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Creation and Evaluation of Wound Dressings Based on Alginate for controlled drug release Levofloxacin

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Abstract

Chronic wounds pose a challenge in the healing process and can lead to infections, requiring extended treatment and endangering patients' lives. Developing a drug release system for stable local release at the wound site is a challenge. Chitosan particles were synthesized and optimized for use in wound treatment. Levofloxacin was loaded into the particles and confirmed through FTIR analysis. A Alginate film with varying concentrations of Genipin cross-linking agent was synthesized and evaluated to find the most effective concentration. The Levofloxacin -loaded particles were incorporated into the Alginate substrate to create a system for controlled and extended drug release. The designed system significantly increased the average drug release time and demonstrated effectiveness in providing controlled and extended release of Levofloxacin for the treatment and management of chronic wound infections.

Keyword: Wound Dressing, Hydrogel, Alginate, Drug Delivery

1. Introduction

The study focuses on developing an advanced wound dressing composed of gelatin integrated with chitosan particles [1]. Gelatin and chitosan are chosen for their biocompatibility, biodegradability, and antimicrobial properties. Levofloxacin, a widely used antibiotic, is incorporated into the dressing to enable sustained and prolonged antimicrobial release at the wound site. The wound dressing is characterized for its physical, mechanical (including tensile strength), and structural properties, which are critical for effective wound healing. Swelling behavior of the dressing is assessed to understand its interaction with wound exudate and moisture management [2]. The in vitro release profile of Levofloxacin is evaluated using UV-Vis spectrophotometry to determine release kinetics and efficiency over time. Antimicrobial properties of the dressing are investigated to confirm its effectiveness against wound pathogens. Cytocompatibility tests with skin cells are conducted to ensure the dressing is safe and does not harm healthy tissue. The integration of gelatin and chitosan enhances both antimicrobial activity and cytocompatibility, making the dressing suitable for clinical applications. Incorporation of nanoparticles further improves drug delivery capabilities, ensuring sustained release of Levofloxacin [3]. The research findings suggest that the developed wound dressing can significantly improve wound management by providing controlled drug release and promoting faster healing. Overall, the study contributes to the advancement of wound dressings with enhanced therapeutic efficacy and safety, potentially improving patient outcomes and quality of life. This particle incorporation can improve the efficacy of the wound dressing by maximizing the release of Levofloxacin and minimizing the frequency of dressing changes [4]. This could lead to better patient compliance and improved wound healing outcomes. The controlled release of **Levofloxacin** from the gelatin-chitosan wound dressing can also minimize the risk of antibiotic resistance and adverse reactions. The research focuses on designing a wound dressing based on gelatin loaded with

chitosan particles containing the antibiotic **Levofloxacin** for treating chronic skin wounds. The main goal is to prevent infection in the wound environment, as infection control is a key factor in wound healing failure. The combination of gelatin, chitosan, and **Levofloxacin** for wound dressings is novel and has not been previously reported in terms of both usage and fabrication techniques. There is a current lack of effective wound dressings in the country that can control infection and promote healing in chronic and infectious wounds, highlighting the need for this development. Chitosan is chosen not only as a carrier for Levofloxacin but also for its inherent antimicrobial properties, enhancing the antibacterial effectiveness of the dressing [5]. **Levofloxacin** is selected due to its strong antibacterial activity, particularly effective against infections in chronic wounds such as burns and diabetic ulcers. The chitosan particles loaded with **Levofloxacin** are embedded in a gelatin film to enable controlled, slow, and continuous drug release, aiming to manage wound secretions and infections over an extended period [6]. Chronic wounds require longer treatment due to their severity and depth, necessitating a system capable of sustained infection control and wound healing through regulated drug release. Alginate hydrogel film serves as a suitable substrate for chitosan particles and contributes additional benefits such as antibacterial properties and moisture retention in the wound environment [7]. The hydrogel film's temperature is potentially lower than that of the wound and skin, which may help alleviate patient pain.

2. Materials & Method

Materials used include medium molecular weight chitosan (190,000–310,000 Da, 75–85% deacetylation), medical-grade Type Alginate, ciprofloxacin hydrochloride, genipin, sodium tripolyphosphate, glacial acetic acid, and glycerol. Chitosan solutions were prepared at concentrations of 1%, 2%, and 3% (w/v) in 1% (w/v) acetic acid, resulting in clear and uniform solutions. A 5% (w/v) sodium tripolyphosphate solution was prepared to enhance the functional properties of chitosan. Chitosan droplets were introduced into the tripolyphosphate solution using two methods: plastic syringe injection and electrospray device [8]. The mixture containing particles was stirred magnetically for 1 hour to allow cross-linking of the particles. After cross-linking, the particles were poured into Petri dishes, washed twice with distilled water, and then dried using incubators and freeze dryers. The optimal chitosan concentration was selected to produce spherical chitosan particles. For drug loading, ciprofloxacin (0.06 g) was first dissolved in the water and acid solution before adding chitosan, following the same particle preparation steps. The process successfully incorporated Levofloxacin into chitosan particles, maintaining the particle formation procedure. The electrospray technique was chosen for microparticle preparation due to its cost-effectiveness, uniformity, simplicity, and ability to control particle size and morphology [9]. **Levofloxacin** -loaded chitosan microparticles (ciprofloxacin-CS MPs) were prepared by dissolving chitosan in deionized water at concentrations of 1 wt%, 2 wt%, and 3 wt%, followed by stirring for four hours to ensure homogeneity. Ciprofloxacin (0.06 g) was gradually added to the chitosan solution, which was then subjected to ultrasonic treatment for five minutes to remove gas bubbles. The electrospray process involved pumping the **Levofloxacin** -Alginate solution at 0.5 mL/min into a 5% w/v tripolyphosphate aqueous solution under a 13kV electrical potential difference [10]. The positive electrode was placed at the nozzle tip, and the negative electrode was connected to the collecting beaker, positioned 100 mm from the nozzle.

