to dry in a hood with Hexamethyldisilazane (HMDS) (Sigma, USA). Prior to SEM analysis, the samples were coated with a gold/palladium (Au/Pd) and images were taken at various magnifications using a scanning electron microscope (Tescan, USA).

### Statistical Analysis

The samples were measured in triplicate, and experimental results are reported as mean ± standard deviation. Statistical analysis was conducted using GraphPad Prism 8 software, with significance levels determined by a t-test for Alamar Blue analysis. A significance level of 95% ($p < 0.05$) was considered to indicate a statistically significant difference.

## RESULTS

### Cross-linked Hyaluronic Acid Gels

#### *The concentration of Hyaluronic Acid*

The linear concentration curve obtained with 4-point calibration is presented in Supporting Figure 1A. The $R^2$ value of the curve was determined to be 0.99102. The FTIR spectra of the HA gel samples were evaluated using the same interface based on the obtained calibration graph. According to the model, the average hyaluronic acid concentration was determined to be 22.52±0.8 mg/mL (Table 1).

#### *BDDE Analysis*

The five point calibration curve of BDDE is given in Supporting Figure 1B. According to LC-MS analysis report, the concentration of BDDE was determined under 1 ppb (Table 1) meaning that there is no toxic BDDE residue in HA gel.

#### *Physical Conditions*

For pH measurements, 1 mL of sample was added to tubes, electrodes were immersed into the tubes, and the mixture was stirred precisely. The average pH value was determined to be 6.95±0.2 based on these measurements. For osmolarity measurements, the sample tube was placed in the device chamber and measurements were taken. The average osmolarity value was determined to be 361.3 ± 2.9 mOsm/Kg (Table 1).

### *Injection Force*

The setup used for the injectability test, performed with a mechanical testing machine, is shown in Figure 1A. The force vs. plunger displacement curve obtained for a sterile HA syringe sample (the first of three repeats) is presented as a representative graph in Figure 1B. The peak force of 1.31 N was reached at a plunger travel distance of 8.32 mm for the HA syringe sample. Additionally, the average injection force was determined to be 1.94±0.7 N.

### *Rheological Evaluation*

Rheological measurements of the HA gels were performed both before and after sterilization. The stress curves obtained against shear strain are shown in Figure 2A and Figure 2B, respectively. The results for G′ (elastic modulus), G′′ (viscous modulus), and phase angles (d) are presented numerically in Figure 2C. The elastic modulus of the cross-linked HA gels significantly decreased after sterilization ($p<0.001$), while the viscous modulus and phase angle values increased ($p<0.05$). This phenomenon is explained by the effect of the temperature applied during sterilization, which alters the physical structure of the gel, transforming it into a more fluid form.

## Cross-linked Hyaluronic Acid Gels Embedded Polyglycolic acid (PGA) Meshes

### *Chemical Characteristics*

The FTIR spectra of HA, PGA mesh, and HA gel-impregnated PGA mesh structures are shown in Figure 3A, 3B, and 3C, respectively. Also, the specific FTIR pe-

**Table 1.** Properties of the cross-linked and sterile hyaluronic acid gel.

| | |
|---|---|
| The concentration of Hyaluronic Acid | 22.52±0.8 mg/mL |
| BDDE | <0.1 ppb |
| pH | 6.95±0.2 |
| Osmolarity | 361.3 ± 2.9 mOsm/Kg |
| Injection Force | 1.94±0.7 N |

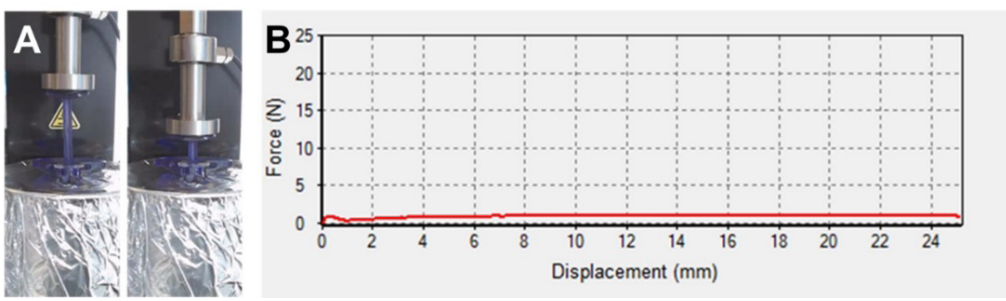
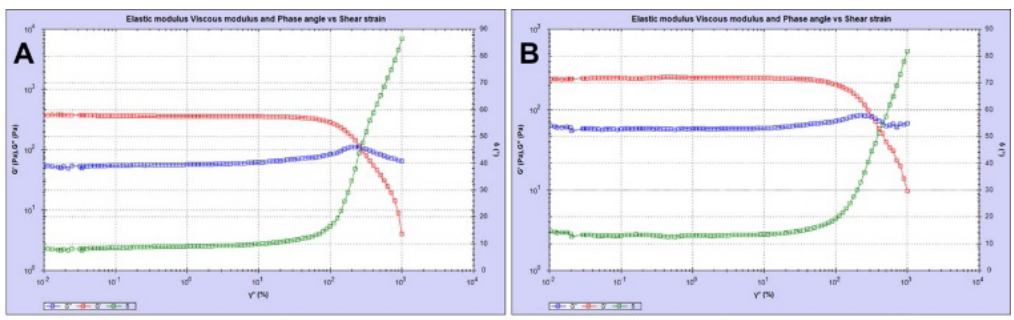



