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# Synthesis and Structural Characterization of Bioactive Chitosan Hydrogels from Lobster Shell Waste

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## ABSTRACT

**Background.** Chitosan is a biopolymer derived from chitin, a polysaccharide abundantly present in the shells of marine crustaceans. It has good antimicrobial and biomedical applications. Chitosan plays a vital role in the production of hydrogels, which retain significant water-holding capacity and antimicrobial properties, making this biomaterial useful for treatment options, particularly in implant coatings and wound dressings. The current study focuses on the use of lobster shell waste to produce a bioactive, chitosan-based, hydrogel biomaterial with a wide range of biological applications. Lobsters provide a rich source of chitin, while the hydrogels act as carriers of pharmaceutical drugs. After structural characterization, these hydrogels were evaluated for their antibiofilm activity and dental applications.

**Objective.** The study focuses on the use of lobster shell waste as a source of chitosan hydrogels. Further, it evaluates the antibacterial and antibiofilm potential of the prepared chitosan hydrogels against dental bacterial isolates.

**Method.** Polysaccharide chitin from lobster shells was isolated by deproteinization, demineralization, and deacetylization to yield chitosan. For hydrogel preparation, the dispersing agent, tween-80, and 2% acetic acid were mixed. Water holding capacity, swelling, and dissolution were monitored for structural evaluation. Agar well diffusion assay was performed against bacterial isolates (*Staphylococcus aureus, Bacillus sp., E. coli, and Pseudomonas pneumoneae*). Antibiofilm and hemolytic assays were also performed for biofilm and biocompatibility purposes.

**Results.** Synthesized chitosan hydrogels showed good water retention (51.25%) and swelling (10.45%), with the highest inhibition zone of 22 mm against *Staphylococcus aureus*, along with strong antibiofilm activity and better biocompatibility. Chitosan hydrogels against dental isolates (*DI2, DI5, DI8, DI9*) exhibited activity comparable to pyodine, suggesting strain-dependent efficacy of the hydrogel.

**Conclusion.** The current study suggests chitosan hydrogel as an effective and promising coating material for dental implants.

**Keywords:** antibacterial, antibiofilm, biomaterial, chitosan hydrogel, implant coatings

## Highlights

- The study analyzes biowaste (crustacean waste) from shrimps as a potential source of chitin extraction and chitosan source.

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- Chitosan was converted into chitosan hydrogels and analyzed for its potential characterization.
- Chitosan hydrogels exhibited antibacterial and antibiofilm activity, strongly suggesting their potential as implant coatings.

## 1. INTRODUCTION

Highly toxic solvents as well as the availability of free active sites of chitosan offer additional advantages over chitin [1]. Exhibiting various properties like biocompatibility, biodegradability, and low toxicity, chitosan has a broad range of applications such as a flocking agent in water treatment, a supplement in food industry and drug delivery systems, and a dehydrating agent in cosmetics. Moreover, its antimicrobial activity against bacteria and fungi have also been studied [2].

Recently, researchers have given much attention to hydrogels because of their unique structure, which is applicable in various fields. The main reason is the presence of hydrophilic functional groups on polymer backbone, which can hold large amounts of water without collapsing, leading to 3D polymer networks [3]. The sol-gel behavior of hydrogels to external stimuli provides them with special features, such as non-toxicity and biocompatibility. Furthermore, their structural properties open avenues for their application in medical and pharmaceutical fields, leading to the synthesis of medical hydrogels [4]. Hydrogels can be prepared from both natural and artificial polymers. As a natural source, it is ecofriendly, less toxic, and has low production cost.

Hydrogels can be synthesized naturally from chitin, chitosan, alginate, starch, and cellulose [5]. Among them, chitosan is the most common source for their synthesis [6]. Biological characteristics, such as antioxidant, antibacterial, and anticancer make chitosan hydrogels different from other biofilm-forming agents [7]. These hydrogels play a major role in wound dressing, but they are also capable of exerting antimicrobial action. Since hydrogels composed of polymer chitosan have intrinsic antimicrobial properties, they can be used for wound treatment and exhibit dual action when incorporated with drugs and nanoparticles [8]. For instance, a hydrogel composed of chitosan derivative o-carboxymethyl embedded lincomycin showed antibacterial activity towards *S. aureus* and *E. coli* [9]. It has been shown that chitosan hydrogel can be used as a component of toothpaste and mouthwash, as a prevention against dental plagues [10]. Chitosan has been shown also to possess a strong affinity for tooth enamel and salivary pellicles, suggesting that it can form a protective covering over these mineralized surfaces [10]. So far, hydrogels have gained more traction as the threat of AMR proceeds. In this regard, biopolymers like chitosan have a multi-target effect and bear the ability of enhanced intrinsic antimicrobial actions. Hence, this work is focused on the characterization of chitosan-based hydrogels and their effect on the sensitivity of bacteria towards them.

## 2. MATERIALS AND METHODS

To isolate chitin, 5 lobsters were collected from a hotel in Lahore in October 2019. These lobsters were of equal size and carried almost the same weight. Total shell weight before processing was 1360 g. The lobsters were defrosted and washed with autoclaved distilled water and 95% ethanol solution to remove all dirt and surface contaminants (Figure 1 B and 1 C). They were de-shelled; the separated shells were re-washed and oven dried at 80°C for 2-3

days. Dried shells were thoroughly crushed using pestle and mortar. The crushed material was stored in an airtight polythene bag.

