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Chitosan Based Periodontal Pocket Inserts – Formulation, *In vitro* and Preliminary Clinical Evaluation

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Abstract

The present paper concerns the *in vitro* and clinical evaluation of chitosan based periodontal inserts of ciprofloxacin hydrochloride (CPH). The effects of polymer concentration, plasticizer, drug loading and type and concentration of cross-linking agents (formaldehyde and glutaraldehyde) on CPH release were studied. The release of CPH followed a Q vs $t^{1/2}$ profile. An increase in the plasticizer concentration (propylene glycol and glycerol) and drug loading resulted in a higher rate of CPH release, but the type of plasticizer did not show any significant effect on drug release, whereas retardation was observed with an increase in polymer concentration and cross-linking. Clinical evaluations of the inserts were carried out in patients suffering from periodontitis with an average pocket depth of > 5 mm. Significant improvements were observed in various clinical indices as: Peridontal Index (PI), Bleeding Index (BI), Periodontal Disease Index (PDI), Shick-Ash Modification of Plaque Criteria (SAPC) and Gingival Index (GI) and microbial parameters (% of G (+)and G (–) bacteria and Total Bacterial Count – TBC) at the device inserted site, thus affirming the therapeutic value of the inserts in the treatment of periodontal pocket formation.

Key Words: Chitosan, Periodontal inserts, Ciprofloxacin hydrochloride

Introduction

The utility of antimicrobial agent-loaded periodontal pocket inserts have been described by various authors (Addy *et al.* 1982; Tanner *et al.*, 1994; Karunakar *et al.*, 1994; Jones *et al.*, 1994; Roskos *et al.* 1995). Of the various polymers that have been found useful in the fabrication of this device, the biodegradable ones are now being investigated vigorously due to their expected non-interference in the process of periodontal tissue regeneration (Agarwal *et al.*, 1993). Naturally occurring biodegradable polymers offer the added advantage of excellent tissue compatibility. Chitosan, the deacetylated derivative of chitin, is an accelerator for wound healing and a non-antigenic (Hirano *et al.*, 1990) compound that has attracted attention for its value in the fabrication of various types of pharmaceutical devices (Inouye *et al.*, 1988; Chandy *et al.*, 1991; Hou *et al.*, 1985). In this report the formulation, drug release aspects and preliminary clinical screening of Chitosan based ciprofloxacin hydrochloride (CPH) inserts are reported.

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Materials and Methods

CPH and Chitosan were generously gifted by Ranbaxy Laboratories Ltd, New Delhi, India and Central Institute of Fisheries and Technology (CIFT), Cochin, India, respectively. All the other reagents used were of analytical grade.

Fabrication of the inserts: Periodontal inserts with different concentrations of chitosan (30 mg and 40 mg / cm^2), with three different drug loadings (300, 500 and 750 µg per insert of 2x6 mm) containing 10 and 20% w/w (w.r.t. polymer) of glycerol or propylene glycol as plasticizer were fabricated using 1 and 2.5% v/v acetic acid as the solvent. Cross-linked inserts using varying proportions of formaldehyde and / or glutaraldehyde were fabricated using 20% w/w (of polymer) of glycerol.

Chitosan was dissolved in the solvent with stirring and the calculated quantity of CPH was incorporated into it, followed by further stirring and degassing. The resultant homogenous solution was casted on leveled glass moulds (6.5 x 6.5 x 0.8 cm). The moulds were placed inside an oven at 50 °C for 24h after wards the films were removed and inserts of 0.12 cm^2 were punched out with a sharp punch, wrapped in aluminium foil and stored in amber coloured glass vials in a desiccator till further use. For the cross-linking, the cross-linking agent(s) were added to the drug-polymer solution, stirred for 1h and the films were casted as above.

Evaluation of insert

Thickness and weight variation: The thickness of the inserts was measured at 10 different randomly selected spots with a screw gauge. For weight variation, 10 inserts were weighed individually and the mean determined.

Drug content uniformity: The inserts were weighed accurately and homogenized using 1 to 2 ml of McIlvaine's buffer (pH 6.6) transferred to amber coloured bottles and shaken for 4h The resultant supernatant was centrifuged and diluted suitably and CPH content was determined by a spectrophotometer (Shimadzu-1601, Japan) at 274 nm.

Moisture vapour transmission (MVT): The MVT through the inserts was determined by a modified procedure of the American Standard Test Method (ASTM Test No. E – 96 – 53 T) as described (Agarwal *et al.*, 1993).

