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Bioactive collagen and spirulina hydrogels: structural and functional characterization for biomedical applications

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ABSTRACT

Polyurethane-crosslinked collagen hydrogels incorporating different concentrations of spirulina were developed to evaluate the effect of microalgal biomass on the structural, physicochemical, and biological properties of the system. The incorporation of spirulina modified the gelation kinetics and promoted an increase in the hydration capacity of the hydrogels, reaching swelling percentages greater than 5000% under certain pH conditions. Likewise, formulations with higher spirulina contents showed an increase in the degree of cross-linking and less deformation during mechanical fatigue tests, indicating an improvement in the structural stability of the matrix. FTIR and SEM analyses revealed changes primarily associated with the physical organization of the system, without substantial modifications to its chemical composition. The incorporation of spirulina also allowed for the modulation of the hydrolytic and enzymatic degradation of the hydrogels, demonstrating that the material's response depends on the interaction between hydration, network organization, and environmental conditions. From a biological standpoint, formulations with higher concentrations of spirulina exhibited reduced hemolytic activity, greater stability in simulated body fluid, and a superior ability to promote the formation of mineralized deposits. The results demonstrate that spirulina acts as a bioactive component capable of modifying multiple properties of the hydrogel, highlighting the potential of these materials as multifunctional platforms for applications in tissue engineering and regenerative medicine.

Keywords: Collagen Hydrogels; Spirulina; Bioactive Polyurethane; Swelling Behavior; Mechanical Stability; Hydrolytic Degradation; Enzymatic Degradation; Cross-linking; Hemocompatibility; Biomineralization; Bioactivity; Tissue Engineering.

1.0. Introduction

Hydrogels are one of the most versatile platforms in the field of tissue engineering and the controlled release of biomolecules. They are highly hydrated three-dimensional networks formed by cross-linked hydrophilic polymers, capable of creating a microenvironment like that of the extracellular matrix. This characteristic makes them ideal for promoting processes such as cell proliferation and differentiation, in addition to allowing for the controlled release of bioactive molecules [1–3]. Furthermore, their biocompatibility and biodegradability facilitate their integration into tissues and help reduce the inflammatory response.

Polymers of natural or synthetic origin can be used for the synthesis of hydrogels. Among these, collagen stands out as the most abundant protein of animal origin, and for its fundamental role in the extracellular matrix [2]. Collagen-based hydrogels have been extensively studied due to their ability to promote processes such as cell migration, proliferation, and signaling, as well as their role in tissue regeneration and their low immunogenicity. A positive effect has been reported on wound healing, skin aging, and osteoarticular pathologies [4,5]. However, collagen-based hydrogels have limitations, primarily related to their low mechanical stability and rapid degradation, which restricts their application in certain biomedical settings [2,5].

On the other hand, spirulina, a filamentous cyanobacterium, is a rich source of proteins, essential nutrients, phenolic compounds, and various bioactive metabolites. Its antioxidant and anti-inflammatory activity, primarily associated with compounds such as phycocyanin, has generated growing interest in biomedical applications [6–8]. These properties give it the ability to modulate cellular processes, reduce oxidative stress, and promote tissue regeneration

[7,9]. In this regard, its incorporation into polymeric matrices represents a promising strategy for providing biomaterials with additional functionalities beyond their structural role.

The combination of collagen and spirulina in hybrid systems could offer an approach for creating hydrogels with enhanced properties, where collagen provides the structural and biological foundation necessary for cellular interaction, while spirulina contributes its bioactive potential [10,11]. However, despite the interest in both components, there is limited information on their integration into hydrogel matrices and on the effect this combination may have on the physicochemical, structural, and biological properties of the system. A schematic representation of the collagen–spirulina hydrogel platform evaluated in this study is presented in Figure 1.

In this context, the objective of this study is to synthesize and characterize collagen hydrogels enriched with spirulina, evaluating their physicochemical, mechanical, and biological properties. The hypothesis is that incorporating spirulina into tyrosine-crosslinked collagen hydrogels will result in a three-dimensional matrix with improved properties, including greater structural stability, more controlled swelling behavior, and a favorable biological response. To this end, hydrogels with different proportions of spirulina were developed and analyzed for their morphology, swelling behavior, stability against degradation, and potential bioactivity.