### 2.1. Production of Chitin

Two primary stages comprise this production process: demineralization and deproteinization [11]. In demineralization, lobster shells were crushed and treated with 1.25 N HCl (4 volumes) for 3 hours and then rinsed and re-incubated overnight with 1.25 N HCL (4 volumes). In deproteinization, the treated shells were mixed with 5% NaOH (5 volumes) and then heated at 70-75°C for 1 hour. The process was repeated until the mixture was protein-free. Finally, the sample was rinsed and then oven-dried at 65°C for 8 hours and ground into chitin.

**2.1.1. Production of Chitosan from Chitin.** Chitin was deacetylated to chitosan [12]. In this process, 40% NaOH was mixed with chitin powder at 5:1 ratio. It was then heated at 100°C for 5-6 hours. After heating, NaOH was removed and the mixture was rinsed with cool distilled water until the pH turned neutral. It was then oven-dried at 65°C for 8 hours. The resultant dried material was ground into fine chitosan powder. To maintain solubility, the chitosan was dispersed uniformly in tween-80 to prevent aggregate formation.

**2.1.2. Preparation of Chitosan Hydrogels.** To make chitosan hydrogels [13], 1.5 g of chitosan powder (as shown in Figure 4) was mixed with 20 mL of 2% acetic acid solution at room temperature, with continuous stirring in a shaker for 24 h. The shaking was continued until a thick yellow solution was obtained. To this solution, 0.5% tween-80 was added with continuous stirring to prevent clumping. The solution was filtered using a glass crucible to get a smooth viscous solution. After filtration, this liquid mixture was poured onto a petri dish and dried for 5-7 days. Finally, the mixture was oven-dried at 45°C for 12 h to remove the residual solvent. The prepared hydrogels varied in their degree of gelation and pore size.

### 2.2. Structural Characterization of Chitosan Hydrogels

**2.2.1 Swelling Test.** To assess the swelling ability [14] of chitosan hydrogels, they were cut into 2x2 cm pieces and their initial dry weight (W0) was measured. Afterwards, an empty petri plate was weighed and the hydrogel piece was placed onto it. Then, 10 ml of PBS solution was added to it. The gel in PBS was incubated for 20 mins. The swollen hydrogels were removed and any excess PBS solution was discarded. The swollen weight (W1) was also recorded.

The swelling ratio was determined by using the following formula:

$$\text{Swelling ratio} = \frac{W1 - W0}{W0}$$

**2.2.2. Assessment of Water Content.** The absorbance rate of water by hydrogels was determined [15] by cutting the obtained hydrogel into 2 cm pieces using sterilized scissors. Afterwards, its dry weight was measured ($W_{dry}$). Then, it was soaked into 10 ml of autoclaved distilled water for 24 hours. Post 24 hours, the swelled gel was again weighed ($W_{swelled}$). The water content or uptake was analyzed using the following formula:

$$\text{Water content ratio} = \frac{Wswelled\ gel - Wdry\ gel}{Wdry\ gel}$$

**2.2.3. Dissolution Ratio.** The rate at which water is dissolved/absorbed in the cross-linked structures of a hydrogel is referred to as its dissolution rate. To measure it, hydrogels were cut into 2 cm thin slices and weighed (W0). These slices were then soaked in distilled water at 80°C for 24 hours. After the required incubation time,

the gels were dried and weighed (W1) again. The dissolution ratio was calculated using the following formula:

$$\text{Dissolution ratio} = \frac{W1}{W0}$$

### 2.3. Bioactivity Analysis of Chitosan Hydrogels

**2.3.1 Bactericidal Effect of Chitosan Hydrogels.** Some biofilm-forming bacterial strains from dental implants were isolated and identified via Gram staining, catalase/oxidase tests, and biochemical characterization. Moreover, 24-hour fresh cultures of Gram-positive strains *Bacillus Spp. and S. aureus*, as well as Gram-negative strains *E. coli and P. pneumoniae* (MMG lab), were used. The strains were then swabbed onto agar plates and wells were created using the back side of Pasteur pipettes. A total of 5 to 6 pieces of chitosan hydrogel were placed onto each plate at equal distances and the plates were incubated (37°C, 24-48 hours). Zones of inhibition were measured in mm around each piece of hydrogel.

**2.3.2 Hemolysis Test.** The hydrogels' hemolytic activity was assessed using Malagoli's method [16]. Fresh human blood was collected in a sterile EDTA vial, centrifuged (14,000 rpm, 5 min), and resuspended in PBS (pH 7.2) erythrocytes/RBCs (200 µL). Approximately, 100 µL RBC suspension was thoroughly shaken and mixed with different concentrations of chitosan hydrogels (50, 100, 150 mg/mL), incubated (35°C, 1h), and centrifuged (10,000 rpm, 10 min). The supernatant was analyzed spectrophotometrically at 540 nm using a microplate reader. The test used RBCs in water as positive control and RBCs in PBS as negative control. Hemolysis percentage was calculated using the following formula:

$$\%\ \text{Hemolysis} = \frac{Sample\ OD - Negative\ Control\ OD}{Positive\ control\ OD - Negtaive\ Control\ OD} \times 100$$

**2.3.3. Tetrazolium Overlay Assay.** This assay assesses bacterial cell viability and respiratory activity. The test was performed following the instructions of [17]. A 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) served as a redox indicator; a change of color to purple indicated viable cells. Firstly, nutrient agar plates with freshly swabbed bacterial strains were prepared. Then, a 2 cm hydrogel piece was added and the plates were incubated at 37°C for 24 hours. Afterwards, the plates were supplemented with TTC-enriched agar, covered with aluminum foil, and again incubated at 37°C for 4-5 hours in darkness. Finally, color changes were observed: yellow (inhibition), purple (live cells).

