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(RESEARCH ARTICLE)

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Assessing the changes in mechanical properties of different Gellan gum hydrogels under tissue engineering conditions

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Abstract

Background: Tissue engineering (TE) seeks to develop biological substitutes that restore, maintain, or improve tissue function. A significant challenge in TE is designing scaffolds that replicate the biomechanical environment of native tissues, especially in load-bearing applications. Hydrogels, particularly Gellan Gum (GG), are promising scaffold materials due to their high water content and tunable viscoelastic properties. Crosslinking agents such as calcium (Ca^{2+}) and spermidine (SPD) play a crucial role in modulating these properties.

Purpose: This study aims to compare the mechanical behavior of GG hydrogels crosslinked with calcium and spermidine over a 28-day period under chondrogenic conditions. The goal is to assess the impact of crosslinker type on mechanical stability and determine their suitability as scaffolds for cartilage tissue engineering.

Method: GG was dissolved at a concentration of 5 mg/mL in HEPES/sucrose buffer and crosslinked using either 10 mM $CaCl_2$ or 2 mM SPD. Polymer and crosslinker solutions were mixed at a 5:1 volume ratio and cast into cylindrical molds (10 mm diameter, 2 mm height). Hydrogels were cultured in chondrogenic media at 37°C and sampled on Days 1, 3, 7, 14, and 28. Stress relaxation tests were conducted using a Mach-1 Micromechanical Testing System with a 3 mm flat-ended cylindrical indenter, applying 5%, 10%, and 15% compression steps followed by 240-second holds. Instantaneous modulus (Einst) and equilibrium modulus (Eeq) were calculated using Hayes' analytical solution with Poisson's ratios of 0.1 and 0.5, respectively. Data were statistically analyzed using Pearson correlation and ANOVA (significance set at $p < 0.05$).

Results: Both GG/Ca and GG/SPD hydrogels remained structurally stable over 28 days. GG/SPD initially exhibited a slightly higher Eeq (0.0015 ± 0.0003 MPa) than GG/Ca (0.0014 ± 0.00009 MPa) on Day 1. However, from Day 3 onwards, GG/Ca hydrogels consistently showed higher stiffness values. Over time, GG/Ca displayed a slight increase in Eeq, while GG/SPD remained unchanged or decreased marginally. Similar trends were observed for Einst. Pearson correlation analysis indicated a weak negative correlation between time and Eeq in GG/SPD ($r = -0.25$), and a weak positive correlation in GG/Ca ($r = 0.08$), neither of which were statistically significant. ANOVA revealed no significant changes in moduli over time for either group ($p > 0.05$).

Conclusion: GG hydrogels crosslinked with calcium or spermidine exhibit stable mechanical properties under prolonged chondrogenic culture. Although GG/Ca hydrogels showed a slight increase in stiffness over time, both formulations maintained mechanical integrity and are viable candidates for cartilage tissue engineering. Further studies are required to explore their biological performance and regenerative potential.

Keywords: Gallen Gum(GG); Hydrogel; Spermidine(SPD); Calcium(Ca); Crosslinker

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1. Introduction

For several years now, one of the most significant areas of medicine in the recent decades has been lauded as Tissue Engineering (TE) in the area of Regenerative Medicine (RM). While the creation of bio-engineered skin replacements was the primary focus of the initial therapeutically relevant TE studies, Tissue Engineering applications have now been progressively broadened to a wide spectrum of tissues and organs. The emergence of either mesenchymal adult stem-cell technology or embryonic stem-cell technology has inspired various attempts to incorporate this potential tool with TE techniques and has united the two areas under the umbrella known as "regenerative medicine." The identification of telocytes, a novel cell type that has been characterized in several organs and identified by electron microscopy, opens another doorway to RM and serves as a classic illustration of translational medicine. In addition to cell-therapy techniques, the use of gene therapy in conjunction with TE has been researched to produce tissues and organs. A vital function is also played by the vascularization of constructions in addition to the matrix and cell replacements. As a result, new in vivo models of vascularization have emerged that enable axial vascularization followed by construct transplantation. Tissue engineering has elements such as cells, scaffold biomolecules, and bioreactors [1–5].

The idea of using cell-culture methods and biomaterials to create living tissue equivalents and/or organs in the lab, later dubbed "Tissue Engineering," was first proposed as a multidisciplinary approach to regenerate lost tissue or organ functions. It was envisioned as the solution to both the organ shortage in transplantation medicine and the donor site morbidity issues that had previously gone unresolved. Clinicians and experts from many different fundamental fields in biology, engineering, and applied sciences have collaborated extensively only on the concept of growing tissue in the lab. Therefore, in order to create biological replacements that will preserve and restore normal function in sick and wounded tissues, researchers working in the fields of tissue TE and RM are currently using the concepts of cell culture and transplantation, material science, and bioengineering.[6–10].

Though there have been great efforts and improvements in standardizing cell culture processes and developing customized biomaterials to replace lost organ functions, the translation of laboratory triumphs into clinical situations has not proven to be as successful as it may be. This is partly due to the three-dimensional orientation of the organs and tissue, which necessitates the existence of a microvascular network in order to sustain the survival of the cells even at the center of any given construct by providing adequate blood flow and oxygenation. Before extrinsic vascularization may occur, an internal vascular network must form. This is an inherent limitation on the ability of cells to survive in three-dimensional scaffolds in the first stages after the implantation of tissue-engineered substitutes into patient structures. [13].