Swelling index studies: Weighed inserts were placed individually in stainless steel wire mesh holders with dimensions 2x8x8 mm and the system was accurately weighed and placed inside vials containing 10 ml of McIlvaine's buffer (pH 6.6). The holders were removed at pre-determined time intervals, dried and weighed. Swelling index was calculated using the following formula:

Swelling index = (Final Wt of insert - Initial Wt of insert) / Initial Wt of insert

In vitro evaluation of the inserts: The inserts were evaluated for drug release kinetics by a modified static, stagnant dissolution method. Weighed inserts were individually placed in stainless steel wire mesh holders of dimensions 2x4x6 mm and suspended in amber coloured vials containing 10 ml of McIlvaine's buffer (pH 6.6) as the dissolution medium. The vials were stoppered and placed in the vial holder (to prevent dislodging) fitted in a water bath thermostated at 37 ± 1 °C. One ml samples were withdrawn at pre-determined time intervals and the dissolution medium was replaced immediately with fresh pre-warmed buffer. The dissolution was carried out for 96-120h and the buffer was changed daily to maintain sink conditions. The withdrawn samples were assayed for CPH content at 274 nm (shimadzu-1601). All the evaluations were done in triplicate. All the inserts remained intact at the end of the study.

Clinical evaluation: The clinical evaluations were conducted in the Department of Dentistry of the host institution. In all, 7 patients (age 34.02 ± 10.06 years, 4 females and 3 males) suffering from periodontitis (pocket depth > 5 mm) participated in the study. Ethical approval was obtained from the ethical committee of the Institute of Medical Sciences, Banaras Hindu University and appropriate consent was obtained from all the patients. All patients received full

mouth supra-gingival scaling and curettage prior to the commencement of the study. Selected batches of inserts, containing 750μg of CPH were placed in the periodontal pockets, and a placebo insert was placed at a different site in the same patient to serve as control, as is the practice followed by other investigators (Noguchi *et al.*, 1984). No periodontal dressing was placed in the pockets. Clinical parameters like GI (Loe *et al.*, 1963), PI and PDI (Russell *et al.*, 1956), SAPC (Shick and Ash, 1961), CC and BI (Ramfjord *et al.*, 1967) and the microbiological parameters like the TBC and the % of Gram (+) and Gram (–) bacteria were monitored at pre-treatment and at predetermined time intervals over a period of 4 weeks.

TBC: Suspension of the plaque sample, collected using a curette, from the device and placebo inserted pockets of the patients were dispersed in 0.5 ml of sterile normal saline. The standard loop volumes of the suspension were placed on a glass slide and a smear was prepared by spreading the suspension over an area of 1 cm^2 (drawn on the back side of the slide). The smear was heat fixed and the bacterial counts were performed after suitable staining, in 30 fields, using a Senior Student Optical Microscope (Model GR 33).

Results and Discussion

Formulation variables and the physicochemical properties of the various batches of the inserts prepared are shown in Tables 1 and 2, respectively. The inserts were smooth in appearance and uniform in thickness and weight and the drug assay values were consistently found to be > 90% of the theoretical drug load. Crystallization of CPH was observed at drug loading exceeding 750μg / insert. The MVT of the inserts was comparatively higher in the first 24h than after 168h.

Table 1. Formulation variables of the batches of inserts fabricated

Batch Code	Chitosan concentration (mg/cm^2)	Volume and concentration of acetic acid (ml, %v/v)	Plasticizer and its concentration (w/w of polymer)	Drug/insert (μg)
B_1	30	40, 1	Glycerol 10%	300
B_2	30	40, 1	Propylene glycol 10%	300
B_3	40	40, 2.5	Glycerol 10%	300
B_4	40	40, 2.5	Propylene glycol 10%	300
B_5	40	40, 2.5	Glycerol 10%	500
B_6	40	40, 2.5	Glycerol 20%	750
B_7	40	40, 2.5	Glycerol 10%	750
B_8*	40	40, 2.5	Glycerol 20%	750
B_9*	40	40, 2.5	Glycerol 20%	750
B_{10}*	40	40, 2.5	Glycerol 20%	750
B_{11}**	40	40, 2.5	Glycerol 20%	750
B_{12}**	40	40, 2.5	Glycerol 20%	750
B_{13}**	40	40, 2.5	Glycerol 20%	750

