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DESIGN AND EVALUATION OF CHLORHEXIDINE LOADED PERIOCHIP

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ABSTRACT

Background: Periodontitis is an inflammatory response to bacteriological infections. The main objective of this study was to design a formulation for local delivery of chlorhexidine to the oral cavity for the management of chronic periodontitis. Chlorhexidine is a broad-spectrum biocide effective drug and chitosan is biodegradable polymer having broad spectrum antimicrobial activity are being selected as choice for formulation of periochip. **Methods and Findings:** Drug loaded periochips were prepared by solvent casting method employing mercury as a substrate and evaluated. Formulated periochips were found to be compatible with normal buccal mucosa within an acceptable pH range of 6.5 to 7. The results of drug content indicated that drug is uniformly dispersed into the periochips. The formulation with higher percentage of chitosan showed slower drug release when subjected to comparative *in-vitro* dissolution studies. The selected periochip formulations showed a broad spectrum of *in-vitro* antibacterial activity for an extended period of time. **Conclusion:** Chlorhexidine incorporated periochips was found to be stable, compatible with extended and better treatment of periodontitis having patient compliance.

KEYWORDS: Chlorhexidine, Chitosan, Periodontitis, Periochip.

INTRODUCTION

Periodontitis is a response to bacteriological infections generally characterized by periodontal tissue inflammation. Periodontitis destroys the attachment apparatus of teeth resulting in periodontal pocket formation and alteration of normal osseous anatomy.[1] The effective use of antimicrobial agent for the treatment of periodontal disease requires an adequate drug

concentration at the site of action and a means to maintain the drug levels for sufficient duration to allow drug to act.[2]

The periodontal chip is a pharmaceutical composition applied to periodontal pocket used for treating local periodontal diseases. It may be in the form of sheet, film or bar like formulation which releases an effective concentration of an active ingredient in a controlled manner at the periodontal pocket for the treatment of plaque and inflammation beneath the gingival margin.[3]

Chlorhexidine, a bis biguanide compound, has been shown to possess a broad-spectrum of topical anti-microbial activity. It has been used by dental professionals for plaque control and for the treatment of gingival inflammation. Chlorhexidine was primarily used in mouth rinses and was recommended in the hygiene phase of treatment as an adjunct to tooth-brushing.[4,5,6]

Chitosan is a well-accepted drug delivery carrier which is stable, biodegradable, nontoxic hydrophilic polysaccharide with significant mucoadhesive properties and permeation improving factors.[7]

The purpose of this study was to develop an extended release chlorhexidine loaded periochip comprising chitosan as the main polymer and PEG 200 as a plasticizer for the management and treatment of periodontal diseases.

MATERIALS AND METHODS

Chlorhexidine base was obtained as gift sample from Cadila Pharmaceuticals Limited, India. Chitosan (≤75 deacetylation) and PEG 200 were procured from Himedia. All the other reagents used were of A.R. grade.

Formulation of Periochip

Formulations of periochip containing drug were prepared by solvent casting technique employing mercury as a substrate. The casting solutions were prepared by dissolving appropriate concentration of drug, polymer and plasticizer in suitable solvent using magnetic stirrer at 800 rpm for 20 minutes to get uniform solution. The solution was then transferred quantitatively onto a known dimension circular ring placed over the surface of mercury in a petriplate. Controlled solvent evaporation was achieved by placing an inverted funnel over the petriplate undisturbed at room temperature for a period of 24hrs.[8] The formed films was

then retrieved intact by slowly lifting the rings from the mercury substrate and stored in the desiccators until further use. The composition of formulations are indicated in Table 1.

Table 1: Formulation of chlorhexidine loaded periochip.

Formulation code		CPC 1	CPC 2	CPC 3	CPC 4
Composition of Periochip	Chitosan (%w/v)	1	2	3	4
	PEG 200	10% w/w of polymer			
	Loading dose /Periochip	2 mg/sq. cm.			