1.1. Instantaneous Modulus (Einst)

The instantaneous modulus, often represented as E, serves as an indicator of a material's stiffness at a specific point along its stress-strain curve, reflecting the slope of the curve at that particular location. Hooke's law describes the stress-strain relationship for an elastic material as follows:

$$\sigma = E \cdot \varepsilon \quad \text{..... (1.6)}$$

Here:

-σ (sigma) signifies the applied stress to the material.

- (E) represents the instantaneous modulus.

- ε (epsilon) denotes the strain (deformation) experienced by the material.

The calculation of the instantaneous modulus involves taking the derivative of the stress-strain curve at the designated point, expressed mathematically as: [116].

$$E = \Delta\sigma / \Delta\varepsilon \quad \text{.....(1.6.1)}$$

The initial instantaneous modulus (Eoinst) is determined at the intersection of the fitted linear line, while the strain-dependent instantaneous modulus (Eεinst) is obtained by measuring the slope of this line at each stress relaxation phase [116]. The overall instantaneous modulus (Einst) is then given by the sum of Eoinst and Eεinst :

$$E_{inst} = E^{\varepsilon}_{inst} + E^{o}_{inst} \quad \text{.....(1.6.2)}$$

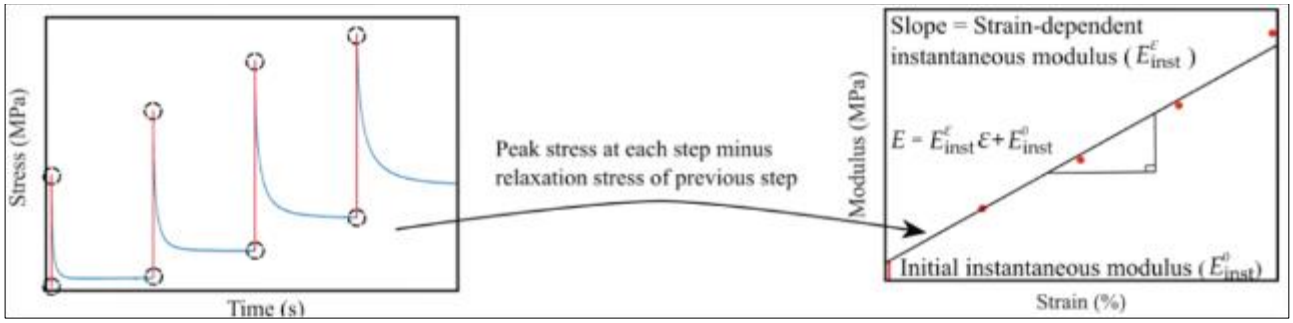

Figure 1 The instantaneous modulus derived from loading in several steps. Each "point" in the right picture is formed by the red rising portion in the left figure [116]

1.2. Equilibrium Modulus (Eeq)

The equilibrium modulus, indicated as Eeq, describes the material's stiffness once it has attained a state of mechanical equilibrium, typically following some deformation. Its computation involves utilizing stress and strain values at a specific point and the origin (unstressed state) [116].

The formula for calculating the equilibrium modulus is as follows:

$$Eeq=\sigma/\varepsilon \quad \text{.....(1.6.3)}$$

Where:

- σ represents the stress at the point where equilibrium is achieved.

- ε stands for the strain at the point where equilibrium is reached.

The equilibrium modulus offers an average stiffness measurement spanning from the origin to the equilibrium point, providing insight into the overall stiffness of the material under specific loading conditions [116].

To characterize the equilibrium modulus, a common approach involves employing multi-step stress relaxation or creep tests. Following the application of displacements or stress to the material, a relaxation period is allowed. [116] The equilibrium modulus is then calculated using the stress-strain ratio, determined by the slope of a linear line fitted to equilibrium points, expressed as:

$$Eeq=\Delta\sigma_{eq}/\Delta\varepsilon_{eq} \quad \text{.....(1.6.4)}$$

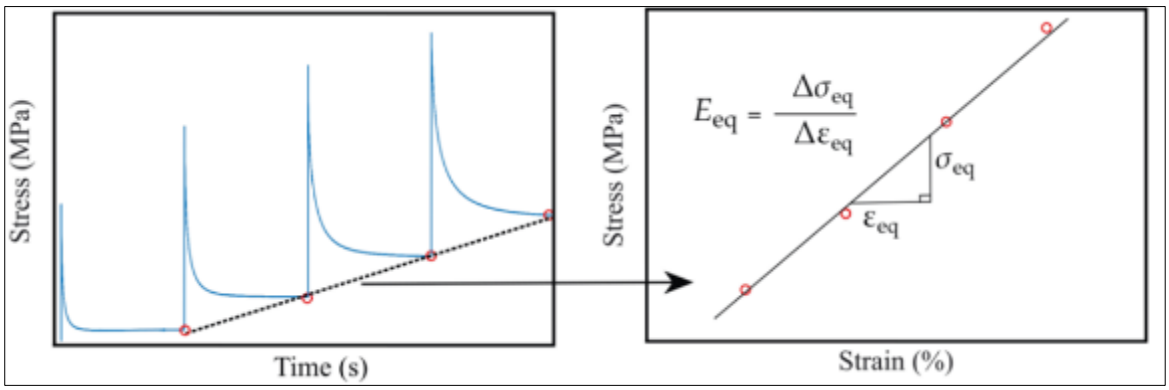

Figure 2 Calculating the elastic equilibrium modulus using stress relaxation [116]

It's important to note that the terms "instantaneous modulus" and "equilibrium modulus" are frequently employed when dealing with linear elastic materials. However, real materials can display more intricate behaviors, including non-linearity, viscoelasticity, or plasticity, necessitating the use of more advanced modeling and testing approaches.

