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Mechanical Characterization of Collagen-Enhanced Chitosan–Alginate Scaffolds for Tissue Engineering

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Abstract

Tissue engineering scaffolds require a delicate balance between porosity, mechanical strength, and biocompatibility. In this study, chitosan–alginate scaffolds reinforced with Type I collagen were fabricated via freeze-drying and ionic crosslinking. The effect of collagen incorporation (0%, 10%, and 20% w/w) on mechanical properties was evaluated using compressive, tensile, and cyclic compression tests, as well as degradation-related stability over 7 days in PBS. Results demonstrated that the addition of 10% collagen (CAC-10) significantly enhanced compressive and tensile strength, elastic modulus, and recovery ratio, while maintaining favorable stability during degradation. Excessive collagen (20%) slightly decreased mechanical performance, likely due to structural heterogeneity and higher water uptake. Swelling behavior increased with collagen content, highlighting the influence of hydrophilic components on scaffold mechanics. Overall, CAC-10 scaffolds exhibited an optimal combination of mechanical strength, elasticity, and stability, making them promising candidates for soft tissue engineering applications.

Keywords: Chitosan–alginate scaffold, Collagen, Mechanical properties, Degradation stability, Tissue engineering.

1 | Introduction

Tissue engineering has emerged as an interdisciplinary field aimed at developing biological substitutes to restore, maintain, or improve the function of damaged tissues and organs. A fundamental component in this approach is the scaffold, which serves as a temporary three-dimensional framework that mimics the native Extracellular Matrix (ECM) [1–5]. Scaffolds not only provide structural support for cell attachment and proliferation but also influence cellular behavior through their physicochemical and mechanical properties. Among these characteristics, mechanical strength plays a crucial role, particularly in applications where the scaffold must withstand physiological stresses and maintain its integrity during tissue regeneration. Natural polymers have been extensively utilized in scaffold fabrication due to their inherent biocompatibility, biodegradability, and similarity to biological macromolecules. Chitosan, a linear polysaccharide derived from the deacetylation of chitin, has attracted considerable attention because of its favorable biological properties,

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including biocompatibility, bioadhesion, and antimicrobial activity. Additionally, its cationic nature allows for electrostatic interactions with negatively charged molecules, making it a versatile material for biomedical applications. Alginate, an anionic polysaccharide extracted from brown seaweed, is another widely used biomaterial known for its mild gelation process, hydrophilicity, and ability to form hydrogels in the presence of divalent cations such as calcium ions [6–8]. The combination of chitosan and alginate results in the formation of polyelectrolyte complexes through ionic interactions between oppositely charged functional groups. These complexes can enhance the structural stability and handling properties of the resulting scaffold. However, despite these advantages, chitosan–alginate scaffolds often exhibit insufficient mechanical strength and poor load-bearing capacity, which limit their applicability in certain tissue engineering fields, particularly those involving mechanically demanding environments such as bone and cartilage regeneration. To address these limitations, various strategies have been explored, including the incorporation of reinforcing agents and bioactive components [9]. Among these, collagen has received significant attention due to its abundance in the ECM and its critical role in providing mechanical strength and structural organization in native tissues. Collagen is a fibrous protein that contributes to tissue elasticity, tensile strength, and cell signaling. Its incorporation into polymeric scaffolds has been shown to enhance not only biological performance but also mechanical properties through the formation of additional intermolecular interactions and improved network architecture. The presence of collagen within a chitosan–alginate matrix can potentially modify the scaffold's mechanical behavior by influencing factors such as stiffness, compressive strength, and elasticity. These changes are particularly important because the mechanical properties of a scaffold can directly affect cell differentiation, tissue formation, and overall regeneration outcomes [10–14]. Therefore, optimizing the composition of composite scaffolds is essential for achieving a balance between mechanical integrity and biological functionality. Although previous studies have investigated composite scaffolds containing chitosan, alginate, and collagen, many of them have primarily focused on biological or morphological characteristics, often relying on techniques such as Scanning Electron Microscopy (SEM) or chemical analyses. In contrast, fewer studies have provided a detailed and focused evaluation of the mechanical properties of these systems, especially in relation to varying collagen content. A systematic investigation of how collagen concentration influences mechanical performance can provide valuable insights for the rational design of scaffolds tailored to specific tissue engineering applications. Therefore, the aim of the present study is to investigate the effect of collagen incorporation on the mechanical properties of chitosan–alginate scaffolds. By evaluating parameters such as compressive strength, elastic modulus, and deformation behavior, this study seeks to determine the extent to which collagen can enhance the mechanical performance of the scaffold. The results of this work are expected to contribute to the development of optimized biomaterial scaffolds with improved mechanical characteristics suitable for use in tissue engineering.