* Formaldehyde cross-linked batches (B_8, B_9 & B_{10} contains 1%, 0.1% and 0.01%w/w w.r.t. chitosan)

** Gluteraldehyde cross-linked batches (B_{11}, B_{12} & B_{13} contains 0.1%, 0.05% and 0.01%w/w w.r.t. chitosan)

Table 2: Physico-chemical properties of the prepared inserts

Batch code	Average weight (mg) ± S.D.	Average Thickness (mm) ± S.D.	Average (%) ± S.D.	MVT (g cm^2 hr^{-1} × 10^{-3})	
				24h.	168 h
B_1	3.35 ± 0.214	0.25 ± 0.01	92.30 ± 2.72	3.23	2.49
B_2	3.58 ± 0.116	0.27 ± 0.01	92.17 ± 3.07	2.55	1.52
B_3	4.13 ± 0.356	0.39 ± 0.01	91.20 ± 4.66	2.11	1.05
B_4	4.08 ± 0.224	0.38 ± 0.04	94.21 ± 2.27	2.54	1.76
B_5	4.71 ± 0.142	0.39 ± 0.02	94.84 ± 2.24	2.25	1.28
B_6	5.38 ± 0.154	0.42 ± 0.02	93.02 ± 3.78	2.34	1.33
B_7	4.93 ± 0.180	0.41 ± 0.03	94.17 ± 2.62	2.30	1.35
B_8	5.61 ± 0.214	0.45 ± 0.01	90.08 ± 4.20	2.34	1.51
B_9	5.52 ± 0.237	0.44 ± 0.02	92.12 ± 3.93	3.18	1.94
B_{10}	5.42 ± 0.148	0.42 ± 0.03	93.73 ± 2.80	2.64	1.55
B_{11}	5.59 ± 0.199	0.44 ± 0.01	92.78 ± 3.32	1.82	0.90
B_{12}	5.53 ± 0.238	0.43 ± 0.02	92.18 ± 3.43	1.98	1.10
B_{13}	5.42 ± 0.187	0.42 ± 0.01	95.61 ± 2.76	2.76	1.61

The swelling studies of the inserts were carried out in McIlvaine's buffer (pH 6.6). The results indicated that the degree of swelling was lesser in the case of batches crosslinked with formaldehyde or glutaraldehyde (Table 3 A & B). In the case of formaldehyde cross-linked batches, least swelling was observed for batch B_8 when compared to batches B_9 and B_{10} and in case of glutaraldehyde cross-linked batches, least swelling was observed for batch B_{11}. The observed decrease in swelling with an increase in the concentration of cross-linking agents may be due to the increase in the extent of cross-linking of the polymeric chains. The comparatively higher degree of swelling of batches B_1 and B_2 may be attributed to the lower chitosan concentration. Increase in the concentration of glycerol resulted in an increase in the degree of swelling, which could be attributed to an increase in the hydrophilicity of the matrix.