**2.3.4. Assessment of Chitosan Hydrogels on Tightly Bound Cells.** A ring test was performed to check the ability of bacterial strains to form biofilms. For this test, a loopful of standardized bacterial culture was inoculated in sterile N-broth and incubated for 24 hours at 37°C. After 24 hours, the optical densities of the cultures were measured at 600 nm using a spectrophotometer (Model U2020, IRMECO Germany UV/VIS Spectrophotometer). The supernatant in tubes was gently transferred into sterile falcon tubes. The glass tubes were left for some time to get air-dried. The falcons were centrifuged at 6000 rpm for 10 mins to obtain a pellet of bacterial cells. The supernatant was discarded and the pellet was resuspended in 200 µl of autoclaved distilled water. Approximately, 200 µl of 0.1% crystal violet solution was added to each pellet. After 5 mins, crystal violet was discarded and the pellet was washed thrice with 0.85% NaCl. Then, 200 µL of glacial acetic acid (33%) was added to the pellet.

Finally, the solution's optical density was measured at 523 nm.

## 3. RESULTS

This study focused on chitin extraction from lobster shell waste using chemical methods. The process used incorporated deproteination and demineralization. By using this method, chitin in white powder form was obtained. Highest quality chitin and chitosan were obtained at high temperatures in a short time. It was determined that the quality of chitosan depends upon time, pH, and temperature. Chitosan extraction is also helpful in reducing shrimp shell waste that was previously dumped. After extraction, some chitin was converted to chitosan (not naturally present) by deacetylation with NaOH at high temperature. Chitosan is a semi-crystalline polysaccharide consisting of D-glucosamine subunits. It has an adhesive property (due to extensive DD subunits) and hemostatic ability (due to positive charges on the chitosan backbone).

### 3.1. Sampling of Marine Waste for Chitin Production

Marine shells, primarily lobster exoskeletons, were collected from a Hotel in Lahore. Lobsters were selected due to their high chitin content.

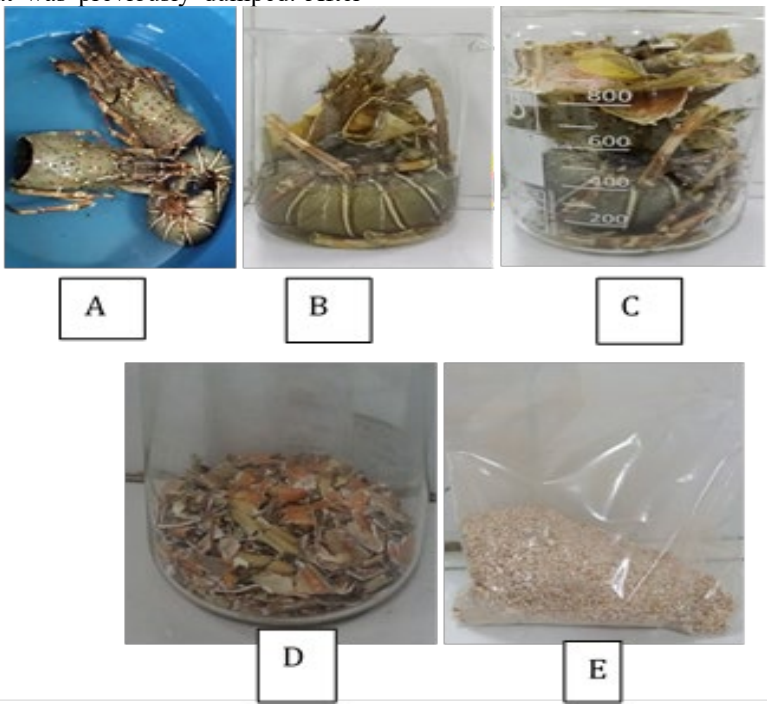


**Figure 1.** Stages of Lobster Shell Waste Processing to Obtain Chitin. A: Shell Waste Sample, B: Shelled Exoskeleton of Lobsters, C: Exoskeleton Washed and Crushed, D: Crushed Exoskeleton E: Chitin Powder

**3.1.1. Isolation of Exoskeleton from Lobsters.** The exoskeleton is the primary source of chitin in lobsters (Figure 1B) and consists mainly of the hard, segmented outer shell and jointed limbs. Initially, the cartilaginous material was separated from

the hard shell, then crushed and oven-dried. Dry weight was determined to be ~50-60% of total marine waste.

**3.1.2. Production of Chitin from Lobsters' Exoskeleton.** The exoskeleton was crushed (Figure 1D) and the resulting powder, that is, Chitin (Figure 1 E) was further subjected to two other chemical processes, namely demineralization and deproteination, to produce chitin.

**3.1.3. Demineralization.** Demineralization removed the calcium carbonate impurities, yielding a chitin-protein mixture. Deproteinization further purified chitin, removing protein impurities (shown in Figure 2).