Additionally, it is crucial to maintain consistency in the units of stress and strain in these formulas, typically using Pascals (Pa) for stress and dimensionless units for strain since it represents a ratio of lengths [116].

By calculating the ratio of the difference between the peak stress in the second step and the equilibrium stress in the first step to the strain amplitude, the instantaneous modulus was found. By evaluating the slope of the linear fit at the equilibrium locations, the equilibrium modulus was computed. The mean values of stress for the last five seconds were used to approximate the equilibrium stresses for each step. Elastic and isotropic behavior of the hydrogel was considered for both the immediate and equilibrium loading phases. Thus, the Hayes model was used to modify the predicted instantaneous and equilibrium moduli. It was assumed that the instantaneous and equilibrium moduli had a Poisson's ratio of 0.5 and 0.1 respectively [118]. In this project work we made use of the same step method to calculate our instantaneous and equilibrium moduli however we determined our instantaneous and equilibrium moduli with Poisson ratio of 0.5

2. Materials and Methods

The materials used for hydrogel preparation were provided by Prof. Kellomäki at the BioMediTech Institute, Faculty of Biomedical Sciences and Engineering, Tampere University of Technology. These materials included sterile-filtered 5 mg/mL Gellan Gum (Gelzan™ CM Gelrite, Sigma-Aldrich) in a HEPES/sucrose solution (HEPES 25 mM, sucrose 100 mg/mL), spermidine trihydrochloride in HEPES/sucrose buffer (2 mM), and calcium chloride (10 mM). Teflon molds (10 mm diameter, 2 mm thickness) were also utilized.

Hydrogels were incubated in chondrogenic media, composed of α-Minimum Essential Medium, 1x Insulin-Transferrin-Selenium +1, 0.3% Penicillin/Streptomycin, 50 µg/mL ascorbic acid-2-phosphate, 55 µg/mL sodium pyruvate, and 23 µg/mL L-proline.

2.1. Hydrogel Preparation

Two types of hydrogels were prepared: (1) Gellan Gum crosslinked with calcium (GG/Ca) and (2) Gellan Gum crosslinked with spermidine (GG/SPD). Both hydrogels were prepared following an identical protocol, maintaining a polymer-to-crosslinker volume ratio of 5:1 (e.g., 1000 µL GG and 200 µL CL). Samples were prepared in a 24-well plate, with 10 technical replicates per plate. The hydrogels were incubated at 37°C and monitored over a 28-day period, with mechanical testing conducted on days 1, 3, 7, 14, and 28.

2.2. Mixing Technique

Hydrogel components were combined and mixed in a glass vial before being transferred into the molds. Additional components were incorporated into the polymer solutions before the addition of crosslinkers to ensure homogeneity. Once crosslinkers were added, the solutions were briefly stirred and transferred to the molds using a pipette. The gelation onset occurred within 30 to 60 seconds. Following confirmation of gel formation, 1 mL of chondrogenic media was added under aseptic conditions. Media was refreshed every three days until mechanical testing.

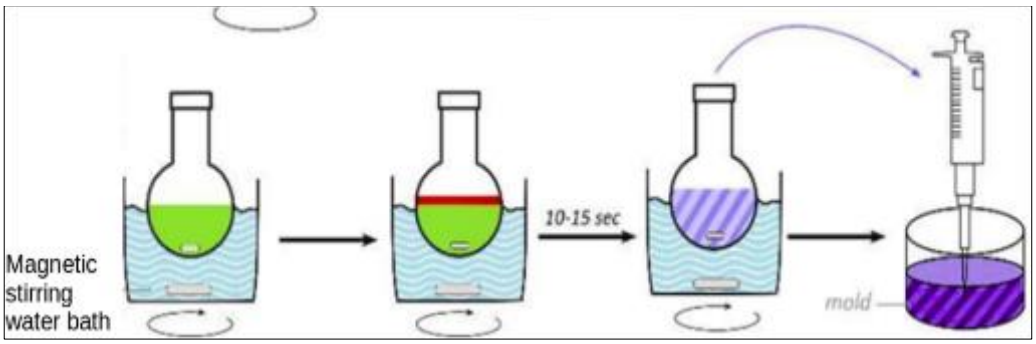

Figure 3 Schematic representation of hydrogel-plated plating gel, ensuring homogenous gelation supported by Teflon at 37 °C. [117]

2.3. Mechanical Studies

A Mach-1 (Micromechanical System, Biomomentum) biomechanical testing device was employed for stress relaxation testing. Data obtained from these tests were analyzed to determine the equilibrium modulus and instantaneous modulus over time. Each hydrogel type (GG/Ca and GG/SPD) was tested using five replicates per well plate.

2.3.1. Mechanical Testing

Hydrogel mechanical properties were assessed using a Mach-1 device comprising a PM500-C Precision Motion Controller (Newport Corporation, USA), a Model V500Css Actuator (Newport), and a 0.15 kg single-axis load cell (Biomomentum, Quebec, Canada). A cylindrical flat-ended indenter (3 mm diameter) was used for indentation testing.

