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Reserch Article

SITE-SPECIFIC DELIVERY OF THE NUTRACEUTICAL COQ10 FOR PERIODONTAL THERAPY

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ABSTRACT

The present study was carried out to develop a site-specific gel-delivery system of coenzyme Q10 (CoQ10) for periodontal therapy. Poloxamer 407 (P407) was employed to confer a thermoreversible gelling character while carbopol 947P (CBP) and sodium alginate (Alg) were also included for their mucoadhesive power giving rise to Gel-A and Gel-B respectively. The gels were evaluated in adult patients with chronic periodontitis and the clinical parameters *viz.* plaque index (PI), gingival index (GI) and probing depth (PD) were recorded over a sufficient period of treatment (1-21 days). Results revealed that the two formulas had gelation temperature ($T_{sol\text{-}gel}$) in the physiological range whereas they differed in their mucoadhesive force being significantly higher for Gel-A. Both gels displayed a shear thinning property suitable for intrapocket periodontal injection; nevertheless, Gel-A had higher thixotropic behaviour. The release profile and kinetics of CoQ10 from the gels indicated zero-order release from Gel-A but Higuchi-type diffusion from Gel-B. Clinical evaluation was performed in ten patients with chronic periodontitis who received a clinical (plaque index, gingival index, probing depth) examination at the baseline and at 1, 7, 14 and 21 days. The results of the clinical study showed that, the group treated with Gel-A demonstrated significantly higher reduction in all clinical recordings compared to the groups treated with Gel-B or CoQ10 suspension. In conclusion, the significant improvement in clinical parameters indicated the potential of site-specific delivery of CoQ10 in an in situ-gel formulation with high mucoadhesive power and optimized rheological properties.

Keywords: Site-specific delivery, Periodontal therapy, Coenzyme Q10, Poloxamer 407, In-situ gel, Mucoadhesion, rheology

INTRODUCTION

Periodontitis is an inflammatory response in which the structural support to the tooth is destroyed. The disease results in resorption of the alveolar bone, detachment of the periodontal ligament supporting the tooth and formation of a periodontal pocket (lesions between teeth and junctional epithelium) [1]. The pocket provides an ideal environment for the proliferation of a variety of pathogenic bacteria. Therapeutic approaches for the treatment of periodontitis include mechanical or surgical methods and administration of systemic antibiotics and/or other therapeutic agents. Systemic therapy has a low benefit to risk ratio [2]. The drugs must be given in high doses to maintain an effective concentration in gingival crevicular fluid (GCF) which may cause side effects such as gastrointestinal disorders, development of resistant bacteria and super-infection. With advances in understanding of the etiology and pathogenesis of periodontal disease, attention has been focused on local drug delivery systems[3]. These include both sustained and controlled release polymeric systems which when inserted into the periodontal pocket, release active agents for a sustained period of time. Thus intrapocket devices have a high benefit to risk ratio[3, 4].

The wonder nutrient: coenzymes Q10 (CoQ10) is a fat-soluble, vitamin-like, benzoquinone compound (Fig. 1) that occurs naturally in the body [5]. It functions primarily as an antioxidant, a membrane stabilizer and a cofactor in the oxidative phosphorylation production of adenosine triphosphate (ATP) [6]. Its beneficial effects include but are not limited to: delaying the ageing process and increasing longevity, improving heart health, boosting body's defence system, supporting immune system, minimising free radical damage, fighting gum disease, reducing migraine frequency & length, increasing stamina and endurance, increasing sperm motility leading to enhanced fertility, the symptoms of Parkinson's disease, coping with the effects of diabetes, improving age-related macular degeneration, and improving exercise tolerance in people with muscular dystrophies [7, 8]. Research shows that the gums of people with periodontal disease are often significantly deficient in CoQ10 [9, 10]. Indeed, gingival and leucocytic deficiencies of CoQ10 have been detected in periodontal patients attributed to a systemic nutritional imbalance and not neglected oral hygiene [9, 11]. It was suggested that oral intake of CoQ10 may be effective in improving the periodontal disease. Barbieri et al. reported that oral CoQ10 was beneficial in periodontal inflammation by virtue of its intracellular antioxidant activity and its ability to enhance macrophages-phagocytic activity and to increase granulocyte proliferation [12]. However, the oral powders formulations of CoQ10 have poor dissolution in aqueous media, resulting in poor oral bioavailability [13]. The market lacks an intrapocket periodontal formulation for CoQ10 and this form is inadequately explored in literature. Therefore, formulations that allow site-specific delivery of CoQ10 i.e. directly in the periodontal pocket are warranted. A recent study reported an intrapocket gel formulations of CoQ10 in chronic periodontitis with promising results both clinically and microbiologically [14]. Intrapocket CoQ10 formulations should improve the solubility and stability of this nutraceutical and allow mixing/dilution with body fluids. In this context, water-soluble organic solvents e.g. propylene glycol as well as surfactants e.g. poloxamers were commonly employed as solubilizing excipients[15]. Indeed, the poloxamer surfactants at high concentrations (17-25%) were frequently explored not only for their solubilizing power [15] but also for thermoreversible gel-formation [16]. From a stability viewpoint, the immobilization of drug molecules in a thixotropic gel preparation favours stability due to a complete rest in a solid gel matrix eventually decreasing the chance for chemical degradation [17]. Benefits of poloxamer-based systems include: long history of safe and effective use in semi-solid formulations [18], low irritancy and non-sensitizing properties with repeated usage [19], compatibility across a broad pH range and with most acidic, basic, and neutral drugs, consistent and reproducible properties due to their synthetic nature, stability against microbial growth and shear thinning properties that facilitate syringeability or extrusion from product packaging [20]. From a periodontist point of view, intrapocket injectable gels are highly convenient since they enable a good filling of the pocket in comparison to solution or solid intrapocket devices and afford improved syringeability with modulated rheological properties [21]. Furthermore, inclusion of mucoadhesive polymers to the in-situ gel would offer, beside their high viscosity, site-specific mucoadhesion[22], increased residence time and hence prolonged therapeutic levels in the GCF [23-25]. In the present study, a site-specific delivery system of CoQ10 was developed for management of periodontitis. The system was evaluated pharmaceutically as well as clinically to assess its beneficial action to patients with chronic periodontitis.