**Figure 1.** (A) Experimental setup for mechanical testing machine and syringe apparatus during injectability tests; (B) Representative graph of applied force (N) and plunger displacement (mm) for hyaluronic acid gel sample.




| C | G' Elastic Modulus (Pa) | G'' Viscous Modulus (Pa) | δ Phase Angle (°) |
|---|---|---|---|
| HA Gel-Before Sterilization | 380.2 ± 17.2 | 52.21 ± 4.1 | 7.82 ± 0.6 |
| HA Gel-After Sterilization | 250 ± 12.1 | 57.98 ± 4.7 | 13.06 ± 0.5 |

**Figure 2.** The determined elastic modulus (G′, red), viscous modulus (G″, blue), and phase angle ( δ , green) curves vs shear strain (%) for (A) non-sterile, (B) sterile hyaluronic acid gel; (C) determined rheological values for non-sterile and sterile hyaluronic acid gels (n=3).

aks for hyaluronic acid and polyglycolic acid (PGA) are presented in detail in Table 2. Accordingly, the peaks at 2959 $cm^{-1}$ and 1736 $cm^{-1}$ are characteristic of PGA, whereas the peaks at 1622-1640 $cm^{-1}$ are characteristic of hyaluronic acid. An increase in the stretching of the -OH and -NH bonds of the PGA mesh due to hyaluronic acid impregnation was observed (3200-3500 $cm^{-1}$). Similarly, the bands at a wavelength of 1640 $cm^{-1}$ correspond to the amide carbonyl groups of the hyaluronic acid incorporated into the PGA mesh.

### *Microscopic Evaluation*

The SEM images of the PGA mesh and HA gel-impregnated PGA mesh structures are presented in Figure 3D and Figure 3E, respectively. In Figure 3D, the fibers of the PGA mesh structure can be observed. The images of the PGA mesh combined with HA gel show that the material surface is coated with the gel, which fills the spaces between the fibers and covers the mesh (Figure 3E).

### *Cellular Viability Assessment*

The cell viability values obtained from the Alamar Blue assay, conducted to evaluate the viability of chondrocyte cells on HA-PGA mesh at specified cell culture days, were analyzed in terms of optical density, and the results are presented in Figure 4. According to these results, a significant increase in cell viability was observed throughout the culture period. The optical density value measured on the 1st day of culture was 0.08±0.005, which increased to 0.12±0.01 on the 4th day ($p< 0.001$) and 0.22±0.007 on the 7th day. By the 14th day of culture, as the cell activity level continued to rise, this value reached 0.30±0.002 ($p<0.0001$). Thus, cell viability increased by 3.7 times by the last day of culture. This indicates that the HA gel-PGA mesh structure positively affects chondrocyte viability and supports cell proliferation. The significant increase in cell viability observed with the culture days demonstrates that the HA gel combined with the PGA mesh does not exhibit any cytotoxicity.

