Synthesize of Sodium Alginate Hydrogel from Iranian Brown Seaweed as a Candidate for Biomedical Applications

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Abstract

Hydrogels, which are cross-linked networks of hydrophilic polymers, are considered promising candidates for various biomedical applications due to their unique physical and chemical properties. This property is especially beneficial in applications such as drug delivery, where ease of administration is crucial. Among these materials, alginate, a natural polysaccharide derived from brown seaweed, can form hydrogels through ionic cross-linking under mild conditions without the need for toxic reagents, making it a popular choice in biomedical fields. A primary source of alginates is brown seaweed, scientifically classified under the group Phaeophyceae. Alginates are polysaccharides that are abundantly found in the cell walls of these brown seaweed species. In this research, brown algae were systematically harvested from the Oman Sea in southern Iran. After collection, the alginate was meticulously extracted and purified process to ensure its quality and suitability for medical applications. A hydrogel primarily composed of purified sodium alginate was then developed. Various techniques were utilized to evaluate the properties of the resulting hydrogel, including detailed assessments of its physical and morphological characteristics through Fourier transform infrared spectroscopy, X-ray diffraction, gel fraction analysis, and rheological studies. To determine the safety and compatibility of the synthesized hydrogel for biomedical applications, a cytotoxicity test was conducted. The results of these studies indicate that the synthesized hydrogel holds considerable promise as a candidate for biomedical applications, particularly in targeted therapies, thereby opening avenues for future research in biomedicine and pharmaceutical innovation. As the field continues to evolve, the implications of this study underscore the importance of exploring natural biopolymers for sustainable biomedical applications.

Keywords: *Nizamuddinia Zarnardinii*, Biopolymer, Algin, Alginic acid, Hydrogel

Introduction

Hydrogels, which are cross-linked networks composed of hydrophilic polymers, are regarded as promising options for various biomedical applications, including drug delivery, tissue engineering, and wound healing (Ahmed, 2015). Their unique ability to retain substantial

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quantities of water, biomacromolecules, or suspensions is largely attributed to their low polymer content, which allows for high theoretical drug loadings. The modular nature of hydrogels in terms of chemistry, structure, and functionality further enhances their versatility, enabling researchers to develop innovative therapies tailored to specific medical needs. Furthermore, hydrogel particles exhibit shear-thinning behavior even at elevated volume fractions, contributing to their advantageous flow characteristics. This property is especially beneficial in applications such as drug delivery, where ease of administration is crucial. Overall, the multifunctional capabilities of hydrogels position them as vital tools in advancing modern medicine and improving patient outcomes (Mahmood et al., 2022).

Alginate is a natural polysaccharide that can effectively form a hydrogel through ionic cross-linking under mild conditions, eliminating the need for toxic reactants. This unique property makes alginate an ideal material for a variety of biomedical applications, including drug delivery systems, wound dressings, and tissue engineering scaffolds (Abbasinia et al., 2024; Lee and Mooney, 2012). Alginate has a proven track record of safe clinical use across numerous biomedical applications, highlighting its versatility and reliability. Structurally, alginate is a linear anionic polysaccharide composed of repetitive units of β-D-mannuronic acid (M-blocks) and α-L-glucuronic acid (G-blocks) in varying proportions. Its characteristics include a non-toxic nature, affordability, and excellent water absorption capacity, which enhance its utility in medical settings. Furthermore, alginate exhibits remarkable biocompatibility, intrinsic non-stick properties, and biodegradability, making it an environmentally friendly option for various applications (Raus et al., 2021).