Table 3A : Swelling characteristics of the prepared inserts

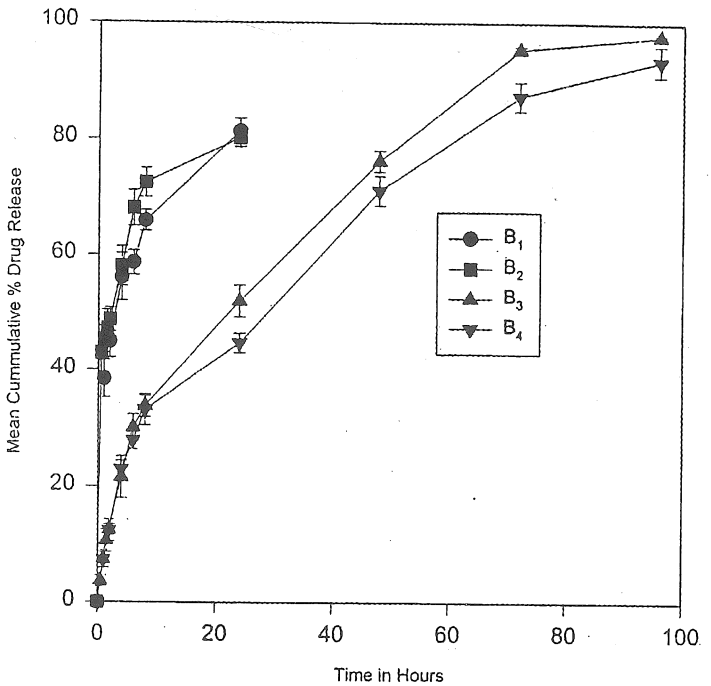
Time (h)	Swelling index (Mean ± S.D.)						
	B_1	B_2	B_3	B_4	B_5	B_6	B_7
0	0	0	0	0	0	0	0
4	0.018 ± 0.001	0.017 ± 0.002	0.011 ± 0.003	0.013 ± 0.003	0.012 ± 0.004	0.016 ± 0.003	0.012 ± 0.002
8	0.019 ± 0.002	0.020 ± 0.001	0.012 ± 0.003	0.014 ± 0.001	0.014 ± 0.002	0.019 ± 0.002	0.014 ± 0.002
24	0.025 ± 0.001	0.029 ± 0.001	0.016 ± 0.003	0.017 ± 0.001	0.017 ± 0.001	0.020 ± 0.001	0.018 ± 0.002
48	0.016 ± 0.001	0.017 ± 0.002	0.009 ± 0.001	0.004 ± 0.001	0.008 ± 0.001	0.013 ± 0.002	0.011 ± 0.002
54	0.016 ± 0.002	0.014 ± 0.002	0.005 ± 0.002	0.003 ± 0.001	0.007 ± 0.001	0.012 ± 0.002	0.010 ± 0.002
72	0.012 ± 0.001	0.016 ± 0.003	0.004 ± 0.003	0.006 ± 0.001	0.004 ± 0.001	0.011 ± 0.003	0.009 ± 0.001
78	0.007 ± 0.001	0.009 ± 0.001	0.002 ± 0.001	0.009 ± 0.002	0.003 ± 0.001	0.005 ± 0.001	0.002 ± 0.001
96	0.012 ± 0.002	0.015 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.008 ± 0.001	0.014 ± 0.001	0.011 ± 0.001
102	0.009 ± 0.001	0.013 ± 0.002	0.003 ± 0.002	0.003 ± 0.001	0.003 ± 0.002	0.019 ± 0.002	0.017 ± 0.003

Table 3B : Swelling characteristics of the prepared inserts

Time (h)	Swelling index (Mean ± S.D.)					
	B_8	B_9	B_{10}	B_{11}	B_{12}	B_{13}
0	0	0	0	0	0	0
4	0.003 ± 0.001	0.010 ± 0.001	0.012 ± 0.002	0.003 ± 0.001	0.006 ± 0.001	0.010 ± 0.001
8	0.005 ± 0.001	0.013 ± 0.002	0.012 ± 0.001	0.003 ± 0.000	0.010 ± 0.003	0.010 ± 0.003
24	0.009 ± 0.002	0.016 ± 0.002	0.016 ± 0.004	0.004 ± 0.001	0.011 ± 0.005	0.010 ± 0.004
48	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.008 ± 0.002	0.010 ± 0.004	0.012 ± 0.008
54	0.008 ± 0.002	0.005 ± 0.001	0.007 ± 0.001	0.006 ± 0.001	0.012 ± 0.007	0.010 ± 0.004
72	0.008 ± 0.003	0.006 ± 0.002	0.002 ± 0.000	0.006 ± 0.002	0.009 ± 0.001	0.011 ± 0.005
78	0.010 ± 0.001	0.002 ± 0.001	0.001 ± 0.000	0.008 ± 0.001	0.009 ± 0.001	0.011 ± 0.006
96	0.004 ± 0.001	0.003 ± 0.000	0.005 ± 0.002	0.009 ± 0.001	0.006 ± 0.002	0.006 ± 0.001
102	0.003 ± 0.002	0.006 ± 0.001	0.005 ± 0.001	0.007 ± 0.002	0.008 ± 0.002	0.007 ± 0.001

The drug release profile from the prepared inserts followed matrix diffusion (Higuchi type kinetics). The results indicated that more than 80% of the drug was released within 24h from batches B_1 and B_2, while the drug release from batches B_3 and B_4, having the same drug loading but a higher chitosan concentration, extended up to 96h (Fig. 1).