2 | Materials and Methods

2.1 | Materials

Chitosan (medium molecular weight, degree of deacetylation ~75–85%) was used as the primary cationic polymer in this study. Sodium alginate, a naturally occurring anionic polysaccharide, was employed to form polyelectrolyte complexes with chitosan. Type I collagen, derived from a biological source, was utilized as a reinforcing biopolymer to enhance the mechanical performance of the scaffolds. Calcium chloride ($CaCl_2$) was used as a crosslinking agent for alginate. All chemicals and reagents were of analytical grade and used as received without further purification. Deionized water was used throughout all experiments.

2.2 | Preparation of Chitosan–Alginate–Collagen Scaffolds

Chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under constant stirring at room temperature until a homogeneous solution was obtained. Separately, sodium alginate was dissolved in deionized water under magnetic stirring to form a uniform solution. The alginate solution was gradually added to the chitosan solution under continuous stirring to form a chitosan–alginate mixture through electrostatic interactions. Subsequently, collagen was incorporated into the mixture at different weight percentages (e.g.,

0%, 10%, and 20% relative to the total polymer content) to investigate its effect on mechanical properties. The mixtures were stirred thoroughly to ensure uniform distribution of collagen within the polymer matrix. The resulting solutions were cast into molds of defined dimensions and subjected to a freezing process at −20 °C for 24 hours, followed by freeze-drying (lyophilization) to obtain porous scaffolds. To further stabilize the structure, the scaffolds were immersed in an aqueous calcium chloride ($CaCl_2$) solution for ionic crosslinking, then washed with deionized water to remove excess ions and finally dried at room temperature [15].

2.3 | Experimental Groups

The scaffolds were divided into different groups based on collagen content:

I. CA: chitosan–alginate scaffold without collagen (control).

II. CAC-10: chitosan–alginate scaffold containing 10% collagen.

III. CAC-20: chitosan–alginate scaffold containing 20% collagen.

All experiments were performed in triplicate to ensure reproducibility of the results.

2.4 | Mechanical Testing

The mechanical properties of the fabricated scaffolds were evaluated using a universal testing machine. Prior to testing, all samples were cut into uniform cubic shapes with approximate dimensions of 10 × 10 × 10 mm. Compression tests were performed at room temperature with a constant crosshead speed of 1 mm/min. The compressive strength and stress–strain behavior of the scaffolds were recorded. The compressive modulus (elastic modulus) was determined from the slope of the initial linear region of the stress–strain curve. The maximum compressive stress at failure and the corresponding strain were also calculated. At least three samples from each group were tested, and the average values were reported [16–18].

2.5 | Cyclic Compression Test

Cyclic compression tests were performed to evaluate the elastic recovery and fatigue resistance of the scaffolds. Samples were subjected to repeated compression cycles up to a predefined strain (e.g., 30–50%) and then unloaded. The loading–unloading curves were recorded to assess hysteresis behavior and energy dissipation. The ability of the scaffold to recover its original shape after repeated loading was also evaluated [19].

2.6 | Tensile Testing

The tensile properties of the scaffolds were evaluated using a universal testing machine. Samples were prepared in rectangular shapes with uniform dimensions. The tests were performed at a constant crosshead speed of 1 mm/min until failure. Tensile strength, elongation at break, and Young's modulus were determined from the stress–strain curves. All measurements were conducted in triplicate [17].

2.7 | Degradation-Related Mechanical Stability

To evaluate the stability of mechanical properties over time, scaffolds were immersed in Phosphate-Buffered Saline (PBS) for different time intervals (e.g., 1, 3, and 7 days). After each interval, samples were removed and subjected to compression testing. The changes in compressive strength and modulus were analyzed to assess the effect of degradation on mechanical performance [20].