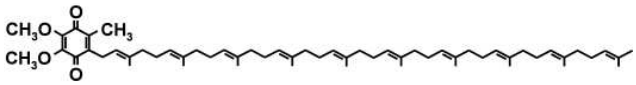

Fig. 1: Chemical Structure of CoQ10

MATERIALS AND METHODS

Materials

CoQ10 was kindly supplied by MepaCo, Egypt; Carbopol® 974P NF (Lubrizol Advanced Materials, Inc. Cleveland, Ohio, USA), poloxamer 407, propylene glycol (ADWIC Co., Egypt).

Equipment

Electric balance (Sartorius, GMBH, Gottingen, Germany); thermostatically controlled magnetic stirrer (Thermolyne Corporation, U.S.A.); Brookfield viscometer (cone and plate; type DVT-2); USP dissolution test apparatus (Pharma test, type PTW, Hainburg, Germany). UV- visible double beam spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan).

Methodology

Preparation of Coq10 gel formulations

The poloxamer 407 gel (Polox) was prepared by the cold method as previously described [16, 20] with some modification. Briefly, the mucoadhesive polymers namely: carbopol 974P (CBP) and sodium alginate (Alg) were dispersed in the calculated amount of distilled water at room temperature at a concentration 2% and stirred using a magnetic stirrer. The dispersions were cooled in the fridge; the Polox (18% w/w) was then added slowly and left to hydrate at 4ºC with occasional stirring on a vortex mixer.

Before incorporation into the gel, a dispersion of Coq10 was first prepared by mixing the drug (200 mg) and propylene glycol (PG; 5%) in a porcelain dish with stirring till smooth paste formation. The cold CBP/Polox and Alg/Polox solutions were added to the paste and mixed with a glass pestle for 2 min until it sets into a gel giving rise to Gel-A or Gel-B respectively. The gels were then packed into plastic wide mouth container and stored at room temperature (Shah and Donovan 2007). To serve as a control, CBP/Polox (Gel-A) and Alg/Polox (Gel-B) were prepared in a similar manner but without Coq10.

Measurement of gelation temperature of the prepared gels

The gelation temperature($T_{sol\text{-}gel}$) was measured by the simple test tube inverting method exactly as mentioned in previous publications [16, 26]. Two ml aliquot of refrigerated tested formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a thermostatically controlled water bath at 25℃. The temperature of the water bath was increased gradually in increments of 10℃ in the beginning of the experiment and then 1℃ increments in the region of sol-gel transition temperature and 0.1℃ when it approaches gelation. The tested formulation was left to equilibrate for 5 minutes at each new setting (or for 2 minutes when temperature was increased with an increment 0.1℃). The gelation temperature is taken as the temperature at which the meniscus would no longer move upon tilting through angle 90°. The maximum accepted sol-gel transition temperature tested was 37℃ which represents the gingival crevicular fluid (GCF) temperature [27]. The tilting rate, amount of solution and the diameter of the test tube were kept constant throughout the experiment

Measurement of mucoadhesive properties of the prepared gels

The mucoadhesive forces of the prepared gels were determined using mucin tablets and a mucoadhesive force-measuring device as previously mentioned [16]. The detachment force (dyne/cm^2) was determined using the following equation

$$\text{Detachment force (dyne/cm}^2) = \frac{m*g}{A} \quad \ldots\ldots\ldots\ldots (1)$$

Where m: the weight of water in grams

g: acceleration due to gravity taken as 980 cm/sec^2.

A: area of the mucin disc (area of contact) and is equal to πr^2 (r is the radius of the mucin disc).