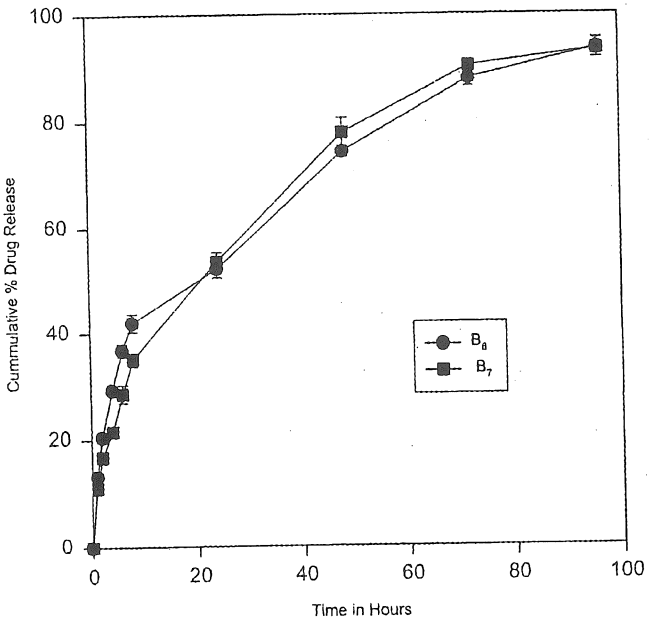
Fig. 1: Effect of Chitosan Concentration and types of plasticizer on *in-vitro* drug release from the prepared inserts in McIlvaine's buffer pH 6.6.



Since the drug release is governed predominantly by matrix diffusion, the path length that the drug has to traverse decreases when the concentration of the polymer used is less (Thanoo *et al.*,1992), as was the case with batches B_1 and B_2. Moreover, the dissolution medium enters the bulk of the insert, resulting in its swelling, which is again influenced by the concentration of chitosan present in the inserts. The intrusion of the dissolution medium and the resultant swelling triggers the degradation of chitosan, leading to the formation of monomeric units of

N-acetyl glucosamine (Felt *et al.*, 1998). The progressive degradation changes the microstructure of the bulk through the formation of pores, resulting in the release of CPH. Our findings are in accordance with those of other investigators (Thacharodi and Pandurangarao, 1993 and 1995), who have attributed the transport of a variety of drug molecules from chitosan matrices, via the pore mechanism.

Fig.2 : Effect of Increased Glycerol Concentration on *in-vitro* CPH release from the inserts in McIlvaine's buffer pH 6.6



Generally the nature and amount of plasticizer used influence the drug release to a great extent, but in this study the plasticizers viz. glycerol and propylene glycol had no significant effect ($P>0.05$) on drug release. The effect of increased concentration of glycerol on CPH release was also studied. The results showed a higher initial release from batch B_6, which could be due to an increase in the concentration of glycerol by 10%, resulting in increased hydrophilicity of the matrix system, as was evidenced by the results of the swelling studies. Apart from an increase in the initial drug release, there was no significant difference in the drug release from batches B_6 and B_7 (Fig 2). The drug release was proportional to the drug loading (batches B_3, B_5 and B_7, Fig. 3).

The percent of drug released during the first 8h. from batches B_3, B_5 and B_7 were 33.8, 40.11 and 41.83, respectively. The initial burst effect seen with increase in drug loading gives an indication of crystallization and presence of CPH in the surface layers of the insert, though visual examination of the inserts did not show crystal formation on the surface of the inserts.

Inserts with varying proportions of formaldehyde and glutaraldehyde were fabricated to retard the drug release, as reported by other investigators (Thanoo *et al.* 1992 and Jameela and Jayakrishnan 1995) and to compare the efficiency of the two cross-linking agents. The maximum concentrations of formaldehyde and glutaraldehyde were restricted to 1% and 0.1% w/w of chitosan, since beyond that concentration the polymer solution became practically unpourable. The results indicate that cross-linking significantly retarded CPH release ($P<0.05$) in comparison to the corresponding non cross-linked inserts. The degree of cross-linking directly influenced drug release from the inserts (Fig. 4).

Fig.3: Effect of CPH Loading on its release from the prepared inserts in McIlvaine's buffer pH 6.6