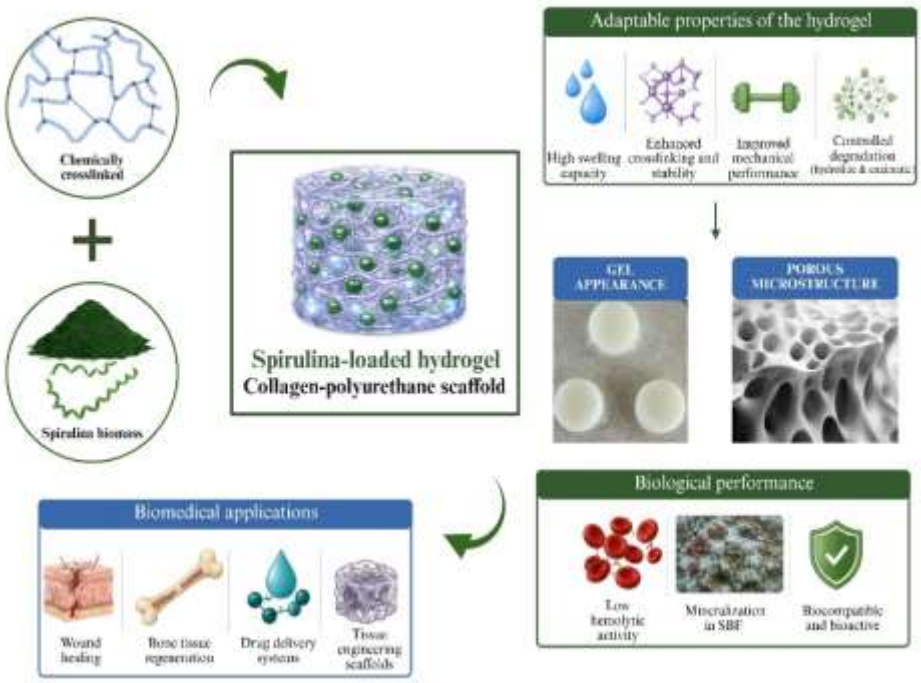

Figure 1. Spirulina biomass was incorporated into polyurethane-crosslinked collagen hydrogels to modulate the structural and functional properties of the matrix, thereby facilitating the development of multifunctional biomaterials for biomedical applications.

1.1. Study Objectives

The main objectives of this study were:

1) Synthesize tyrosine-crosslinked collagen–spirulina hydrogels, varying the spirulina concentration to obtain stable matrices.

2) Characterize the physicochemical properties of the hydrogels, including gelation behavior, degree of crosslinking, swelling capacity, mechanical properties, and structural analysis via Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).
3) Evaluate the stability of the hydrogels through studies of hydrolytic and enzymatic degradation.
4) Analyze the effect of incorporating spirulina on the structural and functional properties of the hydrogel.
5) Evaluate the biological properties of the material, including its hemocompatibility via hemolysis testing and its potential bioactivity.
6) Compare the different formulations to identify trends in the physicochemical and biological behavior of the developed hydrogels.

2.0. Materials and Methods

2.1. Materials

Type I collagen was extracted from porcine dermis using a protocol previously optimized in the laboratory [12]. The spirulina used was commercial-grade powdered biomass (Nature's Heart), purchased from a local store in Saltillo, Mexico, and used without further purification. Hexamethylene diisocyanate (HDI), glycerol ethoxylates (GE, Mw ~1000 $g \cdot mol^{-1}$), and L-tyrosine were used as precursors for the synthesis of the cross-linking agent [13]. The reagents used for physicochemical characterization, including ninhydrin, sodium hydroxide, and analytical-grade salts, were obtained from commercial suppliers. For degradation and biological evaluation studies, enzymes such as pepsin, as well as calcium salts and other necessary reagents, were used; all were of analytical grade and used without further purification.

2.2. Hydrogel Preparation

The synthesis of collagen–spirulina hydrogels was carried out under controlled conditions. Initially, the collagen solution (6 mg/mL) was dispensed into 24-well plates and kept at 4 °C to prevent premature gelation. Subsequently, spirulina was incorporated at different concentrations (17.89, 35.78, and 71.57 µg/mL), ensuring uniform dispersion within the matrix through gentle stirring. The formulations were identified as CS-200, CS-400, and CS-800, according to their spirulina content, while the system without spirulina was considered the control (CS-0). The composition of all hydrogel formulations is summarized in Table 1. Once the spirulina was incorporated, the cross-linking agent was added in a fixed proportion relative to the collagen content (30% w/w) [14,15]. The mixtures were homogenized and subsequently incubated at 37 °C for 24 h to allow hydrogel formation.

Table 1. Composition of polyurethane-crosslinked collagen hydrogels containing different spirulina concentrations used throughout the study.

Formulation	Collagen (mg/mL)	Polyurethane crosslinker (mg/mL)	Estimated spirulina content (%)
Control	6.0	1.8	0.00
CS-200	6.0	1.8	17.89
CS-400	6.0	1.8	35.78
CS-800	6.0	1.8	71.57

Finally, the resulting hydrogels were stored for subsequent characterization.

2.3. Physicochemical Characterization

The physicochemical and structural properties of collagen-spirulina hydrogels were evaluated using various characterization techniques. Fourier-transform infrared spectroscopy (FTIR) was used to identify present functional groups and analyze possible interactions within the polymer network. Scanning electron microscopy (SEM) was employed to examine the morphology of the hydrogels, using previously dried samples. In addition, the mechanical behavior of the hydrogels was evaluated through fatigue testing under dynamic conditions, with continuous agitation at 300 rpm for 72 h [16]. Finally, the structural integrity of the systems was analyzed to determine their response to repetitive stress.

2.4. Evaluation of the formation, stability, and degradation of hydrogels

The rate and degree of gelation of collagen-spirulina hydrogels were determined by measuring changes in turbidity over time, with absorbance recorded at a wavelength of 410 nm, over a 2-hour period. The percentage of water absorption was determined gravimetrically with the hydrogel in both the dry and hydrated states and corresponds to the material's swelling capacity. The swelling capacity of the hydrogels was determined at 37 °Cover 3 days in media with pH 4.0, 7.0, and 8.0, to evaluate the behavior of the matrices under conditions associated with different physiological and pathological environments. The degree of crosslinking of the hydrogels was evaluated using a ninhydrin assay, which allows for the quantification of free amine groups in the matrices. The decrease in these groups compared to non-crosslinked collagen was associated with an increase in the crosslinking density of the hydrogel network [14–17].

The stability of the hydrogels was determined through hydrolytic and enzymatic degradation assays. For hydrolytic degradation, the matrices were incubated in buffered solutions at pH 4.0, 7.0 and 8.0, allowing their behavior to be evaluated under conditions representative of various physiological and pathological microenvironments. Enzymatic degradation was performed in proteolytic media containing pepsin (14 U per hydrogel) at pH 4.0 and 7.0, simulating acidic and physiological conditions, respectively. The change in mass of the samples was monitored during the evaluation period to determine degradation kinetics and the stability of the hydrogel network [14–17].

