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Extraction, characterization and wound healing property of chitosan extracted from *Penaeus indicus* shells on mouse fibroblast (L929) cell line

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ABSTRACT

The current study aimed to characterize the properties of chitosan, extracted from *Penaeus indicus*, on the mouse fibroblast cell line (L929) for wound healing purposes. Chitosan is well known for its wide variety of biological characteristics. Shrimp exoskeletons were obtained from a local fish market in Chengalpattu, Tamil Nadu. Chitosan was extracted through demineralization, deproteination, and deacetylation processes. It was characterized using X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy. The characterized chitosan was processed into nanoparticles and evaluated for cytotoxicity on cell lines. The cell line was treated with concentrations ranging from 25 to 500 µg/mL for 4 h. The results demonstrated 99% cell viability across all concentrations. The study primarily focuses on evaluating the ability of chitosan to repair wounds. Mouse fibroblast cell lines were seeded on a microtitre plate at a density of 1×10^5 cells per well. Results indicated that the chitosan-treated groups exhibited significantly greater wound closure, with 10% to 20% more growth compared to the control groups. These findings underscore the potential of chitosan derived from *P. indicus* shrimp waste as a valuable pharmaceutical compound. Such applications could significantly contribute to the nation's socioeconomic development.

Keywords: chitosan; L929 cell line; shrimp shell; wound healing

1. INTRODUCTION

Chitin, a mucopolysaccharide, is one of the most abundant natural polymers, ranking second only to cellulose. Composed of repeating β(1,4)-N-acetylglucosamine units, it forms a linear polymer found in the exoskeletons of crustaceans, mollusks, and insects (Kang et al., 2010). Chitosan, derived through the alkaline deacetylation of chitin, is a copolymer consisting of β-(1→4)-linked 2-acetamido-2-deoxy-d-glucopyranose and 2-amino-2-deoxy-d-glucopyranose units (Seenuvasan et al., 2020). It is moderately reactive and available in various forms,

including fiber, powder, film, and paste. While structurally similar to cellulose, chitosan has garnered less attention due to its perceived inert nature, resulting in its underutilization as a resource for acids (Lim & Halim, 2010).

Chitosan can dissolve in small amounts of malic, lactic, acetic, succinic, and formic acids when in aqueous solutions. At a pH below 6, it becomes polycationic, allowing it to interact readily with negatively charged molecules (Lim & Halim, 2010; Klinkesorn, 2013). Additionally, chitosan specifically binds metal ions such as calcium, copper, iron, and magnesium (Ahmad et al., 2015). Its unique properties, including biodegradability, non-toxicity, antibacterial effects, and biocompatibility, have made it an area of significant interest in biomedical research.

Chitosan and its oligomers exhibit various biological activities, including antioxidant, anti-inflammatory, cholesterol-lowering, immunomodulatory, antitumor, neuroprotective, antimicrobial, antifungal, and hypoglycemic properties (Muhamad, 2022; Varun et al., 2017). These attributes make chitosan oligomers valuable for enhancing animal health. Additionally, chitosan promotes wound healing by stimulating hemostasis and accelerating tissue regeneration (Ueno et al., 2001). Natural materials like chitosan are preferred in biomedical research for their superior biocompatibility compared to synthetic materials. Its biodegradability and ability to be metabolized by human enzymes further enhance its appeal (Rodríguez-Vázquez et al., 2015). Moreover, its hydrophilic nature makes it a promising candidate for tissue engineering scaffolds (Thein-Han et al., 2008). Notably, hyaluronic acid, an extracellular macromolecule vital for wound healing, shares N-acetylglucosamine as a monomeric unit with chitosan (Keong & Halim, 2009). In recent years, hydrogels synthesized from chitosan nanoparticles have been widely used as antibacterial agents to prevent wound infections (Hou et al., 2020; Shafique et al., 2020).

The Indian white shrimp (*P. indicus*) is abundant in the coastal waters of India and is extensively farmed for aquaculture, contributing significantly to the seafood industry in the Middle East and Southeast Asia (Liu, 1985). Despite this, substantial quantities of shrimp waste, rich in chitin, are discarded daily without recognizing their potential. This study aimed to extract chitosan from *P. indicus* shells, characterize it, and evaluate its wound-healing properties on mouse fibroblast cells (L929).

Chitosan's wound-healing capabilities, such as its hemostatic effects and ability to promote immune cell migration (e.g., neutrophils and macrophages) during early wound repair, are well-documented (Patrulea et al., 2015). Previous studies have investigated the extraction and physicochemical characterization of chitosan from shrimp shells, including *P. indicus* (Öğretmen et al., 2021; Mittal et al., 2020). These studies provide valuable insights into extraction methods and properties of chitosan from various shrimp species, forming the basis for understanding the unique characteristics of chitosan derived from *P. indicus* shells.

The use of chitosan in wound care has also been extensively studied, demonstrating its role in wound closure, re-epithelialization, and tissue regeneration (Azad et al., 2004; Mohanasrinivasan et al., 2013; Abo Elsoud & El Kady, 2019). By specifically focusing on chitosan from *P. indicus* shells for wound healing in the L929 cell line, this study provides new insights into chitosan's application in regenerative medicine and wound management. The unique approach of exploring the wound-healing potential of chitosan from this specific shrimp species can offer a valuable contribution to the field of wound care and regenerative therapies.

2. MATERIALS AND METHODS

2.1 Materials

We obtained Indian white shrimp (*P. indicus*) shells from a local fish market in Chengalpattu district, Tamil Nadu, India. MTT reagent, H_3BO_3, HCl, and NaOH (all from Merck, Germany) were procured from Scientific Advance Company, Chennai, India. Dulbecco's Modified Eagle Medium (DMEM) (Himedia, India), fetal bovine serum (Thermo Fisher), phosphate-buffered saline (Thermo Fisher), and DMSO (Thermo Fisher) were obtained from Southern India Scientific Corporation, Chennai, India. The L929 cell line was sourced from the Cell Repository at the National Centre for Cell Science (NCCS), Pune, India.

2.2 Sample process

The shrimp shells were thoroughly washed with fresh water, followed by rinsing with distilled water to ensure complete cleaning. The cleaned shells were then sun-dried and finely powdered. The powdered shells were stored at low temperatures for subsequent extraction.