A primary source of alginates is brown seaweed, scientifically classified under the group Phaeophyceae. Alginates are polysaccharides that are abundantly found in the cell walls of these brown seaweed species. These polysaccharides play a crucial role in providing a flexible mechanical structure that helps protect the seaweed from various environmental stresses, particularly damage caused by strong water movements and turbulent ocean currents. In marine ecosystems, brown seaweed serves as an essential habitat for numerous marine organisms, and the alginates found in their cell walls contribute to the overall health and resilience of these underwater environments. Moreover, the extraction and utilization of alginates from brown seaweed are of significant interest in sustainable practices, as they provide a renewable resource that can be harvested with minimal impact on marine life (Bojorges et al., 2023; Saji et al., 2022; Ye et al., 2024). *Nizamuddinia Zarnardinii* represents a significant species of brown algae found along the northern shores of the Oman Sea. This unique alga is distinguished by possessing the highest concentration of alginate, a natural polysaccharide, following *Sargassum* algae, making it particularly valuable for various industrial applications. The alginate extracted from this species is sought after for its use in

food, pharmaceuticals, and biotechnology due to its thickening and gelling properties. This particular alga is endemic to the region and has not been documented in any other location globally, existing solely along the northern coastline of the Oman Sea. Its distribution extends from Chabahar in Iran to Karachi in Pakistan, showcasing a narrow habitat range that highlights its ecological significance (Abkenar, 2021).

In this comprehensive research study, brown algae were meticulously collected from the Oman Sea, a unique marine ecosystem located in southern Iran. Following the collection, the alginate—a biopolymer derived from the algae—was carefully extracted and subjected to a thorough purification process to ensure high quality. Subsequently, a hydrogel composed primarily of the purified sodium alginate was fabricated. Various methods were employed to assess the characteristics of the resulting hydrogel, including rigorous evaluations of its physical and morphological attributes. These evaluations were conducted using advanced analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD), which provided detailed insights into the chemical properties of the hydrogel. Furthermore, gel fraction and rheological analyses were carried out to understand the hydrogel's mechanical performance and stability under different conditions. To assess the safety and compatibility of the synthesized hydrogel for biomedical applications, a cytotoxicity test was performed. The findings from these investigations suggest that the synthesized hydrogel exhibits significant potential as a promising candidate for biomedical applications, paving the way for future research in biomedicine and pharmaceutical development.

Materials and methods

Algae collection and identification

Nizamuddinia Zarnardinii were gathered from the Tang Bay, Chabahar Port, Oman Sea, located in the southern region of Iran (25°14'49" N and 59°48'02" E).

Sodium alginate extraction and purification

Brown algae are washed with fresh water to eliminate impurities and then sun-dried at ambient temperature to preserve their biochemical integrity. The dried algae were subsequently milled into a fine powder, passed through a 1 mm mesh sieve to ensure uniformity, and stored in sealed bags at room temperature to maintain their quality and prevent moisture absorption. The extraction of alginate was performed using previous reports (Calumpong et al., 1999; Khajouei et al., 2018; Yazdani et al., 2015), which is known for its efficiency and effectiveness. A quantity of 25 g of the dried algae was soaked in 800 mL of 2 % (v/v) formaldehyde at room temperature for 24 hours while stirring continuously, to eliminate phenolic compounds and pigments that could interfere with the extraction process. After this initial treatment, the insoluble residue was carefully washed three times with MilliQ water to remove any remaining formaldehyde and impurities. Following this, the residue was treated with 800 mL of 0.2 M HCl and incubated at 60 °C for three hours while stirring at 250 rpm.

The resulting suspension was centrifuged at

10,000 g for 20 minutes at 20 °C, leading to the formation of pellets, which contained the alginic acid. These pellets underwent thorough washing three times with MilliQ water to ensure purity. They were then soaked in a 3 % (w/v) Na_2CO_3 solution for 2.5 hours at 60 °C to facilitate the extraction of sodium alginate. After this soaking period, the mixture was centrifuged again at 10,000 g for 30 minutes at 20°C, separating the supernatant, which was then precipitated using three volumes of 96 % (v/v) ethanol to isolate the sodium alginate effectively. The resulting pellets containing sodium alginate were dissolved in MilliQ water and precipitated with ethanol as previously described, ensuring that all excess impurities were removed (Calumpong et al., 1999; Khajouei et al., 2018). This purification process was repeated twice to maximize yield and achieve high purity, after which the sodium alginate was collected and freeze-dried for long-term storage and future applications.