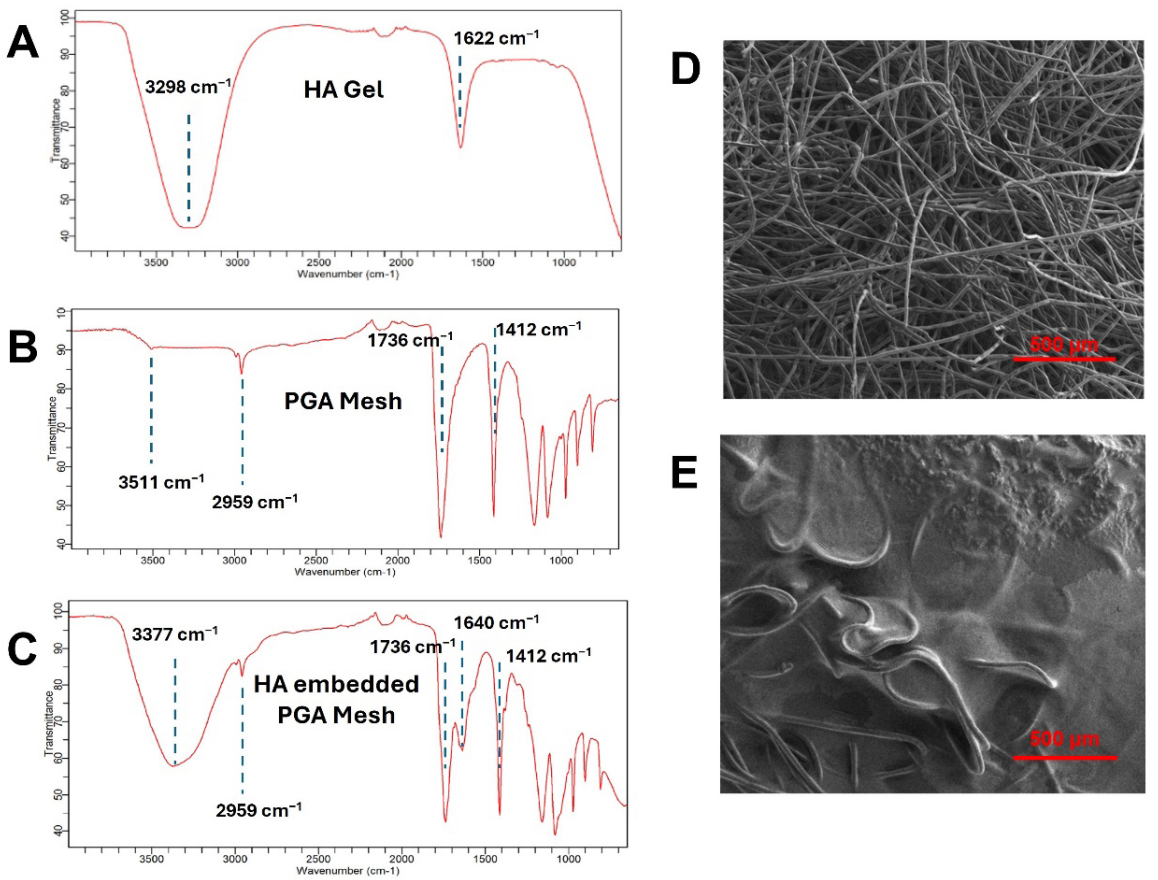



**Figure 3.** FTIR spectra of (A) hyaluronic acid gel, (B) poly(glycolic acid) mesh, (C) hyaluronic acid embedded PGA mesh; SEM images of (D) PGA mesh and (E) hyaluronic acid embedded PGA mesh (Scale bar: 500 µm)

**Table 2.** FTIR spectral band assignments of hyaluronic acid gel, poly(glycolic acid) mesh and hyaluronic acid embedded PGA mesh[18, 19].

| Functional Group | Absorption Band |
|---|---|
| –OH stretch vibration, | <0.1 ppb |
| –NH symmetrical vibration | 3200-3500 $cm^{-1}$ |
| -C-H stretching vibration | 2959 $cm^{-1}$ |
| C=O stretching vibration | 1736 $cm^{-1}$ |
| C=O amide I carbonyl | 1622-1640 $cm^{-1}$ |
| C–O–H deformation vibration | 1412 $cm^{-1}$ |