2.5. Hemocompatibility and Bioactivity Assessment

The hemocompatibility of the hydrogels was evaluated using hemolysis assays in accordance with ASTM F756-08. For this purpose, the samples were incubated with human erythrocytes at 37 °C under controlled conditions. Subsequently, the suspensions were centrifuged, and hemoglobin release was determined spectrophotometrically based on the absorbance of the supernatant. The percentage of hemolysis was calculated using positive and negative controls as a reference, allowing the compatibility of the matrices with blood to be determined [18].

The bioactivity of the hydrogels was evaluated through in vitro mineralization assays using a body fluid simulant (BFS) solution, prepared to mimic the ionic composition of human plasma. The pre-weighed samples were immersed in BFS and incubated at 37 °C for one week under controlled conditions. At the end of the incubation period, changes in mass were determined gravimetrically to assess the deposition or loss of mineral material.

Additionally, surface mineralization was analyzed using alizarin red staining, allowing for the identification of calcium-rich deposits [18,19]. Finally, the surface of the hydrogels was examined under a microscope to assess the presence of mineral structures associated with mineralization processes.

2.6. Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation. Statistical differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A significance level of $p < 0.05$ was considered statistically significant.

3.0. Results and Discussions

3.1. Network Formation, Swelling Behavior, and Mechanical Stability

The gelation profiles of the collagen-spirulina hydrogels are shown in Figure 2a. In general, all formulations exhibited a characteristic sigmoidal behavior associated with the nucleation, growth, and stabilization stages of the polymer network. However, differences were observed in the gelation rate and the maximum absorbance reached as a function of the incorporated spirulina content.

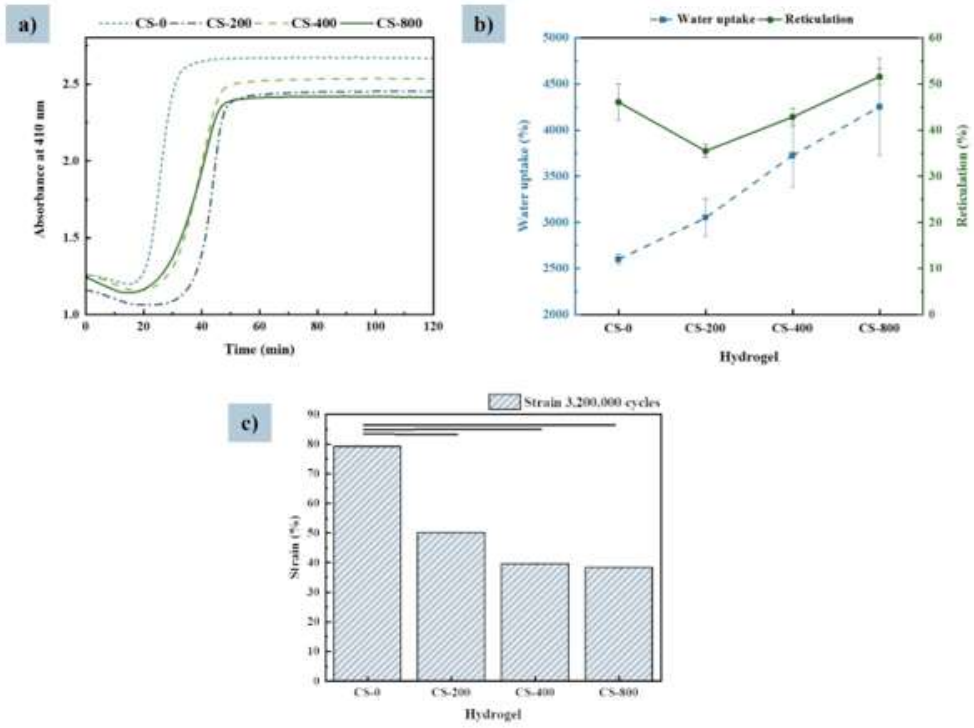

Figure 2. Effect of spirulina incorporation on the physicochemical and mechanical properties of collagen hydrogels. (a) Gelation profiles showing nucleation and network formation times. (b) Swelling ratio and crosslinking degree. (c) Mechanical deformation after 3,200,000 fatigue cycles, illustrating the influence of spirulina on structural stability.

The control formulation (CS-0) exhibited the earliest onset of the sol-gel transition, as evidenced by a sharp increase in absorbance around 20 minutes and subsequent stabilization of the network near 35 minutes. In contrast, the incorporation of spirulina caused the growth phase to shift to later time points. The CS-200, CS-400, and

CS-800 hydrogels exhibited longer gelation times, reaching the plateau between 45 and 55 minutes. Among the formulations containing spirulina, CS-200 showed the most pronounced delay in network formation, while CS-400 and CS-800 exhibited very similar gelation profiles.

This behavior suggests that the presence of spirulina alters the kinetics of collagen network assembly. Spirulina biomass contains proteins, polysaccharides, and other bioactive compounds capable of interacting with collagen fibrils during the self-assembly process, which could temporarily hinder the molecular reorganization necessary for the formation of a fully developed network [20,21]. As a result, a delay in the sol-gel transition and a decrease in final absorbance compared to the control are observed.

Despite these differences, all formulations achieved a stable structure in less than an hour, indicating that the incorporation of spirulina does not prevent hydrogel formation. From the perspective of biomedical applications, these results are significant, as they allow for the incorporation of high amounts of microalgal biomass without compromising network formation, while maintaining appropriate gelation times and exploring potential applications in tissue engineering and wound healing.

