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Design and *in vitro*/*in vivo* evaluations of a multiple-drug-containing gingiva disc for periodontotherapy†

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In the current work, we set out to develop and evaluate a gingiva disc of cellulose acetate phthalate and poloxamer F-127 for the simultaneous delivery of multiple drugs, namely minocycline, celecoxib, doxycycline hyclate, and simvastatin, to abolish infection, impede inflammation, avert collagen destruction, and promote alveolar bone regeneration, respectively. *In vitro* release studies revealed the sustained release profiles of the drugs for 12 h and that they were active against *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans*. The *in vivo* bioactivity levels of these drugs were assessed by comparing the number of colony forming units during different phases of a study on Wistar rats, and the results showed a reduction in the number of bacterial colonies with the applied formulation. A mucosal irritation study conducted on Wistar rat gingiva confirmed the non-irritancy of the optimal gingiva disc. Hence, this customized, non-invasive polymeric gingiva disc displaying a sustained release of drugs can be a useful tool to treat acute to moderate stages of periodontitis.

Introduction

Periodontitis is the commonest and most challenging progressive dental ailment and exhibits a wide spectrum of symptoms such as gingival puffiness, inflammation, bleeding, and detachment of tooth bone often leading to pocket formation of 3–4 mm.[1] The progression is often accompanied by a deposition of biofilm, leading to deeper pockets and edentulism if left untreated. The conventional dental therapeutics revolves around mechanical debridement of the periodontal pocket with plaque control measures to remove bacterial infection and biofilm deposits.[2] This treatment strategy, however, is time consuming, involves consumption of a high dose of antibiotics, is expensive, and is often marred with significant patient non-compliance. Many novel drug delivery systems have been proposed for this issue, but most such systems are associated with various limitations as they do not address the complexity of periodontitis, which demands instead an all-encompassing therapeutic approach capable of mitigating all of the associated disease symptoms.[3–6]

The success of any drug delivery system also relies upon the choice of the target site. An acute phase periodontitis involving relatively shallow pockets can be better treated with a system that could adhere to the gingival tissue and bathe the gum line with the drugs and arrest the disease. The gingival delivery of drugs involves immobilizing a drug delivery device, namely a polymeric disc, on a specific site for targeted release in the oral cavity, eliciting intimacy and a specific duration of contact.[7] Thereby on the gingiva, when using the proposed disc, the risk is much lower than the benefit.

A gingiva disc should simultaneously deliver antimicrobial, anti-inflammatory, anti-bone resorptive and osteogenic agents to achieve a complete reversal of acute phase periodontitis. The assemblage of drugs chosen in the current work included minocycline, an antibiotic against the plaque bacteria,[8,9] celecoxib, a selective COX-II inhibitor for treating infection-associated inflammation,[10] doxycycline hyclate as an inhibitor of matrix metalloproteinases (MMP-1, MMP-8) for circumvention of collagen destruction[11–13] and simvastatin to promote the expression of bone morphogenic protein (BMP-2) and aid in alveolar bone regeneration.[14,15]

For the matrix of the gingiva disc, a system including an association of the polymers cellulose acetate phthalate and

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poloxamer F-127 (CAP-P) was selected, as it has been shown to release the drugs in a controlled manner. To impart mucoadhesion characteristics onto the gingiva disc, a thin coating of hydroxyl-ethyl cellulose solution was applied. The CAP-P system has been exploited in various studies to achieve a controlled and programmed drug delivery by altering the ratio of the amounts of the two polymers.[16–19]

The present study aimed to deliver antimicrobial, anti-inflammatory, anti-bone resorptive and osteogenic agents from the gingiva disc for the complete reversal of acute phase periodontitis.

Experimental

Materials

Cellulose acetate phthalate (CAP) and poloxamer F-127 were obtained from Jubilant Life Sciences (New Delhi, India) *ex gratia*. Hydroxyl-ethyl cellulose was procured from SRL India. Isotonic phosphate buffer of pH 6.8 was prepared according to USP XX (1980). Acetone and isopropyl alcohol were procured from E. Merck (India) Ltd (Mumbai, India). A Shimadzu (Japan) UV-1601 UV spectrophotometer was used for spectrophotometric analysis. A Shimadzu (Japan) LC-10 HPLC system was also used.

Preparation of the polymeric gingiva disc

Each disc was prepared using the pressed pellet method.[14] Various components and the four drugs minocycline, doxycycline, celecoxib and simvastatin in each formula were mixed by carrying out trituration in a glass pestle and mortar. All four drugs were present in a single disc in combined form. The mixture was then compressed using a 13 mm-diameter die on an infrared hydraulic press (Spectra Lab-SL-89, Mumbai) using a compression force of 5 tons and a compression time of 15 s. The prepared disc was then coated with a 2% (w/v) slurry of hydroxyl-ethyl cellulose in a mixture of acetone : isopropyl alcohol (65 : 35).[20]

Optimization of the gingiva disc

Discs were prepared using the pressed pellet method and optimized using Design Expert® version 10 software.[21] A 2^3 factorial design was applied to study the effect of two independent factors, namely CAP concentration [*A*] and poloxamer concentration [*B*], in three levels (coded as −1, 0, +1). Four drugs were incorporated in the formulation and the amount of each drug was fixed at 6 mg. A total of eleven formulation batches were prepared in which 2% hydroxy-ethyl cellulose was used as a coating polymer. All eleven formulations were tested to determine mucoadhesion time, tensile strength, bioadhesive force, and *in vitro* drug release, and these results were compared to each other.