**Figure 2.** Demineralization and Deproteination of Chitin

**3.1.4. Preparation of Chitosan from Chitin.** Chitosan production involved deacetylating chitin powder, yielding 34% chitosan from the total exoskeleton weight (Table 1).

The obtained chitin and chitosan had zero lipid content. Color was brownish-white in chitin to bright off-white in chitosan, while pH was 7.3 in chitin and 7.8 in chitosan. Chitosan and chitin are not soluble in 1% acetic acid. Both products were stored in refrigerator for future use.

**Table 1.** Weight Loss of Deacetylated Chitin Powder

| Initial Weight of Exoskeleton (g) | Weight after Deacetylation of Chitin (g) | Percentage Decrease in Weight (%) |
|---|---|---|
| 1360 | 462.4 | 34 |

**3.1.5. Formation of Chitosan Hydrogels from Chitosan.** Chitosan hydrogels were obtained as yellowish, semi-solid, gel-like substance (Figure 3). The deacetylation of chitin produced semi solid chitosan hydrogels which were further dried to obtain solid hydrogels.

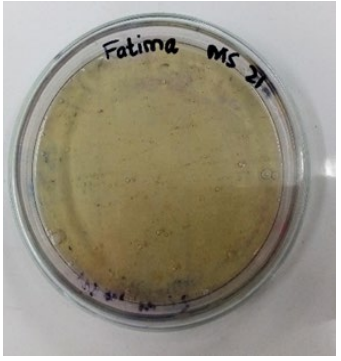


**Figure 3.** Prepared Chitosan Hydrogels

Chitosan hydrogels were prepared using the already available procedure. Each factor was handled carefully to achieve a better effect on the swelling ability and other properties of chitosan hydrogels. In addition to molecular structure, gel structure, and the degrees of crosslinking, the quality and state of water in the hydrogel are important physicochemical qualities for this gel [5].

### 3.2. Structural Characterization of Chitosan Hydrogels

**3.2.1. Swelling Test.** Hydrogels exhibited a high swelling capacity and a significant weight increase (Table 2).

**Table 2.** Swelling Capacity of Hydrogels

| Sr. No | Weight of Hydrogels (g) | | Swelling Ratio |
|---|---|---|---|
| | Initial Weight of Hydrogels ($W_0$) | Weight after 24 Hours ($W_1$) | |
| 1. | 0.059 | 0.734 | 11.4 |
| 2. | 0.044 | 0.465 | 9.5 |
| Mean Ratio | $\bar{x}$= 0.0515 | $\bar{x}$= 0.2692 | $\bar{x}$= 10.45 |

The swelling ratio of 10.45 showed that the prepared chitosan hydrogels had a good cross-linked structure. The prepared hydrogels were tested and characterized for their swelling and water-holding capacity. Hydrogels are hydrophilic due to their 3D structure. It gives them the swelling ability. Swelling contributes to medicine production and drug delivery. The swelling ratio of these chitosan hydrogels was around 10.45 gm.

**3.2.2 Water Content.** Hydrogels with high water content and high mechanical strength are desired for their biomedical properties. Water content is the amount of water a hydrogel can absorb or retain. The results (Table 3) showed significant weight increase after PBS soaking, indicating excellent water-absorbing and retaining potential.

**Table 3.** Water Content of Chitosan Hydrogels Prepared from Lobsters Waste

| Weight of Hydrogels (g) | | Water Content Ratio |
|---|---|---|
| Dry Weight | Wet Weight | |
| 0.036 | 1.734 | 47.16 |
| 0.026 | 1.465 | 55.34 |
| $\bar{x}$= 0.031. | $\bar{x}$= 1.599 | $\bar{x}$= 51.25 |

The average water content in the prepared hydrogels was calculated to be above 50%, as indicated in Table 3.

**3.2.3. Dissolution Test.** The dissolution behavior of gelatin from the chitosan/gelatin membrane was calculated using the following equation:

Dissolution ratio = (W1/W0)

The dissolution rate was calculated to be around 22.5%, as mentioned in Table 4.

**Table 4.** Dissolution Ratio of Chitosan Hydrogels Prepared from Lobster Shell Waste

| Weight of Hydrogels (g) | | Dissolution Ratio |
|---|---|---|
| Before Incubation (W1) | After 24 Hours of Incubation (W2) | |
| 0.046 | 0.797 | 17.32 |
| 0.044 | 1.235 | 28.06 |
| $\bar{x}$= 0.045 | $\bar{x}$= 1.016 | $\bar{x}$= 22.5 |

The hydrogels do not dissolve in water, although their dissolution can be achieved by enzymatic reactions or hydrolysis by esters. Dissolution behavior is useful in medical and other fields, as it removes hydrogel after its function.

### 3.3. Bioactivity Analysis of Chitosan Hydrogels

**3.3.1. Anti-Bacterial Potential of Chitosan Hydrogels.** The antimicrobial activity of chitosan and its hydrogels was determined using agar well diffusion method. Previously identified strains of gram-positive and gram-negative bacteria were inoculated on N-agar plates. The hydrogels were placed on the agar in the form of small equal pieces, while pyodine was used as a positive control and standard to compare the zones of inhibition.

After incubation, the plates showed different zones of inhibition all around hydrogels, as depicted in Figure 4. However, chitosan hydrogels showed maximum inhibition against *S. aureus* and *E. coli* (Figure 4). Pyodine, a commercially available chemical routinely used for wound cleaning, is known as a strong antimicrobial agent against several viruses,

bacterial strains, fungi, and protozoa. The results showed chitosan hydrogels creating significant zones when compared with pyodine. The anti-bacterial properties of obtained hydrogels are comparable to that of pyodine (Figure 4).