Measurement of rheological properties of the prepared gels

The rheological properties of the gel formulations were studied using cone and plate (Brookfield) viscometer (cone and plate; type DVT-2 spindle 52) as previously reported [26]. Briefly a sample (0.5 ml) of sol was applied to the lower plate of the viscometer using a spatula. The measurements were made at 25 and 37±0.1°C using spindle 52 at different shear rates ranging from 5 to 400 rpm. The shear rate (γ) in s^{-1} and the viscosity (η) in centipoises (cps) were determined from the instrument reading and fitted to the power law constitutive equation [28]:

$$\eta = m\,\gamma^{n-1} \quad \ldots\ldots\ldots\ldots (2)$$

The two dimensionless quantities: the consistency index (*m*) and the flow index (*n*) characteristic for each formulation were obtained. If *n*=1 this indicates Newtonian behavior while if (*n*) is less than 1, this corresponds to shear thinning flow. The lower the value of (*n*) the more shear thinning the formulation [29-31] The thixotropic behaviour of the gels was evaluated by calculating the area of the hysteresis loop formed from the shift of the up and down curves of the rheograms of the 2 intrapocket gel formulations using the trapezoidal rule and calculating the coefficient of variation (C.V.%) at all rates of shear for each formula. Measurements were done in triplicates and statistical tests of significance were done using Student's *t*-test at $P < 0.05$.

In vitro drug release study

The in vitro release of CoQ10 from the gel formulations was studied using a modified USP paddle method as previously described [32]. Briefly, a glass cylinder (2.5 cm dia), with a cellulose membrane (Mwt cutoff 12,000) tied to one end, was attached to the metallic drive shaft of the dissolution apparatus and immersed in 250 ml of phosphate buffer pH 7.4 maintained at temperature of 37◦ C. An accurately measured volume of the formula (5ml), was transferred to the glass tube The shaft was allowed to rotate at a constant speed (100 rpm). Aliquots of 5 ml were withdrawn at time intervals 0.5, 1, 2, 4, 8, 10, 12 and 14 days and replaced by an equal volume of fresh buffer. The drug content in the withdrawn samples was determined spectrophotometrically at 275 nm and quantified via a standard curve[32]. For comparison, release of Coq10 from an aqueous suspension prepared with Tween 80 as wetting agent was determined similarly.

Analysis of drug release data

The data obtained from the *in vitro* release experiments were analysed by the following commonly used exponential equation [33].

$$\frac{M_t}{M} = kt^n \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\text{Log} \quad \frac{M_t}{M} = \log k + n \log t$$

Where is $\frac{M_t}{M}$ the fraction of released drug at time t

k: release constant incorporating structural and geometric characteristics of the drug/polymer system.

n : release exponent indicative of the release mechanism. When n is equal to 0.5, the drug is released from the polymer with a Fickian diffusion mechanism (Higuchi model). If 0.5 < n <1 this indicates anomalous or non-Fickian release, while if n=1 this indicates zero-order release.

Clinical Study

Patient selection

Ten healthy patients with chronic periodontitis, (ages 33-45) were included in this study. All patients attended to Oral Medicine and Periodontology department, College of Dentistry, Al-Azhar University and were given information about the proposed treatment and were asked to sign a formed consent. Each patient had at least three contralateral periodontal defects with probing depth > 5 mm and/or clinical attachment loss > 5 mm. Root planning and subgingival debridement was performed to thirty periodontal pockets in a split mouth design; which were then divided into three

groups; group I included ten periodontal pockets that received site-specific delivery of Gel-A, group II received site-specific delivery of Gel-B while group III received site-specific delivery of CoQ10 suspension

All participants were given detailed instructions in self performed plaque control measures.

Clinical assessment

Clinical recordings were assessed immediately before treatment (baseline, day 0) then at day 1,7, 14 and 21 as follows: (1) Plaque index (PI), is an index for estimating the effectiveness of oral hygiene by measuring dental plaque which occurs in areas adjacent to gingival margin and is evaluated on a scale basis as follows: 0 for no plaque, 1 for separate flecks of plaque at the cervical margin of the tooth, 2 for a thin continuous band (up to 1 mm) of plaque at the cervical margin of the tooth, 3 (severe plaque) for a band of plaque wider than 1 mm, at four sites (buccal, lingual, mesial and distal) of each tooth. (2) Gingival index (GI), is a measure of gingival inflammation based on visual inspection of gingiva by periodontist that takes into consideration the color and firmness of gingival tissue along with the presence of blood during probing and recorded on scale basis as follows: score 0 for normal gingiva, 1 for mild inflammation, slight edema, no bleeding on probing, 2 for moderate inflammation, redness, edema, bleeding on probing, 3 for severe inflammation, marked redness, edema, ulcerations and spontaneous bleeding. (3) probing depth (PD), defined as the distance (in mm) from the gingival margin to the bottom of the pocket and is considered as a measure of the severity of periodontal disease [21, 34, 35].

