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Research Article

Comparative Study to Investigate the Effect of Meloxicam or Minocycline HCl *In Situ* Gel System on Local Treatment of Periodontal Pockets

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Abstract. *In situ* gelling formulations allow easy application to the target area. Gelation is induced by physiological stimuli at the site of application where the formula attains semisolid properties and exerts sustained drug release. *In situ* gelling formulations containing either 3% meloxicam (Mx) or 2% minocycline HCl (MH) were prepared for local application into the periodontal pockets. Gel formulations were based on the thermosensitive Pluronic® (Pl) and the pH-sensitive Carbopol® (C) polymers. C gels were prepared in combination with HPMC (H) to decrease its acidity. The total percent drug released from Pl formulae was 21.72% after 1 week for Mx and 85% after 3 days for MH. Their release kinetics data indicated anomalous non-Fickian behavior that could be controlled by both diffusion and chain relaxation. Addition of MH to C/H gels (1:2.5) resulted in liquefaction, followed by drug precipitation. Regarding C/H gel containing Mx, it showed a prolonged release rate up to 7 days with an initial burst effect; the kinetics data revealed Fickian-diffusion mechanism. The *in vitro* antibacterial activity studies for MH gel in Pl revealed that the drug released exceeded the minimum inhibitory concentration (MIC) of MH against *Staphylococcus aureus* ATCC 6538; *placebo* gel showed no effect on the microorganism. Clinical evaluation of Pl gels containing either Mx or MH showed significant improvement in chronic periodontitis patients, manifested by decrease in pocket depth and gingival index and increase in bone density.

KEY WORDS: antiinflammatory; antimicrobial; Carbopol®; clinical evaluation; periodontitis; pH-sensitive; Pluronic®; thermosensitive.

INTRODUCTION

Periodontal diseases comprise a group of inflammatory conditions of the teeth supporting tissue that are initiated by the microorganisms that colonize on the tooth surface and infect the surroundings. The extension of inflammation from the marginal gingiva into the supporting periodontal tissues marks the transition from gingivitis to periodontitis (1).

Progression of periodontal disease could be halted by blocking the inflammatory pathways important in periodontal tissue destruction. Nonsteroidal antiinflammatory (NSAI) drugs were found to slow the progression of periodontal disease (2). Meloxicam (Mx) is a potent NSAI with favorable preferential inhibition of COX-2 enzyme (3). Mx has been reported to be not only a potent inhibitor of acute exudation in periodontal tissues but also an intense inhibitor of bone and cartilage destruction (3,4). In addition, it prevented the alveolar bone loss in experimental periodontitis in rats, when administered subcutaneously (5). Moreover, it reduced the gingival crevicular fluid (GCF) level matrix metalloproteinase 8 (MMP8), a main factor in connective tissue destruction, following oral administration, when used as adjunct to scaling and root planning (6).

Antimicrobial agents are mainly used in treatment of periodontal pockets. Minocycline HCl (MH), a tetracycline analog, has proven to be very effective in eradicating periodontal pathogens implicated in periodontitis (7). It shows more lipid solubility than tetracycline HCl and thus passes directly through the lipid bilayer of the bacterial cell wall (8). Apart from its antibacterial activity, MH shows additional significant pharmacological effects for the management of periodontal diseases, including collagenase inhibition, antiinflammatory action, bone resorption inhibition, and ability to promote the attachment of fibroblasts and connective tissue to the root surface (9).

Local intra-pocket drug delivery, as compared to oral administration, can provide an effective drug concentration at the site of action with avoidance of undesirable side effects. The success of this treatment is measured by its ability to control and prolong the release rate of the drug (10). The injectable *in situ* gelling systems are good candidates for intra-pocket drug delivery; being fluid before and during application, they ensure easy application using an intra-pocket

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syringe that allows the formulation to get access to the entire pocket. Under the physiological conditions, the sol form is easily transformed into a viscous gel, which resists removal by the GCF flow (11).

Gelation occurs *in situ* after exposure to certain *stimuli*, as solvent exchange, UV irradiation, ionic cross-linkage, pH change, or temperature modulation (12). Recent researches focused on ionic cross-linkage (13), change in pH (13,14), or change in temperature (15,16).