Evaluation of the polymeric gingiva disc

Weight and weight uniformity. Ten discs of 13 mm diameter from each formulation batch were selected and weighed using a Shimadzu balance with a sensitivity as low as 0.0001 g (Shimadzu, Tokyo, Japan) and the weight variation was calculated.[14]

Thickness and thickness uniformity. Ten discs from each formulation batch were selected and the thickness of each was measured using a micrometer screw gauge, and the average was determined.[14]

Surface pH. The surface pH of each disc was determined using a surface pH meter. The discs were first allowed to swell in already solidified agar media (2%). The surface pH was determined by bringing a combined glass electrode near the surface of the disc and allowing the electrode to equilibrate for 1 min.[14] The surface pH of the formulation was determined in order to avoid the possibility of irritating the gingiva with acidic and alkaline pH. Hence an attempt was made to keep the surface pH close to neutral.

Swelling index. A swelling index study was carried out to measure the hydration capacity of each polymer disc. Individual discs were weighed (designated as M_1) and separately placed in a Petri plate of solidified agar (2% solution) for one hour (1 h). The (increasing) weights of the discs were noted at each time interval until a constant weight was obtained. Discs were removed from the Petri plate, and filter paper was used to absorb the excess water from the discs.[14] The swollen discs were reweighed (M_2). The degree of swelling was calculated using the formula

$$\text{S.I.} = [(M_2 - M_1) \times 100]/M_1,$$

where S.I. is the swelling index.

***Ex vivo* mucoadhesion time.** *Ex vivo* mucoadhesion time was determined after applying the gingiva disc on the freshly cut goat buccal mucosa. Goat buccal mucosa was fixed, with help of cyanoacylate glue, onto the inner side of the beaker 2.5 cm above the bottom of the beaker. For pasting the disc onto the buccal mucosa, one side of the disc was moistened with a drop of isotonic phosphate buffer (pH 6.8) and a small force was applied with a fingertip for 30 seconds. The beaker was then filled with 500 ml of isotonic phosphate buffer (pH 6.8), and a temperature of 37 ± 10 °C was maintained. To simulate the environment of a buccal cavity, 150 rpm stirring was applied.[14] The time taken for the disc to detach from the goat buccal mucosa was recorded as the mucoadhesion time. The study was performed in triplicate ($n = 3$).

***In vitro* bioadhesive force and tensile strength.** A TA.XT2 Texture analyzer (Stable Micro System, Haslemere, Surrey, UK) equipped with a 5 kg load cell was used to determine the bioadhesive strength of the gingiva disc. Goat buccal mucosa was used as a model membrane. The mucosal membrane was fixed between two circular discs supported from below by a piece of Perspex. The upper circular disc, which had a diameter of 12.7 mm and to which the mucosal membrane was attached, was exposed to the probe. The other circular disc had a diameter of 13 mm and was attached to another piece of Perspex. Before commencing the experiment, the exposed surface of the gingiva disc was wetted with phosphate buffer (pH 6.8). A relatively low 0.5 mm s^{-1} probe speed with a 90 g load and contact time of 120 s was maintained. Also the probe was removed at a speed of

2 mm s^{-1}. Texture Pro CT V 1.3 Build 14 software was used for data collection and calculations.[14] The bioadhesive strength was used to estimate the bioadhesive force of the disc. Bioadhesive force (N) was calculated using the formula

$$\text{Bioadhesive force } (N) = \text{bioadhesive strength (g)} \times 9.81 \div 1000.$$

A similar method was used to assess the tensile strength of the disc, and the force at disc break was measured. Results here are reported as the mean (±SD) of three replicates ($n = 3$). The tensile strength was calculated using the formula

$$\text{TS (kg mm}^{-2}) = \text{force at break (kg)/initial cross-sectional area of sample (mm}^{-2}).$$

***In vitro* release study.** Drug release rate was determined using a USP dissolution apparatus 5 (Paddle over disc) with modifications. The dissolution assembly consisted of 500 ml of dissolution medium containing 2.25% glycoproteins (simulated isotonic phosphate buffer pH 6.8) and a paddle operating at a rotation speed of 50 rpm. The glycoproteins here were included in order to have the dissolution media simulate the salivary fluid, and the percentages reported were calculated according to the composition of human saliva and mucus.[22] A temperature of 37 ± 0.5 °C was maintained throughout the study. The disc was placed in the basket of the apparatus. Samples each having a volume of 5 ml were collected at time points of 0, 0.5, 1, 2, 3, 4, 6, and 12 h. These samples were analyzed using high-performance liquid chromatography (HPLC).[14] Release studies for all eleven formulations were performed separately, each in triplicate ($n = 3$), and the results were compared to each other.