EVALUATION STUDIES

Thickness of the film

The thickness of periochip was measured at three different places using a digital micrometer and average values were calculated.[9]

Uniformity of weight of the films

The prepared patches were dried at room temperature before testing. A specified area of film was cut at different parts of the film and weighed in digital balance. The average weights of individual formulations were calculated.[10]

Surface pH

Periodontal films were allowed to swell for 1 hour on the surface of 2% (w/v) agar gel. The surface pH of all the formulated periochip was measured by placing the probe of digital pH meter on the surface of the swollen film.[9,11]

Folding endurance

Folding endurance was determined by repeatedly folding the sample film at the same place till it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.[12]

Tensile strength of the films

The films were cut into periochips (2cm X 2cm). In order to determine the tensile strength, fabricated tensile strength instrument was used. The apparatus consisted of a base plate with a pulley aligned on it. The periochips were fixed to insert holder at one end of base plate and another end was fixed with help of forceps having triangular end to keep the film straight during stretching. A string was tied to the triangular end and passed over the pulley, to which a small pan was attached to hold weights. A small pointer was attached to the thread that

travels over the graph paper affixed on the base plate. The weights were gradually added in increments at definite time intervals to the pan till the films rupture. The weights necessary to break the periochip was noted as break force and tensile strength was calculated by using the formula and expressed in N/mm^2.

Tensile strength (N/m^2) = (load at break) (original width)/(original thickness)

Drug content uniformity of films

Sample films (size of 10 x5 mm^2) was taken from different areas of the formulation and introduced into a 25 ml volumetric flask, to which artificial saliva was added to dissolve and volume was made. Samples was withdrawn, diluted suitably and absorbance measured against blank at the λ_{max} of 259.5nm using UV- Visible spectrophotometer (Shimadzu U600).[2,13]

***In vitro* drug release studies**

Static dissolution method was used to study the drug release from the periodontal chip. Films of known weight and dimensions (size of 10×5 mm^2) were placed separately into small test tubes containing 2 ml of simulated saliva. The test tubes were sealed with aluminum foil and kept at 37°C. The medium was drained off and replaced with fresh 2 ml of simulated saliva during sampling interval. The samples were taken at 2, 4, 6, 8, 24 and 48 hr. The concentration of drug in the medium was measured against blank at the λ_{max} of 259.5nm using UV- Visible spectrophotometer (Shimadzu U600).[3,14,15]

***In vitro* antibacterial activity**

Nutrient agar medium was prepared by weighing 2.8g of nutrient agar, dissolving in distilled water, volume made upto 100ml and sterilized by autoclaving. Under aseptic condition, known volume of agar medium was transferred to sterile petriplate and allowed to solidify. After solidification of nutrient agar medium, a lawn was made with 0.1 ml microorganism i.e. *S.aureus* and *E.coli* in separate petriplates, over that the films were placed and incubated at 37^oC for a period of 48hrs. The zone of inhibition was measured using "Hi Antibiotic Zone Scale".[3,9]

RESULTS AND DISCUSSION

Thickness of the films

The film thickness of each formulation was measured and uniformity in the thickness in different areas of the same film was observed. The mean thickness of CPC 1, CPC 2, CPC 3 and CPC 4 was found to be 0.075± 0.0002, 0.15 ± 0.0008, 0.225 ± 0.0019 and 0.315 ± 0.0007 mm respectively as indicated in Table 2. The mean thickness of the prepared periochip increased with increase in the concentration of polymer concentration. The order of thickness was found to be CPC 4>CPC 3>CPC 2>CPC 1.

Uniformity of weight of the films

Uniformity of weight in periochip cut from same film was observed in all the formulations. The average weights of formulated films of CPC 1, CPC 2, CPC 3 and CPC 4 was found to be 5.8 ± 0.006, 7.6 ± 0.005, 8.5±0.003 and 10.7±0.004 mg respectively (Table 2). As the proportion of the polymer in the formulation was increased, the weight of the periochip also increased.

Surface pH

Surface pH of films was taken into consideration so that the films do not cause irritation to the buccal mucosa. The pH values of CPC 1, CPC 2, CPC 3 and CPC 4 was found to be 6.8 ± 0.13, 6.9 ± 0.42, 6.8±0.76 and 6.8±0.55 respectively (Table 2). The pH values of all the formulations were within the range of neutral pH of the oral cavity and hence no periodontal pocket irritation expected.No significant difference was observed in surface pH for different formulations.

Folding endurance

The values of the folding endurance is been reported in Table 2. It was found to be above 300 for CPC 1 and for other formulations CPC 2, CPC 3 and CPC 4, 271 ± 3.11, 228 ±3.68 and 117 ± 3.84 respectively. The results of the folding endurance showed highest value for formulation CPC 1 in contrast to other formulations due to its lesser polymer concentration and its flexible mobility within the film.