Hydrogel fabrication from sodium alginate

Initially, a 2 % w/v alginate solution was meticulously prepared by dissolving alginate in a 1% V/V acetic acid solution. This process

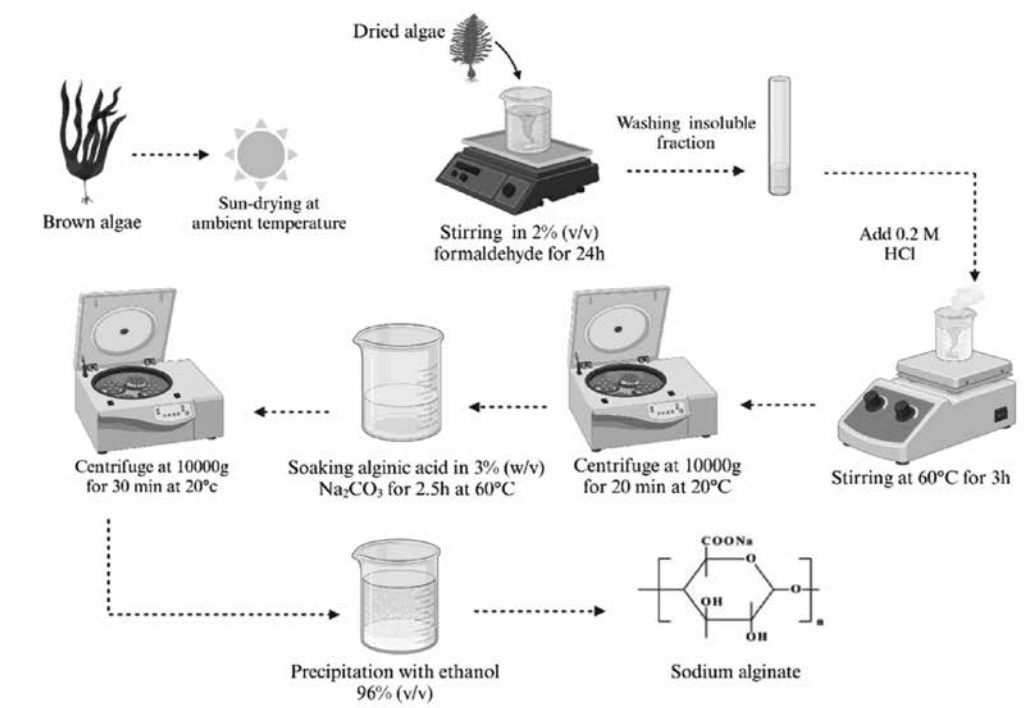

Fig. 1. Schematic extraction and purification of sodium alginates

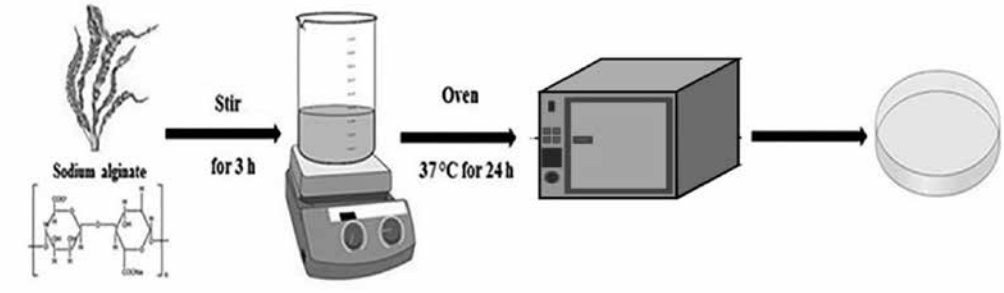

Fig. 2. Schematic hydrogel fabrication of sodium alginate

ensured the alginate was fully hydrated, allowing for optimal gel formation later in the experiment. Subsequently, to enhance the purity of the alginate solution, any impurities present were effectively eliminated through centrifugation. This step was conducted for 3 hours at a controlled temperature of 25 °C and a speed of 4000 rpm, ensuring that the heavier particles settled at the bottom while the clearer supernatant could be collected for further use. Following this purification step, the hydrogel was then dried in an oven set at 37 °C for a continuous duration of 24 hours (Monfared-Hajishirkiaee et al., 2023). This drying phase was crucial as it allowed for the removal of excess moisture, thereby facilitating further characterization and analysis of the hydrogel's properties. Figure 2 illustrate a schematic of the extraction and purification of alginates. The resulting dried hydrogel would be essential for various applications in biomedical fields, including drug delivery and tissue engineering.