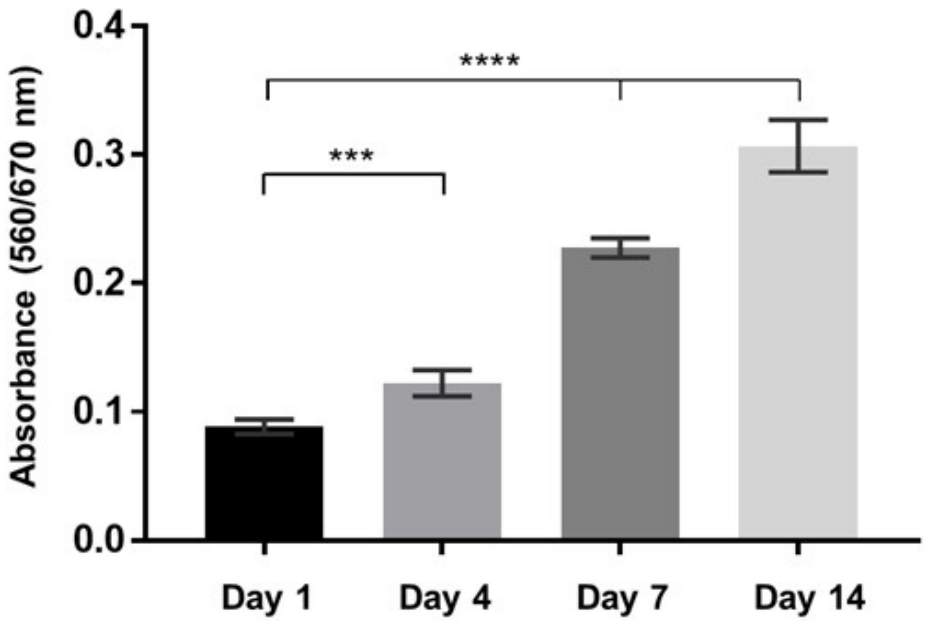



**Figure 4.** Alamar Blue analysis chondrocyte cells seeded-composite structures ($p^{***} < 0.01$, $p^{****} < 0.001$, n = 3).

Additionally, the cellular behaviors of chondrocyte cells during the culture period were evaluated, and the relevant images are presented comparatively in Figure 5A. On the 1st day of culture, chondrocyte cells were observed to be in a spherical form and isolated on the gel structure. By the 4th day, a significant increase in cell number was noted. On the 7th day of culture, besides cell proliferation, the cells lost their spherical forms, spreading over the surface and acquiring a characteristic appearance. By the 14th day of culture, the cells showed a more homogeneous distribution, with increased adhesion to the scaffold and beginning to cover the fiber surfaces. The results of SEM analysis confirm the findings of the Alamar Blue assay in terms of supporting cell proliferation by the HA gel combined with PGA mesh.

## DISCUSSION

The concentration of hyaluronic acid (HA) plays a role in regulating proteoglycan synthesis during the maturation of joint cartilage and in the repair processes that occur in degenerative joint disease[20]. In a healthy synovial joint, the concentration of HA ranges from 1 to 4 mg/mL, which is a key parameter affecting its viscoelastic properties[21]. Solutions with higher concentrations exhibit greater viscosity. During viscosupplementation, the HA injected into the joint capsule should restore the rheological properties of healthy synovial fluid. The best results are achieved with synovial fluids mixed with cross-linked HA[22, 23]. In this study, the concentration of the cross-linked HA obtained was 22.52 ± 0.8 mg/mL. HA can be cross-linked using physical and chemical methods, with butanediol diglycidyl ether (BDDE) being the most commonly used cross-linking agent for the stabilization of HA-based filler materials. BDDE's properties,

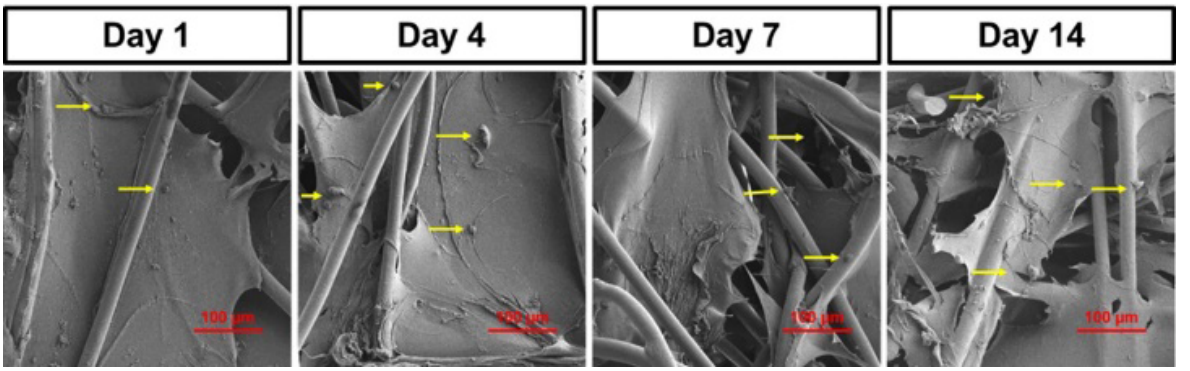



**Figure 5.** SEM images of chondrocyte cells seeded-composite structures (Scale bar:100 µm).

such as biodegradability, toxicity profile, and stability, make it advantageous compared to other cross-linking agents[24]. The maximum permissible level of BDDE in 1 mL of HA gel, as determined by the FDA's safety risk assessment, is <0.002 mg (<2 ppm)[25]. In this study, the BDDE content in the obtained HA gel was determined to be below 1 ppb. Additionally, the pH, osmolality values of the obtained cross-linked HA gels were found to be consistent with literature studies and approved commercial fillers[26]. According to the European Pharmacopoeia, these values for medical devices are specified as pH 7.2 ± 0.4 and osmolality 200-400 mOsm/kg[27]. These values are close to those of blood plasma in joint formulations and are tolerable[28].

