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Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery

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Abstract

Topical delivery of antimicrobial agents is the most widely accepted approach aimed at prolonging active drug concentrations in the oral cavity. As most antifungals do not posses inherent ability to bind to the oral mucosa, this is best achieved through improved formulations. Chitosan, a partially deacetylated chitin, which is a biologically safe biopolymer, prolongs the adhesion time of oral gels and drug release from them. Chitosan also inhibits the adhesion of *Candida albicans* to human buccal cells and has antifungal activity. The antifungal agent, chlorhexidine gluconate (Chx), also reduces *C. albicans* adhesion to oral mucosal cells. The aim of this study was to design a formulation containing chitosan for local delivery of Chx to the oral cavity. Gels (at 1 or 2% concentration) or film forms of chitosan were prepared containing 0.1 or 0.2% Chx and their in vitro release properties were studied. The antifungal activity of chitosan itself as well as the various formulations containing Chx was also examined. Release of Chx from gels was maintained for 3 h. A prolonged release was observed with film formulations. No lag-time was observed in release of Chx from either gels or films. The highest antifungal activity was obtained with 2% chitosan gel containing 0.1% Chx. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Chitosan; Chlorhexidine gluconate; Oral mucosal delivery; Antifungal

1. Introduction

A major difficulty for the successful eradication of fungal infections of the oral cavity is the dilution and rapid elimination of topically applied drugs due to the flushing action of saliva. The delivery system in which the drug is incorporated is therefore an important consideration and should be formulated to prolong retention of the drug in the oral cavity.

Various bioadhesive polymers like polyacrylic acids, e.g. Carbopol®934 and polymethyl–methacrylate used in gel forms prolong the residence time on oral mucosa (Ishida et al., 1983; Gandhi et al., 1994). The use of bioadhesive gels

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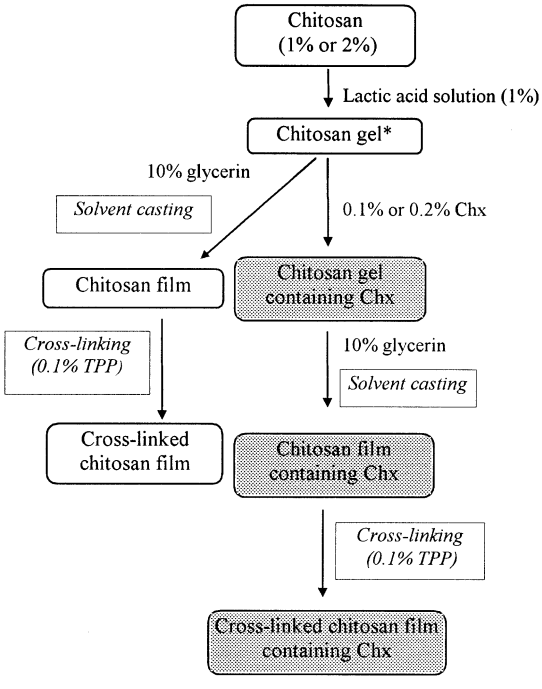
may also reduce the frequency of application and the amount of drug administered, which might improve patient compliance and acceptance. Chitosan is biologically safe and has been proposed as a bioadhesive polymer for oral mucosal delivery (Knapczyk, 1994; Miyazaki et al., 1994). Studies showed that chitosan hydrogels prolong both retention times on the oral mucosa and drug release from gels (Needleman et al., 1997). In addition to its bioadhesive property, chitosan inhibits the adhesion of *Candida albicans* to human buccal cells, and thus helps to prevent the development of mycosis (Knapczyk et al., 1992).

In this study chlorhexidine gluconate (Chx) was chosen as the candidate drug. It is used widely in clinical dental practice as an antiseptic oral rinse due to its activity against a wide range of microbial species (Salem et al., 1987). Chx has anti-Candida properties (Ferretti et al., 1988; Barkvoll et al., 1989; Giuliana et al., 1997) and reduces the adhesion of *C. albicans* to oral mucosal cells (Audus et al., 1992; Darwazeh et al., 1994).