Tensile strength of the films

The tensile strength measures the ability of a periochip to withstand rupture. The tensile strength of the films for CPC 1, CPC 2, CPC 3 and CPC 4 formulations was found to be 6.29 ± 0.032, 2.99 ± 0.028, 2.74±0.053 and 1.52±0.062 N/mm^2 respectively (Table 2). The films

exhibited good physical and mechanical properties. It was revealed that as the concentration of polymer increased in the formulation, there was decrease in tensile strength.

Drug content uniformity of films

Drug content values as indicated in Table 2 for CPC 1, CPC 2, CPC 3 and CPC 4 formulations was found to be 98±0.95, 98.5±0.89, 99±0.87 and 97.5±0.97% respectively. The results percentage drug content of the prepared formulations indicated that the process employed to prepare the films in this study was capable of producing films with a uniform drug content.

Table 2: Evaluation studies of the formulations.

Formulation code	Thickness (mm)	Uniformity weight (mg)	Surface pH	Folding endurance	Tensile strength (N/mm^2)	Drug content (%)
CPC 1	0.075±0.0002	5.8±0.006	6.8±0.13	> 300	6.29±0.032	98±0.95
CPC 2	0.15±0.0008	7.6±0.005	6.9±0.42	271 ± 3.11	2.99±0.028	98.5±0.89
CPC 3	0.225±0.0019	8.5±0.003	6.8±0.76	228 ±3.68	2.74±0.053	99±0.87
CPC 4	0.315±0.0007	10.7±0.004	6.8±0.55	117 ± 3.84	1.52±0.062	97.5±0.97

***average of three readings**

***In vitro* drug release studies**

In vitro drug release profile showed an initial burst release (Figure 1), which is expected to kill most of the periodontal organisms, followed by controlled release, sufficient to inhibit the growth of micro-organisms. At the end of 48 hours the percentage *in-vitro* drug release for CPC 1, CPC 2, CPC 3 and CPC 4 formulations was found to be 72.24, 67.73, 62.29 and 56.85 respectively. The differences of drug release profile may be due to differences in concentration of polymer. The formulation which is having high concentration of chitosan CPC 4 showed the better controlled release of drug in comparison to lower concentration of chitosan. All the formulations showed a prolonged release of drug which aids in treatment of periodontitis.

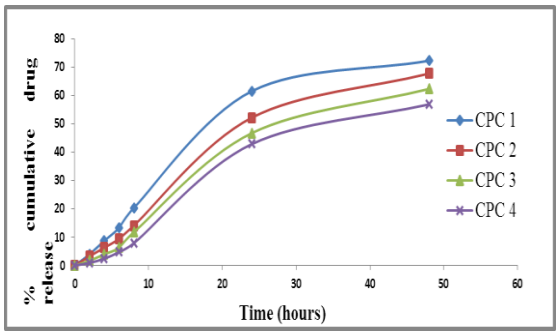

Fig 1: Comparative *in-vitro* drug release profile of Periochip formulations (CPC 1 – CPC 4).

***In vitro* antibacterial activity**

In vitro antibacterial activity was performed using the microorganisms *S.aureus* and *E.coli*. The zones of inhibition values of the prepared formulations are indicated in Table 3 and represented in Figures 2 (a) and (b). The zone of inhibition values for formulations CPC 1 (15mm and 16mm), CPC 2(14mm and 15mm), CPC 3(13mm and 14mm) and CPC 4 (12mm and 12mm) were recorded using gram positive organism *S.aureus and* gram negative organism *E.coli* respectively. The study indicated that the formulated periochip exhibited a broad spectrum of antibacterial activity over a period of time. The zone of inhibition values decreased with increasing concentration of chitosan in the formulation due to controlled drug release of from the film.

Table 3: Antimicrobial studies of prepared periochip formulations.

Formulation code	Zone of inhibition in mm*	
	Staph. aureus	***E. coli***
CPC 1	15	16
CPC 2	14	15
CPC 3	13	14
CPC 4	12	12

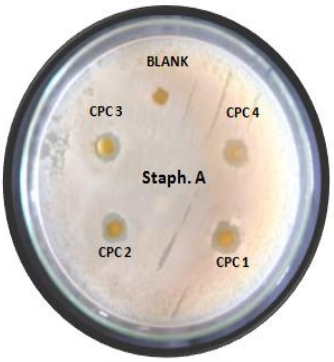

Fig: 2(a)

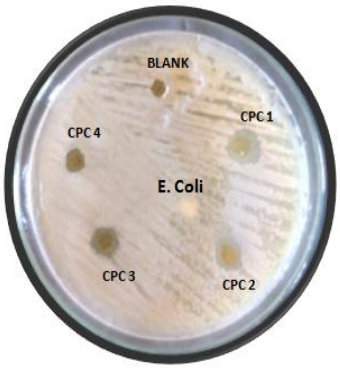

Fig: 2(b)

Fig 2 (a) & (b): *In-vitro* antimicrobial studies of prepared periochip formulations.

