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 around mechanical debridement of the periodontal pocket with untreated. The conventional dental therapeutics revolves of bio compliance. Many novel drug delivery systems have been is expensive, and is o consuming, involves consumption of a high dose of antibiotics, such as gingival pu

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c9ra09569a
polymer 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 Jullibant Life Sciences (New Delhi, India) ex grata. 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 spectrophoto-

metric 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).28

Optimization of the gingiva disc

Discs were prepared using the pressed pellet method and optimized using Design Expert® version 10 software.31 A 2³ 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 M₁) 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 (M₂). The degree of swelling was calculated using the formula

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

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 cyanoacrylate 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.\(^4\) The bioadhesive strength was used to estimate the bioadhesive force of the disc. Bioadhesive force (\(N\)) was calculated using the formula

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

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 (\(\pm \text{SD}\)) of three replicates (\(n = 3\)). The tensile strength was calculated using the formula

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

\textit{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 isotonic phosphate 

\begin{align*}
\text{bu} & \text{dissolution medium containing 2.25\% glycoproteins (simulated}\n\text{ according to the composition of human saliva and mucus.}\text{ The}\n\text{ salivary} & \text{ included in order to have the dissolution media simulate the}\n\text{ bu} & \text{closal membrane. The di} \text{ffusion cell was placed in phosphate} & \\
\text{fusion cells, and} & \\
\text{adhesive tape, and then coated with a thin layer of gold and} & \\
\text{observed with a scanning electron microscope (EVO LS 10, Carl} & \\
\text{Zeiss, Germany).}\n\end{align*}

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

\textit{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 (\textit{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 \textit{et al.}\(^2\) 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. \textit{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 \(\pm\) 0.5 °C for 24 h, and then CFUs were counted and compared.\(^2\) All of the studies were performed in triplicate (\(n = 3\)) and the results are presented as the mean of the three (mean \(\pm\) SD).

\textit{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.\(^2\)\(^6\) These specimens were dehydrated, embedded in paraffin, and stained with hematoxylin and eosin.
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 116.82–136.32 mg. Table 2 shows the means (±SD) of the obtained weight values. The low SD values reflected the uniform thickness.

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 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$^{-1}$, 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:

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

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      | 33                   | 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              | 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                                             |

* 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).
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 so ware 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

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

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 bufer.

**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 goat 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 doxycycline and 5.22% for simvastatin.

| 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                                        |
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.

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.

Acknowledgements

The authors thank the Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, India, for providing essential facilities for this research. The authors are also thankful to the Department of Microbiology and Department of Dentistry HIMSR for providing guidance in the research.
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