Metronidazole and Pentoxifylline films for the local treatment of chronic periodontal pockets: preparation, in vitro evaluation and clinical assessment

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Objective: Periodontitis is one of the most important chronic inflammatory dental diseases arising from the destructive actions caused by a variety of pathogenic organisms presented in the oral cavity. The aim of this study is the preparation and in vitro evaluation of films for the local treatment of periodontal pockets.

Methods: The prepared films contained either metronidazole (Mtr), for its antimicrobial effect in periodontal diseases, using a mixture of polymers namely hydroxypropyl methyl cellulose, Carbopol 934 or locally applied Pentoxifylline (PTX), for its anti-inflammatory activity, using chitosan. All films were prepared using solvent casting technique and were evaluated for their physical characteristics, drug content uniformity, surface pH, swelling behavior, mechanical properties and in vitro release. Further characterization was done on the selected formulations using differential scanning calorimetry and scanning electron microscopy for surface structure. Clinical evaluation tests were also performed.

Result: Appropriate physical characteristics and mechanical properties for most formulations and their suitability for periodontal application were observed. In vitro drug release from most films showed a burst release rate for both Mtr and PTX during the first 2 h after which the release rate was markedly decreased. Clinical trials on patients revealed the advantageous use of Mtr and PTX as an adjunct treatment with traditionally used dental techniques.

Conclusion: The effectiveness of the co-therapy of either drug could add benefit in the eradication of chronic periodontal hazards.

Keywords: chitosan, clinical assessment, hydroxypropyl methyl cellulose, in vitro release, local drug delivery, metronidazole, pentoxifylline, solvent casting technique


1. Introduction

Oral hygiene is considered one of the most effective aspects in the general well-being of individual’s health. In most Arabian countries, the public education for oral hygiene is still a way behind, especially among rural population. Among the most serious consequences of this problem is the development of chronic oral inflammatory diseases that may in turn affect seriously other body organs including the heart [1-4].
Periodontal disease or periodontitis is interchangeable terms of one of the most important oral inflammatory infectious diseases affecting the oral cavity [5]. However, some authors consider periodontitis as a more advanced condition of periodontal diseases that involves bone resorption and periodontal ligaments destruction [6]. Such destruction of the dental supporting tissues will subsequently result in tooth loss. Periodontal pockets caused as a result of periodontitis may reach in depth up to 5 mm. The pockets formed act as a rich field for the growth of several pathogenic organisms including anaerobic and micro aerophilic types [6-8]. The severe inflammation and immune response due to pathogenic organisms stimulate the release of inflammatory mediators and cytokines among which is TNF-α [5,9].

In the past decade, the use of locally applied antimicrobial drug delivery systems has received enormous attention due to the various reported disadvantages of their systemic administration. Both the antimicrobial agents, metronidazole (Mtr) and chitosan (CH) polymer, have been reported in the management of periodontitis [10,11]. Mtr is considered one of the most effective chemotherapeutic agents that have been used for the eradication of many anaerobic species presented in the inflamed periodontal pockets [12-14]. On the other hand, CH is a natural polymer that has proven to be safe and effective against several pathogenic organisms in the oral cavity for the management of several pathological conditions [15]. It also possesses a local hemostatic action and has been used in many medical devices and bandages for such purpose [16,17]. The diverse actions of CH make it an excellent candidate for local drug delivery in the oral cavity. Among the inflammatory reactions associated with periodontitis is the excessive release of inflammatory cytokines among which is TNF-α, which by turn amplifies the expression of other inflammatory cytokines [18]. Pentoxifylline (PTX) is a methylxanthine phosphodiesterase inhibitor that has both hemorheological and an anti-inflammatory activity, which suppresses the synthesis of pro-inflammatory cytokines including TNF-α [19,20]. It has been used orally in the treatment of several oral inflammatory periodontal and osteogenic related conditions [20,21]. However, oral use of drugs in the treatment of periodontitis requires high-dose regimen, which by turn may cause the evolution of several severe undesired side effects [6,22].