2.8 | Swelling Behavior

The swelling properties of the scaffolds were evaluated to assess their water absorption capacity, which can influence mechanical performance. Dry scaffold samples were weighed (W_0) and then immersed in PBS at room temperature. At predetermined time intervals, the samples were removed, gently blotted to remove

excess surface liquid, and weighed again (W_t) [16]. The swelling ratio was calculated using the following equation:

Swelling Ratio (%) = $((W_t - W_0) / W_0) \times 100$

All measurements were conducted in triplicate.

2.9 | Statistical Analysis

All data were expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test to determine significant differences between groups. A value of $p < 0.05$ was considered statistically significant.

3 | Results and Discussion

3.1 | Compressive Properties

The compressive properties of the scaffolds were evaluated to determine the effect of collagen incorporation on mechanical strength and stiffness. The stress–strain curves revealed a typical porous scaffold behavior, consisting of an initial linear elastic region followed by a plateau region associated with pore collapse. As shown in *Fig. 1*, the compressive strength of the scaffolds increased with the incorporation of collagen up to a certain concentration. The CA (control) scaffold exhibited the lowest compressive strength, which can be attributed to its relatively weak polymeric network. The addition of 10% collagen (CAC-10) significantly improved the compressive strength, suggesting enhanced intermolecular interactions and better load distribution within the scaffold matrix. However, further increasing the collagen content to 20% (CAC-20) did not result in a proportional improvement and, in some cases, led to a slight reduction in strength. This behavior may be due to the excessive presence of collagen disrupting the uniformity of the chitosan–alginate network, leading to structural heterogeneity. A similar trend was observed for the compressive modulus, indicating that an optimal collagen concentration exists for achieving maximum mechanical performance. These findings are consistent with previous studies reporting that moderate collagen incorporation enhances mechanical properties, while excessive amounts may weaken the structural integrity.

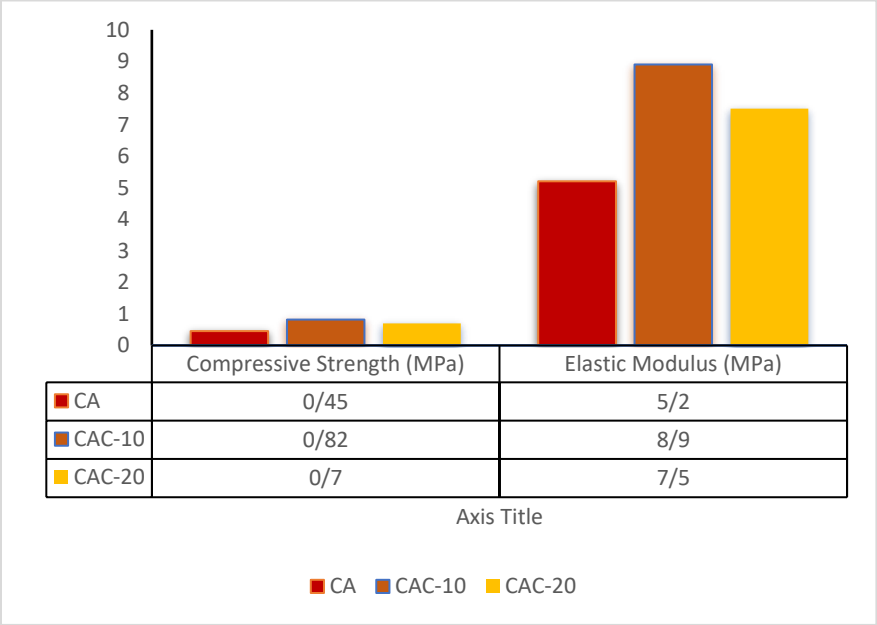


Fig. 1. Compressive properties of scaffolds.

3.2|Tensile Properties

The tensile properties of the scaffolds were investigated to evaluate their stretching forces. As presented in *Table 1*, the incorporation of collagen significantly influenced tensile strength and elongation at break. The CA scaffold showed relatively low tensile strength and limited flexibility, which is typical for hydrogel-based materials. With the addition of collagen, the tensile strength increased noticeably in the CAC-10 group, indicating improved structural cohesion and load transfer between polymer chains. Moreover, elongation at break increased with collagen content, suggesting enhanced flexibility due to the presence of collagen fibers, which can act as reinforcing elements within the matrix. However, similar to compressive behavior, excessive collagen (CAC-20) led to a slight decrease in tensile strength, possibly due to phase separation or poor dispersion within the matrix. These results demonstrate that collagen not only improves strength but also contributes to ductility, which is essential for soft tissue engineering applications.