Statistical Analysis

All results obtained were statistically analyzed with statistical program SPSS ver 16.0* (Statistical package for scientific studies). Statistical tests of significance were performed using one-way ANOVA followed by LSD method for multiple comparisons. The differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

The use of wonder nutrient, Coenzyme Q10 in an intrapocket gel system necessitates significant formulation efforts prior to clinical studies. In this study, the formulation strategy adopted was: first, to optimize the rheological behaviour of the intrapocket gel to achieve site-specific gelation, high thixotropy and physical stability. Second, mucoadhesive property by incorporation of renowned mucoadhesive polymers and third, hopefully a zero-order release profile of CoQ10 from the intrapocket gel to maintain sufficient amounts of CoQ10 in the GCF.

The gel formulations exhibited a sol-gel transition temperature in the physiological range where Gel-A and Gel-B recorded a $T_{sol\text{-}gel}$ of 32°C and 34.5 °C respectively (figure 2). These results were in agreement with our previous results [26]. The thermo- reversible behaviour is desirable for intrapocket delivery system for good filling of the periodontal pocket and for ease of syringeability [21]. Regarding mucoadhesion, results in figure 2 reveals that Gel-A (containing carbopol) exhibited significantly higher mucoadhesive power than Gel-B (containing sodium alginate). These results are in accordance with previous results [22, 36]. The higher mucoadhesion might result in more prolonged residence time in the pockets of patients allowing higher local concentration of CoQ10 in the GCF.

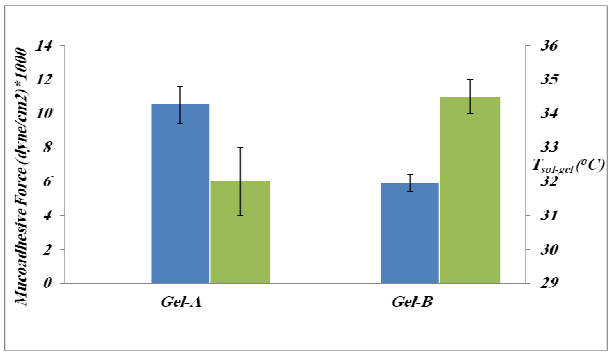

Fig. 2: Gelation temperature ($T_{sol\text{-}gel}$, °C; green bars) and mucoadhesive force (blue bars) of CoQ10 intrapocket gels.

Fig. 3 depicts a representative viscosity versus shear rate profile showing the viscosity changes that occur by temperature variation. At 25 °C the intrapocket gel was in a liquid form and exhibited a Newtonian behaviour (no notable changes in the viscosity over a broad range of shear rates). Conversely at 37 °C, a dramatic shear-thinning behaviour was observed in the corresponding profiles of the intrapocket gels indicating temperature-induced gelation [26].

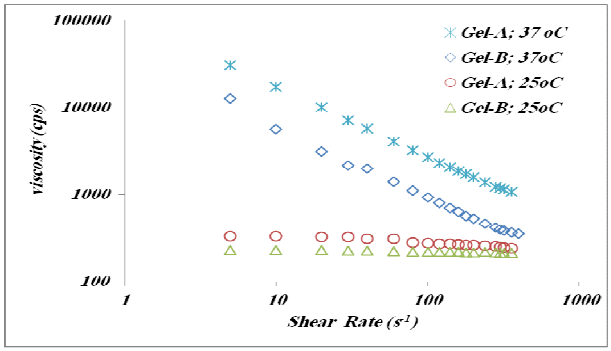

Fig. 3: Viscosity curves of CoQ10 intrapocket gels at different temperatures

According to equation (3), the values of the flow index (n) at 25 °C were 0.92 and 0.98 for Gel-A and Gel-B respectively (i.e. close to 1, confirming a Newtonian behavior) while the respective consistency index (m) values were 407.5 and 241.2 indicative of lower viscosity at shelf condition (Table 1). However, when the temperature was increased to 37 °C, the (n) values decreased. The lower the value of (n), the more shear thinning the formulation [28-30].

Table 1: Rheological Data of CoQ10 Intrapocket Gels.

Mulation	Consistency index (m)	Flow index (n)	Area of Hysteresis loop (cm²/dyne.s)
Gel-A at 25°C	407.5	0.92	NA
Gel-A at 37°C	104584	0.209	309115
Gel-B at 25°C	241.2	0.98	NA
Gel-B at 37°C	36785	0.204	150858

NA: Not applicable

The rheogram shown in figure 3 reveals the higher thixotropic behaviour of Gel-A compared to Gel-B formulation which is revealed by a larger area of the hysteresis loop (i.e. loop formed by the up and down curve in the rheogram). The value for the area calculated by the trapezoidal rule for Gel-A (309115 cm²/dyne.s) was almost double that for Gel-B (150858 cm²/dyne.s) as shown in table 1. Thixotropic behaviour is a desirable phenomenon where the gel exhibits a shear thinning flow (i.e. becoming less viscous upon agitation/injection from periodontist needle).

Once injected in the pocket, the 3-dimensional structure of the gel reforms and consistency was restored conferring rigidity which results in sustained drug release and stabilization of drug molecules against chemical degradation [37, 38].