The swelling behavior and crosslinking percentage of the hydrogels are shown in Figure 2b. The incorporation of spirulina resulted in a progressive increase in the water absorption capacity of the matrices. The control hydrogel (CS-0) exhibited a swelling percentage of approximately 2600%, while the CS-200, CS-400, and CS-800 hydrogels reached values of approximately 3050%, 3700%, and 4250%, respectively. The differences became more evident as the spirulina content increased, with a directly proportional trend observed between the concentration of incorporated biomass and the system's hydration capacity.

The increase in water absorption could be related to the composition of spirulina, which contains proteins, polysaccharides, and other hydrophilic compounds capable of promoting interaction with water molecules. Additionally, the gelation results showed that the supplemented formulations exhibited slower network formation than the control, which could favor a less compact structural organization and facilitate the diffusion of the aqueous medium into the matrix [6,22,23].

Regarding the crosslinking percentage, the hydrogels exhibited values ranging from approximately 35% to 52%. The CS-200 formulation showed the lowest degree of crosslinking, while CS-0, CS-400, and CS-800 exhibited higher values, with CS-800 being the system with the highest percentage of crosslinks. These results suggest that low concentrations of spirulina may partially interfere with the interaction between collagen and the crosslinking agent, temporarily reducing the efficiency of the process. However, increasing the amount of biomass could generate additional interactions between the components present in spirulina and the polymer network, promoting the formation of a more stable structure.

The simultaneous increase in swelling and crosslinking percentage observed for CS-800 indicates that these two parameters are not inversely related in these systems. This behavior suggests that water absorption capacity does not depend solely on crosslinking density, but also on the hydrophilic nature of the incorporated components and the structural organization of the formed network. These results indicate that the incorporation of spirulina modifies

both the interaction with water and the internal architecture of the hydrogel, generating matrices with a higher hydration capacity without compromising the structural stability of the system.

The structural stability of the hydrogels under dynamic conditions was evaluated using a mechanical fatigue test, the results of which are shown in Figure 2c. Significant differences in the percentage of deformation were observed among the formulations evaluated. The control hydrogel (CS-0) exhibited the greatest deformation, with values close to 79%, while the incorporation of spirulina progressively reduced this parameter. The CS-200, CS-400, and CS-800 hydrogels exhibited deformations of approximately 50%, 40%, and 39%, respectively, demonstrating greater resistance to repetitive mechanical stress compared to the control.

This behaviour suggests that the incorporation of spirulina promotes the stability of the polymer network under conditions of continuous deformation. Although the supplemented hydrogels exhibited higher swelling percentages than the control formulation, the observed decrease in deformation indicates that the incorporated biomass may contribute to strengthening the internal structure of the system. This effect could be related to the presence of proteins and polysaccharides capable of establishing additional interactions with the collagen matrix, promoting a more efficient distribution of mechanical stresses within the network [6,22].

The results also show a trend consistent with the crosslinking values obtained previously. Formulations with higher percentages of spirulina exhibited both an increase in the degree of crosslinking and a reduction in deformation, suggesting that a more stabilized network may limit the dimensional changes induced by continuous agitation. CS-800 combined the highest crosslinking percentage with one of the lowest deformation values, indicating that the incorporation of high concentrations of spirulina does not compromise the structural integrity of the hydrogel.

These results demonstrate that spirulina not only modifies the hydration capacity of hydrogels but also helps improve their mechanical stability under dynamic conditions. This characteristic is particularly relevant for biomedical applications, where biomaterials must maintain their structure in the face of mechanical stimuli and constant movements within the physiological environment.

3.2. Molecular Interactions and Morphology

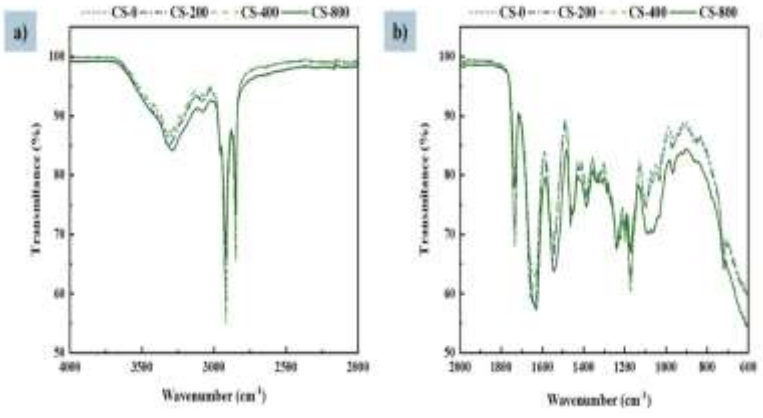

Figure 3. (a) FTIR spectra of the hydrogels showing the characteristic functional groups of the system. (b) Zoom-in of the fingerprint region showing changes associated with the incorporation of spirulina.

The FTIR spectra of the collagen-spirulina hydrogels are shown in Figure 3a. Overall, all formulations exhibited similar spectral profiles, indicating that the incorporation of spirulina did not significantly alter the overall chemical composition of the matrix. Characteristic bands associated with the stretching vibrations of hydroxyl and amino groups were observed between 3600 and 3200 cm^{-1}, as well as signals corresponding to aliphatic C–H groups around 2950–2850 cm^{-1} [24]. Likewise, the amide I and amide II bands were identified at approximately 1650 and 1540 cm^{-1}, respectively, which are characteristic of protein-based materials and confirm the presence of the collagen network [25]. Other signals observed within the fingerprint region were associated with C–O, C–O–C, and C–N vibrations originating from the different components of the system.