The aim of the present work was to develop and evaluate simple *in situ* gelling systems containing either the antiinflammatory drug, Mx, or the antimicrobial agent, MH, for local treatment of periodontal pockets. Local minocycline devices for the treatment of periodontal pockets are available on the market in the form of lipid-like gel, e.g., Periocline® and Dentomycine® and injectable microspheres Arestin®. On the other hand, no commercial products for intra-pocket application containing Mx are available. In addition, limited studies dealing with local periodontal delivery of Mx are reported (17,18). The present work offers an easy and unexpensive way for preparation and application of Mx and MH *in situ* gels to be injected into the periodontal pockets. Evaluation of the prepared formulae includes *in vitro* release study, clinical evaluation for the selected formulae, and microbiological evaluation of the formula containing MH.

MATERIALS AND METHODS

Materials

The materials used in this study were meloxicam (Delta Pharmaceuticals Co., Cairo, Egypt), minocycline hydrochloride (Pharaonia Pharmaceuticals Co., Alexandria, Egypt), poloxamer 407 (Pluronic® F127), and hydroxypropylmethyl cellulose 4,000 cp (HPMC) (El-Amryia Pharmaceutical Ind. Co., Alexandria, Egypt) and Carbopol® 934 (Goodrich Chemicals Co., Cleveland, OH, USA) and agar, CM0003 Nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, England). Standard *Staphylococcus aureus* ATCC 6538 was supplied from the Department of Microbiology, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt. All other chemicals were of analytical or pharmaceutical grade.

Methods

Preparation of Pluronic®-Based Thermo-Sensitive Gels

Medicated Pluronic® (Pl) gels containing either 3% Mx (18) or 2% MH (19) were prepared. First, *placebo* Pl gels were prepared in a concentration of 35% *w/v* according to the reported cold method (20). Pl powder was added to cold distilled water (5–10°C) with gentle stirring till formation of a homogeneous dispersion; then, left overnight in the refrigerator (4–5°C), a clear sol was obtained. The calculated amount of either Mx or MH was added to quarter the amount of the final volume of distilled water, mixed for 1 h using sonifier so that homogeneous slurry was obtained. The cold polymer sol was added to the calculated amount drug slurry and mixed in an ice bath prior to use.

Preparation of Carbopol®-Based pH-Sensitive Gels

A combination of Carbopol® (C) and HPMC (H) was prepared in a ratio of 1:2.5 (21). Colloidal dispersion of C (1%) was prepared by adding the polymer gradually to distilled water with gentle stirring. The H gel (2.5%) was obtained by addition of the polymer to 1/3 volume of distilled water maintained at 90°C with gentle stirring; the remaining volume of water was then added, followed by overnight refrigeration. The C/H combination was obtained by mixing calculated amounts of polymeric dispersions. The resultant sol was thoroughly mixed, and the pH was adjusted to 4±0.1 with 0.5 M NaOH (22). The drug slurry was prepared as previously mentioned and was added to the C/H polymeric system immediately before use.

In Vitro Drug Release Studies from Pl and C/H Polymer System

The medicated gels were gently stirred, and 100 mg was filled into small circular stainless-steel cups (7 mm internal diameter and 4 mm depth) in triplicate and covered with polyester gauze, which acts as a mechanical barrier to prevent gel escape without interfering with drug release (23). The gauze was fixed by means of stainless-steel well-fitting circular holders. The cups were then introduced into 5-ml beakers containing 2-ml Sørenson phosphate buffer, pH 6.6 to simulate gingival fluid pH (24), previously warmed at 37°C. The release systems were placed in a thermostatically controlled water bath maintained at 37°C, without shaking. Aliquots of 2 ml were withdrawn at specified time intervals and replenished with the same volume of pre-warmed fresh release medium. The samples were analyzed spectrophotometrically at λ_{max} 362 nm (25) and 245 nm (26) for Mx and MH, respectively. Drug concentrations were calculated by reference to the appropriate calibration curves constructed in the same buffer.

Kinetic Analysis of Release Data

To determine the mechanism of drug release from different gel formulations, the release data were analyzed using the simple semiempirical equation proposed by Peppas (27):

$$M_t/M_\infty = kt^n$$

where M_t/M_∞ is the fractional release of the drug,

- t is the release time
- k is a constant incorporating structure and geometric characteristics of the controlled release device
- n is the release exponent, indicative of the mechanism of drug release.