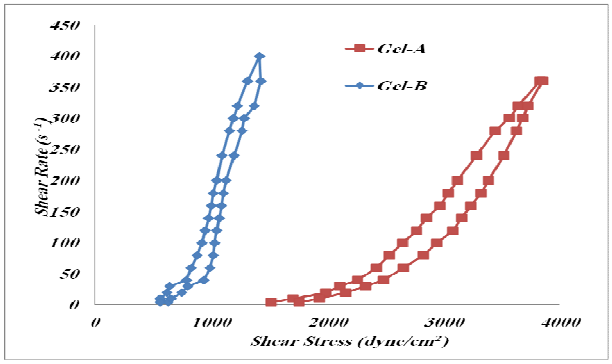

Fig. 4: Rheogram of CoQ10 intrapocket gels

Regarding the release of CoQ10, figure 3 depicts the release profile of CoQ10 from the prepared formulations. For the data it could be observed that Gel-A gave almost a linear release profile with 100% drug release in 21 days while the suspension formulation and the Gel-B formulation showed an initial fast release of the drug. Kinetic data in table (1) revealed that the release exponent (n) of equation (3) had value 0.9 which indicates a zero order kinetics for release whereas a Higuchi diffusion mechanism for Gel-B and CoQ10 suspension was demonstrated by n values near 0.5 [39-41]. These results agree with those previously reported by Prabhushankar et al. for levofloxcin periodontal gel[3].

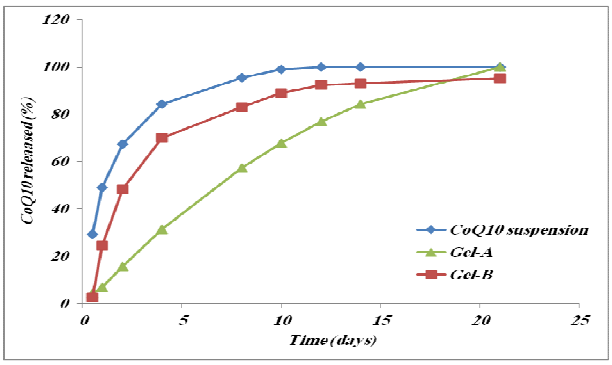

Fig.5. release profiles of Different Intrapocket formulations of CoQ10 Table (2): Kinetic Data of CoQ10 Release from different gel formulations

Formulation	Release Exponent (n)	Kinetic Constant (k,%/min.ⁿ)	Correlation Coefficient (r)
CoQ10 suspension	0.454	1.69112	0.9921
Gel-A	0.90067	0.91	0.9951
Gel-B	0.4283	1.491	0.9937

In the clinical study, the gel formulations were compared to CoQ10 suspension, figures (4-6) summarize the changes in mean plaque index (PI), gingival bleeding index (GI) and probing depth in group I (periodontal pockets that received Gel-A); group II (periodontal pockets that received Gel-B) and group III (periodontal pockets that received CoQ10 suspension).

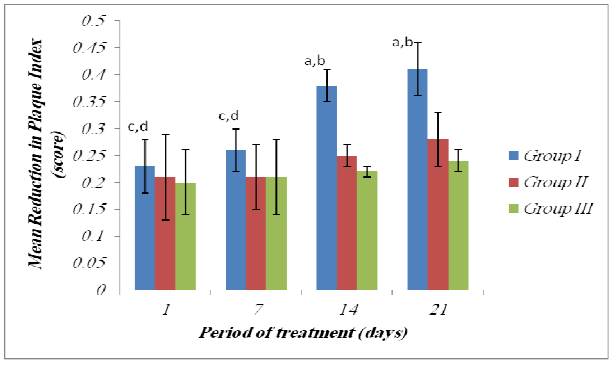

Fig. 6: Mean reduction in plaque index in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment For every group of pockets, a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at *P* < 0.05 using one-way ANOVA followed by LSD test for multiple comparison

The statistical analysis of the results of the mean reduction in plaque index (PI) showed non significant changes in PI in the study period between day 1 and day 7. On the other hand, the changes in this parameter were statistically significant at later study periods namely day 14 and day 21 only for group I (receiving Gel-A) but not group II (receiving Gel-B) or group III (receiving CoQ10 suspension). It worthy to note that Group I (receiving Gel-A) demonstrated the highest reduction in PI after 21 days of treatment where it recorded 0.41± 0.05 as compared to Group II (receiving Gel-B) and Group III (receiving CoQ10 suspension) with mean reduction in PI 0.28 and 0.24 respectively

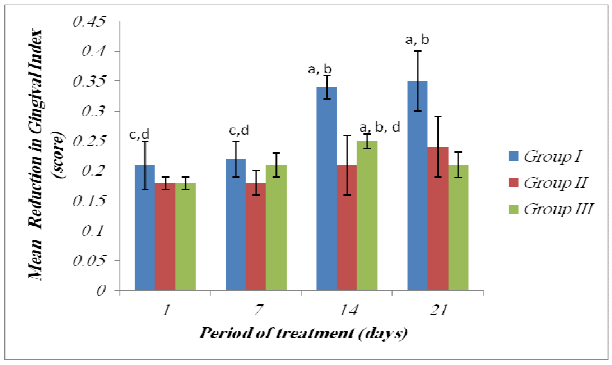

Fig. 7. Mean reduction in gingival index in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment For every group of pockets treated a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at *P* < 0.05 using one-way ANOVA followed by LSD test for multiple comparison