***Ex vivo* drug permeation and retention study.** The disc was evaluated for drug permeation using Franz diffusion cells, and these experiments were carried out in triplicate ($n = 3$). Goat buccal mucosa was taken as the barrier membrane and phosphate buffer (pH 6.8) as the medium. The disc was placed on the mucosal membrane. The diffusion cell was placed in phosphate buffer, maintained at 37 ± 2 °C. The receptor compartment was filled with 50 ml of phosphate buffer (pH 6.8), and the hydrodynamics was maintained by stirring with a magnetic bead at 300 rpm. Samples each having a volume of 2 ml were withdrawn at time points of 0.5, 1, 2, 3, 4, 6, and 72 h and replaced with a 2 ml of fresh medium. Samples were analyzed using HPLC. After 72 h, a mucosal drug retention study was performed. For this study, buccal mucosa was removed, blotted dry with tissue paper, accurately weighed and subjected to homogenization by adding to it 500 μL of each sample. To the resulting mixture, a volume of 200 μl of acetonitrile was mixed in, and the resulting sample was vortexed at 500 rpm for 5 min. To the resulting vortexed sample, a volume of 5 ml of extraction solvent (ethyl acetate) was added, and the resulting mixture was shaken and centrifuged (10 000 rpm, 10 min, 4 °C). A volume of 2 ml of the resulting supernatant organic layer was dried and reconstituted in 100 μL of mobile phase, and a volume of 20 μL of the resulting reconstituted sample was injected into the HPLC system for analysis.[14]

Scanning electron microscopy (SEM). SEM imaging of the optimized formulation was performed to study the surface of the uncoated and hydroxyl-ethyl-cellulose-coated polymeric disc. The sample was fixed on an SEM stub using double-sided adhesive tape, and then coated with a thin layer of gold and observed with a scanning electron microscope (EVO LS 10, Carl Zeiss, Germany).

***In vitro* microbiological assay.** The antimicrobial efficacy of the optimized gingiva disc was tested against *Staphylococcus aureus* (CC25923), *Escherichia coli* (CC25922) and *Streptococcus mutans* (CC25175) using the agar well method in triplicate ($n = 3$). The samples collected from the *in vitro* release study at the various time points were filtered through sterilized Millipore membrane filters (0.2 μm). The wells were carefully filled with ten microliters of the samples. The samples were allowed to diffuse for 2 h at room temperature, and were then incubated.[23] The diameter (mm) of the zone of growth inhibition surrounding each agar well was measured with a zone finder.

***In vivo* antimicrobial activity study.** The experimental study protocol was approved by the Institutional Ethical Committee of Animal Research, Jamia Hamdard, New Delhi, India (Protocol approval no. 1255) and adhered to the "principles of laboratory animal care". Eighteen male Wistar rats (*Rattus norvegicus*) of average weight, 180–210 g, were selected and grouped. These eighteen animals were assigned into three groups (six rats per group), with group 1 including healthy animals (sham group), group 2 including periodontitis-induced animals (negative control), and group 3 including treated animals. Experimental periodontitis was induced in them by using the procedure described previously (6) and adopted by Xu *et al.*[24,25] For this purpose, a non-absorbable sterile surgical silk ligature 3/0 (Ethicon, Johnson & Johnson Ltd., Baddi, Himachal Pradesh, India) was placed around the gingival crevices of the first left lower molar teeth. The ligatures were tightly applied and all loose ligatures were replaced.

For eight weeks, all experimental periodontitis-induced rats were fed with a 10% w/v sucrose solution. When the disease developed, the group 3 animals were treated with the gingiva disc. After eleven days of treatment, the different groups were examined for microbiological studies. *In vivo* antimicrobial activity of the disc was studied by comparing colony forming units (CFUs) on the blood agar media. Before disease induction, after disease development and after disease treatment with gingiva disc, mouth swabs were taken of the rats and applied onto the surface of blood agar media by using the streaking method. The plates were then incubated at 37 ± 0.5 °C for 24 h, and then CFUs were counted and compared.[24] All of the studies were performed in triplicate ($n = 3$) and the results are presented as the mean of the three (mean ± SD).

Mucosal irritation studies. In order to assure non-irritancy of the gingiva disc on the rat mucosa, the gingival segments of the healthy animals (group 1) and treated animals (group 3) were fixed in 10% v/v formalin solution and demineralized in 7% nitric acid for 24 h.[26] These specimens were dehydrated, embedded in paraffin, and stained with hematoxylin and eosin

(HE). Sections with thicknesses of 6 mm were evaluated using light microscopy (40× magnification). Gingiva of the two groups were compared.[24]

Results & discussion

Preparation and optimization of the gingiva disc

Various discs were prepared using different amounts of the polymers cellulose acetate phthalate and poloxamer F-127, and a fixed amount of hydroxyl-ethyl cellulose as given in Table 1. A schematic representation of the disc is shown in Fig. 1. Furthermore, Design Expert® version 10 software was used to optimize the disc on the basis of tensile strength and maximum percentage drug release.

