International Journal of Biological Macromolecules 119 (2018) 166–171

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Carboxymethyl chitosan as an antifungal agent on gauze

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ARTICLE INFO

Article history:
Received 30 January 2018
Received in revised form 8 July 2018
Accepted 10 July 2018
Available online xxxx

Keywords:
Antifungal activities
Carboxymethyl chitosan
Gauze

ABSTRACT

Chitosan is a biopolymer that has antifungal activity against *C. albicans*. Chemical modification of chitosan can provide it with new functional properties for a wide range of biological and biomedical applications. Carboxymethyl chitosan is a derivative of chitosan obtained by etherification of alkaline chitosan with monochloroacetic acid. Carboxymethyl chitosan has a higher solubility than chitosan; therefore it is more readily applicable for use in various fields. Chitosan also has antifungal activity against *C. albicans*. This study evaluated carboxymethyl chitosan as a gauze-coating material to be used for its antifungal properties. This study also optimized the coating process. Gauze was coated with carboxymethyl chitosan then characterized by Fourier Transform Infra-Red Spectrophotometer (FTIR), X-ray diffraction and scanning electron microscopy (SEM). The antifungal activities of gauze-coated samples were then tested by the diffusion method. The results show that the optimum conditions for the process of coating gauze with carboxymethyl chitosan are dipping ten times at a concentration of 1% for 50 s. Antifungal activities of carboxymethyl chitosan-coated gauze as measured by the diameter of the growth inhibition area are 0.30 cm higher than chitosan-coated gauze, which has a growth-inhibition diameter of 0.12 cm.

1. Introduction

Candida causes infections in immunocompromised people and in the general population when the natural microbiota is altered [1]. Among *Candida* species, *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida kruisei*, *Candida scotti*, and *Candida guilliermondii* are the most prevalent ones [2]. *C. albicans* lives as saprophytes within the mouth, intestine, and vagina. *C. albicans* is the most common species of human *Candida* pathogen and can cause both mucosal and deep-tissue infections [3].

Fabrics are essential in human life, in both industrial and medical applications [4]. Fabric with antifungal activities may prevent skin disease caused by *C. albicans* fungi. Prior research [5] on the antibacterial activity of chitosan-coated fabric and [6] on the antifungal activity of chitosan on *C. albicans* showed that the antifungal characteristics of chitosan and its derivates can be applied to fabric, especially gauze. Gauze acts as an absorbent for blood, plasma, and other liquid [7]. Gauze has a texture similar to sanitary napkins. However, gauze cannot protect from microorganism infection and contamination [7]. Addition of an antimicrobial agent serves to slow the growth of microbes on the fabric surface [4].

Chitin is a major structural component of crustacean shells and arthropods and is primarily obtained as a byproduct of the fishing industry [1]. Chitosan is a β-1, 4-D-glucosamine polymer derived from the alkaline *N*-deacetylation of chitin [8]. Chitosan is a polymer that has antimicrobial activity against fungi, yeast, and bacteria [1]. Chitosan was highly active against *C. albicans* [9] and can potentially be used as an active anti-Candida agent capable of acting upon *C. albicans* infections [1]. Chitosan acts on plasma membranes and fungal cell walls, chelating trace metals and inhibiting mRNA synthesis [8]. Chitosan is commonly used as an antimicrobial agent and blended with other polymer films to produce antimicrobial films [10]. Chitosan has attracted much attention from researchers due to its many biological functions. However, the use of chitosan as a coating is limited by the low water solubility at neutral pH [11].

Chemical modification can provide a new function for different biologic and biomedical applications of chitosan. Carboxymethyl chitosan is the carboxymethylated soluble form of chitosan, carrying a glycine amino acid on most of its glucosamine units [12]. Carboxymethylation can change chitosan into a more water-soluble form and provide better functional characteristics [13]. Carboxymethyl chitosan has more prominent antifungal activity than chitosan [14]. The purpose of this study

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https://doi.org/10.1016/j.ijbiomac.2018.07.038
0141-8130/

was to synthesize carboxymethyl chitosan and use it as an antifungal agent on gauze.

2. Materials and methods

2.1. Materials

The materials used in this research were gauze, distilled water, potatoes, jelly, acetate acid (Merck), methanol (Merck), monochloroacetic acid (Merck), and sodium hydroxide (Merck). *Candida* strains were obtained from the collection of the microbiology laboratory and Faculty of Biology at Jenderal Soedirman University in Indonesia. Chitosan was purchased from CV. Chemix Pratama Indonesia with degrees of deacetylation 76.14%.

2.2. Analytical methods

A Fourier transform infrared spectrophotometer (FTIR 8201 Shimadzu), X-ray diffractometer (XRD 6000 3 kW Shimadzu), and scanning electron microscope (SEM JSM-6510LA) were used.

2.3. Preparation and characterization of carboxymethyl chitosan

Preparation of carboxymethyl chitosan was previously reported [15]. Chitosan powder was alkalized using sodium hydroxide 40% (b/v) for 15 min. The mixture was added to monochloroacetic acid at a ratio of 1:7 and stirred for 4 h at 80 °C. The mixture was then neutralized with acetate acid 10% (v/v) and excess 70% methanol. Afterward, the mixture was filtered and washed using methanol. The remaining sediment in the filter was the modified chitosan product. Finally, the precipitate was dried at 55 °C. Characterization of carboxymethyl chitosan includes analysis with FTIR and a solubility test in 0.5% acetic acid.