Fourier transform infrared (FTRI)

FTIR (Tensor 27, Bruker, Germany) was employed to identify specific chemical groups within the synthesized hydrogel. This technique is particularly useful for characterizing materials, as it provides detailed information about the molecular structure and functional groups present in the sample. The FTIR spectrum was obtained within the range of 400–4000 cm^{-1} utilizing KBr pellets, commonly used in FTIR spectroscopy due to their transparency in the infrared range. Furthermore, it is crucial to maintain controlled environmental conditions during the measurement process to ensure the accuracy and reliability of the results. The resulting spectrum can reveal significant insights into the chemical composition of the hydrogel, aiding in understanding its properties and potential applications in various fields, such as biomedicine and materials science.

X-ray Diffraction (XDR)

XRD (Philips PW1730) was employed to thoroughly examine the crystal structure of fabricated sodium alginate. The analysis was conducted with a scanning speed of 3° per minute, allowing for detailed observations of the crystalline features. The scanning range extended from 0 to 80°, which provided a comprehensive insight into the diffraction patterns and helped identify the arrangement of atoms within the sodium alginate structure. The temperature during the analysis was maintained at a stable 25 °C, ensuring that any temperature-induced changes did not affect the results.

Gel fraction

A direct immersion method was employed to assess the gel fraction of the hydrogels, ensuring a comprehensive analysis of their properties. Initially, the hydrogels were carefully dried to a stable weight to eliminate excess moisture content, which could skew the results. After achieving this stable weight, the hydrogels were accurately weighed using a precision balance. They were then placed in individual beakers and immersed in distilled water to facilitate water absorption into the hydrogel structure. To maintain the integrity of the measurement process, the water in each beaker was replaced every four hours. This process was continued until the hydrogels achieved a stable weight, indicating that they had fully

absorbed water and reached equilibrium. Once this was verified, the hydrogels were weighed again after being subjected to a vacuum oven at 37 °C, where they were dried until they reached a consistent weight. This step is critical for ensuring that all excess water is removed before the final measurement. The formula used to calculate the gel fraction is as follows:

$$F\ (\%) = (W_1/W_o) \times 100\% \quad (1)$$

where F (%) indicates the gel fraction, W_o represents the initial weight of the hydrogels, and W_1 denotes the final weight of the double-layer hydrogel films. This meticulous procedure allows for accurately determining the gel fraction, which is essential for understanding the hydrogels' performance in various applications.

Rheological analysis

The rheological characteristics of the hydrogels were assessed at a controlled temperature of 37 °C, a standard physiological condition, to ensure that the results would be relevant for potential biomedical applications. This assessment was conducted using a sophisticated rotary rheometer equipped with a 25 mm diameter parallel plate, model MCR300 SN599139, featuring the firmware version FW2.04 and configured with Slot5 for optimal performance. Using a rotary rheometer allows for precise measurements of the viscosity and viscoelastic properties of the hydrogels, which are crucial for understanding their behavior under various shear conditions. This information is vital for applications in drug delivery, where the mechanical properties of hydrogels play a significant role in their functionality and effectiveness. By thoroughly analyzing these rheological characteristics, we can gain deeper insights into how these materials will perform in biomedical applications, ultimately leading to improved designs and applications.