* For preparation of films, chitosan gel at 1% concentration was used.

Fig. 1. Preparation of chitosan gels and films.

The aim of this study was to design a formulation containing chitosan for local delivery of Chx to the oral cavity. Gel and film formulations of chitosan incorporating Chx were developed, and investigated in vitro for release properties and antifungal activities.

2. Material and methods

2.1. Preparation of chitosan gels and films

Chitosan-H (Lot 337) (Dainishiseika Colour and Chem. MGF Co. Ltd, Japan) and chlorhexidine digluconate (Lot 65H0427) (Sigma Chem.Co., St.Louis, MO) were used. For the preparation of films, glycerin was used as a plastisizer at 10% and tripolyphosphate pentasodium salt (TPP) (Sigma Chem. Co., St. Louis, MO) as cross-linking agent at 0.1% (Bolgül et al., 1997). All other chemicals were of analytical grade. A flow diagram illustrating the preparation of gels and films is shown in Fig. 1.

2.2. Film thickness

Thickness of the films were 400 μm which was achieved by using 0.5 g of casting solution per 1 cm^2 of surface area.

2.3. Water absorption capacity

Films were shaken in distilled water at 37°C in a horizontal shaker until maximum weight was reached.

2.4. Viscosity

Viscosity measurements of gels were performed on a Brookfield digital viscometer (Model DV-II, cone-SD1) at room temperature.

2.5. In vitro release of Chx

Release from gels was assessed through dialysis membranes which were placed in continuously-stirred 50 mL volumes of distilled water at 37°C. Release from films was studied using Franz diffu-

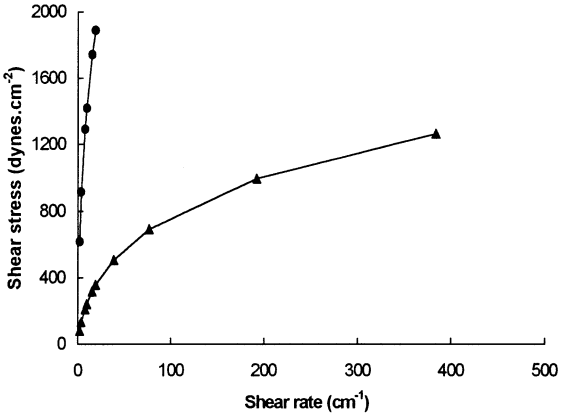


Fig. 2. Flow curves of chitosan gels (▲, 1% chitosan; ●, 2% chitosan).

sion cells with 2.52 cm^2 diffusion area and 20 mL receptor volume. Uniform mixing of the receptor medium was provided by magnetic stirring. Samples of 1 mL were taken from the medium at certain time intervals and replaced with the same amount of distilled water. The samples were filtered and assayed for Chx at 254 nm using a spectrophotometer (UV 160A Shimadzu Spectrophotometer).

2.6. Antifungal activity

A strain of *C. albicans* was isolated from a patient suffering from candidiasis and identified using a Candifast Diagnostic Kit (International Microbio, France). The isolate was subcultured on Sabouraud agar (Difco Laboratories Detroit, MI). Antifungal activities of the formulations were determined by the macrodilution method. *C. albicans* was grown in Yeast Nitrogen Base medium (YNB; Difco Laboratories, Detroit, MI) overnight at 37°C. The suspension of *C. albicans* was diluted with YNB and the final concentration was confirmed by colony forming unit method. A volume of 1 mL of the adjusted inoculum ($2.5 \times 10^3 - 3.5 \times 10^3$ cfu/mL) was added to each tube already containing 1 mL of samples in the dilution series and a positive control tube containing only YNB. The tubes were incubated at 35°C for 18–24 h. After incubation, 0.1 mL of each visual turbidity tube was subcultured to blood sheep agar. All plates were incubated at 35°C for 18–24 h. The MIC values for chitosan gel and Chx as well as their combinations were determined after 18–24h incubation at 35°C.