The local administration of Mtr and PTX in the presence of CH could be advantageous in decreasing their systemic side effects, decreasing the healing time while increasing the possibility of quick regeneration of the destructed tissues. The objective of this study was to prepare and in vitro evaluate films containing either Mtr or PTX using different polymers. Films were investigated for the properties that ensure the ease of their application in periodontal pockets. Subsequently, clinical evaluation was performed on the selected formulations in an attempt to prove their efficiency.

2. Materials

Mtr and PTX were kindly supplied by Pharaonia Pharmaceuticals Co. (Alexandria, Egypt). CH, maximum granule size 0.2 mm, degree of acetylation > 80%, was purchased from CarboMer, Inc. (San Diego, CA, USA). Carbopol 934 was obtained from Goodrich Chemical Co. (Akron, OH, USA) and polyvinyl alcohol (PVA), Mowiol®, 40 – 88, was purchased from E.I. du Pont de Nemours & Co. (Wilmington, DE, USA). Hydroxypropyl methyl cellulose (HPMC) 4000 cp, Methocel®, was kindly supplied by Pharaonia Pharmaceuticals Co. (Alexandria, Egypt), polyvinyl pyrrolidone (PVP), Kollidon® 25 was purchased from BASF Aktiengesellschaft (Ludwigshafen, Germany). Other chemicals were of analytical and pharmaceutical grade.

3. Methods

3.1 Preparation of periodontal films

Film formulations were prepared using the solvent casting technique (Table 1) [23,24]. For HPMC: CP/Mtr films (HP films), the calculated amount of HPMC was added gradually while stirring to half of the required volume of distilled water at 90°C. PVA was dissolved in the minimum amount of hot water at 90°C then added to the HPMC gel and stirred. The mixture was then allowed to cool and the CP solution prepared 24 h before was added to it and mixed thoroughly. The calculated amount of Mtr to provide total drug-polymer ratio of 1:6 was then added after its levigation with 3% glycerol in order to maintain the film flexibility. Calculated amount of triethanolamine (0.3%) was added to the final gel before casting to adjust the pH.

For CH films [25], CH was dissolved in the appropriate amount of 0.5% v/v acetic acid and stirred for 48 h for complete dissolution. Hydrophilic additives, namely gelatin (GEL) and PVP, were first dissolved in the minimum volume of distilled water, and then added to the filtered CH solution. The calculated amount of PTX to produce total drug-polymer ratio of 1:4 was incorporated in the polymeric solutions after levigation with 3% v/v glycerol, which was used as a plasticizer to impart adequate flexibility to the produced films.

The medicated gels were left overnight at room temperature to get rid of any bubbles formed while stirring. The gels were casted into glass Petri dishes and allowed to dry in a leveled oven maintained at 40°C for HP films or at room temperature in case of CH films, a period of time enough to produce flexible, dry film with constant weight. The dried films were cut into circular patches of 8 mm diameter, packed in aluminum foil and stored in amber glass containers maintained at room temperature in well-closed desiccators.
Metronidazole and Pentoxifylline films for the local treatment of chronic periodontal pockets

Table 1. Composition of metronidazole and pentoxifylline films.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Polymer concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPMC</td>
</tr>
<tr>
<td>HP1</td>
<td>2</td>
</tr>
<tr>
<td>HP2</td>
<td>2</td>
</tr>
<tr>
<td>HP3</td>
<td>2</td>
</tr>
<tr>
<td>HP4</td>
<td>2</td>
</tr>
<tr>
<td>CH1</td>
<td>-</td>
</tr>
<tr>
<td>CH2</td>
<td>-</td>
</tr>
<tr>
<td>CH3</td>
<td>-</td>
</tr>
<tr>
<td>CH4</td>
<td>-</td>
</tr>
</tbody>
</table>

CH: Chitosan; HP: Hydroxypropyl.