2.1. Characterization Technique

After making the wound dressing containing drug-carrying particles, various tests were put on the agenda to check the behavior and properties of the obtained samples, which are described below [11].

Scanning Electron Microscope (SEM):
In order to investigate the surface morphology of chitosan particles and choose the optimal concentration of chitosan, after the process of synthesizing particles with different weight percentages, the samples were coated with gold under vacuum in the central laboratory of Tarbiat Modares University of Technology and the laboratory of Islamic Azad University and then examined with a scanning electron microscope, they got [12].

Attenuated Infrared Spectroscopy (ATR-IR):
In order to investigate the interaction of chitosan polymer and gelatin film with each other, the attenuated infrared spectrometry test has been used. ATR-IR of gelatin film and gelatin film containing chitosan particles, each was performed separately in the wavelength range of 4000-650-1 cm and the resulting spectrum was drawn using GraphPad Prism software [13].

2.2. Cell Culture Test

The research involved preparing L929 mouse fibroblast cells from a cell bank and culturing them in RPMI-1640 medium supplemented with fetal calf serum and antibiotics under controlled conditions (37°C, 85% humidity, 5% CO2). After achieving confluency, cells were detached using trypsin, suspended at a concentration of 2 x 10^4 cells/mL, centrifuged, and re-suspended in fresh medium before being incubated. Samples measuring 5 x 5 mm were sterilized using ultraviolet light for one hour to assess cell viability and morphology. These samples were placed in 24-well plates with cell suspensions at 2000 cells/mL, incubated for 48 hours, and then subjected to an MTT assay to evaluate cell viability. The assay involved adding MTT dye, incubating for 4 hours, washing, dissolving the formazan crystals in DMSO, and measuring absorbance at 570 nm using an ELISA reader. Each sample was tested in triplicate, and the average absorbance values were calculated to determine the effect of sterilization on cell viability and morphology [14].

3. Result and Discussion

After studying the influence of different parameters in the synthesis process of chitosan particles, in order to investigate the effect of chitosan concentration, SEM images

were examined before choosing the optimal concentration of chitosan. As shown in pictures 1 (a), (b) and (c), with the increase in chitosan concentration, the surface morphology of the particles has improved; Because the low concentration of the polymer solution reduced the viscosity of the solution, which caused the inability of the viscoelastic force to overcome the surface tension, and particles with a more uneven surface were obtained.

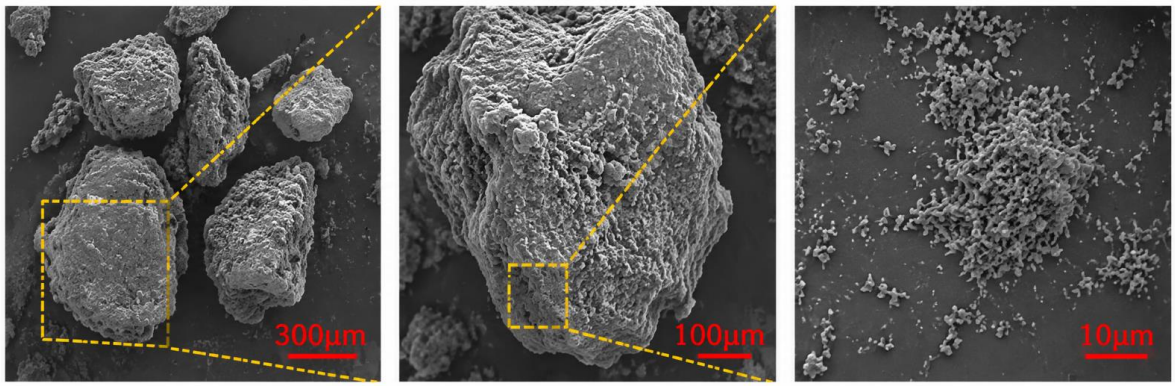

Figure 1: Scanning Electron Microscope Images of Chitosan Particles With 50/100 And 300 Micrometer Magnification