Weight and weight uniformity

Weights of ten discs of each formulation were measured and were found to be in the range 116.82–136.32 mg. Table 2 shows the means (±SD) of the obtained weight values. The low SD values reflected the weight uniformity amongst the various formulations of each batch.

Thickness and thickness uniformity

The thicknesses of ten discs of each type of formulation were measured and were found to be in the range 0.75–0.86 mm. Table 2 shows the means (±SD) of the obtained thickness values. The low SD values reflected the uniform thickness.

Surface pH. Surface pH values for the formulations F01–F09 were found to be in the range 7.02–7.23 as shown in the Table 2. Since these pH values were all similar to the pH of saliva (6.8–7.2), no mucosal irritation was expected from any of the developed formulations. Moreover, there was no clear effect of any of the formulation variables on the surface pH.

Swelling index. Swelling indexes of the discs were found to be in the range 29.70–45.24, and the swelling index was found to increase with increasing cellulose acetate phthalate concentration (Table 2). This property of the disc can have a direct influence on the release of drug. As the poloxamer concentration was increased, the swelling index decreased a little. These results may be attributed to a greater swelling displayed by cellulose acetate phthalate than by poloxamer.

***Ex vivo* mucoadhesive time.** All eleven formulation batches were subjected to testing of mucoadhesion time in triplicate. Mucoadhesion times obtained for all the formulations were in the range 9.0–12.50 h, as shown in Table 2. Since a uniform coating of hydroxyl-ethyl cellulose was applied on all the formulations, the differences between the mucoadhesion times might have been the outcome of the different ratios of the amount cellulose acetate phthalate to that of poloxamer.

***In vitro* bioadhesive force and tensile strength.** Mean bioadhesive forces and tensile strengths of all eleven formulations were studied. The obtained adhesion force values did not exhibit much variation, as shown in Table 2. This lack of variation was attributed to the uniform concentration of coating agent applied for all of the formulation batches.

Mean tensile strengths of all eleven formulations are shown in Table 3. These strength values were all more than 7 kg mm^{-1},[2] indicative of good mechanical strength. Moreover, the tensile strength increased with increasing poloxamer mass. Using design expert software, a quadratic model was derived and an F-value of 62.02 ($p < 0.0500$) was obtained, which implied that the model was significant. The relationship between tensile strength and the independent factors was determined to follow the equation

$$\text{Tensile strength} = +14.68 + 1.56A + 2.92B - 0.84AB + 0.69A^2 + 1.21B^2.$$

ANOVA results showed A, B, AB, A^2, B^2 to be significant model terms with $p < 0.05$. Factors A (CAP mass) and B (poloxamer mass) were concluded from this equation to have positive effects on tensile strength. Interaction terms (AB) showed negative effects on tensile strength whereas higher-order terms (A^2 and B^2) showed positive effects on tensile strength. Also the effects of the two independent variables on the tensile strength were studied by producing a 3D response surface plot that is shown in Fig. 2a.

***In vitro* release study.** As described above, the polymeric drug delivery system included a mixture of four drugs, namely minocycline, celecoxib, doxycycline and simvastatin, each with

Table 1 Compositions of various gingiva disc formulations

Formulation code	Total polymer (mg)	CAP (mg)	Poloxamer F-127 (mg)	Each drug (mg)
F01	110	70	40	6
F02	100	65	35	6
F03	110	60	40	6
F04	95	65	30	6
F05	90	60	30	6
F06	100	70	30	6
F07	95	60	35	6
F08	105	65	40	6
F09	105	70	35	6
F10	100	65	35	6
F11	100	65	35	6