3.2 In vitro evaluation of the prepared films

3.2.1 Physical characterization and content uniformity test

Assessment of weight and thickness was done on six randomly chosen film patches from each formulation using a sensitive balance (Electronic balance, Sartorius AG, weighting technology, BL-210S, Germany) and a digital micrometer (Tricircle micrometer, China), respectively. The mechanical properties of the prepared films were determined by counting the number of times each film patch can fold at the same point without breaking [23]. Determinations were performed in triplicate. Drug content uniformity was tested on six randomly selected film patches of each formulation. Each drug-loaded patch was allowed to dissolve in 100 ml Sørenson phosphate buffer pH 6.6. The concentrations of Mtr and PTX in the patch were determined spectrophotometrically at 320 and 274 nm, respectively. UV standard curves were constructed over a concentration range of 4 - 20 and 5 - 25 µg/ml for Mtr and PTX, respectively. All results are presented as mean ± standard deviation.

3.2.2 Surface pH

Film patches were allowed to swell for 2 h on the surface of agar plates prepared in Sørenson phosphate buffer pH 6.6. The surface pH was determined using pH paper placed on the surface of the swollen patch. A mean of three readings were recorded [26].

3.2.3 Swelling and water uptake study

The sponge method [27] was adopted with slight modifications. The soaking set was composed of a (7 × 3 × 3 cm) sponge immersed in Sørenson phosphate buffer pH 6.6, then placed into a plastic box (10 × 12 × 6.5 cm) containing 100 ml of the buffer to maintain the sponge soaking conditions and to be leveled 1 cm from the bottom. A presoaked rectangle-shaped filter paper (3 × 4 cm) was placed onto the top of the sponge, then the whole set was covered for 30 min for equilibrium. The accurately weighed patches were then placed on the wet filter paper and the box was covered. The water uptake was determined by monitoring the increase in weight of the patches at different time intervals and normalized to the initial dry film weight. The experiment was done in a thermostatic oven (Kotttermann, D3165 Hanigsen/W, Germany), adjusted at 37°C. The swelling index (S.I.W) was calculated at determined time intervals using the following equation that also expressed the water uptake by the films:

\[
S.I.W = \frac{(W_t - W_o)}{W_o}
\]

where S.I.W is the calculated swelling index, \(W_t\) the weight of the swollen patch at time t and \(W_o\) is the patch weight at zero time.

3.2.4 Mechanical properties of the films

The mechanical properties of the prepared films were determined by measuring the tensile strength (TS), percentage elongation at break and elastic modulus using Universal testing machine (Shimadzu AG-1S, Japan) [12,28]. The film strips (50 × 20 mm) were held between clamps and the force and elongation were measured when the strip broke. The instrument was operated at an extension rate of 10 mm/min. [29]. Triplicate measurements for all tested properties were conducted. The following equations were applied to calculate the different measured parameters:

\[
TS(N/mm^2) = \frac{\text{Breaking force}}{\text{Cross sectional area}}
\]

\[
EB\% = \frac{\text{Increase in length} \times 100}{\text{Original length}}
\]

\[
EM(N/mm^2) = \frac{\text{Force at corresponding strain} \times 1}{\text{Cross sectional strain}}
\]

3.2.5 In vitro release study

Each disc of 8 mm diameter was weighed and placed into a 5 ml vial containing 2 ml Sørenson buffer of pH 6.6, previously warmed at 37°C. The closed vials were placed in a thermostatically controlled water bath (Type 1083 GFL Gesellschaft fuer Labortechnik m.b. H. & Co., Burgwedel, Germany) preset at 37°C, until the end of the experiment. The whole volume was withdrawn at predetermined time intervals (0.5, 1, 2, 3, 4, 6, 8, 24, 48 and 72 h) and replaced by fresh warmed buffer solution [30,31]. The samples were assayed for Mtr and PTX contents spectrophotometrically (Pharmacia LKB Ultrospec III double beam, England) at \(\lambda_{max}\) 320 and 274 nm, respectively, and the cumulative drug concentrations were calculated. All experiments were done in triplicate and the values were presented as the mean ± standard deviation. Blank films were also subjected to the release study to detect the contribution of the polymers used, if any, to UV absorption.
3.2.6 Scanning electron microscopy
Surface morphology of the chosen films was studied by scanning electron microscope after gold vacuum coating and visualized at acceleration voltage of 80 kV (JEOL JSM-5300, Japan).