3.1. The Results of Infrared Spectroscopy Analysis (FTIR)

Confirmation of the molecular structure of chitosan using the main bonds of this polymer revealed in the FTIR spectrum is shown in Fig. The index peaks of chitosan particles with absorption at cm-11650, corresponding to the bending vibration of N-H bond of chitosan type II amide, the stretching vibration of C-O-C bonds also appeared in the region of 1030-1060 cm-1. The peaks that appear are consistent with the previous studies done for chitosan. Also, the main bonds of ciprofloxacin have been observed in the FTIR spectrum. The index peak with absorption at cm-11718 shows the stretching vibration of the carbonyl group C=O, the C-F peak is determined with a wave number of cm-1 1016 and the peak in the region of cm-1 1600-1650 is assigned to N-H. As seen in the figure, the main peaks of Levofloxacin appeared in chitosan particles. C=O peak with 1680 g-cm-1 Mo number (stretching vibration) is related to carboxylic acid in Levofloxacin. In addition, the cm-1069 peak, which overlaps with the asymmetric H-O stretch, is related to the stretch of the C-F group. And finally, the overall expansion of the peaks in the range of 3400 cm-1 indicates the hydrogen bond between Alginate and the drug. As a result, the FTIR spectrum clearly shows the drug-particle interaction

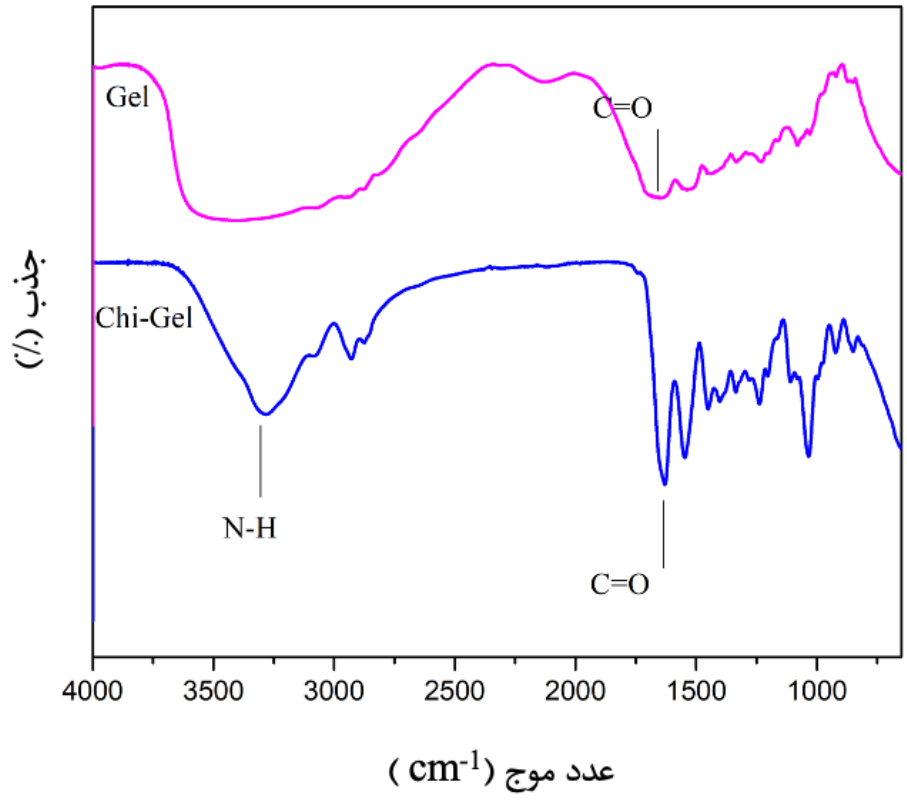

Figure 2: ATIR Transmission Spectrum of Antibiotics Levofloxacin Pure Chitosan Particles

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4. Conclusion

Particles with varying chitosan concentrations were prepared and dried using oven drying and freeze-drying methods. Chitosan at 3% (w/v) concentration was selected as optimal based on electron microscope images showing consistent, uniform surface morphology and particle structure. Freeze-dried particles exhibited a higher drug release percentage (99%) compared to oven-dried particles (96%), attributed to better surface morphology and avoidance of heat-induced drug degradation in freeze drying. Levofloxacin -loaded particles released up to 99% of the drug within less than 48 hours. Antibacterial tests showed a 30 mm inhibition zone, effectively preventing growth of both Gram-positive and Gram-negative bacteria. Cytotoxicity tests indicated high biocompatibility, with up to 96% cell viability after exposure to the particles. Water absorption tests revealed that samples with 0.5% (w/v) chitosan absorbed an average of 279% water, significantly higher than samples with 1% and 2%, important for wound dressing applications to control infection and secretions. Degradation tests demonstrated that gelatin films with 0.5% (w/v) genipin degrade slowly over 20 days, aligning with wound healing timelines and allowing sustained Levofloxacin release. Mechanical strength tests showed that gelatin films with 0.5% genipin have mechanical properties closely matching human skin, making them suitable for wound treatment. The combined system (chitosan particles with drug and gelatin wound dressing) showed enhanced antibacterial activity, with an inhibition zone diameter increasing to 60 mm compared to individual components. Cytotoxicity of the combined system was excellent, with 99% cell viability, surpassing the toxicity profiles of individual components.

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