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Natamycin loaded chitosan microspheres for periodontal therapy

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Introduction

Periodontal diseases are inflammatory conditions, affecting the structural organs supporting the teeth. The gingiva becomes detached from the tooth to form periodontal pockets, providing an ideal ecological niche for the proliferation of anaerobic bacteria. The main purpose of periodontal treatment is to cure the inflamed tissue and reduce the number of pathogenic bacteria. Systemic therapy of periodontal diseases with antibiotics and anti-inflammatory agents is inefficient due to low amounts of drug that reach to the site of infection, therefore a local therapy is preferred. The undesirable side effects caused by systemic administration can be reduced as well by delivering drugs locally directly into the periodontal pocket, which minimises the distribution of the therapeutic agents in the body [1].

An ideal formulation for local delivery of drugs into the periodontal pocket should exhibit ease of delivery, a good retention at the application site, and a controlled release [2]. Recently, microparticulate systems have been proposed as potential carriers for local delivery of active substances into the periodontal pocket [3]. As the inflamed peridontium is sensitive tissue, the locally applied delivery system should minimise the patient's pain and comfort and must be retained in the pocket for the desired period. A degradable system introduces a new concept in pharmaceutical dentistry with several advantages over the nondegradable; for example there is no necessity for removal of the system from the periodontal pocket when treatment ends, and it would not be an obstacle during the reattachment of the periodontal tissues to the tooth.

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In dentistry and oral medicine, chitosan offers various applications due to its favorable properties such as biocompatibility and biodegradability besides its antifungal and wound healing activity [4,5]. The aim of this study was to develop a microparticulate drug delivery system for periodontal therapy using chitosan. An antifungal drug, natamycin was chosen as the candidate compound [6].

Materials and Methods

Materials

Natamycin (Yamanouchi), chitosan (Protasan CL212, DA: 73 %, Pronova-Biomedical, Norway), lactic acid and acetic acid (E.Merck, Germany), glutaraldehyde 25% (w/v) aqueous solution, Sigma, USA) were used as provided.

Preparation and characterization of microspheres

Natamycin loaded chitosan microspheres were prepared by emulsion-polymerization method with an initial drug loading of 8% (w/v) (Figure 1) [7].

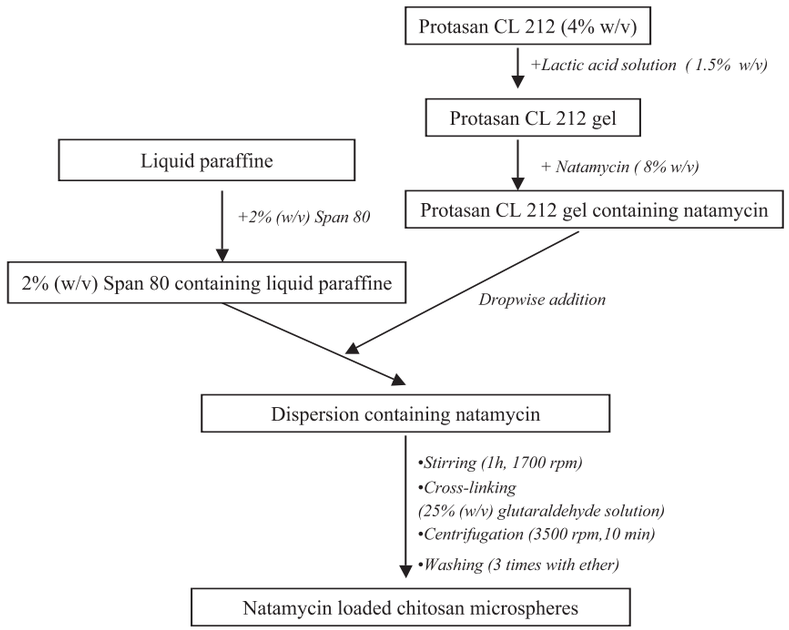

Figure 1
Schematic diagram of preparation of natamycin loaded chitosan microspheres

Particle Size Analysis: It was performed on samples of microspheres suspended in Isoton® using a Coulter-Counter (Coulter Multisizer II, England).

Determination of encapsulation efficiency: 50 mg of chitosan microspheres loaded with natamycin were transferred into dilute acetic acid solution (1% v/v). This mixture was kept in ultrasonic bath until natamycin was completely dissolved (12 h). The concentration of filtered samples was determined spectrophotometrically at 304 nm.

Microscopic evaluation: The samples were examined in a Jeol Scanning Electron Microscope (SEM ASID-10, Japan) at an acceleration voltage of 80 kV. Microspheres were mounted on metal stubs with conductive silver paint and than sputtered with a 150 A° layer of gold in a BIORAD (England) sputter apparatus.