3.2.7 Differential scanning calorimeter
Differential scanning calorimetry (DSC) was carried out on pure drug, polymers, physical mixtures and selected drug-loaded films. Accurately weighed samples (3 mg each) were placed in aluminum pans and sealed. The runs were conducted over a temperature range from ~50 to 400°C at a heating rate of 10°C/min under nitrogen using Perkin Elmer Pyris Series DSC6 (USA).

3.3 Clinical evaluation
Clinical evaluation of the selected film formulations (HP4 and CH2) was carried out according to the World Medical Association Declaration of Helsinki (ethical principles for medical research involving human subjects) (32). All patients recruited in this study signed a written consent before the start of treatment procedures. The study was conducted on 30 patients of both sexes (16 females and 14 males) whose age ranged between 35 and 55 years. They were selected from the out-patient clinic of the Oral medicine and Periodontology Department, Faculty of Dentistry, Pharos University and 6th October University, Egypt. The selection was based on several criteria including the following: being non-smokers, non-pregnant or lactating females, have not received antibiotic therapy or any drugs throughout the past 6 months, do not suffer from any known allergic reaction to both drugs under study and are diagnosed to have moderate to severe periodontitis (with a pocket depth (PD) > 5 mm). They were blindly divided into three equal groups, I, II and III, where all groups underwent full-mouth supra and subgingival debridement. All patients received complete periodontal and gingival examination as well as probing measurements. After complete dryness of the experimental area, each group was treated differently as follows: group I received a suitable size of Mtr films (HP4) fitted to the pocket and a periodontal dressing (Coe-pack) was used to secure the film in place; group II received PTX films (CH2) with the same procedure as group I and both groups were subjected to the same procedure after 1 week. Group III was kept as a control group with no treatment for the purpose of comparison. Patients in all groups were recalled monthly for oral hygiene reinforcement. Each patient was asked to report any sign of discomfort or allergy occurring before the next visit.

3.3.1 Clinical assessment
Clinical parameters applied in the assessment study for every patient were measured before treatment (baseline) and 3 months after treatment. They comprised plaque index (PI), gingival index (GI), probing PD and clinical attachment level (CAL). The concentrations of TNF-α and osteocalcin (taken as markers for the healing process) in the collected gingival crevicular fluid, GCF, were determined using a bioassay method applying commercially available ELISA kit obtained from Diacalone, Tepnel Research, for TNF-α and from BioSource HOST-ELISA Kit for osteocalcin. The assays were performed using the manufacturer’s instructions.

3.3.2 Statistical analysis
Regression analysis using repeated-measures analysis of variance (ANOVA) was used to compare between the groups and studying the effect of time on the different variables. One-way ANOVA was used for comparison between percentage changes in the different variables among the three groups. Tukey’s post hoc test was used for pair-wise comparison between the means when ANOVA test is significant. The significance level was set at p ≤ 0.05. Statistical analysis was done using SPSS 16.0® (Statistical Package for Scientific Studies) for Windows.

4. Results

4.1 Physical characteristics and content uniformity
All films under study were prepared by solvent casting method (Table 1). For comparative purposes, all films were cut equally into circular patches of diameter 8 mm. Physical characteristics and drug content of all formulations were determined (Table 2). HP film patches ranged from 28.47 to 40.24 mg weight and from 0.24 to 0.41 mm thickness, while CH film patches ranged from 32.68 to 55.93 mg weight and from 0.23 to 0.77 mm thickness. The folding endurance study revealed a general increase in the flexibility of films prepared by increasing the CP content in HP formulations and GEL content in CH films. However, it was observed that the absence of CP in HP1 formulation rendered the film brittle and slight sticky, which urged its exclusion from the study. The same behavior was observed with CH4, which contained 6% GEL, showing an absolute increase in stickiness. As for CH1, the contrary was observed as the film was too hard with sharp ends on cutting that would not render the film applicable in periodontal pockets. The surface pH of all HP formulations was about neutrality as adjusted by the addition of triethanolamine before casting and ranged for CH films from 5.5 to 6. Percentage drug content indicated drug uniformity and distribution all over the prepared films as indicated by the relatively small values of standard deviations.