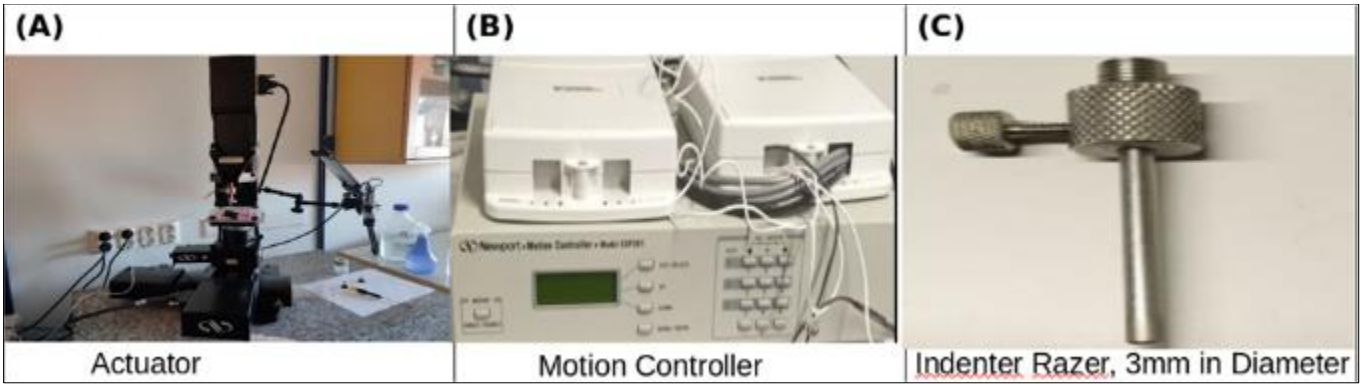

Figure 4 (A) Actuator, (B) Motion Controller, (C) Indenter Razer, dia. 3mm

Stress relaxation tests were conducted at room temperature under wet conditions. Samples were retrieved at designated time points (days 1, 3, 7, 14, and 28) and subjected to indentation testing. The well plates were placed on the Mach-1 device stage, and the indenter was aligned with each hydrogel sample. Each sample underwent a three-step indentation process, consisting of 5% compression per step, resulting in a total indentation of 15%, followed by a 240-second relaxation period per step, yielding a total test duration of 270 seconds per sample. Force and indentation depth data were continuously recorded and analyzed to determine instantaneous and equilibrium modulus values.

Figure 5 Basic template for the biomechanics setup application

2.4. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Each experiment included five replicates per sample group. Group differences were analyzed using an Excel spreadsheet (R package 'stats' version 4.2.1), followed by t-tests (Tukey's test) for multiple comparisons. The Pearson correlation coefficient was employed to assess correlations between modulus values and time. ANOVA was used to evaluate fluctuations in modulus values over time, with statistical significance set at $p < 0.05$.

Equilibrium modulus values were averaged and compared between GG/SPD and GG/Ca samples over time to assess stability, stiffness, and indentation resistance. For example, equilibrium modulus values (± SD) for GG/Ca were 0.0014 ± 0.00009 MPa on day 1 and 0.00137 ± 0.0002 MPa on day 7, which were lower than those observed for GG/SPD (0.0015 ± 0.0003 MPa on day 1 and 0.0014 ± 0.0002 MPa on day 7).

2.5. Expected Outcome

This study aims to provide fundamental insights into the evolution of hydrogel mechanical properties over the incubation period. The findings will contribute to a deeper understanding of hydrogel behavior in long-term culture, informing future studies conducted by the Biophysics of Bone and Cartilage (BBC) research group.

3. Results

3.1. Hydrogel Appearance and Dimensions

Hydrogels were prepared using molds with dimensions of 2 mm in thickness and 10 mm in diameter (Figure 6). Figure 6A presents a schematic representation of hydrogel formation in well plates, while Figure 6B illustrates the mold and its dimensions. Figures 6C and 6D depict the hydrogels within the mold and after removal, respectively, following overnight incubation in culture media. The pink coloration of the gel resulted from the presence of phenol red in the culture medium.