Regarding the gingival index (GI), the results revealed that the mean reduction in GI was not significant between day 1 and day 7 in the three groups. However, the reduction in this parameter were statistically significant at later study periods i.e. day 4 and day 21 only for group I (receiving Gel-A). For group II (receiving Gel-B) or group III (receiving CoQ10 suspension) the changes were not significant throughout the study period (i.e. from day 1 to day 21). Similar to PI, the highest reduction in GI after 21 days of treatment was observed in Group I (receiving Gel-A). Concerning the probe depth (PD), the effect of period of treatment was evident and significantly different among the various days of treatment. At any study period, Group I (receiving Gel-A) displayed the uppermost reduction in PD which was significantly different from that displayed in case of Group II and group III.

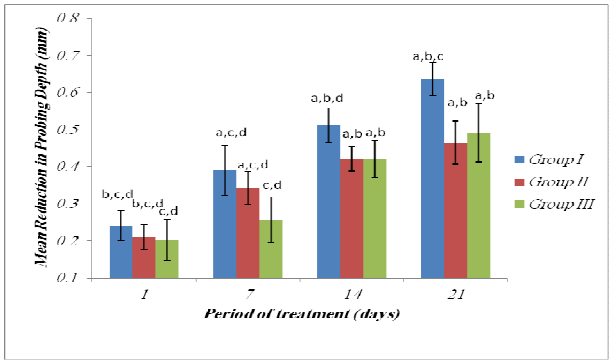

Fig. 8. Mean reduction in probing depth in group I patients (receiving Gel-A), group II patients (receiving Gel-B) and group III patients (receiving CoQ10 suspensions) at different times of treatment. For every group of pockets, a, b, c and d are significantly different from day 1, 7, 14 and 21 respectively at *P* < 0.05 using one-way ANOVA followed by LSD test for multiple comparison

Regional impairment in immune function in the gingiva was demonstrated in patients with periodontitis. An effective way to reduce and/or to control microorganisms in the gingiva of patients with periodontal disease is to increase the efficacy of the immune system of the host. A consensus report by the American Academy of Periodontology stated that 4 to 6 weeks interval seems appropriate to assess the initial response to nonsurgical periodontal therapy, based on evidence of periodontal wound healing [42, 43]. Hence, we evaluated the clinical effect of local delivery of the two gel formulation of coenzyme Q10 as compared to suspension formulation on chronic periodontitis for a study period of 21 days. The results of this study demonstrated the clinical superiority of Gel-A as an adjunctive therapy to scaling and root planing in the three groups of pockets during this the study period where it resulted in significant reduction of PI, GI and PD. These findings were in agreement with those previously reported [44] which suggested that oral administration of CoQ10 may decrease the degree of bleeding tendency in elderly people. Earlier report demonstrated an increase in the mean value of coenzyme Q10 in gingival biopsies during treatment with oral CoQ10 which correlated with extraordinary healing [45]. Moreover, the results of the present study corroborated those reported by Hanioka et al. [46] which evaluated topical application of coenzyme Q10 in periodontal pocket with and without subgingival debridement. The study demonstrated a significant improvement in bleeding on probing and probing depth at the experimental sites and suggested that topical application of CoQ10 in adult periodontitis not only as a sole treatment but also in combination with traditional nonsurgical periodontal therapy. Recent studies on local application of CoQ10 in chronic periodontitis revealed the superiority of CoQ10 in combination with root planning and subgingival scaling over treatment based on root planning and subgingival scaling alone. Previous studies have shown that different formulations of the same drug affected the clinical response [47, 48] and/or the pharmacokinetic profile in GCF, saliva and blood [49, 50]. The superior clinical outcomes observed with Gel-A formulation compared to the Gel-B (containing alginate) and CoQ10 suspension could be attributed to its higher mucoadhesive power, optimum release profile, gel-like consistency and the combined solubilizing effect of poloxamer 407 and propylene glycol. For recapitulation, we developed an intrapocket periodontal gel formulation of CoQ10 convenient for application by the periodontist. The formulation showed optimized physical and rheological properties, high mucoadhesive power for maximized residence in the pocket as well as a zero order drug release profile. Such formulation proved beneficial in treatment of chronic periodontitis and should be considered as an adjunctive treatment with current dental practice.