4.2 Swelling and water uptake study
In general, the HP/Mtr films showed less swelling ability compared to CH/PTX films starting from the second hour to the end of the experiment (Figure 1). HP films showed a decrease in water uptake by the increase in the CP content while, on the contrary, CH films showed an increase in water uptake and swelling index by the increase of the GEL content.
4.3 Mechanical properties of the films
The TS in MPa, % elongation at break, tear force in N and the elastic modulus in N/mm were determined (Table 3). It could be seen that the maximum values of the studied parameters are confined mainly to HP4, CH2 and CH3 formulations.

4.4 In vitro release study
A burst release of Mtr (50 – 60%) and PTX (35 – 40%) from both types of films was observed throughout the first 2 h (Figures 2 and 3). This effect was followed by a decrease in the release rate for the next 10 h, then by a marked decrease in rate to the end of the study.

4.5 Surface morphology of selected films
Scanning electron microscopy (SEM) was performed for surface characterization of the selected films, HP4 and CH2, which were chosen based on their physical, mechanical properties and release pattern (Figure 4).

4.6 Differential scanning calorimeter
The thermal analyses for the films, polymers used, drugs and the physical mixtures of the selected films were done (Figure 5). It was observed that Mtr in HP4 film showed a slight shift to a lower temperature (from 162.007 to 110.162°C) and broader peak when compared to the peaks of the drug alone and its physical mixture with the polymers used. As for the CH2 film, there was a slight, insignificant change in the thermal peak of drug when compared to that of PTX alone or the physical mixture, indicating no possible interaction.

4.7 Clinical evaluation and assessment
Selected films containing Mtr and PTX were inserted in the periodontal pockets of groups I and II, respectively, after scaling and root planning (SRP) procedure (Figure 6). The clinical results are presented for all groups as mean % reduction of the tested parameters after 3-month treatment relative to the baseline values (Figure 7). It was observed that all studied parameters were decreased by the end of the study for all groups. ANOVA results showed that the time had a

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Table 2. Physical characteristics of the prepared films.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Weight* (mg ± SD)</th>
<th>Thickness* (mm ± SD)</th>
<th>Folding endurance* (number ± SD)</th>
<th>Surface pH</th>
<th>Drug content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>28.47 ± 1.19</td>
<td>0.237 ± 0.004</td>
<td>170 ± 3</td>
<td>7</td>
<td>4.74 ± 0.24</td>
</tr>
<tr>
<td>HP2</td>
<td>31.7 ± 1.04</td>
<td>0.346 ± 0.016</td>
<td>178 ± 2</td>
<td>7</td>
<td>5.28 ± 0.21</td>
</tr>
<tr>
<td>HP3</td>
<td>35.23 ± 1.27</td>
<td>0.372 ± 0.004</td>
<td>215 ± 6</td>
<td>7</td>
<td>5.87 ± 0.26</td>
</tr>
<tr>
<td>HP4</td>
<td>40.24 ± 0.77</td>
<td>0.410 ± 0.001</td>
<td>271 ± 4</td>
<td>7</td>
<td>6.70 ± 0.16</td>
</tr>
<tr>
<td>CH1</td>
<td>32.68 ± 0.98</td>
<td>0.227 ± 0.004</td>
<td>60 ± 3</td>
<td>5.5</td>
<td>8.17 ± 0.30</td>
</tr>
<tr>
<td>CH2</td>
<td>38.8 ± 0.79</td>
<td>0.463 ± 0.012</td>
<td>&gt; 300</td>
<td>5.5 – 6</td>
<td>9.69 ± 0.24</td>
</tr>
<tr>
<td>CH3</td>
<td>40.63 ± 0.89</td>
<td>0.576 ± 0.025</td>
<td>&gt; 300</td>
<td>5.5 – 6</td>
<td>10.12 ± 0.27</td>
</tr>
<tr>
<td>CH4</td>
<td>55.93 ± 0.65</td>
<td>0.770 ± 0.012</td>
<td>-</td>
<td>6</td>
<td>13.98 ± 0.20</td>
</tr>
</tbody>
</table>