In Vitro Determination of the Antibacterial Activity of MH in Pl Gel

The standard curve and the minimum inhibitory concentration (MIC) of MH against *S. aureus* ATCC 6538 was first determined. Different concentrations of MH solutions were prepared in sterile distilled water and tested against *S. aureus*

ATCC 6538 using "cup-agar plate diffusion technique" (28). Nutrient agar plates were inoculated with the tested microorganism using sterile cotton swabs. Wells equidistant from each other were made in the inoculated nutrient agar plate using a sterile cork borer (7 mm in diameter). The wells were carefully filled with 50 µl of the different MH concentrations. After incubation at 37°C for 24 h, the inhibition zone diameters in millimeter were measured. Logarithms of the concentrations of MH solutions were plotted against the corresponding inhibition zone diameter. The MIC of MH against *S. aureus* ATCC 6538 was determined by plotting the square of inhibition distance against the corresponding log drug concentrations.

The MH gel in Pl was tested for its *in vitro* antibacterial activity using cup-agar diffusion technique against *S. aureus* ATCC 6538. The inoculated nutrient agar plate was prepared as previously mentioned. Three cups (7 mm diameter) were made in the inoculated nutrient agar plate using a sterile cork borer. One hundred milligrams of the gel was introduced into each cup using a sterile microsyringe (weight by difference). After incubation at 37°C for 24 h, the mean of the inhibition zone diameters and the standard deviation were calculated. The antibacterial activity of placebo gel was also tested.

Comparative Clinical Evaluation of Thermosensitive Pl Gels Containing Either Mx (3%) or MH (2%)

Both Mx (3%) and MH (2%) gels based on 35% Pl were selected for the clinical study. Protocol of clinical trials was approved by the Ethics Committee of the University of Alexandria.

Patients who participated in this study were systemically healthy nonsmoker females aged 23–40 years with mild to moderate chronic periodontitis. The patients exhibited periodontal pockets that bled upon gentle probing and clinical probing depth ≤6 mm and clinical attachment level ≤4 mm. They were informed of the benefits and risks involved, their consent was obtained, and they were instructed to carry on all routine oral hygienic procedures and a special dietary pattern. Periodontal pockets chosen as experimental sites were isolated with cotton rolls and exposed to curettage to remove dead cells and scaling to remove plaque, then dried with cotton pellets and an air syringe (29). Seven patients were tested by scaling and root-planning followed by local application of either Mx in Pl (group I) or MH in Pl (group II) gels into the periodontal pockets. The gel applications were repeated at 2 and 4 weeks after the first application. The gel was applied into the periodontal pocket by means of periodontal syringe adopted with a cannula (N.B. the formula was kept in ice bath till the time of use to avoid its gelation at room temperature). The pockets were then covered with periodontal pack to prevent the escape of the gel to the oral cavity (Fig. 1). Patients were instructed to keep normal oral hygiene but not to brush or floss the tested area for 1 week. They were also instructed to avoid chewing or using antibacterial mouth rinse during the study period. Assessment of some parameters was carried out at the baseline and after 1, 3, and 6 months. These parameters were pocket depth, gingival index, and radiographic examination.

Pocket depth is a measure of the severity of the periodontal disease; it was measured in millimeters using a sterile metered periodontal probe immediately before gel application.

Gingival index measurement was done according to Loe (30); the tissues around each tooth were divided into four gingival scoring units. A periodontal probe was used to assess the bleeding potential of the tissues. Each of the four gingival units was assessed according to the following criteria:

Score 0 Normal gingiva
Score 1 Mild inflammation, slight changes in color, slight edema, and no bleeding upon probing
Score 2 Moderate inflammation, redness, edema, glazing, and bleeding upon probing
Score 3 Severe inflammation, marked redness, edema, ulceration, and tendency to spontaneous bleeding

Radiographic examination (31) was performed to follow up the bone regeneration process up to 6 months after gel application.

The paired *t* test was used as a parametric test for comparison between a quantitative variable during periods of follow-up in relation to the baseline values.

Two sample *t* tests were used for comparison between the two groups during the different follow-up periods using the percentage change.