2.3 Extraction of chitin

Chitin extraction was carried out in two main steps: demineralization and deproteinization of the powdered shrimp shells (Percot et al., 2002). For demineralization, 500 mL of 1 M HCl was mixed with 50 g of shrimp shells (Nawaz et al., 2023). The reaction was carried out at room temperature with continuous agitation at 250 rpm for 2 h. The demineralized shells were then filtered and washed with distilled water until the pH reached neutral. After bleaching by immersing the shells in ethanol for 10 min, they were dried in a hot air oven at 70°C. To deproteinize the dried demineralized shells, 1 M NaOH was added at a solid/liquid ratio of 1:10 (g/mL) and heated to 80°C with agitation for 3 h (Omar et al., 2021). The resulting substance was filtered and rinsed with distilled water until neutral pH was achieved. The chitin was then subjected to a final drying process in the hot air oven at 70°C, following a 10-minute immersion in ethanol for additional bleaching (Figure 1).

2.4 Extraction of chitosan

The isolated chitin was deacetylated by treating it with 12.5 M NaOH at a solid/liquid ratio of 1:15 (g/mL) (Kumar & Rajasulochana, 2021). The reaction mixture was kept at – 80°C for 24 h (Figure 1c). After this, the mixture was heated to 115°C, and the reaction was continuously agitated at 250 rpm for 6 h. Following this, the mixture was filtered and rinsed with distilled water to neutralize the pH of the resultant chitosan. Finally, the chitosan was dried at 70°C in a hot air oven (Panchal & Desai, 2022) (Figure 1).

Figure 1. Overview of chitosan extraction process

2.5 Characterization of chitosan

Fourier transform infrared spectroscopy (FTIR) analysis was performed to characterize the chitosan (Espinosa-Andrews et al., 2010). The IR spectrum of lyophilized (freeze-dried) chitosan was recorded using a Thermo Scientific spectrometer (USA) at room temperature, covering a wave number range from 400 to 4000 cm^{-1}. The spectrum was obtained by averaging 32 scans with a resolution of 8 cm^{-1}. The entire experiment was conducted at a room temperature of 21°C.

An XRD analysis of the freeze-dried chitosan was carried out using a Siemens D5000 XRD with a CuKα radiation source (λ = 0.154 nm) as described by Salari et al. (2018). The generator operated at a voltage of 40 kV and a current of 30 mA, with a step size of 0.03°. The scan speed was set to 0.043°/s, and the scan range covered 2θ from 2° to 80°. Additionally, scanning electron microscopy (SEM) was performed to investigate the morphology, topography, and composition of the extracted chitosan.

2.6 Chitosan nanoparticles synthesis

In this study, we adopted the approach described by Ngan et al. (2014), employing the spray drying method to produce nanoparticles from the extracted chitosan. This method was used because particles within the size range of 10–1000 nm are classified as nanoparticles (Nawaz et al., 2023). In the process, chitosan was first dissolved in aqueous acetic acid, and then nanoparticles were generated by passing this solution through a nozzle at elevated temperatures ranging from 120°C to 150°C.

2.7 Preparation of mouse fibroblast cell line (L929)

2.7.1 Preparation of chitosan solution

A total of 0.618 g of boric acid (H_3BO_3) was dissolved in approximately 800 mL of distilled water in a 1-liter volumetric flask. The solution was stirred until the boric acid was completely dissolved, and then the volume was adjusted to 1 liter with distilled water. Chitosan solution of varying concentrations (w/v) were subsequently prepared by stirring the mixture on a magnetic stirrer with gentle heating to facilitate dissolution of the chitosan.

2.7.2 Cytotoxicity assay

To assess the cytotoxicity of chitosan, the L929 cell line was plated in 96-well plates at a density of 1×10^4 cells per well. The cell culture procedures were carried out according to standard protocols (Basha et al., 2018; De Bari et al., 2001). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Himedia, India) supplemented with 10% fetal bovine serum and 1X antibiotic solution and incubated at 37°C with 5% CO_2. After reaching confluence, the cells were washed with 200 µL of 1X phosphate-buffered saline (PBS).

The cells were then exposed to different concentrations of chitosan in serum-free medium, with 30% DMSO (dimethyl sulfoxide) as a positive control and 10 mM boric acid buffer as a negative control. The cells were incubated for 24 h. After the treatment period, the medium was aspirated, and 0.5 mg/mL MTT solution prepared in 1X PBS was added. The cells were incubated in a CO_2 incubator for 4 h at 37°C. After incubation, the media containing MTT was removed, and the cells were washed with 200 µL PBS. The formazan crystals were dissolved in 100 µL of DMSO, and the resulting purple-blue color intensity was measured by absorbance at 570 nm (Anjaneyulu et al., 2016).

For the scratch wound healing experiment, L929 cells were plated in twelve-well plates (Tarsons, India) at a density of 1×10^5 cells per well (Kanimozhi et al., 2022). Once the cells formed a monolayer, a sterile 200 µL pipette tip was used to create a scratch. Each well was then treated with a 500 µg/mL chitosan solution and

incubated at 37°C. Images of the wound areas were taken at 0, 24, and 48 h using an OPTIKA IM-3FL4 microscope (Optika, Italy). The percentage of wound closure was quantified using the ImageJ tool (Suarez-Arnedo et al., 2020).

2.8 Statistical analysis

The experimental data were statistically analyzed using ANOVA and Student's t-test to compare the variables at the $p<0.05$ level, using SPSS 21.0 software.

3. RESULTS AND DISCUSSION

3.1 Characterization of chitosan

In the present study, the total yield percentage of extracted chitin from *P. indicus* was found to be 13.23 ± 0.32 %. From this extracted chitin, approximately 72 % chitosan was obtained, constituting over 9.33 ± 0.23 % of the shell. Previous studies (Raja et al., 2012; Iber et al., 2021) reported similar compositions of chitosan extracted from crabs and prawns. However, Varma and Vasudevan (2020) reported a much lower composition of chitosan when extracted from the chitin of horse mussels, suggesting that prawns, shrimp, and crabs are the best sources for chitosan extraction.

Chitin yield is typically measured as the percentage ratio of the dry weight of chitin to the dry weight of the source material (Wu et al., 2004). The extraction and physicochemical characterization of chitin and chitosan from various sources, including crustacean shells and insects, have been extensively studied, emphasizing the significance of chitin as a bio-polysaccharide (Ibitoye et al., 2017). Studies have shown that chitin extraction can be optimized using techniques such as ultrasound-assisted extraction, with reported yields of up to 34% achieved within a short duration (Singh et al., 2019). Additionally, alternative sources of chitin and chitosan, such as fungal species and insect exoskeletons, have been explored, demonstrating the potential for sustainable production (Kasongo et al., 2020; Kim et al., 2017). Various methods, including fermentation and the use of deep eutectic solvents, have been investigated to improve chitin extraction efficiency and yield (Xie et al., 2021; Zhang et al., 2024). Furthermore, the application of chitin and chitosan in diverse fields, such as antimicrobial coatings and food preservation, underscores the importance of efficient extraction methods (Zaghloul & Ibrahim, 2019; Troudi, 2022).