In vitro release studies

The release of natamycin from the microspheres was studied in lactic acid solution (1% w/v) using the USP XXIII rotating paddle apparatus (500 mL, 37 ± 0.5°C, 100 rpm). 50 mg of microspheres was added to the dissolution medium, samples of 5 mL were taken at predetermined time intervals and replaced with fresh medium. The samples were filtered and assayed for natamycin at 304 nm using a spectrophotometer (Shimadzu UV 160 A, Japan). The microscopic evaluation of the microspheres at the end of the release studies was also done.

Results and discussion

Chitosan microspheres with a yield value of 40.77 % were prepared within the particle size range of 10 to 100 μm. The encapsulation efficiency of the drug was 21.8%.

The surface morphology of natamycin loaded chitosan microspheres, using SEM, is shown in Figure 2(a-b). About 50% of the microspheres had a mean particle size of 60.5±10.5μm in size and spherical in overall shape. The surface of the microspheres was also shown to be porous with a rough surface and close inspection of the electron micrographs revealed no drug particles adhering to the surface that had been removed by washing and filtration of the microspheres

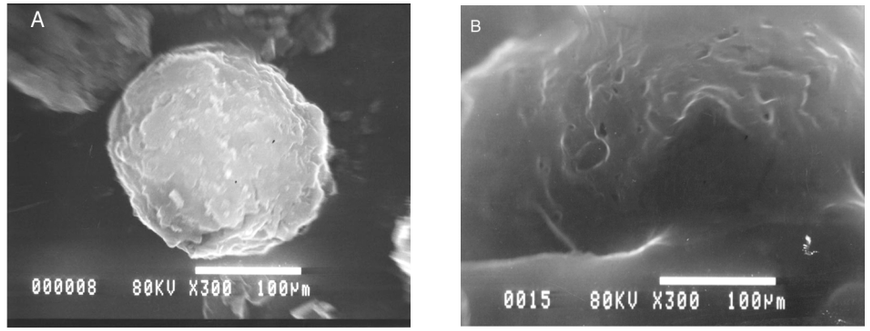

Figure 2
Scanning electron micrographs of natamycin loaded microspheres:
a) the surface; and b) the cross-section.

during the recovery process. Drug loading did not cause any change in the shape, size or surface morphology of the microspheres.

An emulsion-polymerization method has been reported earlier for preparation of chitosan microspheres because it is easy and requires simple equipment[8]. This technique, when applied to the preparation of natamycin loaded microspheres, resulted in 21.8% of drug entrapped in the microspheres. Failure of drug entrapment may be reduced by increasing the polymer concentration in the formulation[9]. Nishioka et al.[10] produced glutaraldehyde crosslinked chitosan microspheres and the encapsulation efficiency increased markedly with an increasing chitosan from 1% to 5%.

The drug content of 21.8% could be sufficient enough for observing the antimicrobial effect of the particles at periodontal pocket. With a particle size of 60.5 µm, the developed microspheres can easily be administered into the periodontal pockets which would allow the release of the drug locally at the site of action. The in vitro release studies indicated that a sufficient amount of drug would be provided in the pocket to obtain an antimicrobial effect.

In vitro release

The percentage release of natamycin from microspheres during 8 h period is summarized in Table 1 and the release profile is shown in

Figure 3. The release kinetic of natamycin from chitosan microspheres by swelling shows a hyperbolic profile. 4.8 ± 1.8% of natamycin is released in the first hour and the maximal release of the incorporated natamycin was 10.8 ± 1.5% in 8h.

Natamycin release from microspheres was studied by simple swelling in lactic acid solution (1% v/v) at pH 2.40. The shape of the chitosan microspheres after swelling is shown in Figure 4(a-b). In general, the slow release from chitosan microspheres is attributed to the extent of swelling and gel-layer formation, which are significantly dependent on the pH of the medium[11,12]. Chitosan microspheres are reported to have a greater swelling and gel layer forming abilities at low pH values than at high pH values[12]. Increased swelling at the low pH would facilitate the movement of drug molecules out of the chitosan microspheres. However, cross-linked chitosan microspheres have rather weak swelling and gel-layer forming properties even at low pH. The cross-linking procedure gives a more sustained release in the release medium because of the denser gel structure after the cross-linking process[7,13]. With the microspheres prepared in our study, a slow release was observed following swelling and gel-layer formation.