*Cytotoxicity assessment*In this study, cytotoxicity was meticulously evaluated using ultraviolet-sterilized sponges that were carefully fragmented into small pieces to maximize their surface area for interaction with the cultured cells. These sponge fragments were subsequently dispersed in Dulbecco's Modified Eagle Medium (DMEM) to create a stable suspension, facilitating the assessment of their impact on cell viability. L929 mouse fibroblast cells, which are widely utilized in cytotoxicity assays due to their sensitivity and relevance, were seeded into 96-well plates at a density of 5×10^3 cells/mL, with 100 μL of cell suspension introduced into each well. Following a 24-hour incubation period, hydrogel suspensions were added to the wells, allowing the cells to adhere and proliferate. Specifically, 100 μL of hydrogel suspension at a concentration of 400 μg/mL was introduced alongside pure phosphate-buffered saline (PBS) as a control, ensuring that any observed effects on cell viability could be accurately attributed to the hydrogels. After an additional 24 hours of incubation, MTT reagent (10 μL) was added to each well, initiating the conversion of MTT to formazan crystals by metabolically active cells. The wells were incubated for approximately 4 hours to allow sufficient time for the reaction to occur. The resulting MTT solution was then carefully replaced with 200 μL of dimethyl

sulfoxide (DMSO), which served to dissolve the formazan crystals, and the mixture was thoroughly mixed for 30 minutes to ensure complete dissolution. The absorbance of the solution in each well was measured at 492 nm, utilizing a reference wavelength of 655 nm, using a microplate reader (Tecan Austria GmbH Untersbergstrasse 1, A-5082, Austria) to quantify the amount of formazan produced. To assess the cytotoxic effects of the hydrogel suspensions, cell viability was calculated using the standard formula:

Cell viability % = (Sample abs 492-655) / (Control abs 492-655) × 100 (2)

This method provided valuable insights into the biocompatibility of the tested hydrogels, crucial for their potential applications in biomedical fields.

Results and Discussion

FTIR analysis

The FTIR spectra of sodium alginate are illustrated in Figure 3. The shoulders observed near 922 cm^{-1} suggest the presence of a C-O-C band, which is closely associated with the saccharide structure of the hydrogel. This band indicates the involvement of glycosidic linkages that are fundamental to the molecular configuration of polysaccharides. Furthermore, the peaks at approximately 3277 cm^{-1}, 1604 cm^{-1}, and 1414 cm^{-1} correspond to the stretching vibrations of O-H and COO- functional groups. The peak at 3277 cm^{-1} highlights the presence of hydroxyl groups, which are crucial for the hydrophilicity of the material, allowing it to absorb water and swell, while the peaks at 1604 cm^{-1} and 1414 cm^{-1} are indicative of carboxylate ions that play a significant role in the ionic interactions and gel formation of the hydrogel system (Pereira et al., 2003; Thu and Ng, 2013; Karaki et al., 2013).

XRD analysis

The spectrum of sodium alginate, illustrated in Figure 4, exhibits a broad peak near 2θ=20°, which signifies an amorphous structure lacking distinct crystal planes or phases. This broad peak indicates the material's disordered arrangement at the atomic level,