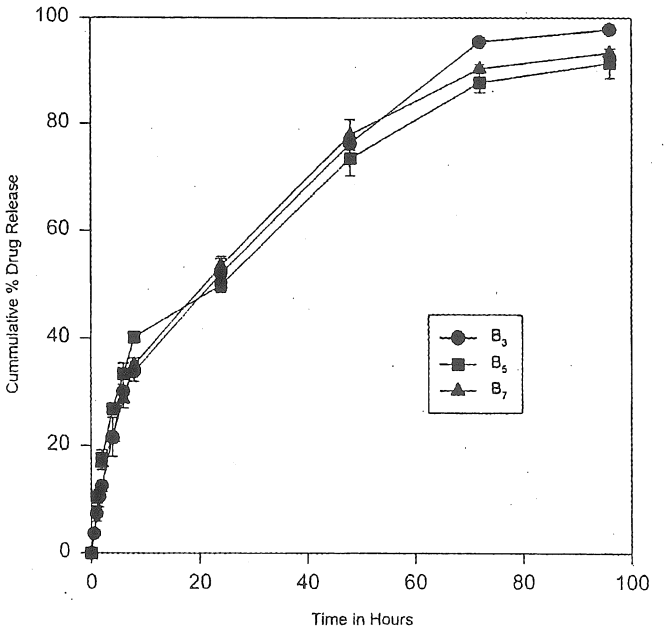
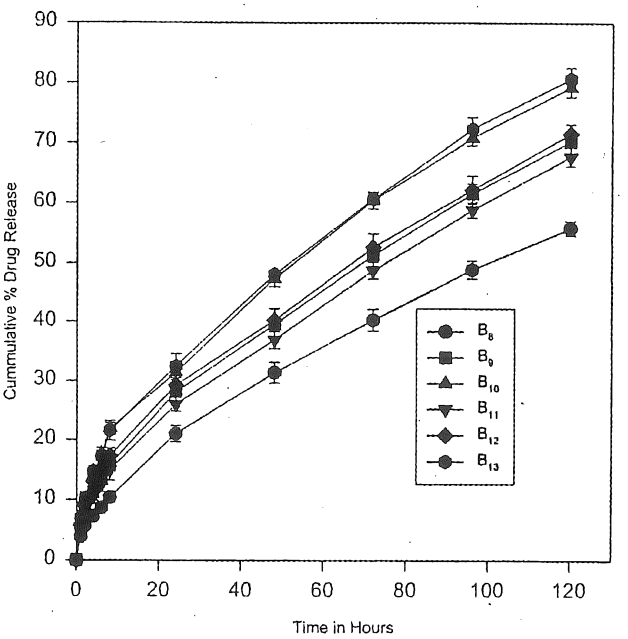


Fig. 4: Effect of Formaldehyde and Gluteraldehyde cross-linking on *in-vitro CPH* release from the inserts in McIlvaine's buffer pH 6.6



Clinical evaluation: The sites of insertion of the drug loaded device (D) and placebo (P) was noted and the various parameters that were monitored during the course of treatment are shown in Table 4 (A and B). The device was found missing in all the patients who had reported for the first follow up, presumably due to complete dissolution in the periodontal pocket, but none of them excepting patient No. 7 was able to inform the exact mode of loss of the device.