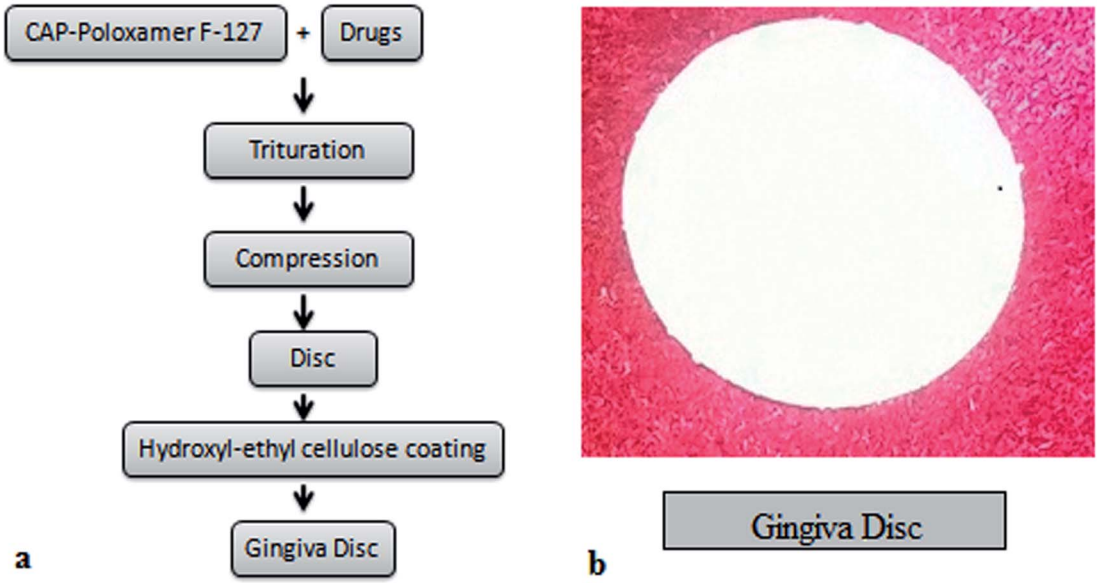

Fig. 1 A schematic presentation of the design and development of the gingiva disc (a). A pictorial presentation of the developed gingiva disc (b).

Table 2 Physical characterization of the formulations (mean ± SD)

Formulation code	Weight (mg) (mean ± SD)	Thickness (mm) (mean ± SD)	Surface pH (mean ± SD)	Swelling index (mean ± SD)	Bioadhesive force (*N*) (mean ± SD)	Mucoadhesion time (h) (mean ± SD)
F01	136.20 ± 0.57	0.86 ± 0.011	7.05 ± 0.58	45.28 ± 1.16	2.738 ± 0.51	11.00 ± 0.15
F02	126.04 ± 0.57	0.81 ± 0.005	7.23 ± 0.58	38.67 ± 1.78	2.543 ± 0.81	10.80 ± 1.10
F03	136.30 ± 0.58	0.75 ± 0.003	7.19 ± 0.57	29.70 ± 1.18	2.198 ± 0.93	09.00 ± 0.78
F04	121.73 ± 0.57	0.84 ± 0.004	7.03 ± 0.58	41.12 ± 1.82	2.621 ± 0.71	11.15 ± 0.84
F05	116.82 ± 0.57	0.79 ± 0.003	7.20 ± 0.58	34.57 ± 1.32	2.423 ± 0.82	10.02 ± 0.18
F06	126.52 ± 0.57	0.83 ± 0.003	7.16 ± 0.57	38.89 ± 1.40	2.557 ± 0.93	12.50 ± 0.95
F07	121.52 ± 0.57	0.80 ± 0.005	7.02 ± 0.57	35.78 ± 1.22	2.501 ± 0.81	10.25 ± 0.77
F08	131.23 ± 0.58	0.85 ± 0.004	7.08 ± 0.57	42.09 ± 1.72	2.672 ± 0.22	12.25 ± 0.52
F09	131.53 ± 0.58	0.77 ± 0.003	7.13 ± 0.57	33.32 ± 1.57	2.392 ± 0.73	09.50 ± 0.31
F10	126.23 ± 0.03	0.80 ± 0.025	7.21 ± 0.41	37.46 ± 1.23	2.538 ± 0.21	10.50 ± 1.90
F11	126.78 ± 0.21	0.82 ± 0.001	7.33 ± 0.81	38.07 ± 1.08	2.563 ± 0.71	10.70 ± 0.23

Table 3 Variable factors and their observed responses for the optimization of the gingiva disc

Formulation code	Factor 1: polymer mass (mg)	Factor 2: poloxamer mass (mg)	Response 1: tensile strength (kg mm^{-2}) (mean ± SD) (n = 3)	Response 2: maximum drug release (%) (mean ± SD) (n = 3)
F01	1	1	20.23 ± 1.86	98.62 ± 0.36
F02	0	0	15.94 ± 0.04	97.50 ± 1.20
F03	−1	1	13.68 ± 0.02	95.01 ± 0.44
F04	0	−1	18.89 ± 0.09	98.04 ± 0.29
F05	−1	−1	14.57 ± 1.01	96.89 ± 0.71
F06[a]	1	−1	17.87 ± 0.32	97.60 ± 0.21
F07	−1	0	15.10 ± 0.51	97.41 ± 0.38
F08	0	1	19.23 ± 0.26	98.26 ± 0.31
F09	1	0	14.01 ± 0.71	96.20 ± 0.82
F10	0	0	15.72 ± 0.23	97.00 ± 0.20
F11	0	0	15.54 ± 0.01	96.90 ± 0.80

[a] F06 was selected as the optimal formulation and used as the subject for further studies.