The injection pressure is directly related to the ejection force and corresponds to the injection force. In clinical practice, the injection force is typically measured by the force applied to the syringe plunger. A study has shown that the ejection pressure corresponding to an injection force of 15 N is at least five times higher than normal blood pressure (<120/80 mm Hg), indicating that an injection force of 15 N can significantly increase blood pressure[29]. Injections using a 27G needle exhibited injection forces ranging from 11 to 14 N, depending on the rheological properties of the materials. In our study, the injection pressure of the HA gel at a concentration of 23 mg/mL with a 27G needle was determined to be 1.94 ± 0.7 N. This is related to the rheological, or viscoelastic properties of the gel.

Viscoelastic materials exhibit both viscous and elastic properties when subjected to shear deformation[30]. HA gels in the body are exposed to various forces due to movement, and the response to mechanical stress can be clinically evaluated through rheological measurements [31]. The elastic modulus (storage modulus) or G', represents a solid-like behavior of HA gel materials when injected, allowing the filler to retain its shape[32]. Reducing the number of cross-links increases the distance between HA molecules, requiring less force to deform the gel, resulting in a softer and less elastic filler (lower G')[33]. The viscous modulus or G'', is related to the gel's fluid behavior after the mechanical stress is removed, allowing the gel to deform and flow to some extent during injection. For any HA filler to be effective, it must be viscoelastic—viscous enough to be injected and initially shaped, but elastic enough to resist shear deformation forces and provide lasting correction after implantation into soft tissue. Lastly, it is important to note that the phase angle or Tan δ allows the determination of whether the filler is more elastic or more viscous but does not provide information about the actual magnitudes of G' and G'' [34]. In our HA gel material, the G' value decreased due to the effect of temperature on cross-link density, resulting in reduced elasticity. This led to an increase in the gel's liquid-phase behavior, i.e., an increase in the G'' value and phase angle. Literature reports indicate that sterilization by heat or stream affects the viscoelastic properties and degradation behavior of HA gels[35]. The G' measurements at a frequency of 5 Hz for commercial HA gels such as Restylane®, Juvéderm®, and Belotero® are reported to range from 40 to 977 Pa, while G'' measurements range from 30 to 198 Pa, influenced by parameters such as HA concentration and cross-link density[35].

The composite structures obtained by impregnating the PGA meshes with HA gels were analyzed using FTIR and SEM. The FTIR results confirmed the presence of peaks corresponding to ester groups (1728 $cm^{-1}$, 1175 $cm^{-1}$, and 1080 $cm^{-1}$) and –CH2–CH2– segments (1412 $cm^{-1}$, 975 $cm^{-1}$, and 900 $cm^{-1}$)[36]. The high water capacity of the HA gel was indicated by the -OH and -NH peaks observed in the 3200-3500 $cm^{-1}$ range. In the composite structure, characteristic peaks of both the

HA gel and the PGA mesh were identified. Additionally, the successful penetration of the HA gel into the PGA mesh was confirmed by SEM images. To further investigate the feasibility of using these composite structures in cartilage tissue engineering applications, the structures were cultured with chondrocyte cells for 14 days. Both synthetic PGA polymer and natural HA polymer are known to exhibit biocompatible and biodegradable properties[35, 37]. The combination of these advantages in the composite structures was shown to support cell adhesion, spreading, and proliferation.

## Conclusion

In this study, a cartilage repair matrix was designed by developing a product reconstituted with cross-linked HA and PGA mesh. The HA gel was cross-linked using BDDE, resulting in a final gel product that exhibited applicable physical, chemical, mechanical, and rheological properties. With this study, the composite structures combined with PGA meshes have been identified in the literature as biomaterials for cartilage regeneration. However, the research is limited to the evaluation of the cellular effects of the HA/PGA biocomposite. It is a promising candidate for potential applications such as in vivo studies and subsequent implantation into cartilage defects.

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