2.7. Morphological characteristics of chitosan films

Morphological characteristics of free and cross-linked films were studied using Scanning Electron Microscopy (SEM). Film samples were mounted on metal stubs with double-sided adhesive band and then sputtered with a 100 Å thick layer of gold in a BIO-RAD apparatus (England). The samples were examined in a Jeol Scanning Electron Microscope (SEM ASID-10, Japan) at an acceleration voltage of 80 kV.

3. Results

3.1. Water absorption capacity

The films were found to have high water absorption capacity showing $180 \pm 10\%$ uptake, and presence of Chx did not cause any changes.

3.2. Viscosity

It was seen from the flow curves (Fig. 2) that chitosan gels exhibit pseudoplastic flow, and viscosity increases significantly with increasing chitosan concentration. Incorporation of Chx into the gels did not change the viscosity.

3.3. In vitro drug release

Maximum Chx release from chitosan gels occurred after 1.5 h (Fig. 3). Increasing the chitosan concentration from 1 to 2% resulted in an increase in Chx release ($p < 0.05$). With the 1% gel formulation, $88 \pm 5\%$ and $77 \pm 9\%$ of Chx was released after 1.5 h from the formulations containing 0.1 and 0.2% Chx, respectively. For 2% chitosan gels, the percent release was $93 \pm 2\%$ and $92 \pm 5\%$, respectively. Changing Chx concentration (0.1 and 0.2%) had no affect on released percent both from 1 and 2% gel formulations ($p > 0.05$). The

release of Chx from free films reached a plateau after 1.5 h. The amount of Chx released was decreased with cross-linking (Fig. 4). A total of 37% of Chx was released from the free film containing 0.1% Chx in 1 h, whereas with the cross-linked film, 14% was released after the same time ($p < 0.05$). When 0.2% Chx was incorporated into films, the percent released from free and cross-linked films was 35 and 20%, respectively. Cross-linking of the films prolonged the time during which the films remained intact from 3 to 4 h. No lag-time was observed in release either from gels or films.

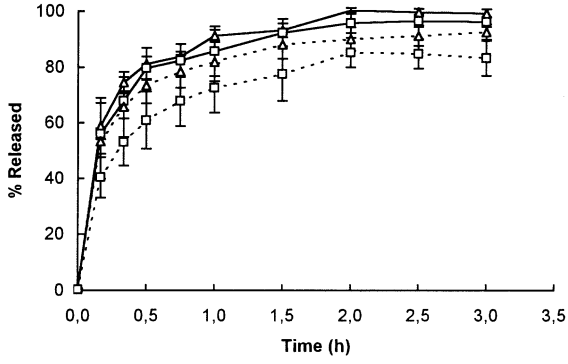


Fig. 3. Release of Chx from chitosan gels containing 0.1% Chx (△), and 0.2% Chx (□); dashed and straight lines indicate 1 and 2% chitosan gels, respectively ($n = 5$).

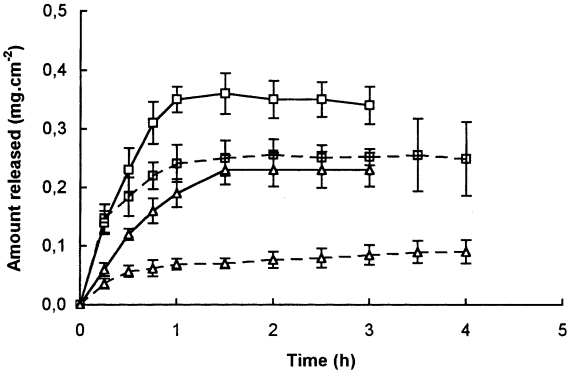


Fig. 4. Release of Chx from chitosan films containing 0.1% Chx (△), and 0.2% Chx (□); dashed and straight lines indicate cross-linked and free films, respectively ($n = 5$).