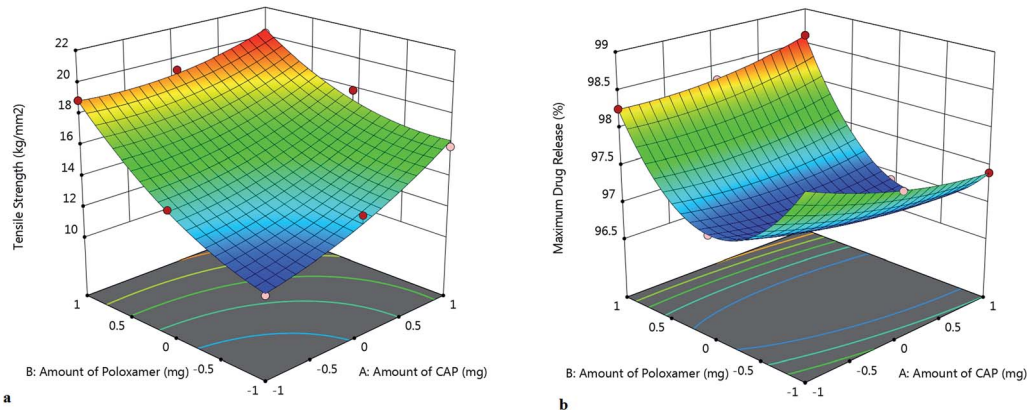

Fig. 2 3D-Response surface plots. As the amounts of polymer and poloxamer were increased, the tensile strength increased (a). As the amounts of polymer and poloxamer were increased, the amount of drug released first decreased and then increased (b).

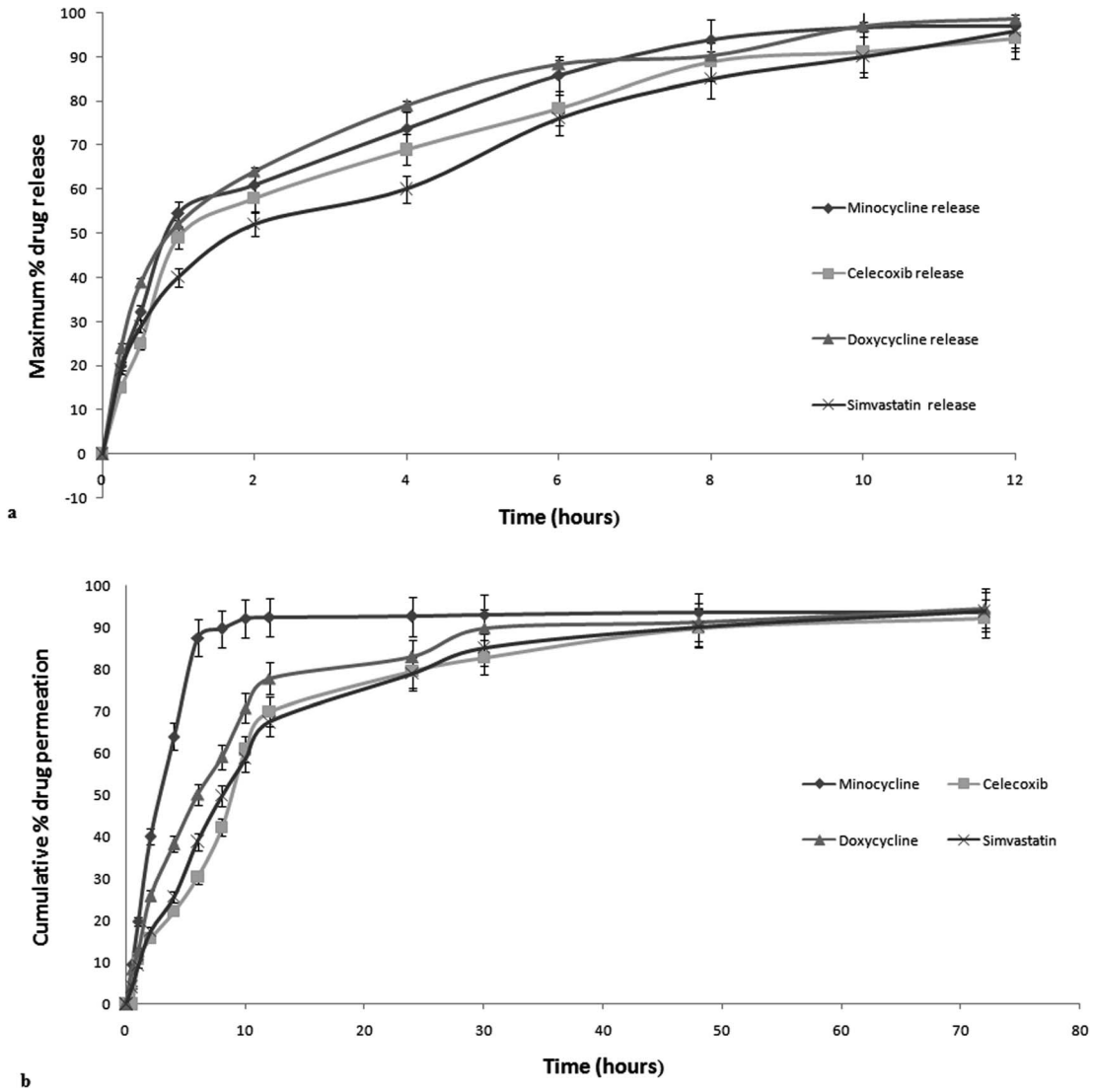

Fig. 3 Drug release and permeation plots. Maximum percentages of drug released (minocycline) from all nine formulations, with F06 showing the highest maximum percentage of minocycline released (a). Cumulative percentage permeations of minocycline, celecoxib, doxycycline hyclate and simvastatin from the F06 formulation (b).