2.4. Dip-coating optimization

Optimizations included factors such as concentration, dipping time and dipping frequency. Gauze samples with a 1.5 cm diameter were sequentially dipped into carboxymethyl chitosan ten times each in solutions of varying concentrations (0.2, 0.4, 0.6, 0.8 and 1% (w/v)). After dipping, the gauze was air-dried then oven-dried at 100 °C for 3 min and cured at 150 °C for 3 min. The coated gauze was stored in a desiccator. Antifungal activity testing of carboxymethyl chitosan-coated gauze was performed to determine the optimum concentration and frequency of dipping.

Gauze samples with a 1.5 cm diameter were also sequentially dipped into carboxymethyl chitosan solution 1% (w/v) ten times each for various dipping times (30, 40, 50, and 60 s). After dipping, the gauze was air-dried then oven dried at 100 °C for 3 min and cured at 150 °C for 3 min. The coated gauze was stored in a desiccator. Antifungal activity testing of carboxymethyl chitosan-coated gauze was performed to determine the optimum frequency of dipping.

Finally, gauze samples with a 1.5 cm diameter were sequentially dipped into carboxymethyl chitosan solution 1% (w/v) for 60 s, at various frequencies of dipping (8, 9, 10, and 11 times). After dipping, the gauze was air-dried, then oven-dried at 100 °C for 3 min and cured 150 °C for 3 min. The coated gauze was stored in a desiccator. Antifungal activity testing in carboxymethyl chitosan-coated gauze was performed to obtain an optimum time of frequency dipping.

2.5. Antifungal activity testing

A growth media solution for fungus was required before antifungal bioactivity was tested. The liquid PDB (*Potato Dextrose Broth*) and solid PDA (*Potato Dextrose Agar*) used as a media. A robust PDA media was made by diluting 200 g potato, 20 g dextrose, and 15 g agar into 1000 mL distilled water. This solution was then sterilized in an autoclave at 121 °C and 20 psi for 20 min. The process of PBD media production was similar to that for PDA media production, but with additional agar.

The procedure of activity testing was as follows [16]: *C. albicans* fungus was grown in PDB liquid media for 24 h. Two hundred (200) μL of liquid culture *C. albicans* was distributed evenly on top of robust PDA media. Then, the sample coated testing gauze was placed on top of the solid media. Each culture was incubated for 24 h at room temperature with triple treatment. Afterward, the inhibition area (the clear zone around the coated gauze) from each *C. albicans* for every sample solution was measured. The clear zone around the gauze showed the coated gauze had antifungal activity.

The data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range tests ($P < 0.05$) using SPSS 18.0.

2.6. Antifungal activity testing and characterization on the coated gauze

Antifungal activities were also investigated on chitosan-coated gauze in optimum conditions with uncoated gauze as a comparison. The optimum coating conditions were obtained at 1% concentration, a dipping time of 50 s, and a dipping frequency of 10 times. The coated gauze was then characterized by IR and SEM.

The clear zones radiating from carboxymethyl chitosan coating on gauze were viewed with a microscope at 400 times enlargement. The carboxymethyl chitosan-coated gauze and uncoated gauze was characterized by XRD.

3. Results and discussion

3.1. Carboxymethyl chitosan

The reaction of chitosan with monochloroacetic acid in alkaline conditions will produce carboxymethyl chitosan. Chitosan powder was alkalized with sodium hydroxide. Na^+ ions from sodium hydroxide react with chitosan to form alkaline chitosan. Chitosan that binds the ion Na^+ reacts with monochloroacetic acid. Chitosan, which releases Na^+ ions, will be reactive towards the carboxyl group from monochloroacetic acid to form carboxymethyl chitosan. Based on the research performed, the yield of carboxymethyl chitosan was 22.68%.

The results from the synthesis of carboxymethyl chitosan were evaluated using FTIR. Carboxymethyl chitosan has a carboxylate group with characteristic function within chitosan. FTIR identification of carboxymethyl chitosan was observed via the presence of this characteristic group. Therefore, the characteristic group may predict whether

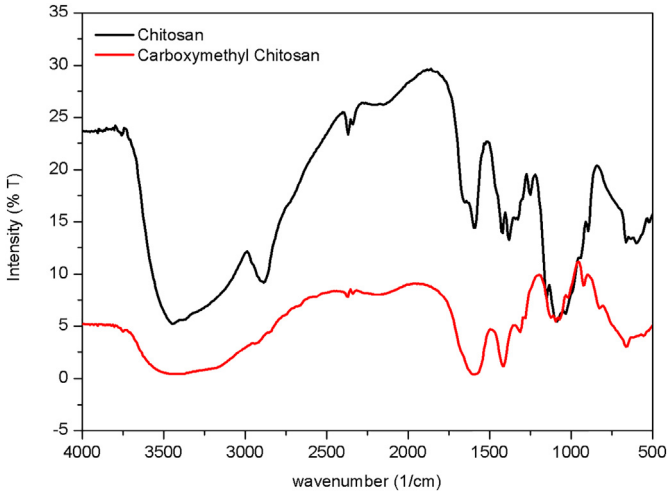

Fig. 1. IR spectra of chitosan and carboxymethyl chitosan.