SEM analysis revealed a folded structure (Rhim et al., 2006), further confirming the chitosan structure (Figure 2). FTIR analysis showed peaks between 1200 and 1225 cm^{-1}, indicating the presence of ether (O) bonds, which are sharp and strong. Additionally, peaks between 3101.31 and 3348.18 cm^{-1} confirmed the presence of amine (NH_2) bonds, consistent with observations made by Cardenas and Miranda (2004). Further peaks in the range of 1080.06 to 1257.50 cm^{-1} indicated the presence of hydroxyl (OH) bonds (Figure 3). XRD analysis of the sample exhibited prominent peaks at 10° and 20° in 2θ (Figure 4), closely resembling the results found in previous studies on chitosan (Tang et al., 2003; Paulino et al., 2005), confirming the consistency of the findings.

3.2 Cytotoxicity on mouse fibroblast

Figure 5 shows the cytotoxicity assay of extracted chitosan on the mouse fibroblast cell line L929, using different concentrations of chitosan. The results indicated that there was no significant mortality among the tested concentrations. However, significant differences were observed between the positive and negative controls and the extracted chitosan groups, ($p<0.05$; Figure 6). Previous studies reported low cytotoxic effects of chitosan and chitosan nanoparticles on cell lines (Li et al., 2021; Frigaard et al., 2022). In contrast, only 50% of the cells were viable in positive control. Anitha et al. (2009) reported no significant difference in the toxicity of chitosan-based nanoparticles across different concentrations, which aligns with our findings. Furthermore, Frigaard et al. (2022) observed that chitosan exhibits lower cytotoxicity in various animal cell types, irrespective of cell type or chitosan concentration. Similarly, Ye et al. (2013) found that chitosan has low cytotoxicity at short exposure times, with cytotoxicity decreasing further as the exposure time increases, suggesting a potential for cell viability recovery.