Table 4A Clinical evaluation of CPH inserts in patients

Age/sex/ Pat. No.	Site of Insertion of device	Clinical parameters	Days of treatment			
			-7	0	7	14
37/F/1	12-D, (31-P)	Periodontal Index (PI)	6 (5)	6 (5)	6 (5)	6 (5)
		Gingival Index (GI)	3 (3)	3 (3)	1 (3)	1 (3)
		Shick-Ash modification of plaque criteria (SAPC)	3 (3)	1 (1)	0 (0)	0 (0)
		Periodontal Disease Index (PDI)	5 (5)	5 (5)	5 (5)	5 (5)
		Calculus criteria (CC)	2 (2)	0 (0)	0 (0)	0 (0)
		Bleeding Index (BI)	3 (3)	2 (2)	1 (2)	1 (2)
		Probing depth	4 (5)	4 (5)	3.5 (5)	4 (5)
38/M/2	18-D, (31-P)	Periodontal Index (PI)	6 (4)	6 (4)	NR	6 (4)
		Gingival Index (GI)	2 (2)	2 (2)	NR	0 (2)
		Shick-Ash modification of plaque criteria (SAPC)	3 (3)	0 (1)	NR	0 (0)
		Periodontal Disease Index (PDI)	5 (5)	5 (5)	NR	5 (5)
		Calculus criteria (CC)	2 (3)	0 (1)	NR	0 (0)
		Bleeding Index (BI)	2 (3)	0 (2)	NR	0 (1)
		Probing depth	6 (6)	6 (6)	NR	5 (6)
28/F/3	34-D, (44-P)	Periodontal Index (PI)	6 (1)	6 (1)	6 (0)	NR
		Gingival Index (GI)	2 (1)	2 (1)	0 (0)	NR
		Shick-Ash modification of plaque criteria (SAPC)	2 (1)	1 (0)	0 (0)	NR
		Periodontal Disease Index (PDI)	5 (1)	5 (1)	5 (0)	NR
		Calculus criteria (CC)	3 (1)	2 (0)	1 (0)	NR
		Bleeding Index (BI)	2 (0)	2 (0)	1 (0)	NR
		Probing depth	5 (3)	4.5 (3)	5 (3)	NR
26/M/4	14-D, (24-P)	Periodontal Index (PI)	2 (6)	1 (6)	0 (6)	0 (6)
		Gingival Index (GI)	2 (2)	1 (1)	0 (1)	0 (0)
		Shick-Ash modification of plaque criteria (SAPC)	2 (2)	1 (1)	1 (0)	0 (0)
		Periodontal Disease Index (PDI)	2 (5)	1 (5)	0 (5)	0 (5)
		Calculus criteria (CC)	0 (5)	0 (0)	0 (0)	0 (0)
		Bleeding Index (BI)	0 (2)	0 (1)	0 (0)	0 (0)
		Probing depth	4.4 (4.7)	4.4 (4.7)	4.14 (4.3)	3.57 (3.57)
22/F/5	27-D, (36-P)	Periodontal Index (PI)	0 (1)	0 (1)	0 (0)	NR
		Gingival Index (GI)	2 (2)	2 (2)	0 (0)	NR
		Shick-Ash modification of plaque criteria (SAPC)	1 (1)	1 (0)	0 (0)	NR
		Periodontal Disease Index (PDI)	2 (5)	2 (5)	0 (5)	NR
		Calculus criteria (CC)	0 (1)	0 (0)	0 (0)	NR
		Bleeding Index (BI)	2 (2)	2 (2)	0 (1)	NR
		Probing depth	1.5 (4)	2 (3.4)	2 (3.6)	NR
52/M/6	16-D, (25-P)	Periodontal Index (PI)	6 (6)	6 (6)	6 (6)	6 (6)
		Gingival Index (GI)	3 (3)	2 (2)	0 (2)	0 (1)
		Shick-Ash modification of plaque criteria (SAPC)	3 (3)	0 (3)	0 (2)	0 (0)
		Periodontal Disease Index (PDI)	6 (6)	6 (6)	6 (6)	5 (6)
		Calculus criteria (CC)	3 (3)	0 (0)	0 (0)	0 (0)
		Bleeding Index (BI)	2 (2)	2 (1)	0 (2)	0 (1)
		Probing depth	6 (6)	6 (6)	5 (6)	5 (6)
38/F/7	36-D, (44-P)	Periodontal Index (PI)	6 (6)	6 (2)	1 (2)	1 (1)
		Gingival Index (GI)	3 (2)	2 (2)	1 (2)	1 (1)
		Shick-Ash modification of plaque criteria (SAPC)	3 (3)	2 (1)	2 (0)	1 (0)
		Periodontal Disease Index (PDI)	6 (5)	6 (6)	6 (6)	5 (6)
		Calculus criteria (CC)	2 (1)	0 (0)	0 (0)	0 (0)
		Bleeding Index (BI)	3 (2)	2 (2)	1 (2)	0 (1)
		Probing depth	6 (6)	6 (6)	6 (5.5)	6 (5.5)

D - Device inserted site P - Placebo inserted site -7 Pre'scaling 0 - Day of insertion of device
7- Fist follow up 14 - 2nd follow up NR - Not reported Reacting in the bracket shows in placebo inserted sites