A more detailed analysis of the region between 2000 and 600 cm^{-1} (Figure 3b) revealed differences primarily related to the intensity of certain bands. The formulations supplemented with spirulina showed slight variations in the regions corresponding to amide I and amide II, suggesting changes in the intermolecular interactions within the matrix. These differences were most evident in formulations with higher spirulina concentrations, particularly in CS-800. Similarly, modifications were observed in the region between 1200 and 800 cm^{-1}, commonly associated with oxygen-containing groups and polysaccharide components [26,27]. This behaviour could be related to the incorporation of compounds specific to microalgal biomass into the polymer network and to changes in the local organization of the hydrogel components.

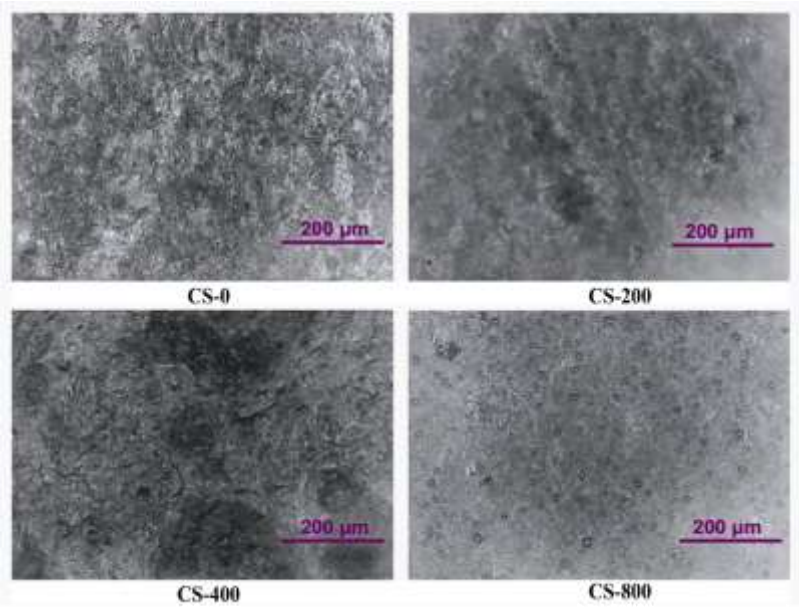

Figure 4. SEM micrographs of CS-0, CS-200, CS-400, and CS-800 hydrogels showing the influence of spirulina concentration on the surface morphology and physical organization of the polymeric network.

The SEM micrographs are shown in Figure 4. In general, all formulations exhibited continuous surfaces with no evidence of fractures or significant structural defects following the incorporation of spirulina. However, differences were observed in the surface organization of the hydrogels. The control hydrogel (CS-0) exhibited a relatively rough and heterogeneous surface, characteristic of collagen-based matrices. In contrast, the CS-200 formulation showed a more uniform and homogeneous appearance. Conversely, CS-400 exhibited regions with greater surface heterogeneity, evidenced by areas of varying contrast distributed throughout the matrix. Finally, CS-800 exhibited

small circular structures scattered across the surface, which could be associated with components of the incorporated microalgal biomass or with domains located within the polymer network.

Although the morphological changes observed were moderate, the results obtained by SEM are consistent with the changes detected by FTIR, suggesting that the incorporation of spirulina primarily influences the physical organization of the matrix without significantly altering its chemical composition. These structural variations could help explain the increase in water absorption capacity, the higher degree of cross-linking, and the improved resistance to deformation observed in the formulations with higher spirulina content.

3.3. pH-Responsive Swelling Behavior and Environmental Stability

The equilibrium swelling profiles of the hydrogels under different pH conditions are shown in Figure 5. In general, all formulations exhibited rapid water absorption during the first day of incubation, followed by a stabilization or network reorganization phase in the subsequent days. However, the extent and evolution of swelling depended on both the pH of the medium and the incorporated spirulina content.

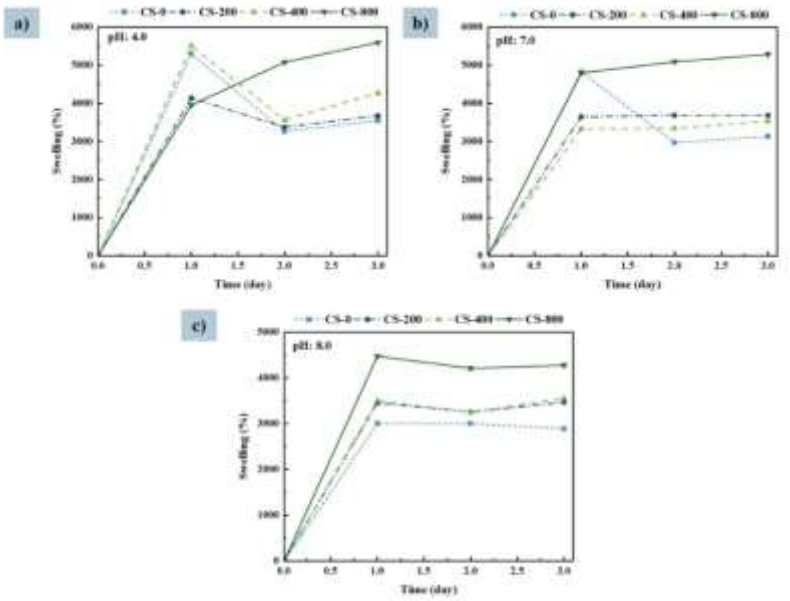

Figure 5. Swelling behavior of hydrogels under different pH conditions: (a) pH 4.5, (b) pH 7.4, and (c) pH 8.5, showing maximum and equilibrium swelling as a function of spirulina content.