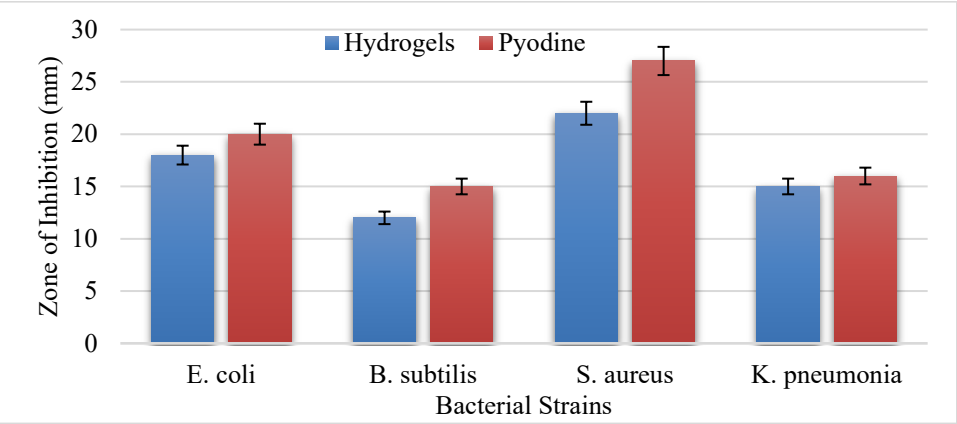


**Figure 4.** Antibacterial Profiling to Evaluate the Bioactivity of Chitosan Hydrogels

**3.3.2. Hemolysis Test.** Hemolytic activity of the hydrogels was tested in order to find their biocompatibility with human blood. Hemolysis percentage induced at different concentrations of test subjects was calculated. The results indicated that chitosan hydrogels are compatible with human blood. It was observed that hemolytic activity of hydrogels increased with the increased concentration of chitosan, suggesting low concentrations of hydrogels as biocompatible. Several known peptide-based hydrogels are biocompatible with human blood cells. Similar results were observed for the prepared chitosan hydrogels (Figure 5). Decreased hemolysis of blood cells was observed to correspond with an increase in chitosan hydrogel concentration. Hence, it remains useful for medicinal and biological purposes.

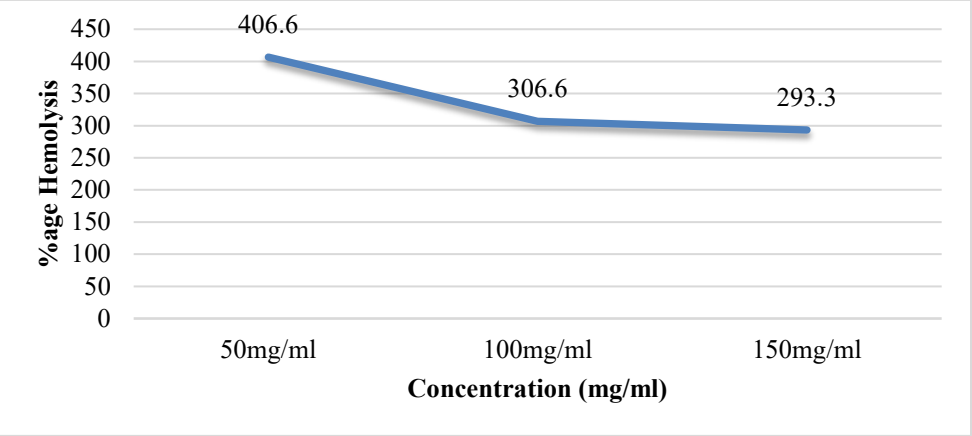


**Figure 5.** Hemolysis Assay to Evaluate the Bioactivity of Chitosan Hydrogels against Bacterial Strains Isolated from Dental Implants

**3.3.3. Biological Activity of Chitosan Hydrogels for Dental Implant Coating.** The bacterial strains present on dental implant coatings are harmful for oral health and dental implant's shelf-life. Unique functional groups of bioactive hydrogels enhance functionality by reducing biofilm formation and increasing cell viability and biodegradation.

***3.3.3.1 Assessment of Tightly Bound Cells.*** The chitosan hydrogels were coated to dental implants. The biofilm-forming ability of bacterial strains present on implants was suppressed. After adding hydrogels, ring formation was less intense, indicating a reduction in biofilm formation. The biofilm formation suppression was indicated by a visible reduction in optical density values, as demonstrated in Figure 6