RESULTS AND DISCUSSION

Evaluation of Thermosensitive "Pl" Gels Containing Either Mx (3%) or MH (2%)

Both Pl formulae described in this study were simply manufactured and allowed easy drug incorporation prior to application. Pl is commercially available poly(oxyethylene)-poly(oxypropylene) block copolymers. The toxicity data for this series of copolymers indicated that Pl F127 is one of the

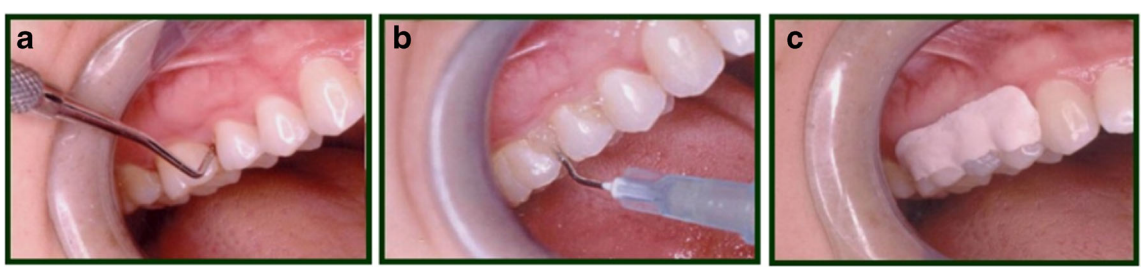

Fig. 1. Clinical application of the medicated Pl gels. **a** Probing depth at test site in case of chronic periodontitis (baseline), **b** *in situ* gel application, and **c** periodontal pack application

least toxic block copolymers (32). The unique character of this polymer is the reversible thermal gelation; concentrated solutions (above 20% *w*/*w*) of the copolymer are fluid (sol) at refrigerator temperature (4–5°C), but form stiff clear gel above its transition temperature (33). This suggests that when injected into the periodontal pocket, the preparation will change into a solid artificial barrier acting as sustained release depot. The present *in vitro* release study was performed using a membraneless dissolution model which allowed the release medium to contact the gels directly (21). Both drugs showed a prolonged release rate extended for several days (Fig. 2). This is probably due to the large number and size of Pl micelles related to high concentration of Pl gel used (35%), resulting in increased gel rigidity and tortuosity in the aqueous phase of the gel structure. The short inter-micellar distances hindered the release of drug molecules through the aqueous channels (34). In addition, a probable micellar entrapment of drugs through the hydrophobic core and/or the hydrophilic shells may explain the prolongation of drug release rates (35). Figure 2 indicates that the release of Mx from Pl formulation had a noticeable slower rate compared to that of MH; they showed 6.35±0.11 and 68.85±2.6% drug release after 24 h, respectively, and the total percent drug released from both formulae were 21.72±0.16% after 1 week for Mx and 85±4.46% after 3 days for MH. This may be attributed to the very poor aqueous solubility of Mx, compared to MH (36).

The release data were analyzed using the semiempirical equation proposed by Peppas. The release exponent *n* which is related to drug release mechanism was found to be 0.76 (till the end of the experiment) for Mx gel indicating anomalous non-Fickian behavior that could be controlled by both diffusion and chain relaxation (37). A comparable result was obtained by Ismail (21), when studying the release kinetics of doxycycline hyclate from 20% Pl gel; value for *n* equal to 0.62 was obtained indicating anomalous non-Fickian behavior, but upon using 25 or 35% Pl, resulted in *n* values <0.5 indicating combined diffusion mechanisms (diffusion partially through a swollen matrix and partially through water-filled pores).

MH followed also anomalous non-Fickian behavior; the *n* value (first 12 h) was 0.8. These findings were in disagreement with those reported by Moore *et al.* (38); they demonstrated that the drug release followed zero order kinetics due to rapid dissolution of Pl in the medium. The observed controversy in results may be attributed to the great influence of the stirring speed and experimental set-up on the mechanism of drug release (38). However, the *in vitro* release study of ceftiofur from Pl gel (25–35%) using a membraneless model without stirring also resulted in zero order kinetics, indicating polymer dissolution controlled release mechanism (34).

Evaluation of pH-Sensitive "C/H" Gels Containing Mx (3%)

The MH gel in C/H polymer system resulted in liquefaction upon addition of drug solution to the polymer system followed by drug coagulation and precipitation. This may be attributed to a possible drug polymer interaction. It was previously reported that the primary thickening mechanism for C is the ionization of the carboxyl groups of the polymer which causes extension of the polymer molecules due to repulsion between adjacent ionized carboxyl groups on the molecule which results in polymer hydration (39). Liquefaction of C/H system upon addition of MH solution may be resulted from the positively charged minocycline, which may disturb the electrostatic repulsion between adjacent ionized carboxyl groups inducing an overall decrease in the polymer hydration. Another reason may be the pH-decreasing effect caused by the acidic drug solution which resulted in decreased polymer ionization and thus decreased hydration.