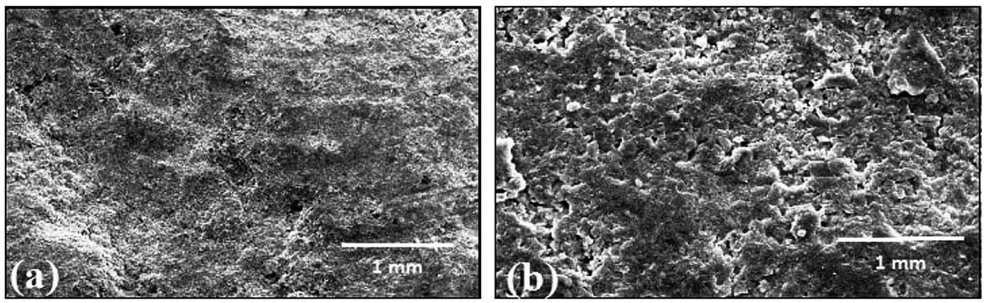

Fig. 4 A scanning electron microscopy (SEM) image of an uncoated disc, showing a smooth surface (a), and of the disc coated with hydroxylethyl cellulose and showing a rough surface (b).

a defined role. Minocycline was primarily used as an antibiotic and a sustained release of this drug generally has an important role in decreasing the oral cavity bio load. Although the *in vitro* drug release study in the current work was done individually for all of the eleven formulations and the maximum percentage release obtained was more than 95% for up to 12 h, we listed in Table 3 the maximum percent release only of minocycline. Our results indicated that almost all of the drug became released from the formulation in 12 h. For *in vitro* release, design expert software was used to derive a quadratic model and an *F*-value of 2254.78 ($P < 0.0500$) was obtained, which implied that the model was significant. The relationship between maximum percentage release and the independent factors was determined to follow the equation

$$\text{Maximum\% drug release} = +96.85 - 0.0683A + 0.3567B + 0.2475AB + 0.1161A^2 + 1.11B^2.$$

ANOVA results revealed A, B, A,^{2} B^2 and AB to be significant model terms. Here we observed that factor A (mass of CAP) produced a significant negative effect on maximum drug release whereas factor B (mass of poloxamer), interaction terms, and higher-order terms produced positive effects on the maximum drug release.

To study the effects of the two independent variables on the *in vitro* drug release, a 3D-response surface plot was constructed and is shown in Fig. 2b. Inspection of the plot indicated that as the masses of CAP and poloxamer were increased, the percentage of the drug released from the formulation first decreased and then increased. This behavior might have been the outcome of an association of the two polymers. By comparing the values of tensile strength and *in vitro* release, F06 (70 mg of CAP and 30 mg of poloxamer) was selected as the optimal formulation and further studies were performed on it. The maximum percentage drug releases of minocycline, celecoxib, doxycycline and simvastatin from this formulation are shown in Fig. 3a and were found to be in the range 94–98%. The differences between the percent releases of the four drugs may have been due to their respective different solubilities in the simulated isotonic phosphate buffer.

***Ex vivo* drug permeation and retention studies.** A permeation profile of the optimal formulation, *i.e.*, F06 (70 mg of CAP and 30 mg of poloxamer) showed that from the goat buccal mucosa, all four drugs started permeating in the initial half hour, a result attributed to the instant release of the drugs from the disc. The cumulative percentage of drug that permeated through the buccal mucosa as a function of time is shown for each drug in Fig. 3b. In each case, most of the drug permeated through the goat mucosa in the initial 14 hours of the study. At the end of the study, *i.e.*, after 72 h, the percent of each drug retained in the mucosa was analyzed using HPLC and found to be 7.33% for minocycline, 6.59% for celecoxib, 8.77% for doxycyline and 5.22% for simvastatin.