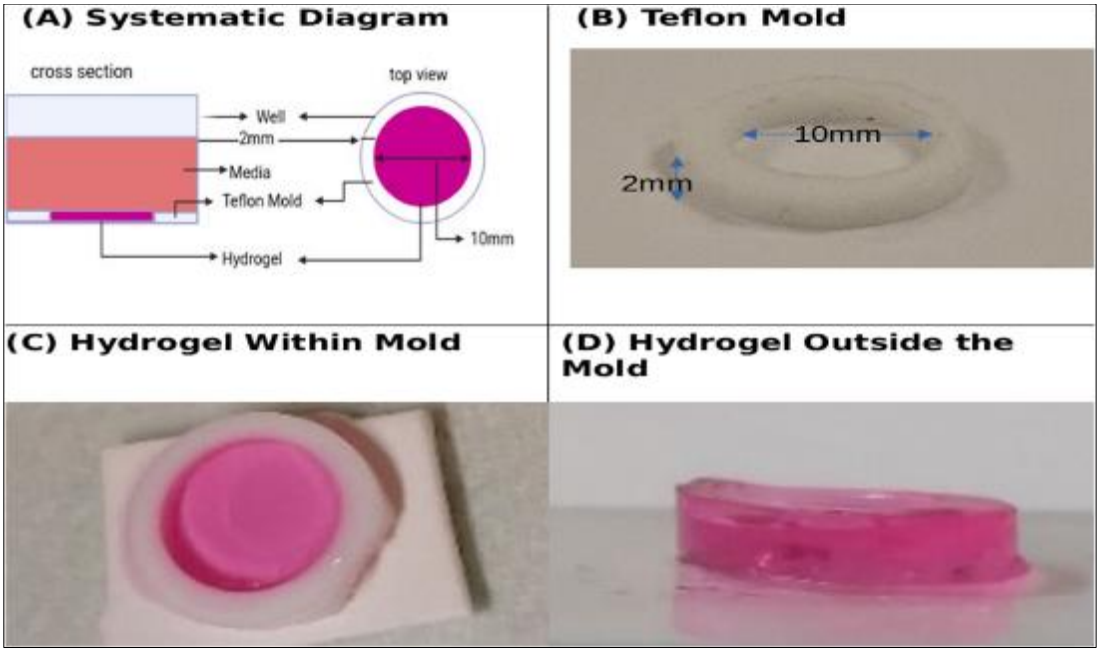

Figure 6 (A) Schematic of gel formation, (B) mold dimensions, (C) hydrogel within mold, (D) hydrogel post-removal retaining mold shape

3.2. Mechanical Properties of the Hydrogels

Indentation testing was conducted using the Mach-1 device to assess the mechanical properties of the hydrogels. A multi-step stress relaxation protocol was applied, in which the indenter compressed the hydrogel samples within a confined space. The applied force at each step is illustrated in Figure 7A. Typically, the equilibrium modulus of hydrogels is characterized using multi-step stress relaxation. However, Figure 7B depicts an invalid result due to an abnormal stress relaxation pattern, inconsistent with the expected three-step protocol validated in Figures 8, 9, and 7A. Consequently, only samples exhibiting expected mechanical responses were included in further analysis.

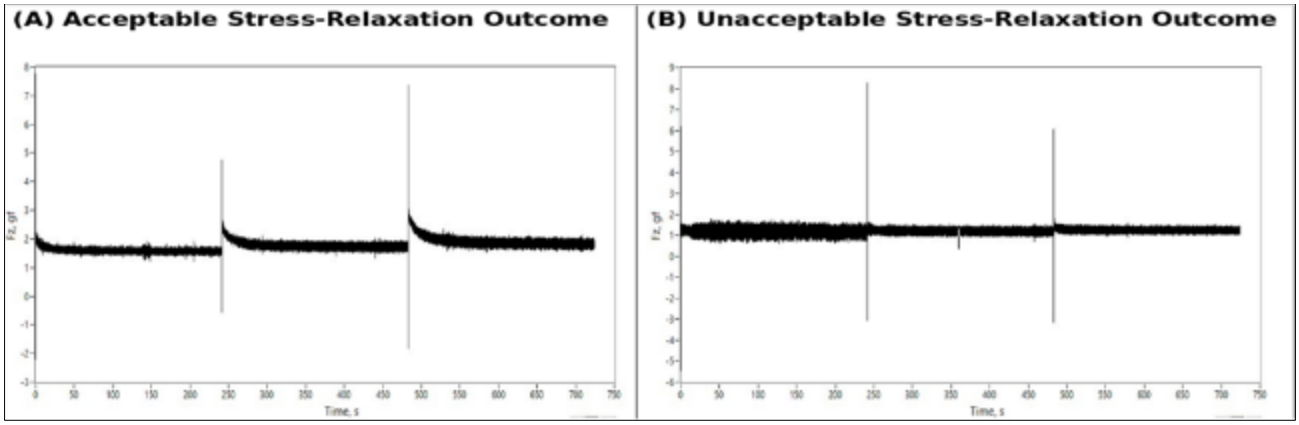

Figure 7 (A) Typical stress relaxation response, (B) Abnormal stress relaxation response (excluded from analysis)

3.2.1. Equilibrium Elastic Modulus of the Hydrogels Over the Incubation Period

The equilibrium modulus values (**Table 1)** indicate that on Day 1, the modulus of GG crosslinked with spermidine (GG/SPD) was slightly higher than that of GG crosslinked with calcium (GG/Ca). By Day 3, the modulus of GG/Ca exceeded that of GG/SPD, but this trend was not sustained, as GG/SPD exhibited a higher modulus on Day 7. From Day 14 onward, GG/Ca demonstrated a consistent increase, surpassing GG/SPD through Day 28.

Despite these variations, statistical analysis revealed no significant difference between the two crosslinkers, as indicated by p-values exceeding the 0.05 significance threshold. This suggests that the observed differences in equilibrium modulus between GG/SPD and GG/Ca are not statistically significant and should not be considered conclusive.

Table 1 Equilibrium Modulus (MPa) for GG/Ca and GG/SPD (Averaged)

Equilibrium Modulus(MPa)						
	Ca			**SPD**		
Time (Days)	Mean (μ)	±Std	Mean (μ)	± Std	Difference	Pvalue
1	0,00142534	9,07142E-05	0,001493667	0,000261774	-0,000068327	0,63508484
3	0,001440113	0,000189421	0,00143656	0,000193493	3,5528E-06	0,979707445
7	0,001378935	0,000170837	0,001412426	0,00028787	-3,3491E-05	0,846391846
14	0,001481232	0,000133703	0,001402372	0,000257827	7,88602E-05	0,601893198
28	0,001452707	0,000241861	0,001300737	0,000121923	0,00015197	0,294345953