Regarding Mx, no visual incompatibility was observed with C/H gel system. Aqueous solutions of C, which is a polyacrylic acid polymer, are acidic and of low viscosity that transform into gels upon an increase in the pH from 4 to ~7.4 (21). However, the amount of C required in the solution to form stiff gel upon periodontal application makes the solution highly acidic, which may induce inflammation. A reduction in C concentration without compromising the *in situ* gelling properties as well as the overall rheological behavior of the system can be achieved by adding a suitable viscosity enhancing polymer, e.g., HPMC which did not show any interaction with C, but its role in the system appeared to be only an inert viscosity enhancing agent (22). The release profile of Mx from C/H polymer system showed a prolonged drug release rate up to 7 days with an initial burst drug release compared to that of Mx gel in Pl (Fig. 3).

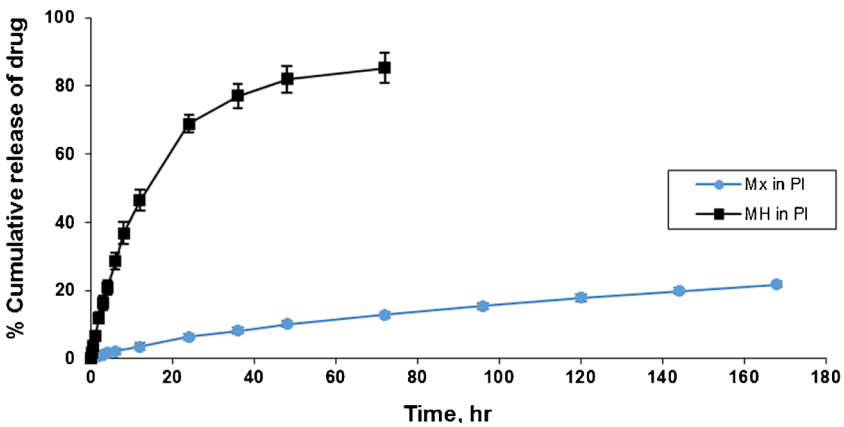

Fig. 2. Release profile of Mx and MH from Pl gel (35%) in Sørenson phosphate buffer, pH 6.6. Each *point* represents the mean±SD (n=3)

After 3 h, the percent drug released was 5.37±0.4 and 1.18±0.008% for Mx in C/H system and Mx in Pl gel, respectively. The release of doxycycline hyclate from both C/H (1:2) and Pl (35%) gels was previously studied (21); the latter was found to show a slower drug release rate (~19% after 7 h) compared to the former (~48%). This may be attributed to micellar entrapment of the drug and the high concentration of Pl used (35%). Such a high concentration of Pl (35%) may probably lead to an increase in the number and size of micelles resulting in increased gel rigidity and tortuosity in the aqueous phase of the gel structure. The short inter-micellar distances may hinder the release of the drug molecules through the aqueous channels (34). On the other hand, the higher release rate of Mx from C/H polymer system may be explained by the fact that the presence of monovalent salts (e.g., potassium in the dissolution medium) reduces the swelling of the polyacrylic acid copolymer (PAA). This may result in the formation of more areas or regions of low microviscosity in the gel microstructure for the drug to channel through, resulting in faster drug release rate. The shielding effect of potassium ion would also reduce the swelling resulted from the repulsion of the negatively charged carboxylate groups in the PAA polymer (40).

The release kinetics for Mx gel in C/H system was studied, and the exponent *n* obtained was 0.49 (till 12 h) and 0.46 (2–7 days), both values ~0.5 indicating Fickian-diffusion mechanism.

In Vitro Determination of the Antibacterial Activity of MH Gel in Pl

The *in vitro* antibacterial activity of MH gel in Pl was tested against *S. aureus* ATCC 6538. The tested gel gave a mean inhibition zone after 24-h incubation of 40 mm which significantly exceeded that of the calculated value for MIC of MH (13.28 mm) against the same microorganism. *Placebo* gel showed no inhibition of growth of the microorganism. These results revealed that the amount of drug released from the formula exceeded the MIC of MH against the tested microorganism (0.9198 μg/ml).