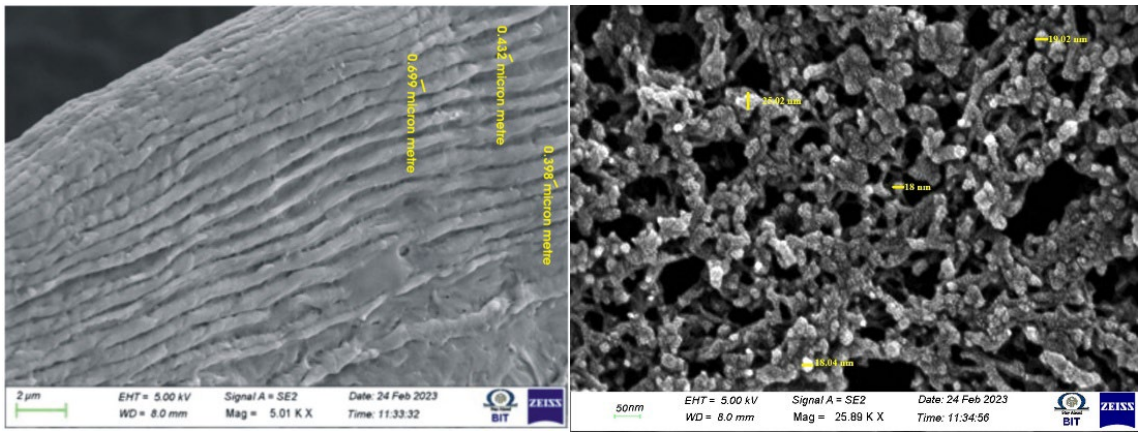

Figure 2. SEM images of extracted chitosan

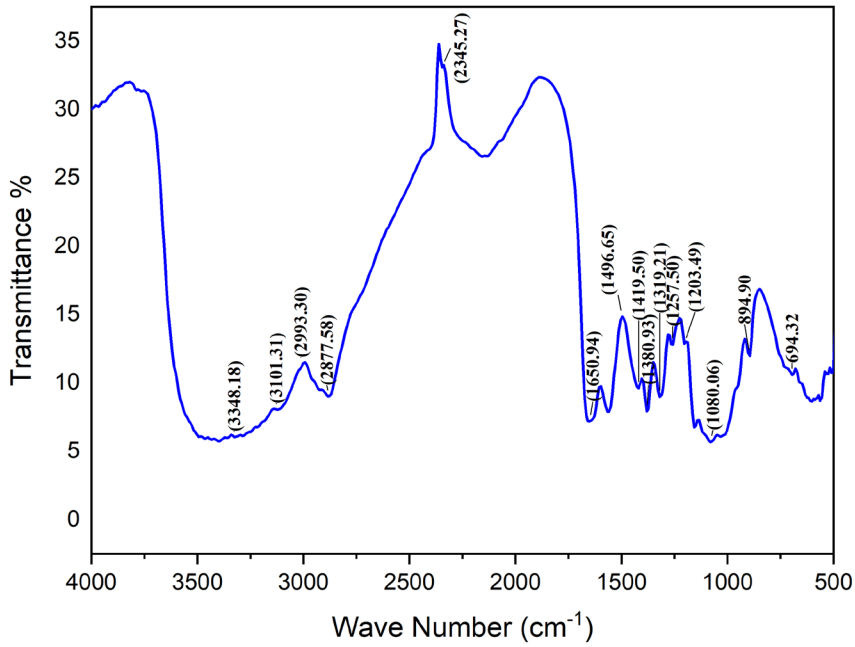

Figure 3. FTIR analysis of extracted chitosan

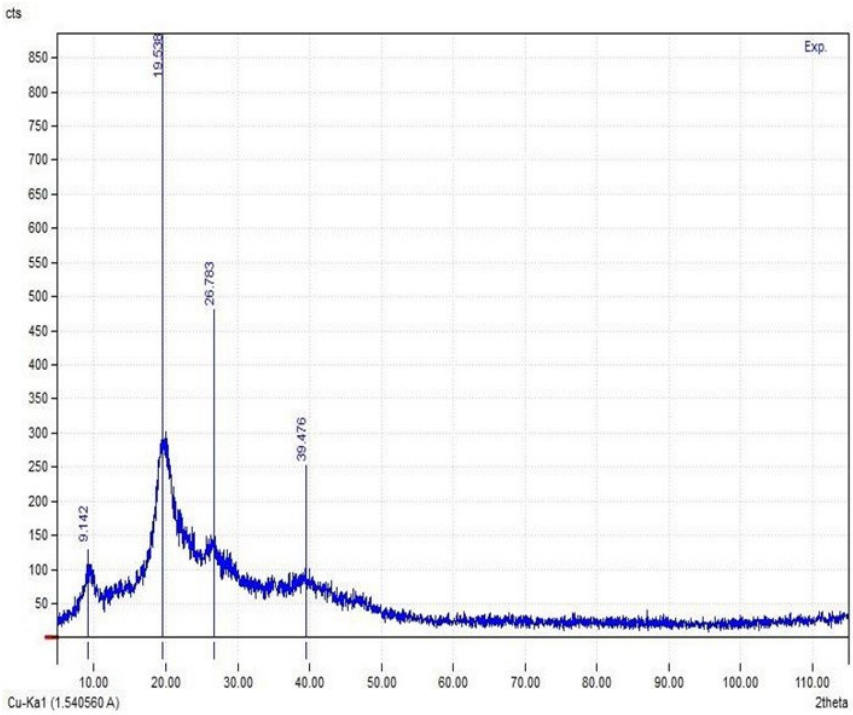

Figure 4. XRD analysis of extracted chitosan

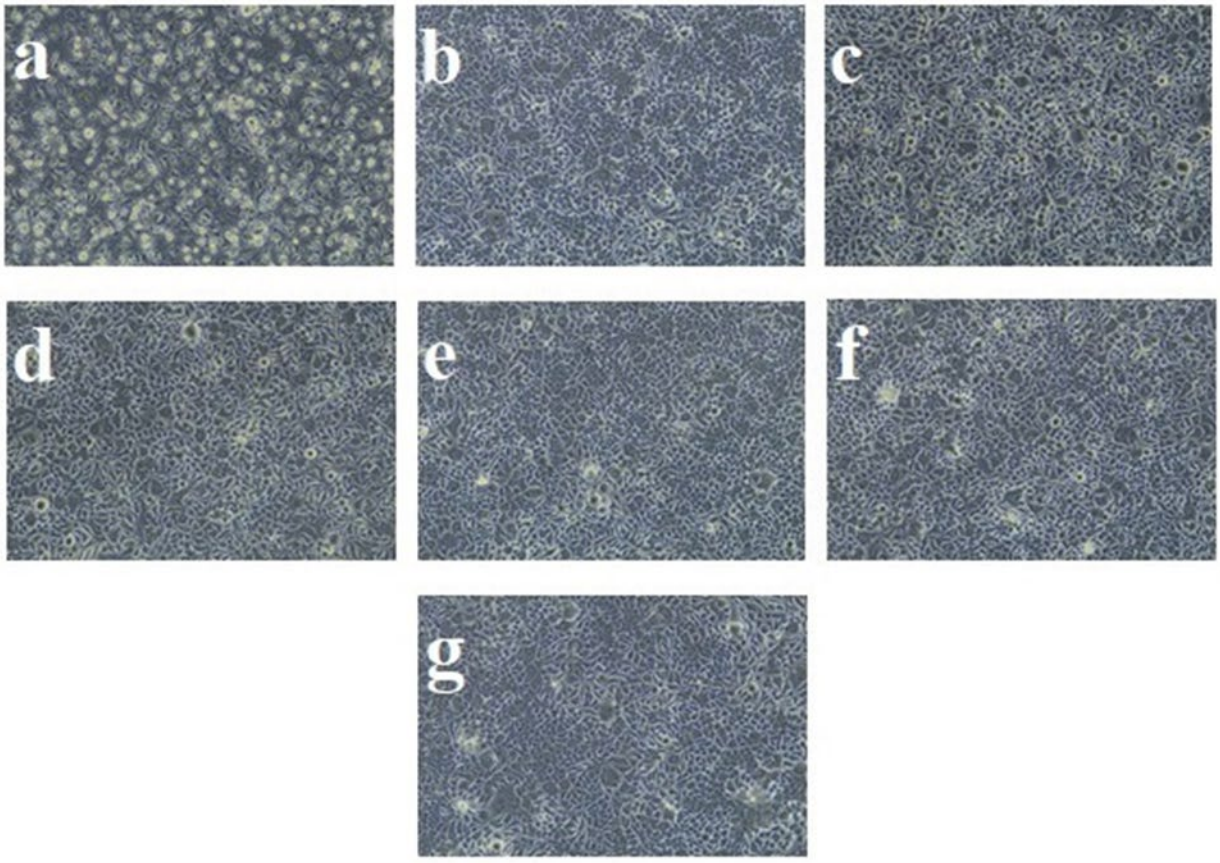

Figure 5. Cytotoxicity assay of extracted chitosan on mouse fibroblast cell line (L929); (a) positive control, (b) negative control, (c) 25 µg/mL, (d) 50 µg/mL, (e) 100 µg/mL, (f) 250 µg/mL, and (g) 500 µg/mL

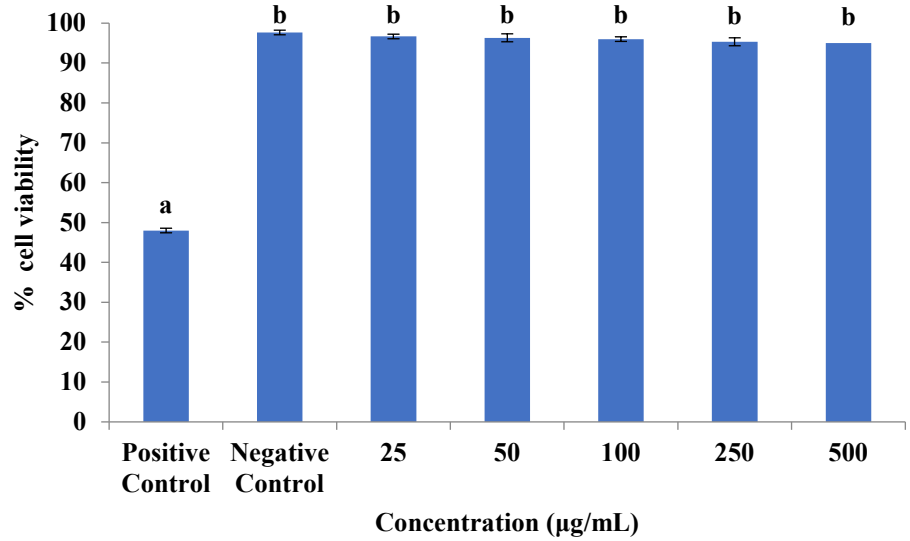

Figure 6. Percentage of cell viability in the mouse fibroblast cell line (L929).
Note: Data are presented as mean ± SD represents from three independent experiments. Statistical analysis was performed using ANOVA followed by Duncan's multiple range test (DMRT). Different superscript letters indicate significant differences between groups at $p<0.05$

Previous research has also demonstrated that chitosan has very low toxicity in cell lines, both in vivo and in vitro (Lim et al., 2018; Mosa et al., 2020; Thai et al., 2020), supporting its potential as an appealing ingredient for wound healing treatments. L929 cells are commonly used in vitro as a sensitive model for evaluating cytotoxicity of biomaterials (Zhang et al., 2019). The MTT assay has commonly been employed to assess the cytotoxicity of chitosan and its derivatives on L929 cells at different concentrations (Reys et al., 2013; Cannella et al., 2020). Additionally, the viability of L929 cells after exposure to chitosan-based structures has also been evaluated using the MTS assay (Mania et al., 2019).