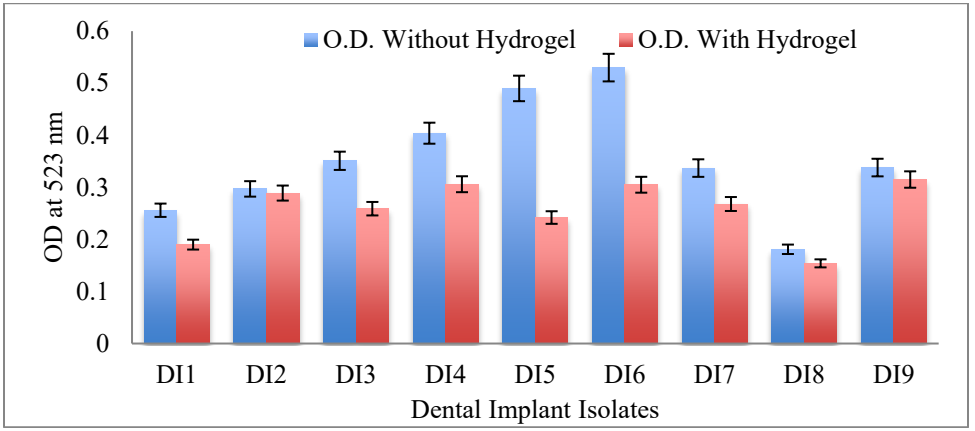


**Figure 6.** Effects of Chitosan Hydrogels on Tightly Bound Cells in Biofilms on Bacterial Strains Isolated from Dental Implants. *$p$<0.01

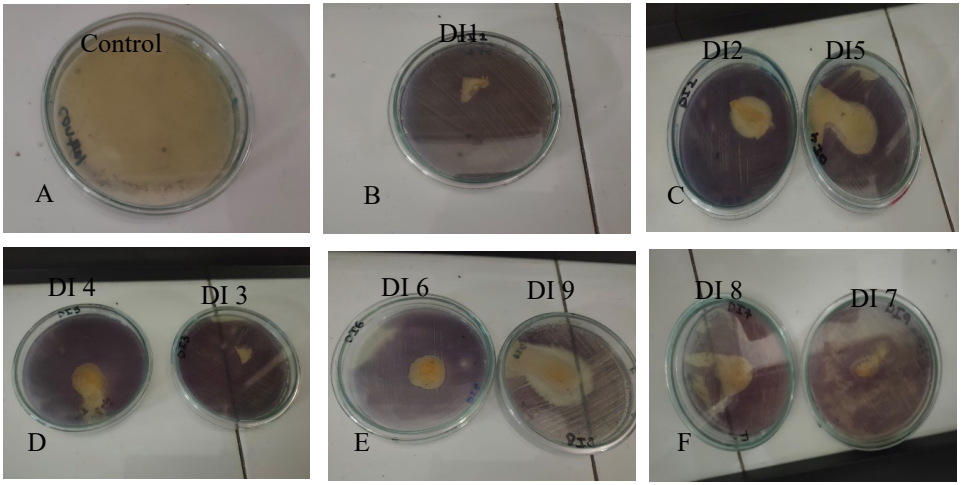


**Figure 7.** Tetrazolium Assay for Bacterial Cell Viability of Chitosan Hydrogels in Bacterial Strains Isolated from Dental Implants. A: Positive Control, B: DI1, C: DI2 & DI5, D: DI4 & DI3, E: DI6 & DI9, F: DI8 & DI7.

The findings suggest the antibiofilm potential of chitosan hydrogels. The results indicated that the prepared chitosan hydrogels have good biocompatibility, in addition to their antimicrobial and antibiofilm activity. The application of chitosan hydrogels caused a statistically significant reduction in O.D. values**,** demonstrating effective biofilm inhibition among tested dental isolates.

**3.3.6. Tetrazolium Dye Overlay Assay.** Tetrazolium dye was used to check the viability of cells. This test was used to check whether hydrogels possessed the ability to inhibit bacterial isolates. Tetrazolium dye was added to plates using an agar overlay method for observation. Pyodine was used as a positive control. The controls having no hydrogel or pyodine showed no viable cells. The results showed hydrogels significantly affecting cell viability but the control indicated much better results (Figure 7).

The results showed a significant difference in antibacterial performance between chitosan hydrogels and pyodine, as indicated in Figure 8, with the control showing slightly higher inhibition. However, several isolates (*DI2, DI5, DI8, DI9*) showed comparable activity, suggesting their strain-dependent efficacy. Figure 8 shows the comparison between pyodine and chitosan hydrogels used against the bacterial isolates of dental implants. Except for DI1, DI3, and DI6, all the other strains created good zones of inhibition, demonstrating that the efficacy of chitosan hydrogels is significant to that of pyodine. This proves that chitosan hydrogels can be used as an alternative after further testing against pyodine, which is a chemical agent.

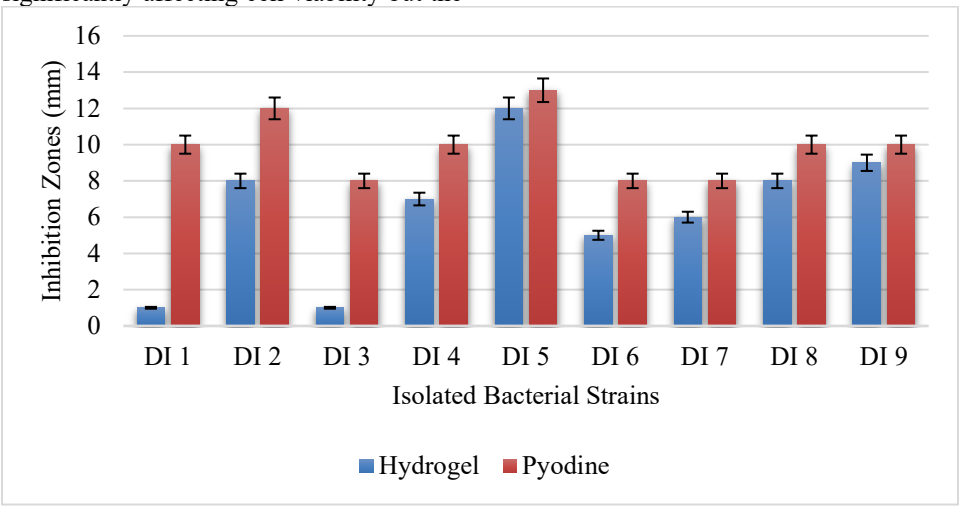


**Figure 8.** Tetrazolium profiling to evaluate the bioactivity of chitosan hydrogels against bacterial strains isolated from dental implants.