The plot below, **Fig.8**, practically revealed how the various crosslinked hydrogel changes through the time of incubation and the application of indentation compression that is from day 1,3,7,14 and 28 respectively. In order to further buttress or prove if there is significant difference between the values recorded with both crosslinkers on GG, we took a step further to elaborate this by looking at the Pearson correlation coefficient (r), where we noticed a decrease in equilibrium of GG/SPD with time and no change for those crosslinked with GG/Ca with r of -0.25 and 0.0837, respectively, which indicate that there is low positive negative correlation between them, we know that Pearson coefficient helps to quantify a correlation, and to show the significance, we now looked at that the P values which are p 0.6907 for GG/Ca and p 0.228 for GG/SPD which are both higher than 0.05, shows that the correlation is not statistically significant as confirmed by their P values. However we derived the Pearson correlation and its P value from the raw data which was plotted (scatter plot) against the independent variable which are the days. Remember that P value helps to assess whether a correlation is real, meaning "r" and the P value should be analyzed together not individually Fig.9 [120].

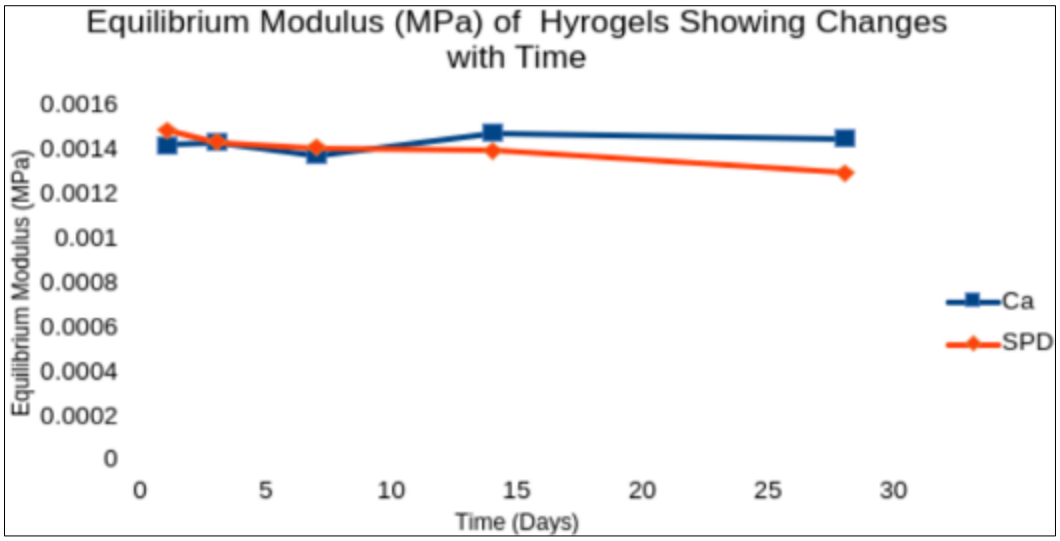

Figure 8 Equilibrium modulus (MPa) of crosslinked GG/Ca and GG/SPD Vs time

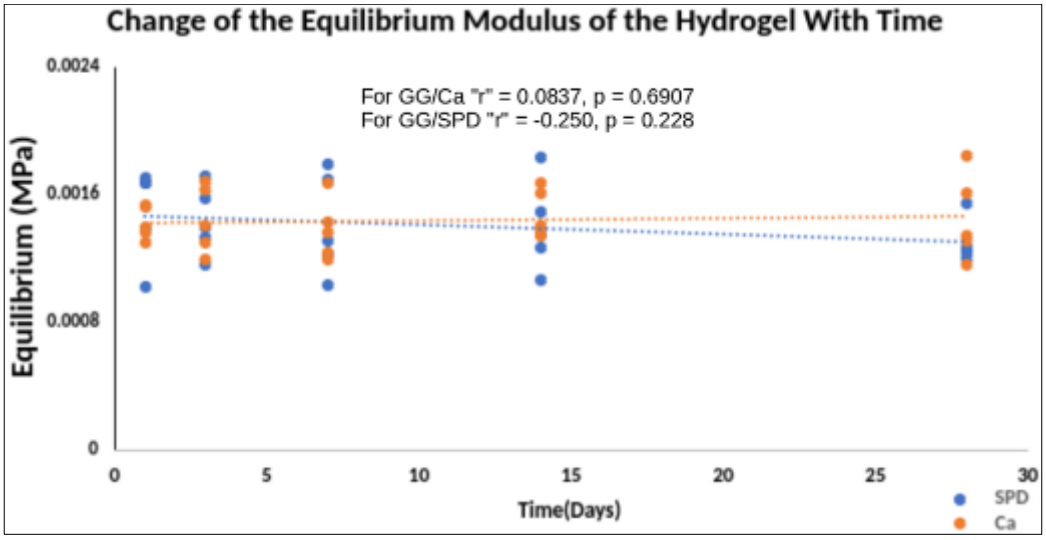

Figure 9 Evaluating Gel's Equilibrium Modulus Changes Over Time Using Pearson Correlation

In Table 1, we previously described the behavior of hydrogels GG/Ca and GG/SPD at a 5:1 ratio over the course of the incubation period under an indentation mechanical compression protocol. The data revealed fluctuations in the modulus values across the days, with notable exceptions on days 14 and 28, where the GG/Ca hydrogel consistently exhibited higher values. Figure 12 further illustrates the relationship between the various hydrogels (GG/Ca and GG/SPD) across the incubation days. To provide a clearer understanding of these trends, we applied Pearson's correlation coefficient and its corresponding p-value to assess the relationship and statistical significance. However, our analysis showed that the observed correlation was not statistically significant.