3.3 Wound healing property of chitosan

The scratch wound assay conducted using extracted chitosan nanoparticles on the mouse fibroblast cell line L929 revealed a significant increase in wound closure in the chitosan-treated groups compared to the control groups at 24 and 48 h (Figure 7). Specifically, the percentage of wound closure in the chitosan-treated group was 32% at 24 h and 63% at 48 h. Statistical analyses confirmed that the wound closure percentage was significantly higher in the chitosan-treated groups compared to the control (Figure 8).

Chitosan, a cationic natural polymer, has been extensively studied for wound management due to its hemostatic properties, healing stimulation, antimicrobial activity, and its nontoxic, biocompatible, and biodegradable nature (Dai et al., 2011). Studies have demonstrated that chitosan-based materials, including chitosan metal nanocomposites, enhance wound healing and control infections through antimicrobial mechanisms (Mohandas et al., 2017). Additionally, combining chitosan with substances like silver nanoparticles has been shown to promote wound healing by accelerating re-epithelialization and collagen deposition in vivo (Liang et al., 2016).

Chitosan nanoparticles loaded with epidermal growth factor have also been developed to enhance the stability and biological activity of the factor, thereby improving wound healing efficacy (Montazeri et al., 2023). The application of chitosan in wound dressings is well-supported by its biocompatibility, biodegradability, cellular binding capability, antimicrobial properties, and wound healing potential (Kang et al., 2010). Several studies have shown that chitosan-based nanoparticles significantly improve wound healing and increase cell viability during long-term exposure (Ye et al., 2013; Ahmed & Ikram, 2016; Dai et al., 2011). Ueno et al. (2001) reported that chitosan promotes wound healing by activating inflammatory cells, such as macrophages and fibroblasts, at the wound site. Bagheri et al. (2022) reported the antibacterial and wound-healing properties of chitosan on bacterial wounds. Fahimirad et al. (2021) also demonstrated the potential of combining chitosan-based nanoparticles with natural compounds enhance their efficacy in treating various wound types, attributable in part to their low cytotoxicity.

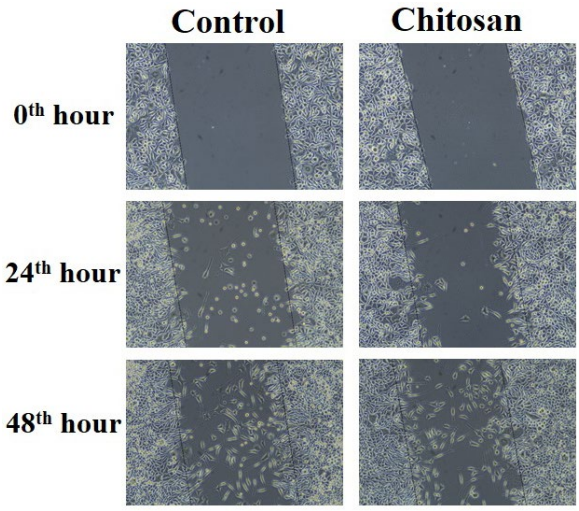

Figure 7. Wound healing property of extracted chitosan on mouse fibroblast cell line (L929)

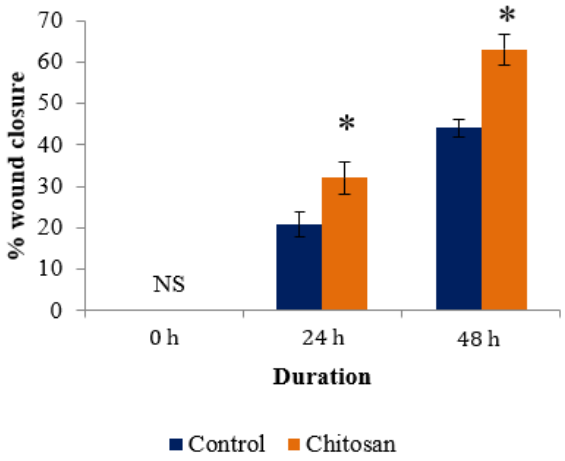

Figure 8. Percentage of wound closure in mouse fibroblast cell line (L929).
Note: Data are presented as mean ± SD represents three independent experiments. *Significantly different at $p<0.05$; NS, not significant ($p>0.05$)

4. CONCLUSION

This study demonstrated that chitosan derived from *P. indicus* shrimp waste is non-toxic to the mouse fibroblast cell line L929 and exhibits significant wound-healing capabilities. The findings revealed a 63% increase in wound closure in the treated fibroblast cell lines after 48 h, highlighting its potential as a valuable biomaterial for wound management. These results underscore the promise of chitosan as a multifunctional medicinal ingredient with profound implications for healthcare and socioeconomic development.

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REFERENCES

Abo Elsoud, M. M., & El Kady, E. M. (2019). Current trends in fungal biosynthesis of chitin and chitosan. *Bulletin of the National Research Centre*, *43*(1), Article 59. https://doi.org/10.1186/s42269-019-0105-y

Ahmad, M., Ahmed, S., Swami, B. L., & Ikram, S. (2015). Preparation and characterization of antibacterial thiosemicarbazide chitosan as efficient Cu(II) adsorbent. *Carbohydrate Polymers*, *132*, 164–172. https://doi.org/10.1016/j.carbpol.2015.06.034

Ahmed, S., & Ikram, S. (2016). Chitosan based scaffolds and their applications in wound healing. *Achievements in the Life Sciences*, *10*(1), 27–37. https://doi.org/10.1016/j.als.2016.04.001

Anitha, A., Rani, V. D., Krishna, R., Sreeja, V., Selvamurugan, N., Nair, S., Tamura, H., & Jayakumar, R. (2009). Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan, O-carboxymethyl and N,O-carboxymethyl chitosan nanoparticles. *Carbohydrate Polymers*, *78*(4), 672–677. https://doi.org/10.1016/j.carbpol.2009.05.028