Table 4 Observed zones of inhibition of bacterial strains resulting from the *in vitro* release of drugs from aliquots of the optimized formulation (F06)

	Diameter of zone of inhibition (mm)		
Time of sampling (min)	*E. coli* (CC25922) (mean ± SD) ($n = 3$)	*S. aureus* (CC25923) (mean ± SD) ($n = 3$)	*Streptococcus mutans* (CC25175) (mean ± SD) ($n = 3$)
15	28 ± 1.0	29 ± 1.3	28 ± 1.24
30	29 ± 1.3	30 ± 2.4	28 ± 0.89
60	30 ± 2.1	32 ± 1.7	30 ± 2.0
120	31 ± 1.5	32 ± 0.9	33 ± 1.03
240	32 ± 1.2	34 ± 1.23	35 ± 0.34
480	34 ± 2.2	35 ± 0.73	37 ± 0.75
720	36 ± 0.8	37 ± 2.1	38 ± 0.31

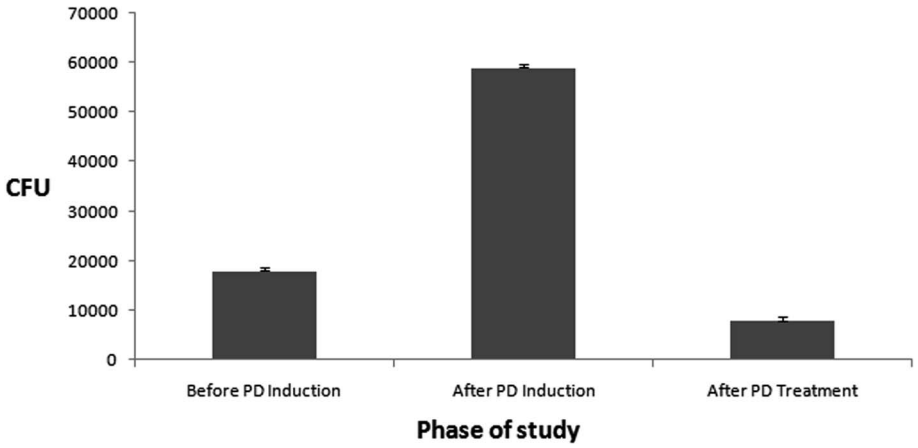

Fig. 5 A bar graph showing the comparisons of colony forming units (CFU) in rat's mouth at different phases of study. Before periodontitis induction, the CFU's in the rat's mouth were much lesser than the CFU's after periodontitis induction, whereas after the disease treatment with formulation the CFU count decreases significantly.

Scanning electron microscopy (SEM). SEM imaging (Fig. 4) of an uncoated disc revealed a smooth surface. In contrast, a coated disc showed a rough surface, which might have helped in the attachment of the coated polymeric disc onto the mucosal surface.

***In vitro* microbiological assay.** The optimized formulation, *i.e.*, F06 (70 mg of CAP and 30 mg of poloxamer) was used as a subject of an antimicrobial efficacy study. Table 4 shows the antibacterial activity of an aliquot of the sample against three bacterial strains. The drug released from the disc was able to inhibit the growth of all three bacterial strains for 12 h. A 38 mm-diameter zone of inhibition of *Streptococcus mutans* (CC25175) was obtained with the aliquot with a 12 h release of sample. Here, fifteen minutes of release also inhibited the bacterial growth, which may have been achieved as a result of the burst of release of drug from the disc.

***In vivo* antimicrobial activity.** Colony forming units (CFUs) were counted in the mouth of each type of rat for each of the different stages of the study (Fig. 5). A greater number of CFUs was found for the experimental periodontitis-induced rat than the control rat in which no disease was induced. The rat treated with the gingiva disc showed fewer CFUs than did the untreated rat. These results indicated the ability of using the gingiva disc to reduce the number of CFUs in a rat's mouth.

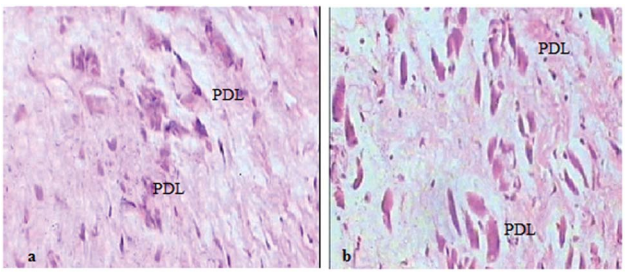

Fig. 6 Images of gingival areas in the mouths of control (a) and treated (b) rats. The gingival cells were observed to be quite similar in the two, suggesting that the formulation did not irritate the gingival tissue.

Mucosal irritation studies. Microscopic images, shown in Fig. 6, revealed that the gingival structure of the control and treated rats were quite similar, with no signs of irritation in terms of redness and inflammation. This result indicated that the optimal gingival disc was a non-irritant of the rat gingiva.

Conclusions

A polymeric gingiva disc displaying muco-retention, the controlled release of embedded drugs, and bioerosion was successfully designed and developed. When subjected to *in vitro* and *in vivo* characterization, it revealed its suitability for completely treating acute-phase periodontitis. The developed formulation is proposed to be an all-encompassing treatment modality for acute phase periodontitis.

Abbreviations

CAP	Cellulose acetate phthalate
MMP	Matrix metalloproteinase
BMP	Bone morphogenic protein
CFU	Colony forming unit
PDL	Periodontal ligament

Conflicts of interest

There are no conflicts to declare.

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