Furthermore, box plots were generated (Fig. 10 and Fig. 11) for each of the hydrogels, GG/Ca and GG/SPD, to further illustrate their relationship over time using the raw data. These box plots indicate that the data did not follow a clear trend relative to the duration of the incubation period.

To provide a more rigorous analysis, we performed an analysis of variance (ANOVA) on the data for each hydrogel, crosslinked with the same crosslinker, as shown in Fig. 10 (GG/Ca) and Fig. 11 (GG/SPD). This was done to assess whether the fluctuations observed in the data held statistical significance, as discussed in paragraph 1 of section 3.2.1. The ANOVA results revealed that the fluctuations were not statistically significant, suggesting that the modulus does not show consistent increases or decreases over time. Specifically, the P-values for GG/Ca (0.940) and GG/SPD (0.831) were both greater than 0.05, indicating no significant fluctuations in the data for either hydrogel.

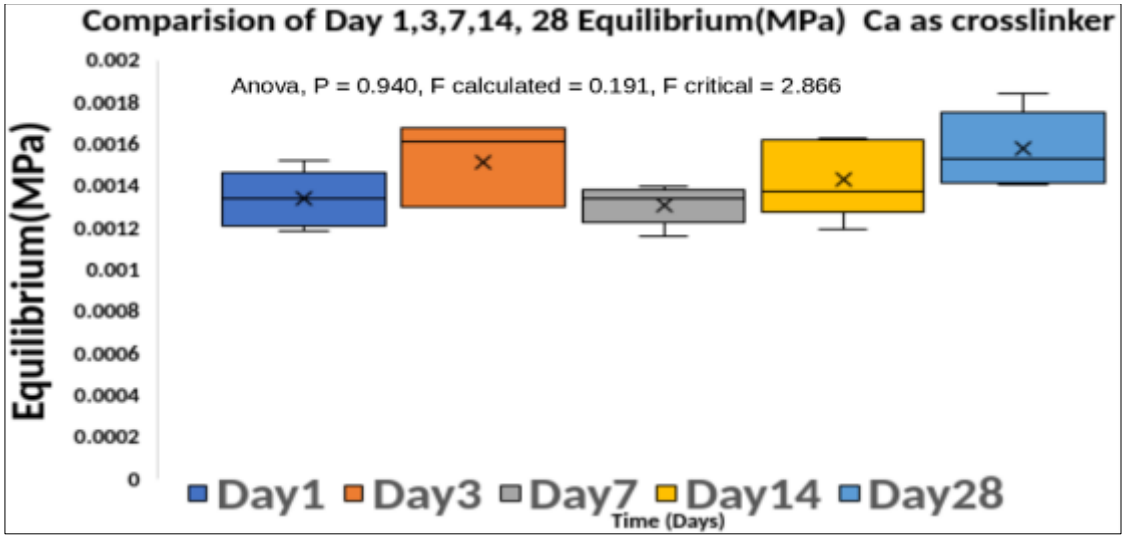

Figure 10 Comparison of equilibrium modulus(MPa) of GG/Ca changes across the days

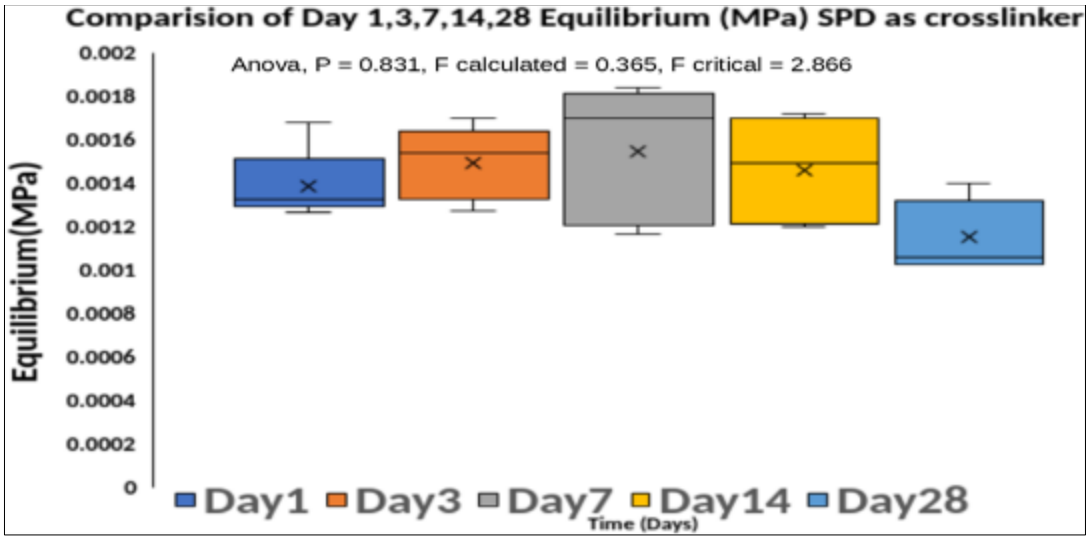

Figure 11 Comparison of equilibrium modulus(MPa) of GG/SPD changes across the days

4. Discussion

Certain inorganic and biological amine cations are known to crosslink negatively charged polymers, forming physical hydrogels via ionotropic gelation [115]. In this study, we employed spermidine, an endogenous polyamine, and calcium, an inorganic cation, as crosslinkers for Gellan Gum (GG) hydrogels. While hydrogels can be formed by simply adding a crosslinker to GG, we also incubated the hydrogel in chondrogenic media. Although not part of our experiment, other studies have included various biological molecules in hydrogels, and GG has shown promise in tissue engineering (TE) applications. The method used here for preparing GG crosslinked with Ca and SPD is flexible, practical, and cost-effective.

