Antibacterial Activity of Chitosan Solutions for Wound Dressing

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Summary: Chitosan has found wide application in the biomedical field due to its interesting biological properties that include: biocompatibility, biodegradability, hemostatic activity and bacteriostatic effect. In this present study, antibacterial activity of chitosan solutions for wound dressing were investigated against *Staphylococcus aureus* (isolated from an activated sludge) and *Escherichia coli* (ATCC 25922) that are potential wound pathogens. Moreover, the effects of plasticizer addition and chitosan concentration on antibacterial activity of chitosan solutions were also evaluated. According to the antibacterial activity study, chitosan solutions, plasticized or not, showed inhibitory activity against *Escherichia coli*. However, they did not inhibit *Staphylococcus aureus* growth, possibly because this bacterium strain would become resistant due to mutations caused by industrial effluent exposure.

Keywords: antibacterial activity; chitosan; wound dressing

Introduction

Chitosan is a cationic copolymer obtained by alkaline deacetylation of chitin, which is a natural polymer derived from marine crustaceans' exoskeletons. Chitosan exhibits numerous interesting properties, such as anti-tumor, immune-adjuvant, hemostatic and antibacterial activities.[1] Moreover, it is considered as a biocompatible and biodegradable polymer and has been shown to facilitate wound healing.[2]

Due to its interesting biological properties, chitosan has been increasingly used in several medical applications such as drug delivery systems, implants, injections and wound dressing.[3–5] Chitosan solutions have been used for treatment of skin wounds, such as skin ulcers, burns and surgical wounds.

The antimicrobial activity of chitosan has been studied extensively. It has been shown that chitosan acts by disrupting the barrier properties of the outer membrane of Gram-negative bacteria.[6]

In this present study, the effects of chitosan concentration and plasticizer addition on the antibacterial activity of chitosan solutions for wound dressing were investigated. D-sorbitol, a biocompatible polyalcohol commonly used along with chitosan, was added as a plasticizer. Besides, the antibacterial activity of chitosan solutions was evaluated against two potential Gram-negative wound pathogens: *Staphylococcus aureus* and *Escherichia coli*.

Staphylococcus aureus, the most common bacteria to cause skin wound infections,[7] was isolated from an activated sludge. The activated sludge process is a wastewater treatment method in which the carbonaceous organic matter of wastewater provides an energy source for the production of new cells for a mixed population of microorganisms in an aquatic aerobic environment. Bacteria constitute the majority of microorganisms present in activated sludge. Both aerobic and anaerobic bacteria may exist in the activated sludge, but the preponderance of species is facultative, able to live in either the presence of or lack of dissolved oxygen.

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In addition, due to the complex environment found in activated sludge, bacteria may suffer mutation, transformation and recombination.

Escherichia coli ATCC 25922, widely applied for antibiotic susceptibility assays, was used as a control Gram-negative bacterium.[8]

Experimental

Materials

High molecular weight chitosan ($M_w \sim$ 100,000), more than 75% deacetylated, and D-sorbitol were purchased from Aldrich Chemical Company (USA). Glacial acetic acid was purchased from Synth (Brazil).

Staphylococcus aureus was isolated from an activated sludge (collected from a Waste Treatment Plant) by Selective Medium Technique and identified by Molecular Method. *Escherichia coli* American Type Culture Collection (ATCC) 25992 was gently supplied by Dr. Marcelo Brocchi from the Department of Microbiology-Institute of Biology at UNICAMP-Brazil.

Preparation of Chitosan Solutions

Solutions were prepared according to Table 1. Acetic acid 1.0% was used as solvent and D-sorbitol 1.0% as plasticizer.

Chitosan flakes were dissolved in acetic acid aqueous solution. Then, the solution was filtered to eliminate insoluble impurities. Plasticized chitosan solution was prepared by adding D-sorbitol to the filtered chitosan solution and the plasticizer solution was prepared by dissolving D-sorbitol in acetic acid aqueous solution.

Table 1.
Solutions contents and concentrations (w/w).

Solution	Chitosan (%)	D-sorbitol (%)
Ac 1.0% (solvent)	0	0
Ch 0.5%	0.5	0
Ch 1.0%	1.0	0
Ch 1.5%	1.5	0
Ch 1.5%-So 1.0%	1.5	1.0
So 1.0%	0	1.0

Antibacterial Activity Study

Both bacteria, *Staphylococcus aureus* and *Escherichia coli*, were independently grown in nutrient broth for 24 hours at 37 °C. The number of Colony-Forming Units (CFU/ml) was determined by the Serial Dilutions Method. According to this method, $3.0^{*}10^{4}$ CFU/ml of *Staphylococcus aureus* and $2.0^{*}10^{3}$ CFU/ml of *Escherichia coli* were counted.

The antibacterial activity study was carried out by agar diffusion technique: 30 ml of PCA (Plate Count Agar) were added to each Petri dish and then inoculated with 0.1 ml of bacterial solution (*Staphylococcus aureus* or *Escherichia coli*). Wells of 0.7 mm diameter were punched in the center of each Petri dish and 0.1 ml of test solution (Table 1) was applied to each well.