Anjaneyulu, U., Swaroop, V. K., & Vijayalakshmi, U. (2016). Preparation and characterization of novel Ag doped hydroxyapatite–Fe3O4–chitosan hybrid composites and in vitro biological evaluations for orthopaedic applications. *RSC Advances*, *6*(13), 10997–11007. https://doi.org/10.1039/c5ra21479c

Azad, A. K., Sermsintham, N., Chandrkrachang, S., & Stevens, W. F. (2004). Chitosan membrane as a wound-healing dressing: Characterization and clinical application. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *69B*(2), 216–222. https://doi.org/10.1002/jbm.b.30000

Bagheri, M., Validi, M., Gholipour, A., Makvandi, P., & Sharifi, E. (2022). Chitosan nanofiber biocomposites for potential wound healing applications: Antioxidant activity with synergic antibacterial effect. *Bioengineering & Translational Medicine*, *7*(1), Article e10254. https://doi.org/10.1002/btm2.10254

Basha, R. Y., Kumar, T. S. S., Selvaraj, R., & Doble, M. (2018). Silver loaded nanofibrous Curdlan mat for diabetic wound healing: An in vitro and in vivo study. *Macromolecular Materials and Engineering*, *303*(9), Article 1800234. https://doi.org/10.1002/mame.201800234

Cannella, V., Altomare, R., Leonardi, V., Russotto, L., Di Bella, S., Mira, F., & Guercio, A. (2020). *In vitro* biocompatibility evaluation of nine dermal fillers on L929 cell line. *BioMed Research International*, *2020*(1), Article 8676343. https://doi.org/10.1155/2020/8676343

Cardenas, G., & Miranda, S. P. (2004). FTIR and TGA studies of chitosan composite films. *Journal of the Chilean Chemical Society*, *49*(4), 291–295. https://doi.org/10.4067/s0717-97072004000400005

Dai, T., Tanaka, M., Huang, Y., & Hamblin, M. R. (2011). Chitosan preparations for wounds and burns: Antimicrobial and wound-healing effects. *Expert Review of Anti-infective Therapy*, *9*(7), 857–879. https://doi.org/10.1586/eri.11.59

De Bari, C., Dell'Accio, F., Tylzanowski, P., & Luyten, F. P. (2001). Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis & Rheumatism*, *44*(8), 1928–1942. https://doi.org/10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P

Espinosa-Andrews, H., Sandoval-Castilla, O., Vázquez-Torres, H., Vernon-Carter, E. J., & Lobato-Calleros, C. (2010). Determination of the gum arabic–chitosan interactions by Fourier transform infrared spectroscopy and characterization of the microstructure and rheological features of their coacervates. *Carbohydrate Polymers*, *79*(3), 541–546. https://doi.org/10.1016/j.carbpol.2009.08.040

Fahimirad, S., Abtahi, H., Satei, P., Ghaznavi-Rad, E., Moslehi, M., & Ganji, A. (2021). Wound healing performance of PCL/chitosan based electrospun nanofiber electrosprayed with curcumin loaded chitosan nanoparticles. *Carbohydrate Polymers*, *259*, Article 117640. https://doi.org/10.1016/j.carbpol.2021.117640

Frigaard, J., Jensen, J. L., Galtung, H. K., & Hiorth, M. (2022). The potential of chitosan in nanomedicine: An overview of the cytotoxicity of chitosan based nanoparticles. *Frontiers in Pharmacology*, *13*, Article 880377. https://doi.org/10.3389/fphar.2022.880377

Hou, B., Qi, M., Sun, J., Ai, M., Ma, X., Cai, W., Zhou, Y., Ni, L., Hu, J., Xu, F., & Qiu, L. (2020). Preparation, characterization and wound healing effect of vaccarin-chitosan nanoparticles. *International Journal of Biological Macromolecules*, *165*(Part B), 3169–3179. https://doi.org/10.1016/j.ijbiomac.2020.10.182

Iber, B. T., Kasan, N. A., Torsabo, D., & Omuwa, J. W. (2021). A review of various sources of chitin and chitosan in nature. *Journal of Renewable Materials*, *10*(4), 1097–1123. https://doi.org/10.32604/jrm.2022.018142

Ibitoye, E. B., Lokman, I. H., Hezmee, M. N. M., Goh, Y. M., Zuki, A. B. Z., & Jimoh, A. A. (2017). Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials*, *13*(2), Article 025009. https://doi.org/10.1088/1748-605x/aa9dde

Kang, Y. O., Yoon, I.-S., Lee, S. Y., Kim, D.-D., Lee, S. J., Park, W. H., & Hudson, S. M. (2010). Chitosan-coated poly(vinyl alcohol) nanofibers for wound dressings. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *92B*(2), 568–576. https://doi.org/10.1002/jbm.b.31554

Kanimozhi, C., Ganesan, S., Gokila, G., & Manimekalai, R. (2022). *In vitro* evaluation of cytotoxicity, anti-inflammatory and wound healing properties of otolith stones from pomadasys maculates. *Journal of Materials Science*, *57*(25), 11899–11910. https://doi.org/10.1007/s10853-022-07385-4

Kasongo, K. J., Tubadi, D. J., Bampole, L. D., Kaniki, T. A., Kanda, N. J. M., & Lukumu, M. E. (2020). Extraction and characterization of chitin and chitosan from *Termitomyces titanicus*. *SN Applied Sciences*, *2*(3), Article 406. https://doi.org/10.1007/s42452-020-2186-5

Keong, L. C., & Halim, A. S. (2009). *In vitro* models in biocompatibility assessment for biomedical-grade chitosan derivatives in wound management. *International Journal of Molecular Sciences*, *10*(3), 1300–1313. https://doi.org/10.3390/ijms10031300

Klinkesorn, U. (2013). The role of Chitosan in emulsion formation and stabilization. *Food Reviews International*, *29*(4), 371–393. https://doi.org/10.1080/87559129.2013.818013

Kim, M., Song, Y., Han, Y. S., Jo, Y. H., Choi, M. H., Park, Y., Kang, S. H., Kim, S., Choi, C., & Jung, W. (2017). Production of chitin and chitosan from the exoskeleton of adult two-spotted field crickets (*Gryllus bimaculatus*). *Entomological Research*, *47*(5), 279–285. https://doi.org/10.1111/1748-5967.12239

Kumar, B. S., & Rajasulochana, P. (2021). Synthesis of chitosan nano particle from chitin and its industrial and clinical application. *Annals of the Romanian Society for Cell Biology*, *25*(4), 17484–17489.