Table 1
MIC values of chitosan gel with or without Chx for *C. albicans* strains

	MIC (mg/cm^3)
1% Chitosan gel	>10.0
2% Chitosan gel	10.0
0.1% Chx in 1% gel	0.50
0.2% Chx in 1% gel	1.00
0.1% Chx in 2% gel	0.25
0.2% Chx in 2% gel	0.50

3.4. Antifungal activity

The results of microbiological studies indicated that *C. albicans* was susceptible to chitosan itself as well as to Chx. Chitosan gel (2%) gave an MIC of 10 mg/cm^3 of gel (Table 1). The MIC of the 1% chitosan gel was higher than this. For Chx, in solution the MIC value was 1.00 mg/cm^3 and when incorporated into 1 and 2% chitosan gels the MIC values were 0.50 and 0.25 mg/cm^3, respectively. Chitosan gel increased the antifungal activity of the Chx.

3.5. Morphological characteristics of chitosan films

Scanning electron micrographs of free and cross-linked chitosan films are shown in Fig. 5(a and b. With incorporation of Chx into films, a change in surface appearance was observed with formation of cracks (Fig. 6(a and b)).

4. Discussion

Chx is effective as an oral rinse, but in comparison to solutions, gels can significantly prolong residence time in the oral cavity and hence improve therapeutic effect. In this study, gel and film vehicles were prepared using chitosan to deliver Chx into the oral cavity. Due to its bioadhesive property and high viscosity, chitosan gel is expected to remain in the oral cavity and release the drug for a long period of time, thus enhancing the clinical effect. The viscosity of 2% chitosan gel was found to be higher than that of 1% gel which makes it more applicable for topical application without causing any difficulty in spreading. More-

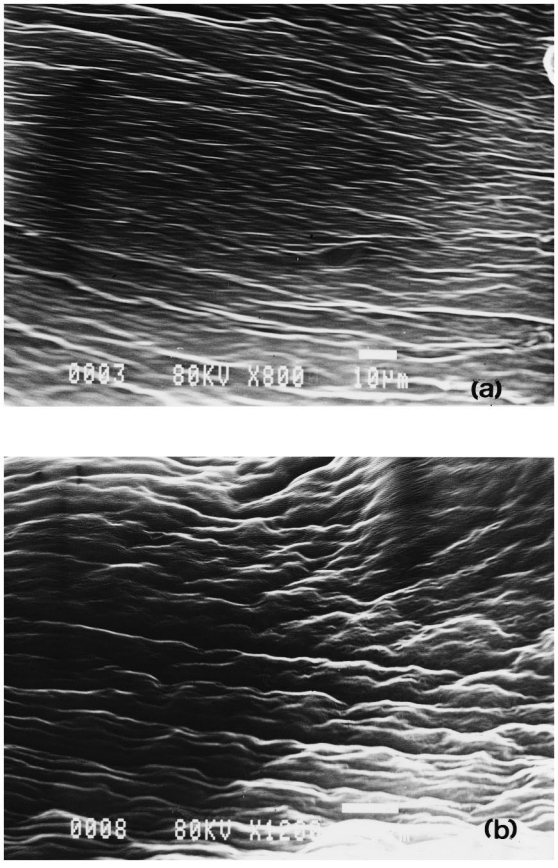


Fig. 5. Scanning electron micrographs of chitosan films: (a) free, and (b) cross-linked.

over, in vitro release results showed that the 2% gel formulation gave higher Chx release for a period of 3 h. No significant difference was observed in release when concentration of Chx was increased from 0.1 to 0.2% indicating that gel formulations would enable application of Chx at lower concentrations. This might be of significant because of the staining effect of Chx on teeth. Gel application might also improve patient compliance by reducing the need for multiple dosing with solutions. In a study which compared the effectiveness of Chx delivered as mouthwash, spray or gel, the gel formulation was found to be significantly more effective than the mouthwash or spray (Francis et al., 1987).