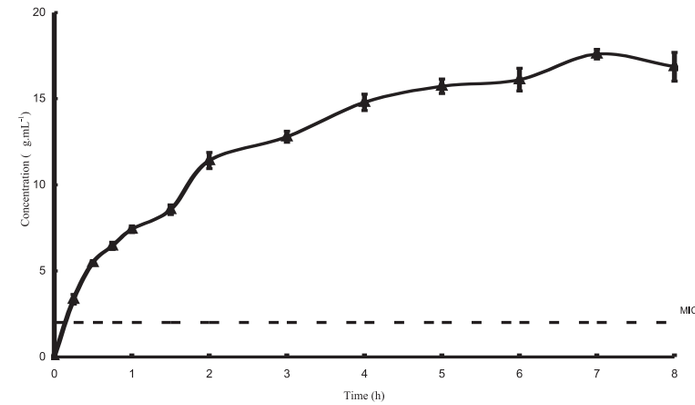

Figure 3
In vitro release profile of natamycin from microspheres in lactic acid solution

TABLE I
Release of natamycin from chitosan microspheres in dilute lactic acid

Time (h)	% Released ± SD* (n=6)
0.5	3.5 ± 1.5
1	4.8 ± 1.8
2	7.3 ± 2.4
4	9.5 ± 2.3
8	10.8 ± 1.5

* SD: Standard deviation

The release of natamycin from the microspheres provided a concentration above its Minimal Inhibitory Concentration (MIC) value (2 μg/mL) within 10 min, and this was maintained during 8 h period at which the dissolution was performed. The MIC value was determined using broth microdilution method in a previous study by Uzunoğlu et al.[14].

The spherical shape of the microspheres remained same as the intact microspheres even after dissolution and a marked swelling was observed in the microspheres (Figure 4a) with an increase in porous structure (Figure 4b). Yet, the increased size of the microspheres due to swelling remained within the acceptable particle size range to stay in the periodontal pocket without giving any discomfort to the patient. Furthermore, chitosan itself has also an additional contribution to the treatment with its antifungal activity besides providing a prolonged delivery.

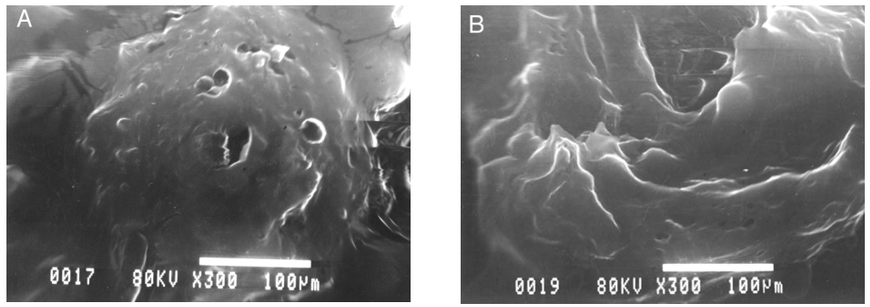

Figure 4
Scanning electron micrographs of natamycin loaded microspheres after 8 h dissolution: a) the surface; and b) the cross-section (note the swelling of the microsphere and the increase in its porous structure)

Conclusion

Microspheres containing natamycin with appropriate physical features and drug content was successfully prepared by using chitosan which provided a drug concentration above its MIC value against *Candida albicans*. The developed formulation can be proposed as a promising system for delivery of antimicrobial agents into periodontal pocket.

Summary

Natamycin Loaded Chitosan Microspheres for Periodontal Therapy

In this study, microspheres for periodontal therapy using chitosan, were developed. An antifungal drug, natamycin was chosen as the candidate compound. Chitosan microspheres with a yield value of 40.77 % were prepared by emulsion-polymerization method. The encapsulation efficiency of the drug was 21.8%. About 50% of the microspheres had a mean particle size of 60.5±10.5 μm in size and spherical in overall shape with a porous and rough surface. A slow release was observed with 4.8% of the drug during the first hour and 10.8% in 8h. The developed formulation which provided a drug concentration above its MIC value against *Candida albicans* can be a promising system for delivery of antimicrobial agents into the periodontal pocket.

Key Words: Chitosan microspheres, Natamycin, Periodontal application, Drug Delivery Systems

Özet

Periodontal Tedavi için Natamisin Yüklü Kitosan Mikroküreleri

Bu çalışmada, kitosan kullanılarak periodontal tedavi için mikroküreler geliştirilmiştir. Antifungal bir ilaç olan natamisin aday bileşik olarak seçilmiştir. Kitosan mikroküreleri %40.77 verim değeri ile emülsiyon-polimerizasyon yöntemi kullanılarak hazırlanmıştır. İlaç tutulma etkinliği %21.8'dir. Mikrokürelerin yaklaşık %50'si 60.5±10.5 μm ortalama partikül büyüklüğüne sahiptir ve poröz, pürüzlü yüzeyli küresel şekillidir. İlk bir saat boyunca %4.8 ve 8 saat'de %10.8 ile yavaş

bir salım gözlenmiştir. *Candida albicans*'a karşı MIC değerinin üzerinde ilaç konsantrasyonu sağlayan bu formülasyon, periodontal cebe antimikrobiyal ilaçların taşınması için ümit vadedici bir sistem olarak önerilmektedir.

Anahtar Kelimeler: Kitosan mikroküre, Natamisin, Periodontal Uygulama, İlaç Taşıyıcı Sistemler

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