## 4. DISCUSSION

This study is focused on the preparation of suitable bioactive materials from lobster shell waste. The hypothesis addressed in this research postulates that marine crustacean waste can be converted into a desirable biomaterial using an eco-friendly approach. The study highlights the production of chitosan-based hydrogels with potential antibacterial and antibiofilm

properties that can be used as dental implant coatings to prevent plaque formation on dental implants.

Chitosan is a biopolymer that can be produced from chitin via an extraction procedure using demineralization, deproteinization, and deacetylation [1]. This biopolymer has superior physiochemical properties with amazing strength and bioactivity [2]. Deacetylation is especially important as it is critical to determine the molecular weight of the synthesized chitosan hydrogels, as well as their solubility, density, and bioactivity [3]. The use of tween-80 further facilitates the proper dispersion of and connections within the hydrogels, which directly affects their water retention, solubility, and swelling capabilities [4].

It was observed that a total of 34% chitosan was produced, which aligns with the conversion rate previously reported by [5] from crustacean wastes. Other physical parameters including color, pH, and solubility all point to the successful conversion of extracted chitin to chitosan [5]. The physical and chemical characteristics of the synthesized chitosan hydrogels showed a water retention capacity of 51.25% and a swelling ability of 10.45%, comparable to the results of [6]. These factors directly influence the cross-linking of polymeric hydrogels which provide structural integrity and also act as barriers in moisture prone areas, a property that is highly desirable for biomaterials used in implant coatings, drug delivery, and wound dressings. In the past, some hydrogels have been demonstrated to hold water up to 80-90% [18]. Water content is also their important characteristic. The higher the water content, the more the absorption and retention of water and the better it can mimic the cell environment [19]. Hence, biocompatibility of hydrogels increases.

The antibacterial effect of chitosan hydrogels was assessed against bacterial strains isolated from dental implants. A strong zone of inhibition was observed against *S. aureus,* comprising 22 mm. This suggests the chitosan's cationic nature that allows it to penetrate bacterial walls through electrostatic interactions [7]. The hydrogels showed antibacterial effects comparable to that of the commercially available pyodine, thus showing their potential to beat a chemically designed antibacterial agent.

Biofilm formation is a critical process in pathogenic and opportunistic bacterial strains. It allows the bacteria to survive a high concentration of anti-bacterial agents, especially antibiotics. It makes them highly resistant and is an increasing concern for implant based biomaterials [8]. The current study isolated bacterial strains from dental implants and assessed the potential anti-biofilm capability of the prepared chitosan hydrogels against these isolates. The results indicated a strong reduction in the number of biofilms. This is extremely important as most of the times biofilms on implants can lead to caries, periodontitis, and peri-implantitis [9]. The use of hydrogels greatly reduced cell adhesion in case of ring test, showing a decrease in the attachment efficacy of bacterial strains. It has been reported that chitosan hydrogels mainly disrupt signaling and inhibit quorum sensing, leading to the disruption of bacterial biofilms [10].

The biocompatibility of the synthesized chitosan hydrogels was assessed using hemolysis assay, which indicated that this biomaterial is highly biocompatible with blood cells. The results are comparable to the previous reports [11] which prove that because of its natural origin, rather than chemical properties, it provides cytocompatibility and biodegradibity and is,

therefore, safe for usage [12]. This strongly supports the use of this biopolymer as a strong competitor for coating material of implants since it provides protection against biofilm formation and also moisture-based destruction.

The utilization of lobster shell waste aligns with the principles of green chemistry and waste valorization by converting an abundant marine byproduct into a value-added biomaterial. Such approaches reduce environmental burden while providing a renewable source of biomedical polymers [5]. Moreover, this strategy supports the circular bioeconomy concept, linking marine biotechnology with healthcare innovation.

### 4.1. Conclusion

The study concludes that the production of chitosan hydrogels from shrimp exoskeletons is an eco-friendly approach. These hydrogels possess superior physicochemical and antimicrobial and anti-biofilm potential. They also prove to be extremely biocompatible, showing their relevance to be used as coatings for implants. They can be exploited also as wound dressings or for drug delivery because of their strong antibacterial potential against *S. aureus*, as well as their water retention ability. However, the study suggests that further evaluations should be done to assess their biocompatibility and biodegradability, hence opening avenues for future research.

#### Author Contribution

**Fatima Zahoor:** data curation, formal analysis, methodology investigation. **Hira Tariq:** writing - original draft. **Muhammad Kashif:** writing - original draft. **Shahmeen Sheikh:** writing - review & editing. **Saiqa Sattar:** writing - review & editing. **Hera Naheed Khan:** conceptualization, funding acquisition, writing - review & editing.

#### Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

#### Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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#### Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research.