GG is FDA- and EMA-approved as a gelling agent in food, cosmetics, and pharmaceuticals, and its use in TE has yielded encouraging results. Hydrogels are categorized into weak gels, which flow under stress, and true gels, which fracture and become self-supporting under severe stress. Our hydrogels are considered true gels, maintaining their structure even when handled with tweezers or cast [109].

We evaluated the mechanical properties of the hydrogels under cultured conditions. Although all materials were based on GG, we assessed the elastic behavior of the crosslinkers (Ca and SPD) using stress relaxation tests, determining the

equilibrium modulus via indentation compression. The goal was to comprehensively examine the mechanical properties of GG hydrogels, which is critical for future cell-based studies. The uniform mixing approach yielded high-quality samples with good repeatability.

Mechanical properties were derived from stress relaxation tests. Using multiple stress relaxation steps allowed for the effective measurement of strain-dependent characteristics related to the equilibrium modulus. However, small sample sizes and site-specific comparisons may have impacted the validity of the results, and the conclusions drawn may not be universally applicable [116].

Hydrogel mechanical characteristics are defined by stresses and strains under applied loads. Standardized mechanical tests on hydrogels subjected to precise load conditions help assess their properties. In TE scaffold design, bio-mimicking materials with mechanical properties similar to relevant tissues are essential. The lack of standardization in mechanical testing across studies has led to variability and difficulty in comparing hydrogel characteristics to tissue properties. Standardizing these tests could improve comparison and interpretation.

In our study, stress relaxation protocols helped define both instantaneous and equilibrium moduli of the hydrogels. Table 1 shows no significant differences between GG/Ca and GG/SPD, with P-values greater than 0.05. Figures 10 and 11 confirm that fluctuations led to non-significant variations. For GG/Ca, the correlation was negligible ($r \approx 0$), while GG/SPD showed a slight inverse relationship ($r = -0.25$), indicating minimal variation across the incubation period. Thus, incubation time did not significantly affect the hydrogels or their differences.

The absence of significant results may be due to the small sample size and noise interference, which affected stress peak measurements and made calculating the instantaneous modulus unreliable. The indenter error, shown in Figure 7B, further compromised the stress relaxation outcomes, resulting in unrealistic modulus values. Consequently, we excluded these data from the analysis, despite their inclusion being a study objective.

GG served as the primary structuring agent in both GG/SPD and GG/Ca mixtures, consistent with existing literature. While crosslinker concentration influences mechanical properties, we used a 5:1 ratio of GG/Ca and GG/SPD in this study. Our results align with previous studies, with modulus values in MPa (converted from kPa). While some differences were noted between the hydrogels, they were not significant. As noted in previous research, polyamines like spermidine are effective crosslinkers at lower concentrations than inorganic cations like Ca^{2+}, which may explain the higher concentrations of calcium (Ca – 10mM) and spermidine (SPD – 2mM) used here [109].

The equilibrium modulus values in **Table 1** reflect the stiffness of the hydrogels at mechanical equilibrium under applied load. This property is essential for understanding how materials respond to external forces and makes GG hydrogels suitable for TE regeneration [112,113]. The limited changes in equilibrium modulus during incubation suggest that the hydrogels remain stable, making them promising for tissue regeneration, as they can integrate effectively with host tissues for long-term functionality

Abbreviations

- GG: Gallen Gum
- SPD: Spamidine
- Ca**:** Calcium
- FDA**:** Food ans Drug Administration
- EMA**:** European Medicines Agency
- ECM**:** Extracellular Matrix
- TE: Tissue Engineering
- RM : Regenerative Medicine

5. Conclusion

This study evaluated the mechanical properties, particularly the equilibrium modulus, of two physical hydrogels GG/Ca and GG/SPD crosslinked via ionotropic gelation. After incubation under tissue engineering conditions, the mechanical properties of these hydrogels were influenced by the constituent components, demonstrating promise for biomedical applications due to their versatile composition, appropriate elasticity, and robust mechanical characteristics.

Calcium (Ca) and spermidine (SPD) serve as effective crosslinking agents for Gellan Gum (GG), and the hydrogels' mechanical properties were evaluated using stress relaxation tests. Despite the limitations of small sample size, our

study found no significant differences between the two crosslinkers in terms of mechanical properties over the incubation period. The degree of crosslinking in GG affected by the concentrations of Ca and SPD can be adjusted to optimize mechanical properties for specific applications. Our findings suggest that GG hydrogels crosslinked with calcium and spermidine are suitable for encapsulating cells due to their gelation ease and favorable mechanical properties.

The hydrogels' mechanical characteristics, such as stability, stiffness, and durability, make them promising candidates for tissue engineering, particularly for cartilage and bone regeneration. Furthermore, these hydrogels allow for the inclusion of biologically relevant molecules, enhancing their potential for biomedical applications, including controlled drug release.

Future studies should focus on minimizing noise interference in measurements and increasing the sample size to improve statistical power, precision, and the reliability of results.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares that there is no conflict of interest regarding the publication of this work. No financial, personal, or professional relationships influenced the outcomes or interpretations presented in this study.

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