The film-forming property of chitosan finds many applications in drug delivery systems (Muzarelli et al., 1988). Free or cross-linked chitosan films were also prepared containing Chx. Incorporation of Chx did not affect the consistency of either chitosan gels or films. Free chitosan films released Chx for 3 h. Less was released from films compared to gels. Chx release from free films was higher than that from films cross-linked with tripoliphosphate. This difference in release could be due to a change in the porosity of the film after cross-linking. No studies on the porosity of films cross-linked with tripoliphosphate have been reported whereas some studies are present for chitosan films cross-linked with glutaraldehyde (Thacharodi et al., 1993; Tomaszewska et al., 1994). Further studies on porosity of the films are needed for a better

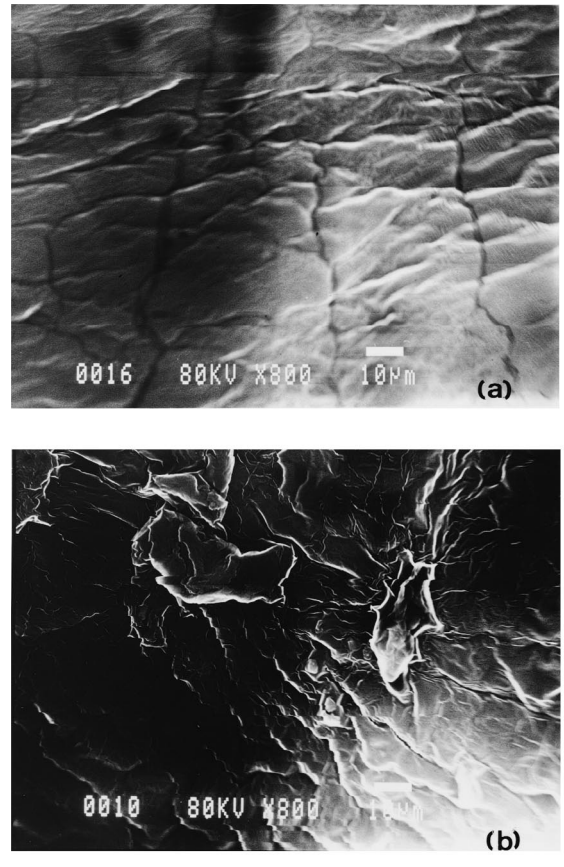


Fig. 6. Scanning electron micrographs of 0.1% Chx incorporated chitosan films: (a) free, and (b) cross-linked (note the cracks).

understanding, as scanning electron micrographs gave no information on porosity. Hydration of the gels was not investigated in this study but water uptake capacities of films were found to be around $180 \pm 10\%$. This would indicate a satisfying bioadhesion, as a direct relationship between the rate of hydration and bioadhesion or retention of chitosan in gel was reported by Needleman et al. (1998).

Chitosan gels were less active than Chx solutions against *C. albicans*. Knapczyk (1994) showed the MIC of 4% chitosan gel on *C. albicans* was 0.1 mg/cm^3. Staroniewicz et al. (1994) has also studied the antifungal activity of chitosan and reported a MIC value of 0.6 mg/cm^3 for *C. albicans*. The MIC values obtained in our study could not be compared with of that study due to the differences in the source of the chitosan used. The antifungal activity of Chx increased with increasing concentration of chitosan in the gels. The highest antifungal activity was obtained with 2% chitosan gel containing 0.1% Chx. This formulation gave a MIC value of 0.25 mg gel/cm^3.

It is concluded that results of these in vitro release and microbiological studies indicate a promising application of Chx at a low concentration (0.1%) incorporated in 2% chitosan gel for candidiasis in the oral cavity. Film formulations gave prolonged release with films remaining intact upto 4 h. This might be advantageous for periodontal therapy.

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