Under acidic conditions (pH 4.0, Figure 5a), all hydrogels reached high swelling percentages during the first day. The CS-0, CS-200, and CS-400 formulations exhibited maximum values close to 5,200 %, 4,100 % and 5,500 %, respectively, while CS-800 reached approximately 3,900 %. After the first day, CS-0, CS-200, and CS-400 showed a decrease in their swelling capacity, followed by a partial stabilization of the network. In contrast, CS-800 exhibited different behavior, progressively increasing its water absorption until reaching values close to 5,500 % on the third day. This behavior could be related to a gradual reorganization of the matrix and the presence of hydrophilic components derived from spirulina biomass that promote water retention over prolonged periods.

At physiological pH (7.0, Figure 5b), a similar trend was observed, although with more pronounced differences between formulations. CS-800 again exhibited the highest swelling capacity, reaching approximately 4,800 % on

the first day and gradually increasing to values close to 5,300% by the end of the test. In contrast, CS-0 and CS-200 showed a decrease after the initial peak, stabilizing around 3,000–3,700%. The CS-400 formulation exhibited intermediate values and a relatively stable response over time. These results suggest that incorporating larger amounts of spirulina promotes interaction with the aqueous medium and helps maintain greater hydration of the matrix under physiological conditions [11,23].

Under alkaline conditions (pH 8.0, Figure 5c), all formulations reached maximum swelling on the first day and subsequently maintained relatively stable values. CS-800 again showed the highest water absorption capacity, with values close to 4,400–4,500 %, while the other formulations remained between approximately 2,900 % and 3,500 %. Although the overall behavior was like that observed at pH 7.0, the lower temporal variation suggests greater network stability under these conditions.

The results demonstrate that the incorporation of spirulina significantly modifies the hydrolytic response of the hydrogels and enhances their hydration capacity, particularly in the CS-800 formulation. This behavior is consistent with previously obtained results from cross-linking, mechanical fatigue, FTIR, and SEM analyses, in which formulations with higher spirulina content exhibited a more stable structural organization and greater resistance to deformation. From the perspective of biomedical applications, the ability to maintain high levels of hydration under different pH conditions is particularly relevant for systems intended for tissue regeneration and wound dressings, where moisture retention is a key factor in promoting tissue repair processes.

3.4. Degradation Behaviour and Environmental Stability

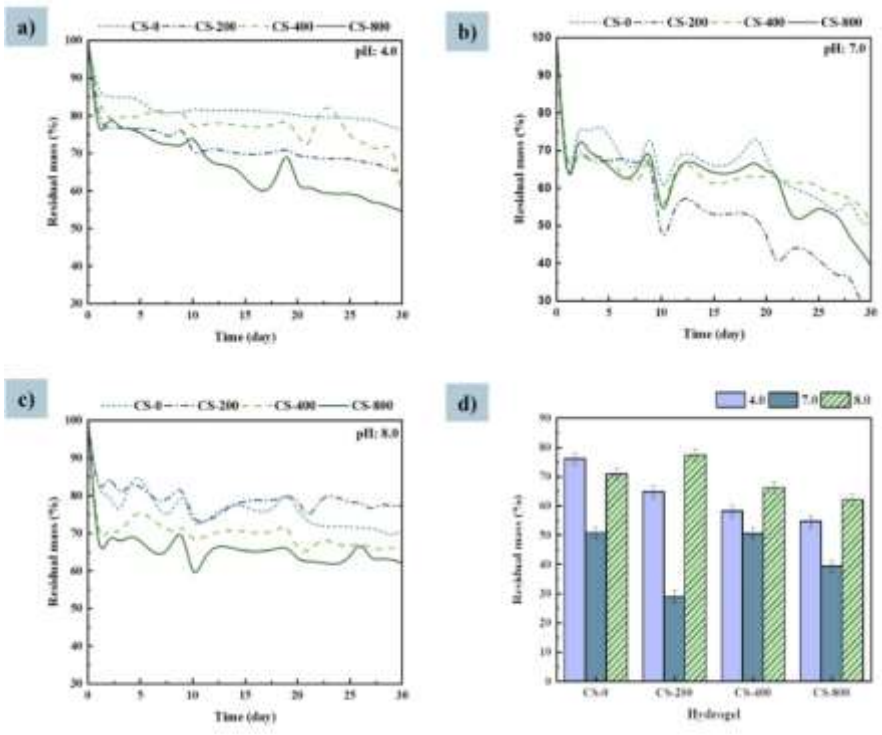

Figure 6. Hydrolytic degradation profiles of collagen–spirulina hydrogels under different pH conditions. (a) pH 4.0, (b) pH 7.0, and (c) pH 8.0 during 30 days of incubation. (d) Residual mass of the hydrogels after 30 days, showing the effect of spirulina content on hydrolytic stability.

The stability of the hydrogels under hydrolytic and enzymatic conditions was evaluated by monitoring residual mass over 30 days; the results are presented in Figures 6 and 7. This evaluation allows us to determine the materials' ability to maintain their structural integrity under various physiological conditions, a critical factor for biomedical applications that require controlled retention of the biomaterial at the implantation site.

Hydrolytic degradation showed a clear dependence on both pH and the formulation being evaluated (Figure 6). Under acidic conditions (pH 4.0, Figure 6a), the control formulation (CS-0) exhibited the highest residual mass percentages at the end of the test, while CS-800 showed the lowest values, indicating greater mass loss during the incubation period. At pH 7.0 (Figure 6b), a general decrease in stability was observed for all formulations, with CS-200 exhibiting the lowest residual mass at the end of the test. On the other hand, under alkaline conditions (pH 8.0, Figure 6c), the hydrogels exhibited greater overall stability, retaining higher residual mass percentages than those observed in the other media. Taken together, these results suggest that the incorporation of spirulina modifies the matrix's hydrolytic response, and that this response depends on both the incorporated concentration and the conditions of the surrounding medium.