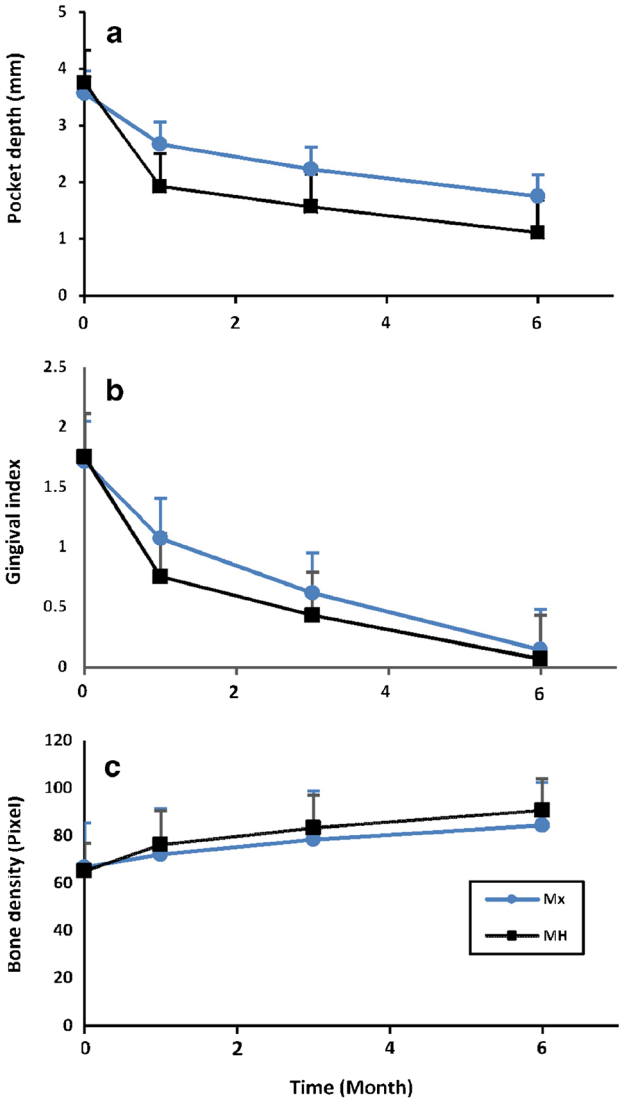

Fig. 4. Follow-up of different parameters in patients after treatment with either Mx or MH Pl gels for a 6-month period. **a** Decrease in pocket depth, **b** decrease in gingival index, and **c** increase in bone density. Each *point* represents the mean±SD (*n*=7)

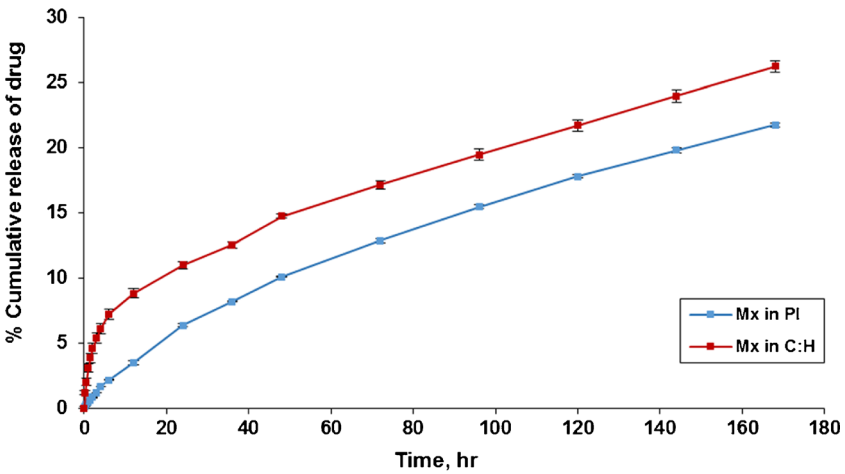

Fig. 3. Release profile of Mx from Pl gel (35%) and C/H gel (1:2.5) in Sørenson phosphate buffer, pH 6.6. Each *point* represents the mean±SD (*n*=3)