*Results are presented as mean ± SD, n = 3.
CH: Chitosan film; HP: Hydroxypropyl methyl cellulose film; SD: Standard deviation

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Figure 1. Swelling behavior of (A) HP films, (B) CH films.
CH: Chitosan film; HP: Hydroxypropyl methyl cellulose film.
significant effect on the mean of the tested clinical parameters including PI, GI, PD and CAL for all groups from the baseline at p-value < 0.001. However, repeated-measures ANOVA indicated insignificant differences between groups under study for the same parameters. For TNF-α level, the results showed that there was a statistically significant difference between its mean values within the three groups. This was confirmed by Tukey’s post hoc test, which showed no statistically significant difference between groups I and II, which have the lowest TNF-α values, while at the meantime, group III showed the statistically significantly highest mean TNF-α value at p ≤ 0.05. As regards CAL, group I showed the statistically significantly highest mean % reduction in CAL. However, there was no statistically significant difference between groups II and III, which showed the statistically significantly lowest % reduction in CAL.

5. Discussion

5.1 Physical characteristics and content uniformity
In all HP films, PVA was added at a concentration of 3.5% before casting to provide consistency and homogeneity to the gel mixtures. The concentration of PVA was selected based on preliminary screening of different concentrations. It was previously reported that PVA had supporting effects on the films’ mechanical properties and flexibility, which would be advantageous for their periodontal applications [29,31]. As for CH films, 1% PVP, as a film-forming polymer, was added to all prepared formulations to improve the flexibility of the films [33]. However, CH1 formulation showed a very hard texture upon drying. All films showed uniform weight and appropriate thickness suitable for periodontal application [9]. The determined pH for all films was suitable for their application in the periodontal environment [34].

5.2 Swelling and water uptake
The water uptake by the films and swelling characteristics are important parameters in their applicability in the periodontal pockets due to the limited pocket size. The marked initial water uptake shown by all films would be of benefit for intra-pocket application, where the film will be hard enough
Figure 4. DSC thermograms of (A) HP4 film, (B) CH2 film.
CH: Chitosan; CH2: Chitosan film; CP: Carbopol 934; DSC: Differential scanning calorimetry; GEL: Gelatin; HP4: Hydroxypropyl methyl cellulose film; HPMC: Hydroxypropyl methyl cellulose; Mtr: Metronidazole; PTX: Pentoxifylline; PVP: Polyvinyl pyrrolidone.

Figure 5. Scanning electron micrographs showing the upper and lower surfaces of the selected periodontal films HP4 and CH2.
CH: Chitosan film; HP: Hydroxypropyl methyl cellulose film.
during intra-pocket insertion, followed by immediate softening by the action of the GCF, thus decreasing the feeling of discomfort. On the other hand, the moderate swelling shown by all films would allow the insertion of a comparatively large piece. However, the clinician should not impact the pocket with excessively large part to afford an additional space for the device to swell. For HP/Mtr films, the rate of swelling decreased after the third hour probably due to the presence of CP. HP4 showed the least swelling characteristics. This finding was in accordance with the previous work, which reported that increasing the CP content would render the film more elastic as well as decrease the extent of swelling of HPMC [35]. Addition of PVP to CH in different ratios has been studied before showing comparable results to ours with respect to the swelling behavior [23]. However, it was observed that the swelling behavior of CH in the presence of 1% PVP was much more pronounced in CH2 and CH3 films probably due to the presence of the water-soluble and swellable GEL. This hydrophilic polymer would in turn increase the surface wet ability and consequently water penetration within the matrix [36]. CH3 showed the maximum swelling properties while the absence of GEL in CH1 limited it.