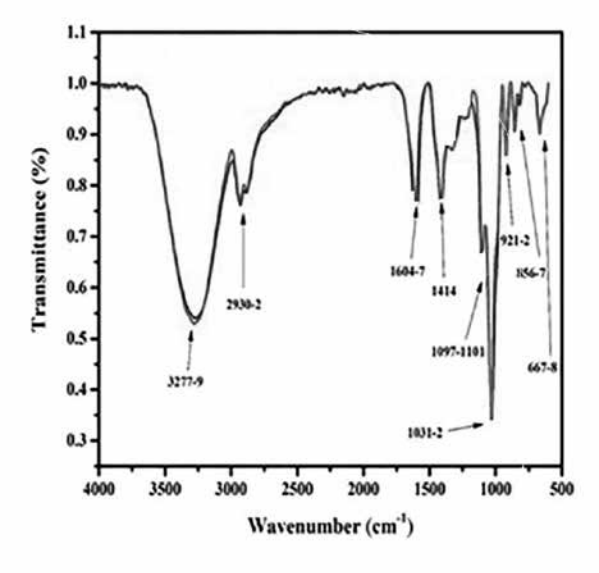

Fig. 3. The FTIR spectra of sodium alginate

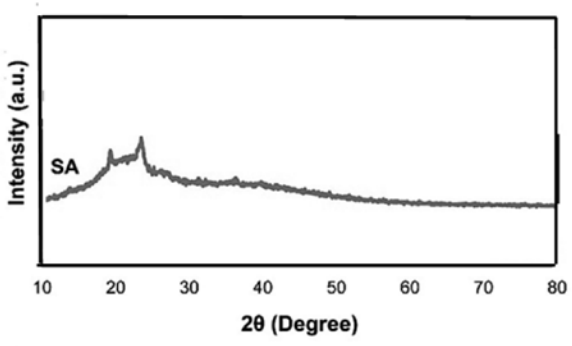

Fig. 4. XRD of sodium alginate

suggesting that the sodium alginate does not possess a well-defined crystalline structure. In contrast to crystalline materials, which have sharp diffraction peaks due to their ordered lattice, the observed peak width in the spectrum reflects a range of interatomic distances and orientations. As a result, this characteristic is often associated with glassy or non-crystalline substances, where the atoms are arranged randomly (Sundarrajan et al., 2012).

Gel fraction test

A gel fraction test is a crucial analytical procedure conducted to assess the efficacy of the cross-linker used in hydrogel formulations. This test measures the proportion of the gel that remains after the hydrogel has been subjected to specific conditions, providing insights into the material's structural integrity and performance. The levels of gel fraction are significantly influenced by both the crystallinity of the network and the extent of crosslinking achieved during the polymerization process. Higher crosslinking generally leads to increased gel fraction values, which can enhance the mechanical properties of the hydrogel. Figure 5 illustrates the results obtained from the comprehensive evaluation of the sodium alginate hydrogel gel fraction. The gel fraction value for this particular hydrogel is determined to be 25%, which indicates its suitability for various biomedical applications. This level of gel fraction suggests that the alginate hydrogel possesses a balanced combination of flexibility and rigidity, making it a promising candidate for use in drug delivery systems, tissue engineering scaffolds, and wound dressings, where both mechanical stability and biocompatibility are essential.

Dynamic rheology analysis

To gain a deeper insight into the intricate characteristics of sodium alginate hydrogels, their rheological properties are meticulously examined under various ambient conditions. This examination is crucial, as it allows researchers to understand how these hydrogels behave under different stresses and strains, which can be significant for their potential applications in various fields such as biomedicine and food science. The loss modulus (G″) represents the viscous response of the material, reflecting its ability to dissipate energy, while the storage modulus (G′) indicates the elastic, solid-like behavior, showcasing the material's capacity to store energy. As illustrated in Figure 6, the G′ values for both samples consistently surpass the G″ values, corroborating findings from earlier research (Wang et al., 2021). This observation concludes that the hydrogels produced can be classified as predominantly elastic, indicating a robust structural integrity. Notably, when the frequency aligns with the reciprocal relaxation timean identifiable characteristic referred to as the gelation time—G″ and G′ will intersect. The findings reveal that gelation times commence at the onset of the process, suggesting that the cross-linked structure of the hydrogels is established swiftly and efficiently. This rapid formation is essential for applications where quick gelation is required, such as in drug delivery systems or tissue engineering, where the mechanical properties of the hydrogels play a pivotal role in their performance and effectiveness.

Cytotoxicity test

A fundamental requirement for medical applications is biocompatibility, which refers to the ability of a material to perform its desired function without eliciting any adverse effects in a biological environment. In this study, we assessed the viability of L929 murine fibroblast cells when in contact with various hydrogels. The evaluation of cell viability was carried out indirectly using the MTT assay. This widely recognized method measures the metabolic activity of cells as an indicator of their health and proliferation. The results of this comparison are illustrated in Figure 7, showcasing a clear differentiation between the impacts of the hydrogels on cell viability. Notably, the positive controls exhibited the highest absorbance levels after 24, 48, and 72 hours of incubation, indicating robust cell viability and confirming the controls' effectiveness used in our experimental setup. This data underscores the importance of selecting biocompatible materials in the design of hydrogels for medical purposes, as it directly affects the safety and efficacy of the resulting biomedical products. The results regarding cell viability demonstrate that the hydrogel exhibits no cytotoxic effects on L929 cells, which are commonly used for such evaluations due to their reliability as a standard cell line. The remarkable