Li, Q., Wang, W., Hu, G., Cui, X., Sun, D., Jin, Z., & Zhao, K. (2021). Evaluation of chitosan derivatives modified mesoporous silica nanoparticles as delivery carrier. *Molecules*, *26*(9), Article 2490. https://doi.org/10.3390/molecules26092490

Liang, D., Lu, Z., Yang, H., Gao, J., & Chen, R. (2016). Novel asymmetric wettable AGNPS/chitosan wound dressing: *In vitro* and *in vivo* evaluation. *ACS Applied Materials & Interfaces*, *8*(6), 3958–3968. https://doi.org/10.1021/acsami.5b11160

Lim, C. K., & Halim, A. S. (2010). *Biomedical-grade chitosan in wound management and its biocompatibility in vitro*. Biopolymers. https://doi.org/10.5772/10256

Lim, L. M., Tran, T.-T., Wong, J. J. L., Wang, D., Cheow, W. S., & Hadinoto, K. (2018). Amorphous ternary nanoparticle complex of curcumin-chitosan-hypromellose exhibiting built-in solubility enhancement and physical stability of curcumin. *Colloids and Surfaces B: Biointerfaces*, *167*, 483–491. https://doi.org/10.1016/j.colsurfb.2018.04.049

Liu, R. (1985). Advances in shrimp culture in China. In Y. Taki, J. H. Primavera, & J. A. Llobrera (Eds.), *Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps* (p. 163). Aquaculture Department, Southeast Asian Fisheries Development Center.

Mania, S., Partyka, K., Pilch, J., Augustin, E., Cieślik, M., Ryl, J., Jinn, J., Wang, Y., Michałowska, A., & Tylingo, R. (2019). Obtaining and characterization of the PLA/chitosan foams with antimicrobial properties achieved by the emulsification combined with the dissolution of chitosan by CO_2 saturation. *Molecules*, *24*(24), Article 4532. https://doi.org/10.3390/molecules24244532

Mittal, A., Singh, A., Aluko, R. E., & Benjakul, S. (2020). Pacific white shrimp (*Litopenaeus vannamei*) shell chitosan and the conjugate with epigallocatechin gallate: Antioxidative and antimicrobial activities. *Journal of Food Biochemistry*, *45*(1), Article e13569. https://doi.org/10.1111/jfbc.13569

Mohanasrinivasan, V., Mishra, M., Paliwal, J. S., Singh, S. K., Selvarajan, E., Suganthi, V., & Devi, C. S. (2013). Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste. *3 Biotech*, *4*(2), 167–175. https://doi.org/10.1007/s13205-013-0140-6

Mohandas, A., Deepthi, S., Biswas, R., & Jayakumar, R. (2017). Chitosan based metallic nanocomposite scaffolds as antimicrobial wound dressings. *Bioactive Materials*, *3*(3), 267–277. https://doi.org/10.1016/j.bioactmat.2017.11.003

Montazeri, S., Rastegari, A., Mohammadi, Z., Nazari, M., Yousefi, M., Samadi, F. Y., Najafzadeh, S., & Aghsami, M. (2023). Chitosan nanoparticle loaded by epidermal growth factor as a potential protein carrier for wound healing: *In vitro* and *in vivo* studies. *IET Nanobiotechnology*, *17*(3), 204–211. https://doi.org/10.1049/nbt2.12116

Mosa, I. F., Abd, H. H., Abuzreda, A., Assaf, N., & Yousif, A. B. (2020). Bio-evaluation of the role of chitosan and curcumin nanoparticles in ameliorating genotoxicity and inflammatory responses in rats' gastric tissue followed hydroxyapatite nanoparticles' oral uptake. *Toxicology Research*, *9*(4), 493–508. https://doi.org/10.1093/toxres/tfaa054

Muhamad, A. (2022). Chitosan oligosaccharides as a nanomaterial platform: Biological properties and applications in the biomedical and pharmaceutical fields. *Makara Journal of Science*, *26*(4), Article 2. https://doi.org/10.7454/mss.v26i4.1337

Nawaz, R. M. A., Raiyaan, G. I. D., Sivakumar, K., & Arunachalam, K. D. (2023). Nanomedicine regulation and future prospects. In R. K. Keservani, R. K. Keshawani, & A. K. Sharma (Eds.), *Advances in novel formulations for drug delivery* (4th ed., pp. 67–80). Wiley. https://doi.org/10.1002/9781394167708.ch4

Ngan, L. T. K., Wang, S., Hiep, Đ. M., Luong, P. M., Vui, N. T., Đinh, T. M., & Dzung, N. A. (2014). Preparation of chitosan nanoparticles by spray drying, and their antibacterial activity. *Research on Chemical Intermediates*, *40*(6), 2165–2175. https://doi.org/10.1007/s11164-014-1594-9

Öğretmen, Ö. Y., Karsli, B., & Çağlak, E. (2021). Extraction and physicochemical characterization of chitosan from pink shrimp (*Parapenaeus longirostris*) shell wastes. *Tarım Bilimleri Dergisi*, *28*(3), 490–500. https://doi.org/10.15832/ankutbd.861909

Omar, B. A., Elmasry, R., Eita, A., Soliman, M. M., El-Tahan, A. M., & Sitohy, M. (2021). Upgrading the preparation of high-quality chitosan from *Procambarus clarkii* wastes over the traditional isolation of shrimp chitosan. *Saudi Journal of Biological Sciences*, *29*(2), 911–919. https://doi.org/10.1016/j.sjbs.2021.10.014

Panchal, K., & Desai, M. (2022). Chitin: Production, purification, and characterization. In D. Amin, N. Amaresan, & S. Ray (Eds.), *Biotic elicitors. springer protocols handbooks* (pp. 87–99). Humana. https://doi.org/10.1007/978-1-0716-2601-6_11

Patrulea, V., Ostafe, V., Borchard, G., & Jordan, O. (2015). Chitosan as a starting material for wound healing applications. *European Journal of Pharmaceutics and Biopharmaceutics*, *97*(Part B), 417–426. https://doi.org/10.1016/j.ejpb.2015.08.004

Paulino, A. T., Simionato, J. I., Garcia, J. C., & Nozaki, J. (2005). Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers*, *64*(1), 98–103. https://doi.org/10.1016/j.carbpol.2005.10.032

Percot, A., Viton, C., & Domard, A. (2002). Optimization of chitin extraction from shrimp shells. *Biomacromolecules*, *4*(1), 12–18. https://doi.org/10.1021/bm025602k

Raja, R., Chellaram, C., & John, A. A. (2012). Antibacterial properties of chitin from shell wastes. *Indian Journal of Innovations and Developments*, *1*(S8), 7–11.