Table 1. Tensile properties of scaffolds.

Sample	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)
CA	0.25 ± 0.03	12 ±0.02	3.5 ± 0.04
CAC-10	0.48 ± 0.05	20 ± 0.03	5.8 ± 0.06
CAC-20	0.40 ± 0.04	24 ± 0.08	5.0 ± 0.05

3.3|Cyclic Compression Behavior

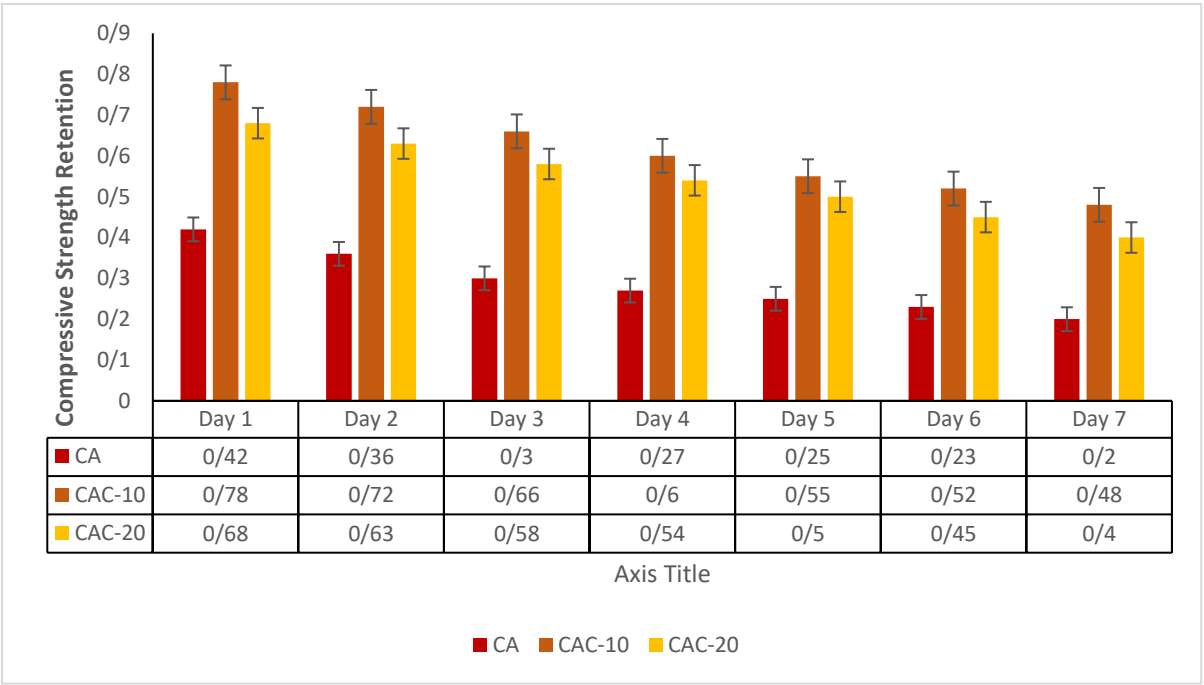
Cyclic compression tests were conducted to assess the elastic recovery and durability of the scaffolds under repeated loading conditions. The loading–unloading curves demonstrated hysteresis behavior, indicating energy dissipation during each cycle. The CA scaffold exhibited poor recovery after repeated cycles, with noticeable permanent deformation. In contrast, collagen-containing scaffolds showed improved elastic recovery, particularly in the CAC-10 group. This improvement can be attributed to the reinforcing effect of collagen, which enhances the structural resilience of the scaffold. The CAC-20 group also showed good recovery; however, slight structural instability was observed after multiple cycles, likely due to network heterogeneity (*Table 2*).

Table 2. Cyclic compression results.

