DESIGN AND EVALUATION OF THIOLATED CHITOSAN BASED FILMS CONTAINING SPARFLOXACIN FOR PERIODONTAL DISEASES

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ABSTRACT

In the present study, broad spectrum antibiotic sparfloxacin has been incorporated in the thiolated chitosan films for periodontal applications. Drug loaded polymeric periodontal films were further evaluated for various physical parameters like thickness, content uniformity, in-vitro static dissolution, mass balance studies, tensile strength and stability studies. Fourier transform infrared (FTIR) spectral studies indicated absence of inter action between drug and polymer. *In vitro* sparfloxacin release studies were conducted in pH 7.2 buffer solution and treated with various mathematical models to understand release behavior. Clinical studies of the fabricated periodontal strips were also carried out in select number of patients 10 in numbers with symptoms of periodontal diseases. Results indicated sparfloxacin loaded films showed significant gain of attachment along with reduction in probing pocket depths.

KEY WORDS: Periodontal films, thiolated chitosan, gingival index, sparfloxacin.

1.INTRODUCTION

Chitosan apart from being a biopolymer also has an excellent antimicrobial action against a wide microbial flora (Zheng,Zhu,2003). Newer derivatives of chitosan with biodegradable property are used to formulate into different devices and have good mechanical properties (Perugini,2003). Presumptive new generation of mucoadhesive polymers are thiolated polymers designated thiomers (Senel,2000). Thiomers are mucoadhesive basis polymers, which display thiol bearing side chains. Based on thiol/disulfide exchange reactions and/or a simple oxidation process, disulfide bonds are formed between such polymers and cysteine-rich subdomains of mucus glycoproteins (Borchard,2001). Hence, thiomers mimic the natural mechanism of secreted mucus glycoproteins, which are also covalently anchored in the mucus layer by the formation of disulfide bonds. In case of the formation of amide bonds the carboxylic acid group of the ligands cysteine and thioglycolic acid reacts with the primary amino group of chitosan mediated for instance by carbodiimides (Thanou,2000). In a study chitosan–thiobutylamidine conjugate displaying 264 µM thiol groups per gram polymer consequently led to a more than 100-fold improvement in mucoadhesion in comparison to unmodified chitosan (Baumann,2001). Bioadhesive natural polymeric films were effectively used treatment of periodontal applications.

Objectives of the periodontal therapy are to remove the bacterial deposits from the tooth surface and destroy the pathogenic microbial flora (Schwach,2000). Various surgical and mechanical debridement of the root surfaces and /or treatment with systemic or local antibiotics (Vyas,2000). Local delivery of antimicrobial agents is popularly practiced since it leads to higher concentration of the drug at the intended site of action by using a lower dose with least side effects when compared to systemic delivery. Various dental delivery systems such as mucoadhesive tablets, dentrifices mouthrinses, dental gels, fibers, compacts, injectable, semisolid systems, irrigation devices, films, inserts and microspheres are available for the effective treatment (Brackett,2006). Many front-line chemotherapeutic agents have been used against anaerobic bacterias namely porphyromonas gingivalis due to their low inhibitory concentration (MIC) values.

In the present study hence broad spectrum antibiotics like sparfloxacin have been incorporated in the thiolated chitosan films. The natural polymeric periodontal strips/films were further evaluated for various physical parameters like content uniformity, *in-vitro* static dissolution, tensile strength and thickness and FTIR spectral studies. A clinical study of the fabricated periodontal strips was also carried out in select number of patients 10 in numbers with symptoms of periodontal diseases.

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2.Experimental:

Materials

Chitosan was purchased from Sigma Aldrich, USA. Glycerin, polyvinyl alcohol, glacial acetic acid, formaldehyde were purchased from S.D.Fine Chemicals, Mumbai. Clinical studies conducted in the present study were cleared from ethical committee, SDM College of dental sciences, Dharwad.