Reys, L. L., Silva, S. S., Oliveira, J. M., Caridade, S. G., Mano, J. F., Silva, T. H., & Reis, R. L. (2013). Revealing the potential of squid chitosan-based structures for biomedical applications. *Biomedical Materials*, *8*(4), Article 045002. https://doi.org/10.1088/1748-6041/8/4/045002

Rhim, J., Hong, S., Park, H., & Ng, P. K. W. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *54*(16), 5814–5822. https://doi.org/10.1021/jf060658h

Rodríguez-Vázquez, M., Vega-Ruiz, B., Ramos-Zúñiga, R., Saldaña-Koppel, D. A., & Quiñones-Olvera, L. F. (2015). Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. *BioMed Research International*, *2015*(1), Article 821279. https://doi.org/10.1155/2015/821279

Salari, M., Khiabani, M. S., Mokarram, R. R., Ghanbarzadeh, B., & Kafil, H. S. (2018). Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. *International Journal of Biological Macromolecules*, *122*, 280–288. https://doi.org/10.1016/j.ijbiomac.2018.10.136

Seenuvasan, M., Sarojini, G., & Dineshkumar, M. (2020). Recovery of chitosan from natural biotic waste. In S. Varjani, A. Pandey, E. Gnansounou, S. K. Khanal, & S. Raveendran (Eds.), *Current developments in biotechnology and bioengineering* (pp. 115–133). Elsevier. https://doi.org/10.1016/B978-0-444-64321-6.00006-9

Shafique, M., Sohail, M., Minhas, M. U., Khaliq, T., Kousar, M., Khan, S., Hussain, Z., Mahmood, A., Abbasi, M., Aziz, H. C., & Shah, S. A. (2020). Bio-functional hydrogel membranes loaded with chitosan nanoparticles for accelerated wound healing. *International Journal of Biological Macromolecules*, *170*, 207–221. https://doi.org/10.1016/j.ijbiomac.2020.12.157

Singh, A., Benjakul, S., & Prodpran, T. (2019). Ultrasound-assisted extraction of chitosan from squid pen: Molecular characterization and fat binding capacity. *Journal of Food Science*, *84*(2), 224–234. https://doi.org/10.1111/1750-3841.14439

Suarez-Arnedo, A., Figueroa, F. T., Clavijo, C., Arbeláez, P., Cruz, J. C., & Muñoz-Camargo, C. (2020). An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays. *PLoS ONE*, *15*(7), Article e0232565. https://doi.org/10.1371/journal.pone.0232565

Tang, E., Huang, M., & Lim, L. (2003). Ultrasonication of chitosan and chitosan nanoparticles. *International Journal of Pharmaceutics*, *265*(1–2), 103–114. https://doi.org/10.1016/s0378-5173(03)00408-3

Thai, H., Nguyen, C. T., Thach, L. T., Tran, M. T., Mai, H. D., Nguyen, T. T. T., Le, G. D., Van Can, M., Tran, L. D., Bach, G. L., Ramadass, K., Sathish, C. I., & Van Le, Q. (2020). Characterization of chitosan/alginate/lovastatin nanoparticles and investigation of their toxic effects *in vitro* and *in vivo*. *Scientific Reports*, *10*(1) Article 909. https://doi.org/10.1038/s41598-020-57666-8

Thein-Han, W. W., Kitiyanant, Y., & Misra, R. D. K. (2008). Chitosan as scaffold matrix for tissue engineering. *Materials Science and Technology*, *24*(9), 1062–1075. https://doi.org/10.1179/174328408x341753

Troudi, D. (2022). Effect of invasive blue crab chitosan coating on extending the shelf life and quality of fresh strawberry fruits. *Icontech International Journal*, *6*(2), 1–9. https://doi.org/10.46291/icontechvol6iss2pp1-9

Ueno, H., Mori, T., & Fujinaga, T. (2001). Topical formulations and wound healing applications of chitosan. *Advanced Drug Delivery Reviews*, *52*(2), 105–115. https://doi.org/10.1016/s0169-409x(01)00189-2

Varma, R., & Vasudevan, S. (2020). Extraction, characterization, and antimicrobial activity of chitosan from horse mussel modiolus modiolus. *ACS Omega*, *5*(32), 20224–20230. https://doi.org/10.1021/acsomega.0c01903

Varun, T. K., Senani, S., Jayapal, N., Chikkerur, J., Roy, S., Tekulapally, V. B., Gautam, M., & Kumar, N. (2017). Extraction of chitosan and its oligomers from shrimp shell waste, their characterization and antimicrobial effect. *Veterinary World*, *10*(2), 170–175. https://doi.org/10.14202/vetworld.2017.170-175

Wu, T., Zivanovic, S., Draughon, F. A., & Sams, C. E. (2004). Chitin and chitosan value-added products from mushroom waste. *Journal of Agricultural and Food Chemistry*, *52*(26), 7905–7910. https://doi.org/10.1021/jf0492565

Xie, J., Xie, W., Yu, J., Xin, R., Shi, Z., Song, L., & Yang, X. (2021). Extraction of chitin from shrimp shell by successive two-step fermentation of *Exiguobacterium profundum* and *Lactobacillus acidophilus*. *Frontiers in Microbiology*, *12*, Article 677126. https://doi.org/10.3389/fmicb.2021.677126

Ye, Y., Xu, Y., Liang, W., Leung, G. P. H., Cheung, K., Zheng, C., Chen, F., & Lam, J. K. W. (2013). DNA-loaded chitosan oligosaccharide nanoparticles with enhanced permeability across Calu-3 cells. *Journal of Drug Targeting*, *21*(5), 474–486. https://doi.org/10.3109/1061186x.2013.766885

Zaghloul, E. H., & Ibrahim, H. H. (2019). Comparative study on antimicrobial activity of commercial and extracted chitin and chitosan from *Marsupenaeus japonicus* shells. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(2), 291–302. https://doi.org/10.21608/ejabf.2019.31536

Zhang, J., Tan, W., Wei, L., Dong, F., Li, Q., & Guo, Z. (2019). Synthesis, characterization, and antioxidant evaluation of novel pyridylurea-functionalized chitosan derivatives. *Polymers*, *11*(6), Article 951. https://doi.org/10.3390/polym11060951

Zhang, L., Wang, J., Zhan, B., Deng, Y., & Yan, L. (2024). Dissolving and efficient fractionation of chitin and synchronous preparation of calcium lactate from crayfish shell waste using amino acid-based deep eutectic solvents. *ACS Sustainable Chemistry & Engineering*, *12*(4), 1548–1560. https://doi.org/10.1021/acssuschemeng.3c06594