Sample	Recovery Ratio (%)	Energy Loss (%)
CA	65 ± 0.05	35 ± 0.10
CAC-10	85 ± 0.04	20 ± 0.03
CAC-20	80 ± 0.02	25 ± 0.03

3.4 | Degradation-Related Mechanical Stability

The mechanical stability of the scaffolds during degradation was evaluated by measuring the compressive strength over a period of 7 days in PBS. The results demonstrated a progressive decrease in mechanical strength for all scaffold groups as a function of immersion time, which can be attributed to water penetration, polymer chain relaxation, and gradual structural disintegration. As presented in *Fig. 2*, the CA scaffold exhibited the fastest decline in compressive strength, indicating its relatively weak structural stability in aqueous conditions. A significant reduction was observed even within the first three days, followed by a continuous decrease until day 7. In contrast, collagen-containing scaffolds (CAC-10 and CAC-20) showed improved resistance to mechanical degradation. The CAC-10 group maintained higher compressive strength throughout the 7 days, suggesting that an optimal amount of collagen enhances intermolecular interactions and stabilizes the polymer network against hydrolytic degradation. Although the CAC-20 scaffold also demonstrated better stability than the control group, its mechanical performance was slightly lower than that of CAC-10 at later time points. This may be due to excessive collagen content leading to structural inhomogeneity and increased water uptake, which accelerates mechanical weakening over time. Overall, the results indicate that collagen incorporation improves the degradation-related mechanical stability of chitosan–alginate scaffolds, with 10% collagen showing the most balanced performance between initial strength and long-term durability.



	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
CA	0/42	0/36	0/3	0/27	0/25	0/23	0/2
CAC-10	0/78	0/72	0/66	0/6	0/55	0/52	0/48
CAC-20	0/68	0/63	0/58	0/54	0/5	0/45	0/4

Fig. 2. Compressive strength during 7 days of degradation (MPa).

3.5 | Swelling Behavior

The swelling behavior of the scaffolds was analyzed to understand their water absorption capacity and its relationship with mechanical properties. As shown in *Table 3*, all scaffolds exhibited significant swelling due to their hydrophilic nature. The incorporation of collagen increased the swelling ratio, which can be attributed to its hydrophilic functional groups. The CAC-20 scaffold showed the highest swelling ratio, indicating increased water uptake capacity. However, excessive swelling may negatively affect mechanical stability by weakening the polymer network. Therefore, an optimal balance between swelling and mechanical strength is essential for scaffold design.

Table 3. Swelling ratio (%).

Sample	Swelling Ratio (%)
CA	180 ± 0.01
CAC-10	230 ± 0.02
CAC-20	260 ± 0.05

4 | Conclusion

This study demonstrated the significant impact of collagen incorporation on the mechanical performance and stability of chitosan–alginate scaffolds. Comprehensive mechanical testing, including compression, tension, cyclic loading, and degradation-related assessment over 7 days in PBS, revealed that the addition of type I collagen markedly enhanced scaffold strength, elasticity, and durability. Among the tested formulations, the CAC-10 scaffold exhibited the most favorable combination of mechanical properties, maintaining higher compressive and tensile strength, improved elastic modulus, and superior recovery ratio under cyclic compression compared to both the control (CA) and the higher collagen content scaffold (CAC-20). The observed improvement in mechanical behavior with moderate collagen content can be attributed to enhanced intermolecular interactions, better load transfer within the polymer matrix, and increased network cohesion. In contrast, excessive collagen (20%) slightly reduced mechanical performance at later time points, likely due to structural heterogeneity and higher water uptake, which may compromise network integrity and accelerate mechanical weakening. Swelling studies further indicated that while collagen increases hydrophilicity and water absorption, an optimal balance is required to prevent adverse effects on scaffold strength. These findings underscore the importance of optimizing collagen content in chitosan–alginate scaffolds to achieve a delicate balance between strength, elasticity, and degradation stability. The CAC-10 scaffold, with its superior mechanical resilience and stability over time, presents a promising candidate for soft tissue engineering applications where sustained structural integrity and mechanical performance are essential.

Authors' Contributions

The author was responsible for all stages of the research and manuscript preparation and approved the final version.

Data Availability

All data are included in the text.

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Conflict of Interest

There are no competing interests to declare.

Consent for Publication

The author confirms consent for the publication of this work.

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants performed by the author.

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