CONCLUSION

In the present study, an attempt was made to load chlorhexidine in the polymeric material as a periochip for use in periodontal infections and characterize the prepared films. The films were smooth, homogenous, non-sticky and flexible. The films were capable of inhibiting the growth of *S. aureus* and *E. coli* strains commonly found in periodontal disease. On the basis of various evaluation parameters and *in vitro* characterization, it was concluded that

chlorhexidine incorporated in chitosan chip proved to be a better system for controlled drug delivery in the treatment of periodontitis.

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REFERENCES

1. Belsen DJ, Manavalan R and Parthasarathy V. (Effective antimicrobial therapy of periodontitis – An overview of dental/periodontal chip). International journal of pharmaceutical development & technology, 2011; 1(1): 11-19.
2. Manoj K, Prabhushankar GL and Satheshbabu PR. (Formulation and *in-vitro* evaluation of periodontal films containing metronidazole). International journal of pharmtech research, 2010; 2(4): 2188-93.
3. Sudeep K, Gnanaranjan G and Preeti K. (Periodontal chip: An adjunct to conventional surgical treatment). International journal of drug research and technology, 2012; 2(6): 411-421.
4. Enel SS, Ikinci G, Kas S¸ Yousefi-Rad A, Sargon MF and Hıncal AA. (Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery). International journal of pharmaceutics, 2000; 193: 197–203.
5. Kevin G, Parth J, Shah M, Ramkishan A and Jaydeep P. (Formulation and evaluation of periodontal *in situ* gel). International journal of pharmaceutical investigation, 2013; 3(1): 29-41.
6. Slots J. (Selection of antimicrobial agents in periodontal therapy). Journal of periodontal research, 2002; 37: 389–98.
7. Fouad H Al-Bayaty, Azwin A. Kamaruddin, Mohd. A. Ismail and Mahmood A. Abdulla, (Formulation and evaluation of a new biodegradable periodontal chip containing thymoquinone in a chitosan base for the management of chronic periodontitis). Journal of nanomaterials, 2013: Article ID 397308, 2013.
8. Bharkatiya M, Nema RK and Bhatnagar M. (Designing and characterization of drug free patches for transdermal application). International journal of pharmaceutical sciences and drug research, 2010; 2(1): 35-39.

9. Ananta C, Sujoy D, Satish D, Sumit K and Abhishak K. (Development and characterization buccoadhesive film of ciprofloxacin hydrochloride). International journal of pharmtech research, 2010; 2(2): 1050-57.
10. Prabhushankar GL, Gopalkrishna B, Manjunatha KM and Girisha CH. (Formulation and evaluation of levofloxacin dental films for periodontitis). International journal of pharmacy and pharmaceutical sciences, 2010; 2(1): 162-8.
11. Umadevi S, Rohini B, Nithyapriya and Sasidhara. (Formulation and evaluation of ciprofloxacin dental films for periodontitis). International journal of pharmacy and pharmaceutical sciences, 2012; 4(6): 2964-71.
12. Rama B, Mukul D, Nargund LVG and Kalyani P. (Formulation and evaluation of felodipine buccal films containing polyethylene oxide) International journal of research in pharmaceutical and biomedical sciences, 2012; 3(3): 1153-58.
13. Belsen DJ and Parthasarathy V. (Formulation and *in-vitro* evaluation of bio-degradable polymer based sparfloxacin periodontal chip). Research journal of pharmaceutical, biological and chemical sciences, 2011; 2(3): 319–25.
14. Preetha A and Banerjee R. (Comparison of artificial saliva substitute). Trends in biomaterials & artificial organs, 2005; 18(2): 178-86.
15. Md. Sajid Ali, Javed Ali, Alka Ahuja and Md. Sarfaraz Alam. (Formulation and characterization of dental film containing ofloxacin). Journal of applied pharmaceutical science, 2012; 2(11): 114-19.