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REFERENCES

1. Greenwell H, Bissada NF. Emerging concepts in periodontal therapy. *Drugs.* 2002;62(18):2581-7.
2. Goodson JM, Offenbacher S, Farr DH, Hogan PE. Periodontal disease treatment by local drug delivery. *J Periodontol.* May 1985;56(5):265-72.
3. Prabhushankar GL, Gopalkrishna B, Manjunatha KM, Girisha CH. Formulation and evaluation of levofloxacin dental gel films for periodontitis. *Int J Pharm Pharm Sci* 2010;2(1):162-8.
4. Soskolne WA. Subgingival delivery of therapeutic agents in the treatment of periodontal diseases. *Crit Rev Oral Biol Med.* 1997;8(2):164-74.
5. Pepping J. Coenzyme Q10. *Am J Health Syst Pharm.* Mar 15 1999;56(6):519-21.
6. Molyneux SL, Young JM, Florkowski CM, Lever M, George PM. Coenzyme Q10: is there a clinical role and a case for measurement? *Clin Biochem Rev.* May 2008;29(2):71-82.
7. Quinzii CM, DiMauro S, Hirano M. Human coenzyme Q10 deficiency. *Neurochem Res.* Apr-May 2007;32(4-5):723-7.
8. Stephen T, Sinatra MD. The Coenzyme Q10 Phenomenon. 1998;Book, McGraw-Hill (Pubs):1-192.
9. Iwamoto Y, Nakamura R, Folkers K, Morrison RF. Study of periodontal disease and coenzyme Q. *Res Commun Chem Pathol Pharmacol.* Jun 1975;11(2):265-71.
10. Lister RE. Coenzyme Q10 and periodontal disease. *Br Dent J.* Sep 23 1995;179(6):200-1.
11. Hansen IL, Iwamoto Y, Kishi T, Folkers K, Thompson LE. Bioenergetics in clinical medicine. IX. Gingival and leucocytic deficiencies of coenzyme Q10 in patients with periodontal disease. *Res Commun Chem Pathol Pharmacol.* Aug 1976;14(4):729-38.
12. Barbieri B, Lund B, Lundstrom B, Scaglione F. Coenzyme Q10 administration increases antibody titer in hepatitis B vaccinated volunteers--a single blind placebo-controlled and randomized clinical study. *Biofactors.* 1999;9(2-4):351-7.
13. Weis M, Mortensen SA, Rassing MR, Moller-Sonnergaard J, Poulsen G, Rasmussen SN. Bioavailability of four oral coenzyme Q10 formulations in healthy volunteers. *Mol Aspects Med.* 1994;15 Suppl:s273-80.
14. Gawish A, Zaki NM, Gomaa O, Sadek HS. Clinical and microbiological assessment of Coenzyme Q-10 gel in treatment of chronic periodontitis. *Egyptian dental journal.* 2011;57 (1):24-33.
15. Strickley RG. Solubilizing excipients in oral and injectable formulations. *Pharm Res.* 2004;21 (2):201-30.
16. Zaki NM, Abd El Hady SS, Mortada N, Awad G, Taha RA. Development of in-site gelling and mucoadhesive mebeverine hydrochloride solution for rectal administration. *Saudi Pharamceutical Journal.* 2003;11 (4):159-71.
17. Wajda R, Zirkel J, Schaffer T. Increase of bioavailability of coenzyme Q(10) and vitamin E. *J Med Food.* Dec 2007;10(4):731-4.
18. Abhaham C. Poloxamer-gel systems with gelling temperatures higher than room temperature. . *Canadian Patent No 1072413.* 1994.
19. Johnston TP, Miller SC. Toxcological evaluation of poloxamer vehicles for intramuscular use. *J Parenter Sci Technol.* 1985;39:83-9.
20. Schmolka IR. "Poloxamers in the pharmaceutical industry". In Polymers in Controlled Drug Delivery. Tarcha, P.J., (ed.), CRC Press; Boca Roton, FL, P. 189-204. 1991.
21. Akncbay H, Senel S, Ay ZY. Application of chitosan gel in the treatment of chronic periodontitis. *J Biomed Mater Res B Appl Biomater.* Feb 2007;80(2):290-6.
22. Bhowmik BB, Nayak BS, Jee A, C. Formulation, development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. *Int J Pharm Pharm Sci.* 2009;1(1):240-57.
23. Bruschi ML, Jones DS, Panzeri H, Gremiao MP, de Freitas O, Lara EH. Semisolid systems containing propolis for the treatment of periodontal disease: in vitro release kinetics, syringeability, rheological, textural, and mucoadhesive properties. *J Pharm Sci.* Aug 2007;96(8):2074-89.
24. Jones DS, Woolfson AD, Brown AF, O'Neill MJ. Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: In vitro release kinetics, syringeability, mechanical and mucoadhesive properties. *Journal of Controlled Release.* 1997;49(1):71-9.
25. Rossi S, Marciello M, Bonferoni MC, et al. Thermally sensitive gels based on chitosan derivatives for the treatment of oral mucositis. *Eur J Pharm Biopharm.* Feb 2005;74(2):248-54.
26. Zaki NM, Mortada ND, Awad GA, Elhady SS. Enhanced bioavailability of metoclopramide hydrochloride by intranasal administration of a mucoadhesive in-situ gel with modulated rheological and mucociliary transport properties. *European Journal of Pharmaceutical Sciences.* 2007;32:296-307.
27. Esposito E, Carotta V, Scabbia A, et al. Comparative analysis of tetracycline-containing dental gels: Poloxamer- and monoglyceride-based formulations. *International Journal of Pharmaceutics.* 1996;142(1):9-23.
28. Tung C. Rheological behavior of poloxamer 407 aqueous solutions during sol-gel and dehydration processes. *Int J Pharm.* 1994;107:85-90.
29. Owen DH, Peters JJ, Katz DF. Rheological properties of contraceptive gels. *Contraception* 2000;62:321–6.