After 24 hours of incubation at 37 °C, bacterial growth's inhibition zones were measured around the wells. This antibacterial activity study was carried out in triplicates.

Results and Discussion

According to antibacterial activity study results presented in Figures 1 and 2, all solutions inhibited *Escherichia coli* growth. It was expected that higher concentration of chitosan would inhibit more bacterial growth. However, Ch 0.5% showed the strongest antibacterial activity, while Ch 1.5% showed the weakest one.

This unexpected behavior can be attributed to the viscosity of chitosan solutions, which increases when chitosan concentration rises. Thus, high viscosity solutions delay chitosan diffusion in agar, while low viscosity solutions permit fast flow.

Plasticizer addition decreases solution viscosity[9] that explains stronger inhibitory activity of Ch 1.5%-So 1.0% when compared to Ch 1.5%. D-sorbitol and acetic acid also showed antibacterial activity against *Escherichia coli*.

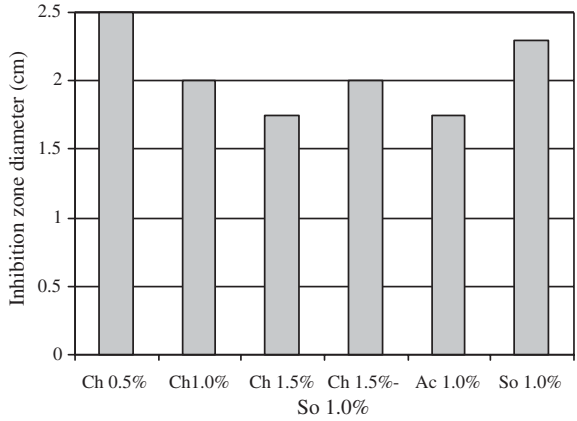

Figure 1.
Inhibition zones diameter (cm) of chitosan, D-sorbitol and acetic acid solutions on *Escherichia coli* growth.

Chitosan kills bacteria through cell membrane damage. It increases the permeability of the outer and inner membranes and ultimately disrupted bacterial cell membranes.[10] These damages are caused by electrostatic interactions between chitosan protonated amino groups and phosphoryl groups of phospholipid components of cell membranes.

For these reasons, it was expected that chitosan solutions would inhibit *Staphylococcus aureus* growth. However, inhibition zones were not observed in Petri dishes inoculated with this bacterium. Probably, this bacterium strain, isolated from an activated sludge, would become resistant to chitosan due to mutations caused by industrial effluent exposure.

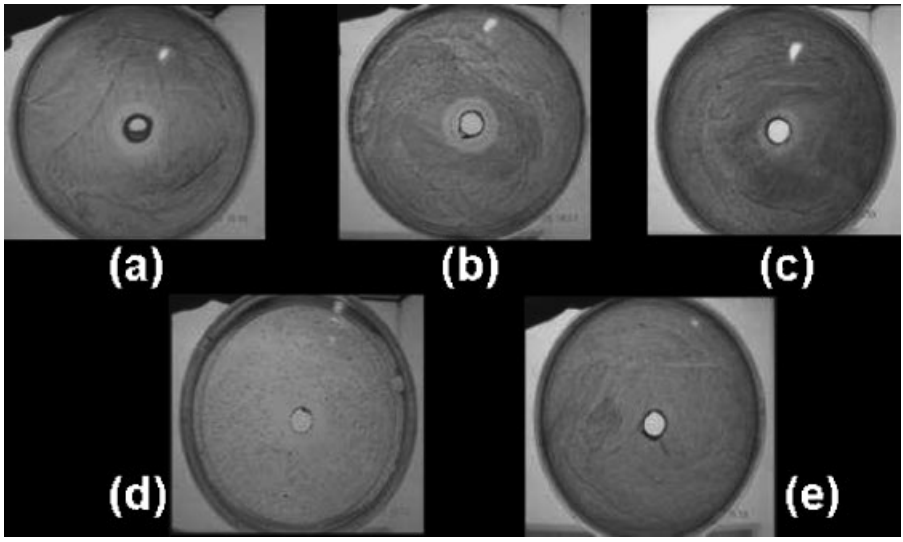

Figure 2.
(a) Ch 1.0% with *Escherichia coli*; (b) Ch. 1.5%-So 1.0% with *Escherichia coli*; (c) Ch 1.5% with *Escherichia coli*; (d) So 1.0 % with *Escherichia coli*; (e) Ch 1.0% with *Staphylococcus aureus*.

Conclusions

Chitosan solutions showed antibacterial activity against *Escherichia coli* and their inhibitory activity was inversely proportional to chitosan concentration. Moreover, D-sorbitol decreased viscosity of chitosan solution and increased its antibacterial activity.

Chitosan did not show inhibitory activity against *Staphylococcus aureus* that probably has became resistant to chitosan due to mutations caused by chemical effluent contact.

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