Preparation of Polymeric Films loaded with Antibiotic drug:

Thiolated chitosan was prepared as per the method explained earlier (Kafedjiiskia,2005). Thiolated chitosan in different ratios was dissolved in 1% glacial acetic acid solution to make 3-5% w/w solution and the resulting polymeric dispersion was stirred for a period of 2-3 hours using a magnetic stirrer. The polymeric dispersion was allowed to stand for some time so as to facilitate the escape of entrapped air. The solution was then transferred to sterile petridishes. The petridishes with cast (Kulkarni,2008) solution were covered with glass funnels with plug of cotton wool in their stems so as to control the rate of drying. After complete drying procedure, the films were cross linked with formaldehyde vapours and subsequently washed with distilled water. The films were further soaked in saturated solutions of drug for almost 1 hour. The films were then taken out and washed with distilled water and dried. The films were further cut 0.5mmX0.5mm size for insertion into the periodontal pocket stored in an air tight container for further use.

Thickness and Tensile Strength:

Thickness of the films was determined using micrometer screw gauge. To determine the tensile strength, one end of the film was clamped at the static end. Another end was attached to a clamp, which was connected to a spring balance. The spring balance was moved to apply pressure on the film till the film was broken to calculate tensile strength.

Clinical Study Protocol

Clinical evaluation studies are to be carried out in healthy human volunteers placing a film(strip) measuring 0.5cmX0.5cm into the periodontal pocket with an intrapocket depth of 5-6mm at several sites anterior to the tooth. A single strip is to be introduced into the pocket with a sterile tweezers. A minimum of 5 patients are selected and their plaque index, gingival index, bleeding index, probing depth. Assessment of pathogenic microbial population is done by gram's staining and fluorescence microscopy. Supragingival and sub gingival plaque samples are obtained pretreatment and at specified intervals after insertion of the strips and suspended in 1 ml of sterile normal saline and slides are prepared.

3.RESULTS AND DISCUSSION

Thiolated chitosan was prepared as per the method explained earlier (Kafedjiiskia,2005) by mixing chitosan with thioglyclic acid for several hours. FTIR spectral studies indicated that new peaks appeared at 2525 cm^{-1} due to S-H stretching vibration of thiol group on chitosan backbone with alterations in the peaks at 1459, 1416 CM^{-1} due to NH substitution. About 3-5% thiolated chitosan containing formulation gave best quality films along with plasticizer and lubricants (Table 1).

Preliminary experiments were conducted to optimize the films. Plasticizers are hydrophilic low molecular weight polymers use to increase the flexibility and elegancy. Glycerin is used to facilitate for easy peeling off from the petridishes. Results of film thickness, content uniformity and tensile strength of all three formulations are shown in Table 2. It was observed that thickness of the film increased with increase in polymer concentration. Drug content was found in between 90-95% w/w found in accordance with thickness of the film thereby increased swelling capacity. The content uniformity of the drug was estimated for all the formulations. For various formulations content uniformity was found to vary between from 0.504 to 0.615 % w/v. Tensile strength range was in the range of 101.2 to 113.3 newtons per square centimeter and in an acceptable range. The percentage cumulative drug retained and cumulative percent drug released by films *in vitro* static dissolution studies were based on the mean content of drug present in the respective film. Among the three formulations prepared, formulation with 4% chitosan gave an extended release of antibiotic drug over a period of 22 days and the same was selected for clinical studies. Results of in vitro dissolution studies are shown in figure 1. 80 % drug were released 10 days for all the formulations.

Clinical evaluations of all the three formulations were done and one implant with drug was placed in the experimental pocket (test side) while another implant without drug was kept in the control pocket. The implants were retained in the pocket without any sutures or periodontal dressings for a period of 21 days. Results

indicated that gingival index at 1st, 7th, 14th and 21st day were 2.9, 2.1,1.62,1.4 and 4.5, 4.3, 3.0, 2.8 for sparfloxacin strips and placebo treated respectively. This clearly indicates the reduction in gingival index in test treated group. Results of probing depth at 1st and 21st days were 6.8 and 2.01mm for test treated where as for placebo treated were 5.02 and 3.12mm respectively. This indicates that there is significant reduction in the probing pocket depth with gain in attachment for chitosan strips loaded with sparfloxacin than the control.