30. Copetti G, Grassi M, Lapasin R, Pricl S. Synergistic gelation of xanthan gum with locust bean gum: a rheological investigation. *Glycoconj J.* Dec 1997;14(8):951-61.
31. Chang JY, Oh YK, Choi HG, Kim YB, Kim CK. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. *Int J Pharm.* Jul 8 2002;241(1):155-63.
32. Nehilla BJ, Bergkvist M, Popat KC, Desai TA. Purified and surfactant-free coenzyme Q10-loaded biodegradable nanoparticles. *Int J Pharm.* Feb 4 2008;348(1-2):107-14.
33. Peppas NA. Analysis of fickian and non-fickian drug release from polymers. *Pharm Acta Helv.* 1985;60:110-1.
34. Akalin FA, Baltacioglu E, Sengun D, et al. A comparative evaluation of the clinical effects of systemic and local doxycycline in the treatment of chronic periodontitis. *J Oral Sci.* Mar 2004;46(1):25-35.
35. Needleman IG, Collins AM, Moles DR. Periodontal flap surgery with 25% metronidazole gel. (1). Clinical outcomes. *J Clin Periodontol.* Mar 2000;27(3):187-92.
36. Shakshak D. Thiomers -mediated drug delivery systems for vaginal infections. *Thesis, Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University.* 2010.
37. Li J, Li X, Ni X, Wang X, Li H, Leong KW. Self-assembled supramolecular hydrogels formed by biodegradable PEO-PHB-PEO triblock copolymers and alpha-cyclodextrin for controlled drug delivery. *Biomaterials.* Aug 2006;27(22):4132-40.
38. Li J, Ni X, Leong KW. Injectable drug-delivery systems based on supramolecular hydrogels formed by poly(ethylene oxide)s and alpha-cyclodextrin. *J Biomed Mater Res A.* May 1 2003;65(2):196-202.
39. Bromberg LE, Buxton DK, Friden PM. Novel periodontal drug delivery system for treatment of periodontitis. *J Control Release.* Apr 28 2001;71(3):251-9.
40. Tonetti M, Cugini MA, Goodson JM. Zero-order delivery with periodontal placement of tetracycline-loaded ethylene vinyl acetate fibers. *J Periodontal Res.* Jul 1990;25(4):243-9.
41. Ho EA, Vassileva V, Allen C, Piquette-Miller M. In vitro and in vivo characterization of a novel biocompatible polymer-lipid implant system for the sustained delivery of paclitaxel. *J Control Release.* May 5 2005;104(1):181-91.
42. Segelnick SL, Weinberg MA. Reevaluation of initial therapy: when is the appropriate time. *J Periodontol.* 2006;77 (9):1598-601.
43. Cianco N, Giannopoulou C, Ugolotti G, Mombelli A. Amoxicillin and metronidazole as an adjunct to fullmouth scaling and root planing of chronic periodontitis. *J Periodontol.* 2009;80 (3):364-71.
44. Nylander M, Nordlund M. Clinical effects on periodontal status after given oral supplement of ubiquinone. *Sweed J Biol Med.* 1991;1:6-11.
45. Wilkinson EG, Arnold RM, Folkers K, Hansen I, Kishi H. Bioenergetics in clinical medicine. II. Adjunctive treatment with coenzyme Q in periodontal therapy. *Res Commun Chem Pathol Pharmacol.* Sep 1975;12(1):111-23.
46. Hanioka T, Tanaka M, Ojima M, Shizukuishi S, Folkers K. Effect of topical application of coenzyme Q10 on adult periodontitis. *Mol Aspects Med.* 1994;15 Suppl:s241-8.
47. Hanes PJ, Purvis JP. Local anti-infective therapy: pharmacological agents. A systematic review. *Ann Periodontol.* Dec 2003;8(1):79-98.
48. Killoy WJ. The clinical significance of local chemotherapies. *J Clin Periodontol.* May 2002;29 Suppl 2:22-9.
49. Kim TS, Klimpel H, Fiehn W, Eickholz P. Comparison of the pharmacokinetic profiles of two locally administered doxycycline gels in crevicular fluid and saliva. *J Clin Periodontol.* Apr 2004;31(4):286-92.
50. Kim TS, Burklin T, Schacher B, et al. Pharmacokinetic profile of a locally administered doxycycline gel in crevicular fluid, blood, and saliva. *J Periodontol.* Nov 2002;73(11):1285-91.