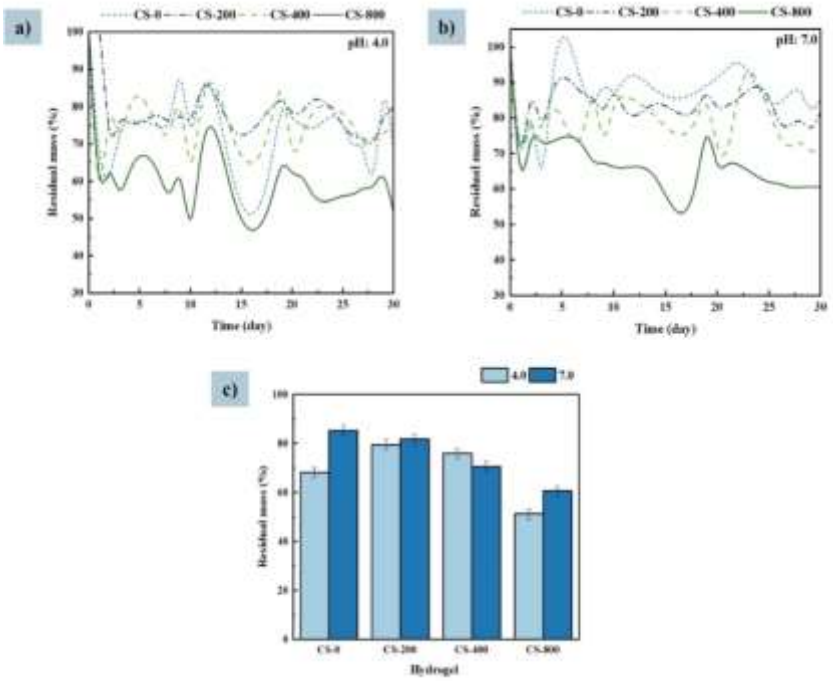

Figure 7. Enzymatic degradation profiles of collagen–spirulina hydrogels in the presence of pepsin. (a) pH 4.0 and (b) pH 7.0 during 30 days of incubation. (c) Residual mass of the hydrogels after 30 days, showing the effect of spirulina content on enzymatic stability.

Enzymatic degradation in the presence of pepsin (Figure 7) exhibited different behavior than that observed in the hydrolytic assays. Under both pH conditions evaluated, the CS-200 and CS-400 formulations exhibited the highest percentages of residual mass at the end of the assay, while CS-800 showed the lowest stability against enzymatic attack. These results indicate that susceptibility to degradation does not depend exclusively on the degree of matrix cross-linking, but also on the accessibility of the network to enzymatic molecules and the structural organization generated by the incorporation of spirulina. The lower residual mass observed in CS-800 could be related to its high

hydration capacity, which would favor the diffusion of the medium into the hydrogel and facilitate the enzyme's action on the components susceptible to degradation.

This behavior is consistent with the previously obtained swelling results, in which CS-800 exhibited the highest levels of water absorption under the various conditions evaluated. Although this formulation also showed higher crosslinking percentages and better resistance to deformation during fatigue testing, the high hydration of the matrix appears to promote greater interaction with the degradation environment. This suggests that the degradation behavior of hydrogels is not governed by a single structural parameter, but rather by a combination of factors such as hydration capacity, network organization, and the accessibility of internal components to the surrounding environment [28,29].

These results demonstrate that the incorporation of spirulina allows for the modulation of hydrogel stability under different degradation conditions. This adjustability is particularly attractive for biomedical applications, as it enables the design of materials with differentiated degradation rates according to the specific requirements of each application, while maintaining favorable hydration and mechanical stability properties.

3.5. Hemocompatibility and Bioactivity

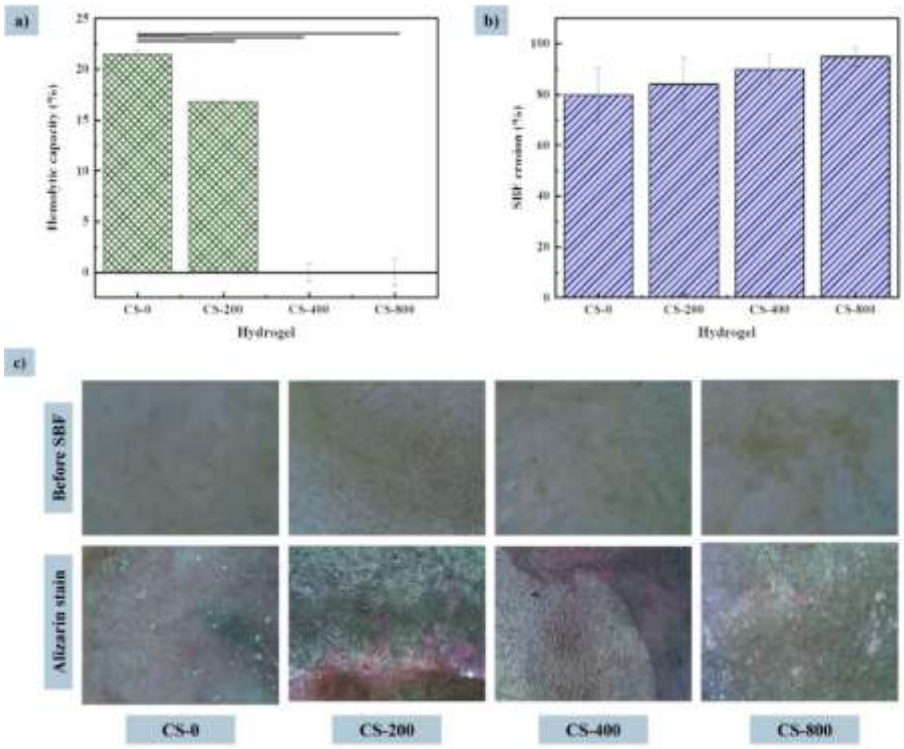

Figure 8. Biological evaluation of collagen–spirulina hydrogels. (a) Hemolytic activity demonstrating the effect of spirulina on hemocompatibility. (b) Surface erosion after 7 days of incubation in simulated body fluid (SBF). (c) Surface morphology before SBF exposure and Alizarin Red staining showing calcium-rich mineral deposition associated with biomineralization.