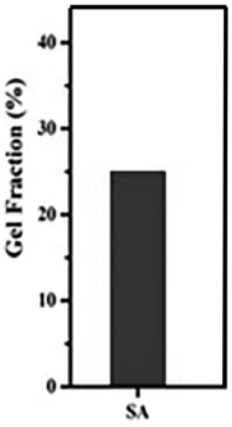

Fig. 5. Gel fraction of the sodium alginate hydrogel

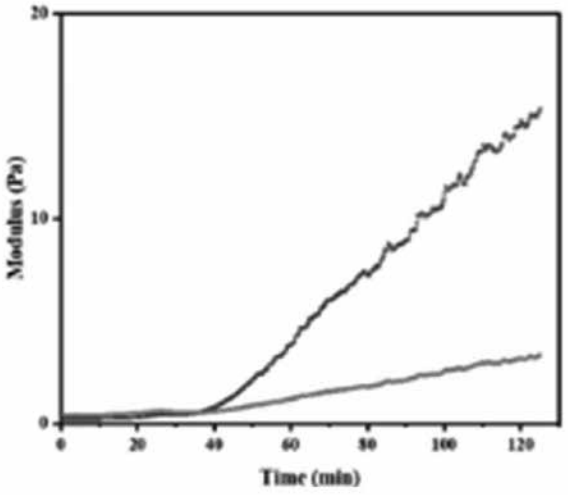

Fig. 6. Dynamic rheology analysis of sodium alginate hydrogel

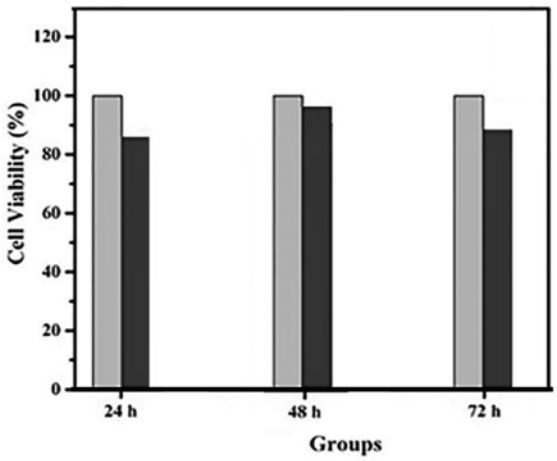

Fig. 7. L929 cells viability treated using sodium alginate hydrogel

biocompatibility of alginate hydrogel likely contributes to its high cell viability, making it a suitable candidate for various biomedical applications. In accordance with the ISO-10993-5-2009 standards, a substance is classified as cytotoxic if less than 70% of the cells exposed to it for a duration of 24 hours remain viable (Liang, 2016). In this study, the hydrogel maintained a cell viability well above this threshold, indicating its safety for potential use in biomedical applications. Further investigations can explore the long-term effects of the hydrogel on cell behavior and its potential applications in clinical settings.

Conclusion

The research highlights the successful extraction and characterization of a hydrogel derived from brown algae from the Oman Sea, demonstrating its substantial potential as a viable candidate for biomedical applications. In this study, brown algae were meticulously collected from the Oman Sea, and the alginate was efficiently extracted using established protocols. Subsequently, a hydrogel composed primarily of purified alginate was carefully fabricated through a series of controlled processes. Various methods were employed to thoroughly assess the characteristics of the resulting hydrogel, including rigorous evaluations of its physical, chemical, and morphological attributes. These evaluations incorporated advanced techniques such as scanning electron microscopy and rheological measurements to capture the hydrogel's unique properties accurately. The findings from these investigations suggest that the synthesized hydrogel exhibits significant potential as a promising candidate for biomedical applications, particularly in targeted therapies. This approach paves the way for future research in biomedicine and pharmaceutical development, opening new avenues for the effective delivery of therapeutic agents while enhancing patient outcomes. As the field continues to evolve, the implications of this study underscore the importance of exploring natural biopolymers for sustainable biomedical applications.

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