Table I. Comparative Clinical Evaluation of Pl *In Situ* Gels Containing Either Mx or MH

Parameter	Group I (Mx)			Group II (MH)		
	1 month	3 months	6 months	1 month	3 months	6 months
% Decrease in pocket depth	24.97	37.38	50.99	48.57	58.11	70.48
% Decrease in gingival index	37.51	64.17	91.65	57.14	75.48	95.94
% Increase in bone density	8.07	17.32	26.11	16.92	27.82	39.06

Mx meloxicam, *MH* minocycline HCl

Comparative Clinical Evaluation of Thermosensitive "Pl" Gels Containing Either Mx (3%) or MH (2%)

Clinical evaluation of patients suffering from periodontal pockets showed that both medicated gels were well tolerated; no signs of inflammation or irritation were recorded by the test subjects. There was a statistically significant decrease in the pocket depth and gingival index and increase in bone density after 1, 3, and 6 months when compared to the baseline values at $p \leq 0.05$ for both groups **(**Fig. 4**)**. Although the release of Mx from Pl was incomplete over the 7 days of the *in vitro* release study period, it showed appreciable clinical results. On the other hand, group II, which received MH in Pl, showed more significant percent reduction in pocket depth, percent decrease in gingival index, and also percent increase in bone density, compared to group I (Table I). Radiographic analysis plays an important role in determining treatment outcomes because it offers the only noninvasive method of evaluating the hard tissue response to therapy (41). Figure 5 shows the significant improvement in bone density in groups I and II. The improvement in clinical results observed in group I is in agreement with previous findings suggesting that oral administration of selective COX-2 inhibitors combined with scaling and root-planning showed significant improvement in all clinical parameters including pocket depth and gingival index (42). Regarding group II, comparable results were obtained after an 18-week follow-up period following local application of minocycline microspheres (Arestin®) in adjunct to scaling and root-planning (43). The observed increase in bone density in group I is in agreement with the observation of Bezerra *et al.* (5) who found that the animals subjected to 7 days of treatment for periodontitis with subcutaneous Mx (0.75, 1.5, 3 mg/Kg) exhibited a dose-dependent inhibition of alveolar bone loss that was significantly different from that of untreated groups. The current study results of group II are in accordance with those of a previous study (44), where minocycline microspheres application diminished bone resorption and increased alveolar bone height.

Results of the current study indicated that both treatment modalities revealed significant improvement of all studied clinical variables in chronic periodontitis patients, manifested by decrease in pocket depth and gingival index and increase in bone density.

CONCLUSION

Injectable *in situ* gelling systems are good candidates for intra-pocket drug delivery. They offer easy manufacture and application and allow the formulation to get access to the entire pocket. In addition, they ensure good residence time and sustained drug release rate. The satisfactory clinical outcomes indicated that the medicated *in situ* gels under test are promising formulae for the treatment of periodontal pockets.

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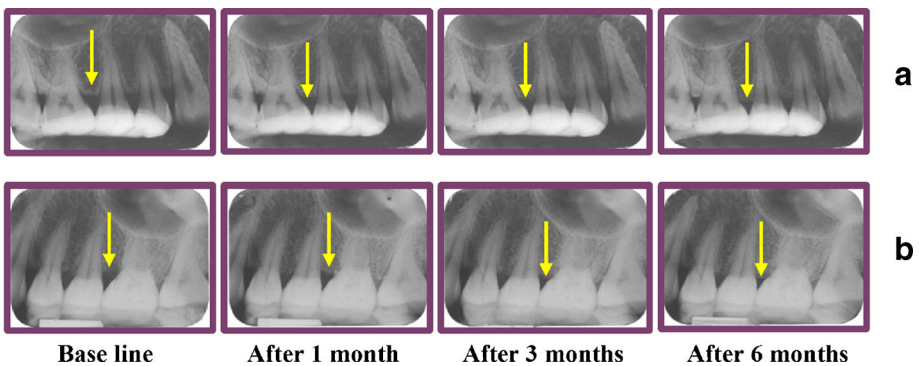

Fig. 5. Follow-up of bone regeneration (peri-apical radiographs) in a patient after treatment with Mx (**a**) and MH (**b**) Pl gels

Conflict of Interest This manuscript has not been published and is not under consideration for publication elsewhere, and we have no conflict of interest to disclose.

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