The hemocompatibility of the hydrogels was evaluated using a hemolysis assay, the results of which are presented in Figure 8a. The control formulation (CS-0) showed a hemolysis rate of approximately 21%, while the

incorporation of spirulina resulted in a progressive decrease in this parameter. The CS-200 formulation exhibited values close to 17%, while CS-400 and CS-800 showed practically zero percentages. This reduction in hemolytic activity suggests that the incorporation of spirulina improves the blood compatibility of the matrix, possibly due to modifications in the surface characteristics of the hydrogel and the formation of a more hydrated interface [30,31]. This behavior is consistent with the swelling and structural characterization results previously discussed, where formulations with higher spirulina content showed greater hydration capacity and changes in the organization of the polymer network.

The bioactivity of the hydrogels was evaluated by incubation in SBF, using surface erosion and mineral deposit formation as indicators of interaction with the physiological environment. After seven days of incubation (Figure 8b), all formulations retained high percentages of residual mass, with an increasing trend observed as the spirulina concentration increased. The values obtained were approximately 80%, 84%, 90%, and 95% for CS-0, CS-200, CS-400, and CS-800, respectively. These results suggest that the incorporation of spirulina promotes the stability of the hydrogels in the presence of SBF and could contribute to a more favorable interaction between the matrix and the ionic components present in the medium.

Microscopic observations conducted before and after incubation in SBF, along with alizarin red staining (Figure 8c), revealed changes on the surface of the hydrogels associated with mineralization processes. After exposure to SBF, surface deposits were observed distributed across the matrices, particularly in formulations with higher spirulina concentrations. This trend was corroborated by alizarin red staining, where CS-400 and CS-800 exhibited more intense staining compared to formulations with lower concentrations. The dye's affinity for calcium-rich deposits suggests greater formation of mineralized phases on these hydrogels. This behavior could be related to the high hydration capacity previously observed, which favors ion diffusion and the formation of mineralization nuclei within the matrix [32]. The results obtained demonstrate that the incorporation of spirulina not only improves the system's hemocompatibility but also promotes processes associated with the material's bioactivity, highlighting the potential of these formulations for applications in tissue engineering and biomedical regeneration.

4.0. Conclusions

This study demonstrates that the incorporation of spirulina into polyurethane-crosslinked collagen hydrogels significantly alters the structural, physicochemical, and biological properties of the system. The presence of microalgal biomass influenced the gelation kinetics, hydration capacity, mechanical stability, and degradation behavior of the hydrogels, demonstrating that the concentration of spirulina plays an important role in the organization and functionality of the polymer network. Formulations with higher spirulina contents exhibited greater swelling capacity, an increase in the degree of crosslinking, and less deformation during fatigue tests, indicating an improvement in the structural stability of the system. FTIR and SEM analyses showed that these changes were primarily associated with modifications in the physical organization of the matrix rather than with substantial chemical transformations.

Furthermore, the incorporation of spirulina allowed for the modulation of the hydrolytic and enzymatic degradation of the hydrogels, demonstrating that degradation behavior depends on the interaction between hydration capacity,

network accessibility, and the structural characteristics of the material. From a biological standpoint, formulations with higher concentrations of spirulina exhibited reduced hemolytic activity and increased mineralization capacity in the presence of SBF, suggesting an improvement in both the hemocompatibility and bioactivity of the system. These results demonstrate that spirulina acts as a bioactive component capable of modulating multiple properties of the hydrogel, highlighting the potential of these materials for biomedical applications related to tissue regeneration and the development of functional biomaterials.

4.1. Future perspectives

1. Despite the results obtained, it is necessary to continue the biological evaluation of these hydrogels to determine their potential more accurately for biomedical applications.
2. Evaluate the cytocompatibility, cell adhesion, and cell proliferation associated with collagen–spirulina hydrogels to further assess their biological performance.
3. Investigate the biological response of the developed hydrogels using more complex in vitro and in vivo models to validate the results obtained in this study.
4. Explore the mechanisms by which spirulina influences matrix organization and promotes mineralization processes within the hydrogel system.
5. Assess the incorporation and controlled release of therapeutic molecules and additional bioactive compounds to expand the functionality of the materials.
6. Determine the long-term stability and degradation behavior of the hydrogels under dynamic physiological conditions.
7. Conduct in vivo studies to establish the potential of these multifunctional hydrogels for tissue engineering and regenerative medicine applications.

Declarations

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Competing Interests Statement

The authors declare that they have no conflict of interest.

Consent for Publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Informed Consent

Not applicable.

Availability of Data and Material

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Institutional Review Board Statement

Not applicable.

Ethical Approval

Not applicable.

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Declaration of Artificial Intelligence

The authors used ChatGPT (OpenAI) as a language assistance tool exclusively for grammar correction, language refinement, and improvement of manuscript structure. The AI tool was not used for data analysis, interpretation of results, generation of scientific conclusions, or preparation of figures and experimental content. The authors reviewed, verified, and take full responsibility for the final content of the manuscript.

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