## REFERENCES

1. Ali G, Sharma M, Salama ES, Ling Z, Li X. Applications of chitin and chitosan as natural biopolymer: potential sources, pretreatments, and degradation pathways. *Biomass Convers Biorefin.* 2024;14(4):4567-4581. https://doi.org/10.1007/s13399-022-02684-x

2. Desai N, Rana D, Salave S, Gupta R, Patel P, Karunakaran B, et al. Chitosan: a potential biopolymer in drug delivery and biomedical applications. *Pharmaceutics.* 2023;15(4):e1313. https://doi.org/10.3390/pharmaceutics15041313

3. Affes S, Aranaz I, Acosta N, Heras Á, Nasri M, Maalej H. Physicochemical and biological properties of chitosan derivatives with varying molecular weight produced by chemical depolymerization. *Biomass Convers Biorefin.* 2024;14(3):4111-4121. https://doi.org/10.1007/s13399-022-02662-3

4. Faghmous N, Bouzid D, Boumaza M, Touati A, Boyron O. Optimization of

chitosan-coated W/O/W multiple emulsion stabilized with Span 80 and Tween 80 using Box–Behnken design. *J Dispersion Sci Technol.* 2021;42(10):1566-1578. https://doi.org/10.1080/01932691.2020.1774387

5. Periyannan K, Selvaraj H, Subbu B, Pallikondaperumal M, Karuppiah P, Rajabathar JR, et al. Green fabrication of chitosan from marine crustaceans and mushroom waste: toward sustainable resource utilization. *Green Process Synth.* 2023;12(1):e20230093. https://doi.org/10.1515/gps-2023-0093
6. Lu G, Ling K, Zhao P, Xu Z, Deng C, Zheng H, et al. A novel in situ-formed hydrogel wound dressing by the photocross-linking of a chitosan derivative. *Wound Repair Regen.* 2010;18(1):70-79. https://doi.org/10.1111/j.1524-475X.2009.00557.x
7. Fasiku VO, Omolo CA, Devnarain N, Ibrahim UH, Rambharose S, Faya M, et al. Chitosan-based hydrogel for the dual delivery of antimicrobial agents against bacterial methicillin-resistant *Staphylococcus aureus* biofilm-infected wounds. *ACS Omega.* 2021;6(34):21994-22010. https://doi.org/10.1021/acsomega.1c02547
8. Stewart PS, Bjarnsholt T. Risk factors for chronic biofilm-related infection associated with implanted medical devices. *Clin Microbiol Infect.* 2020;26(8):1034-1038. https://doi.org/10.1016/j.cmi.2020.02.027
9. Colombo APV, Tanner ACR. The role of bacterial biofilms in dental caries and periodontal and peri-implant diseases: a historical perspective. *J Dent Res.* 2019;98(4):373-385. https://doi.org/10.1177/0022034519830686
10. Nag M, Lahiri D, Mukherjee D, Banerjee R, Garai S, Sarkar T, et al. Functionalized chitosan nanomaterials: a jammer for quorum sensing. *Polymers.* 2021;13(15):e2533. https://doi.org/10.3390/polym13152533
11. Zhou HY, Zhang YP, Zhang WF, Chen XG. Biocompatibility and characteristics of injectable chitosan-based thermosensitive hydrogel for drug delivery. *Carbohydr Polym.* 2011;83(4):1643-1651. https://doi.org/10.1016/j.carbpol.2010.10.022
12. Jia Y, Wei Z, Fu W, Huo M, Li F, Zhong C, et al. Biocompatibility evaluation on a bio-hydrogel composed of bacterial cellulose and chitosan. *J Biomater Tissue Eng.* 2014;4(2):118-125. https://doi.org/10.1166/jbt.2014.1142
13. Al Hoqani HAS, Noura AS, Hossain MA, Al Sibani MA. Isolation and optimization of the method for industrial production of chitin and chitosan from Omani shrimp shell. *Carbohydr Res.* 2020;492:e108001. https://doi.org/10.1016/j.carres.2020.108001
14. Felinto MC, Parra DF, da Silva C, Angerami J, Oliveira M, Lugão A. The swelling behavior of chitosan hydrogels membranes obtained by UV- and γ-radiation. *Nucl Instrum Methods Phys Res B.* 2007;265(1):418-424. https://doi.org/10.1016/j.nimb.2007.09.025
15. Ankur G, Harish K. Synthesis and swelling behavior of superabsorbent hydrogels acquired from CMC for efficient drug delivery. *Res J Chem Environ.* 2018;22:19-26.

16. Malagoli D. A full-length protocol to test hemolytic activity of palytoxin on human erythrocytes. *Invertebr Surviv J*. 2007;4(2):92-94.

17. Castro-Concha LA, Escobedo RM, de Miranda-Ham ML. Measurement of cell viability in in vitro cultures. In: Loyola-Vargas VM, Vázquez-Flota F. eds. *Plant Cell Culture Protocols*. Springer; 2006:71-76.

18. Chopra I, Sharad S, Kumar S, et al. A comprehensive review on hydrogels. *Curr Drug Deliv*. 2021;19(6):658-675. https://doi.org/10.2174/1567201818666210601155558

19. Wang Y, Zhang Q, Li H, et al. The synergistic effects of 3-D porous silk fibroin matrix scaffold properties and hydrodynamic environment in cartilage tissue regeneration. *Biomaterials*. 2010;31(17):4672-4681. https://doi.org/10.1016/j.biomaterials.2010.02.006