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Table 1: Formulation details of the various thiolated chitosan films loaded with sparfloxacin

Ingredient (% W/V)	F1	F2	F3
Thiolated Chitosan	2-5	3	4
Glacial Acetic Acid	1	1	1
Polyvinyl Alcohol	0.5	0.5	0.5
Glycerine	0.1	0.1	0.1
Drug	0.5	0-5	0-75

Table 2: Results of thickness, content uniformity and tensile strength of various formulations

Formulation	Thickness (mm)	Drug Content (%w/w)	Tensile Strength (N/cm^2)
F1	0.430	0.504	108.0
F2	0.450	0.615	113.3
F3	0.353	0.612	101.2

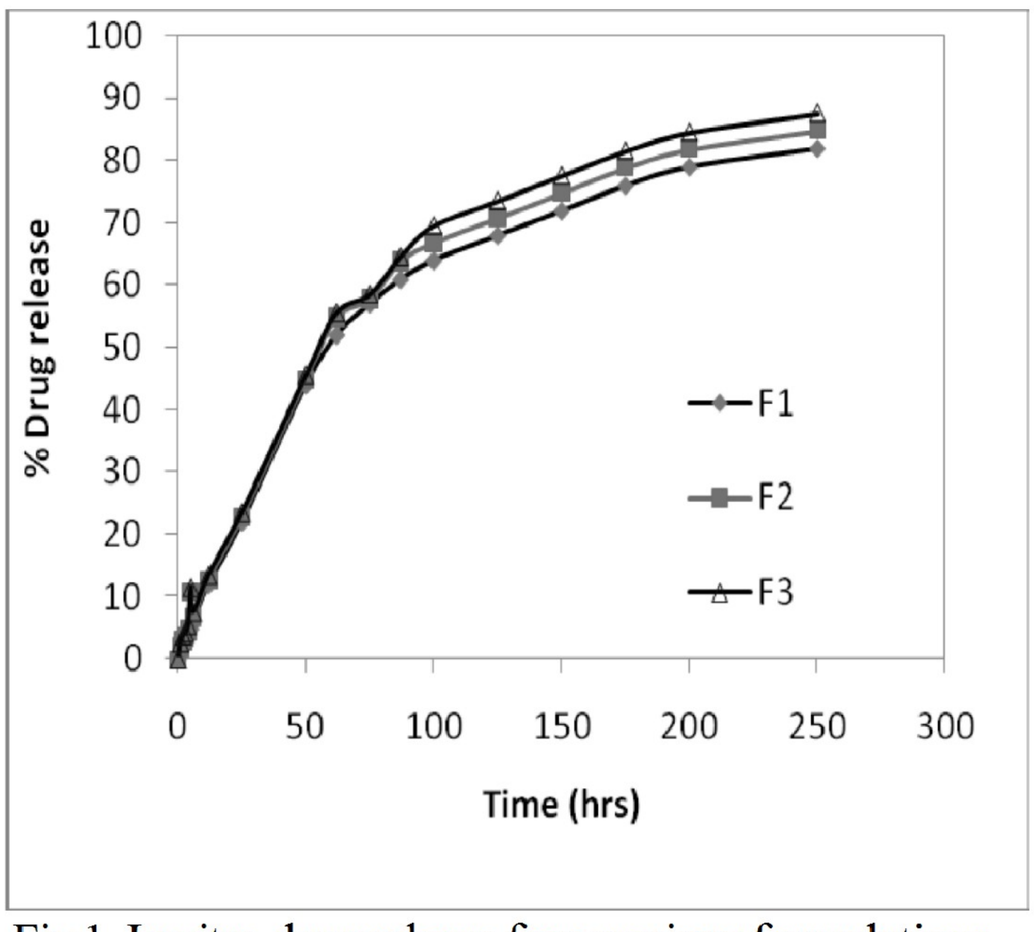

Fig 1. In vitro drug release from various formulations.

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