Table 4B: Microbiological evaluation of CPH inserts in patients*

Patient No.	Disease classification (PI score)	Microbiological parameters	Days of treatment		
			0	7	14
1	Established destructive disease (2.2)	TBC	321569(401960)	194118 (381962)	315687 (400000)
		% G+ve Cocci	57.9 (66.34)	59.59 (62.9)	75.7 (68.5)
		% G+ve Rods	18.9 (5.12)	27.27 (18.18)	19.25 (19.2)
		% G+ve Cocci	17.68 (14.14)	10.1 (15.55)	4.9 (10.92)
		% G+ve Rods	5.48 (4.39)	3.03 (3.29)	0 (1.38)
2	Established destructive disease (4.3)	TBC	315687 (319608)	NR	243138 (258824)
		% G+ve Cocci	58.38 (66.8)	NR	77.4 (70.4)
		% G+ve Rods	20.49 (16.5)	NR	20.96 (10.6)
		% G+ve Cocci	13.66 (9.8)	NR	1.61 (7.5)
		% G+ve Rods	7.45 (6.7)	NR	0 (2.2)
3	Established destructive disease (3.15)	TBC	427450 (359242)	376471 (296470)	NR
		% G+ve Cocci	66.9 (76.6)	63.5 (64.9)	NR
		% G+ve Rods	17.43 (8.3)	27.6 (23.1)	NR
		% G+ve Cocci	11.09 (10)	6.7 (9.27)	NR
		% G+ve Rods	4.5 (5)	2.08 (2.65)	NR
4	Established destructive disease (3.9)	TBC	439216 (333333)	298040 (286275)	339215 (378431)
		% G+ve Cocci	65.6 (71.7)	66.4 (70.5)	73.98 (75.1)
		% G+ve Rods	20.98 (12.35)	23.02 (19.1)	26.01 (21.2)
		% G+ve Cocci	8.03 (10)	9.21 (10.95)	0 (3.6)
		% G+ve Rods	5.35 (5.88)	1.31 (2.05)	0 (0)
5	Simple gingivitis (0.4)	TBC	327450 (323530)	311765 (294118)	NR
		% G+ve Cocci	65.8 (58.78)	70.44 (65.3)	NR
		% G+ve Rods	21.5 (26.6)	19.49 (24.6)	NR
		% G+ve Cocci	11.3 (12.72)	10.06 (10)	NR
		% G+ve Rods	1.19 (1.84)	0 (0)	NR
6	Established destructive disease (3.9)	TBC	439215 (390196)	305883 (298040)	287540 (332256)
		% G+ve Cocci	65.17 (64.32)	78.2 (72.3)	59.96 (69.98)
		% G+ve Rods	19.64 (18.09)	20.51 (21.05)	38.22 (16.22)
		% G+ve Cocci	9.82 (12.06)	1.28 (6.63)	2.22 (10.52)
		% G+ve Rods	5.35 (5.52)	0 (0)	0 (3.28)
7	Established destructive disease (4.6)	TBC	452944 (247059)	311764 (286275)	385538 (342887)
		% G+ve Cocci	65.36 (67.46)	75.47 (73.2)	63.06 (53.25)
		% G+ve Rods	21.64 (19.05)	22.07 (19.86)	28.52 (30.98)
		% G+ve Cocci	11.25 (11.10)	2.51 (6.85)	8.42 (14.22)
		% G+ve Rods	1.73 (2.38)	0 (0)	0 (1.55)

* Sites of insertion of drug loaded and placebo inserts same as table 4A0-Day of insertion of device 7-First follow up 14-Second follow up NR - Not Reporte Reading in bracket shows scores in placebo inserted sites

In case of patient No. 1 there was no significant improvement in the clinical parameters in both the device and placebo inserted sites, but there was marked improvement in the microbiological parameter as evidenced by the decrease in TBC in the device inserted site during the 1st follow up. TBC showed an increase during the 2nd follow up, which may be due to the absence of device at the site. Patient No. 2 did not report for the first follow up, but reported for the second follow up. However, a decrease in the TBC was observed in both the drug treated and placebo inserted sites. Some of the clinical parameters showed improvements in both the sites. Improvements in the clinical parameters were noted in both the device and placebo inserted sites in case of patient No. 3 who failed to report for the second follow up. A significant decrease in the percent Gram – ve organisms was observed in the device-inserted site. The clinical parameter profile of patient No. 4 was similar to patients to 2 and 3, showing a considerable decrease in TBC and % Gram – ve organism in the device inserted site. But the TBC showed an increase in both the sites during the 2nd follow up. Patient No. 5 did not report for the 2nd follow up. Considerable improvements in the clinical parameters were observed in the device-inserted site than the placebo-inserted site. There were dramatic improvements in the clinical parameters of patient No 6 and 7 in the device-inserted sites. Patient No. 7 had reported loss of the device during brushing on the third day after insertion. The devices were tolerated well by the patients and none reported any discomfort or pain or taste related problems due to the presence of the device in the periodontal pocket.

Conclusion

The CPH delivery device provided an initial high release followed by moderate release on later days *in vitro*. Even though the number of patients used in this study was small and of shorter duration and the survival time of the inserts were not monitored, the clinical results leave no doubt whatsoever to the effectiveness of the treatment. Long term clinical trials, comparative studies with other modes of the treatment and the effectiveness of the device as an adjunct to conventional scaling and root planning and the probability of development of resistant strains are currently in progress.

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