5.3 Mechanical properties of the films
The mechanical properties of the prepared films were studied in order to determine the most appropriate films for the clinical assessment. The film to be inserted in a periodontal pocket must have an appreciable strength and resistance to tear as well as being flexible enough without being too hard, with sharp ends or too sticky. The mechanical properties were studied for all prepared films except CH4 formulation due to its sticky consistency and inapplicability. The ratio of CP to HPMC concentration is said to be related to the TS increase [37]. However, in the present study, all HP films showed relatively higher values than the reported ones. This could be attributed to the presence of PVA, which has contributed in adjusting the mechanical properties of the prepared films. For CH films, it was reported that the mechanical properties of CH films used alone or in combination with other polymers depend on several aspects including the pH and molecular mass of the film-forming polymer and on the degree of deacetylation of CH used [38,39], the type of acid used [40], drying conditions of films [41] and the amount of water content [42]. In case of mixed polymer films containing CH, the ratio of the components used may also have remarkable effect on its properties.

5.4 In vitro release study
Release of Mtr and PTX from HP/Mtr films and CH/PTX films was performed in 2 ml buffered system at pH 6.6 to simulate the small space available of the periodontal cavity, the un-sink condition and pH. The initial burst release effect observed from all films could be explained by the fact that the drug may exist in the finely divided state after solvent evaporation during film casting and their deposition on the surface of the films after drying as proved by the SEM photos (Figure 4) [37,43,44]. Two main targets are to be achieved when inserting a medicated film in the periodontal pocket, to release an initial high dose of drug in order to produce an immediate therapeutic effect, followed by small doses to maintain the therapeutic level throughout a longer period of time [45]. The general initial increase of the dissolution of Mtr from HP films could also be attributed to the presence of the water-soluble PVA, as being suggested previously [46]. For CH/PTX films, the accelerated dissolution could also be attributed to the presence of water-soluble hydrophilic additives in these films that would dissolve rapidly introducing porosity [47]. The formed voids will in turn allow for the entrance of the release media and its diffusion through the film.

5.5 Surface morphology of selected films
The surface structure of HP4 film clearly shows that the upper surface was rough with well-structured small drug crystal deposition, while the lower surface was smoother with smaller crystals probably due to the casting of the film in a glass Petri dish, which by turn rendered the lower surfaces smoother in all prepared films. As for CH2 the upper and lower surface of the film was smoother with well-distributed minute drug crystals.

5.6 Differential scanning calorimeter
For HP4 film, a possible interaction of PVA, CP or HPMC with Mtr through H-bonding may have caused the thermal shift of the drug peak to a lower temperature [29]. The broader peak may be explained on the concept that the drug during formulation procedure is partially transferred to the amorphous form thus acquiring lower temperature for the melting process as previously been suggested in similar studies [48,49].

5.7 Clinical evaluation and assessment
The remarkable decrease in TNF-α values in groups I and II compared to the control group could be explained on the
basis of the therapeutic action produced by Mtr in reducing the existing pathogens and thus decreasing the inflammation as well as the action of PTX in its direct effect on increasing the local flow of blood in the inflamed pocket and reducing the level of TNF-α [50]. As for the marked decrease of CAL level in group I, it could be explained on the basis of the strong antimicrobial effect of Mtr on the periodontal pathogens as being previously reported in a similar study [51]. From the clinical study, it could be concluded that although the traditional technique, SRP, applied for the treatment of periodontal pockets is so effective as being previously reported [52], data from the two groups treated by combined therapy in this study revealed higher improvement compared to the group treated by SRP alone.

### 6. Conclusion

From this study it could be concluded that Mtr and PTX films can be prepared by a simple method using a combination of polymers that provide appropriate physical characteristics as well as a sustained drug release. The local delivery of both PTX and Mtr in a sustained-release formula enhances the therapeutic effect of SRP as demonstrated by the clinical parameters measured and the tested biochemical markers of inflammation and bone turnover. The GCF levels of OC and TNF-α as measured in this study insure the effects of locally delivered PTX and Mtr as adjuncts to SRP in treatment of chronic periodontitis.

### 7. Recommendations

Further studies on larger patient populations are indicated to investigate more thoroughly the effects of these drugs and the value of these markers in the local periodontal management.

### Acknowledgments

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. 166-012-D1434. The authors, therefore, acknowledge with thanks DSR for technical and financial support. The authors would also like to express their gratitude to all the medical dental staff and patients involved in the study.

### Declaration of interest

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. 166-012-D1434.
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