Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jpfa



Clinical evaluation of thermoresponsive and mucoadhesive Chitosan in situ gel containing Levofloxacin and Metronidazole in the treatment of periodontal pockets – A split-mouth, clinical study

Monika Bansal [a,*], *Neelam Mittal* [a], *Sarita Kr Yadav* [b], *Gyasuddin Khan* [b], *Brahmeshwar Mishra* [b], *Gopal Nath* [c]

[a] *Faculty of Dental Sciences, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India*
[b] *Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi 221005, India*
[c] *Department of Microbiology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India*

ARTICLE INFO

Article history:
Available online 27 October 2016

Keywords:
Chitosan
In situ gel
Mucoadhesive
Periodontal pocket
Thermoresponsive

ABSTRACT

Objectives: Recently, mucoadhesive formulations have become popular as drug delivery for periodontal pocket and oral mucosa. The objective of this study was to evaluate the clinical efficacy of thermoresponsive and mucoadhesive chitosan in situ gel in treatment of chronic periodontitis.

Methods: Vehicle and medicated in situ gel containing Levofloxacin and Metronidazole was applied in ten systemically healthy subjects (20–50 yrs) with chronic periodontitis adjunctive to scaling and root planing (SRP) in comparison to SRP alone. Clinical parameters were plaque index (PI), gingival index (GI), and bleeding on probing (BoP), which were recorded at baseline and 15th, 30th, 60th, and 90th days, as well as probing pocket depth (PPD), clinical attachment level (CAL), and gingival recession (GR), which were recorded at baseline and 30th, 60th, and 90th days.

Results: The results suggested that changes in clinical parameters were significant from baseline for all groups on the 90th day. On the 90th day, PI was 0.7 ± 0.48, 0.4 ± 0.51, and 0.3 ± 0.48; GI was 0.6 ± 0.051, 0.5 ± 0.52, and 0.3 ± 0.48; BoP was 0.5 ± 0.52, 0.3 ± 0.48, and 0.2 ± 0.42; PPD reduction was 2.5 ± 0.52 mm, 3.4 ± 0.69 mm, and 4.6 ± 0.51 mm; CAL gain was 1.2 ± 0.42 mm, 2.6 ± 0.84 mm, and 3.8 ± 0.42 mm; and GR was 1.1 ± 0.73 mm, 1.0 ± 0.00 mm, and 0.5 ± 0.52 mm for SRP, vehicle, and medicated in situ gel groups, respectively. Reduction in PPD and gain in CAL was also significant after comparison between the treatment groups.

Conclusion: Vehicle and medicated in situ gel showed better clinical outcomes as compared to SRP alone, which could be attributed to the presence of chitosan that is not only effective in treatment of periodontitis but also acts as a good carrier to deliver drug into periodontal pockets.

© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

* *Corresponding author*. Fax: +91 05422367568.
E-mail address: dr.monika97@rediffmail.com (M. Bansal).

http://dx.doi.org/10.1016/j.jpfa.2016.10.003

0970-2199/© 2016 Pierre Fauchard Academy (India Section). Published by Elsevier, a division of RELX India, Pvt. Ltd. All rights reserved.

1. Introduction

A healthy periodontium is mandatory to prevent the loss of teeth as well as to minimize the risk of certain systemic diseases through periodontal infections. With the advancement in science and technology, evidences are there that periodontitis acts as a risk factor for the systemic diseases directly or indirectly.[1] Periodontitis is caused by a wide diversity of microorganisms including the gram +ve, gram −ve, facultative anaerobic, microaerophilic, obligate anaerobic, cocci, rods, and spirochetes. Antimicrobial therapy acts as an adjunct to the scaling and root planing (SRP) with the aim to inhibit the growth or kill the periodontopathic microorganisms in various situations like inapproachable sites such as invasion of bacteria into the dentin, cementum, alveolar bone, connective tissue, aggressive periodontitis, moderate-to-severe chronic periodontitis, refractory periodontitis, and periodontitis as a manifestation of systemic diseases. Periodontitis is a mixed infection; therefore, combination therapy is preferred over the single agent to eliminate the pathogens because a single agent fails to eliminate all pathogenic microorganisms from the deep periodontal pockets.[2–5]

Metronidazole (MZ), a nitroimidazole derivative, has been widely used for the treatment of periodontal disease and is the drug of choice because it has selective antibacterial efficacy against obligate anaerobes and exhibits good concentration in serum as well as in gingival crevicular fluid (GCF).[6–8] Levofloxacin (LVF), a fluroquinolone, is an active isomer of ofloxacin, which is active against both aerobic gram-positive and gram-negative organisms and facultative anaerobic bacteria.[9–11]

Bioavailability of drug in GCF, which is administered systemically, is low and requires a higher dose for it to be effective and is associated with inherent side effects. Direct application of drug into the periodontal pocket increases the bioavailability of drug, but its concentration is rapidly reduced due to constant GCF flow and it is estimated that GCF is 40 times replaced in a hour.[12] Local drug delivery is known as site-specific therapy and subgingival application of drug needs a vehicle and a limiting agent, which increase the substantivity of the drug for prolonged action. The ideal requisite for the local drug delivery should be biocompatible, biodegradable, mucoadhesive, nonallergenic, and release of the drug in a controlled manner.

Chitosan is a natural mucoadhesive polysaccharide obtained from the deacetylation of exoskeleton of crustaceans, e.g. shrimps, crabs, and lobsters. Chitosan is the material of choice as a controlled drug delivery vehicle in biomedical application because it can be formulated into various forms like film, gel, beads, sponges, microparticle, nanoparticle, solutions, fiber, and membranes, and it is also known for its various biological properties: mucoadhesive, biocompatible, biodegradable, antibacterial, wound healing, nontoxic, economical, and widely distributed in nature.[13,14]

Subgingival delivery can be done in film, fiber, injectable gel, in situ gel, strips, microparticle, and nanoparticle form. In our previous study, chitosan-based film cross-linked with gluteraldehyde having LVF, and MZ was formulated with solvent casting method that exhibited sustained release up to 7 days, good antibacterial activity, and decreased clinical parameters in subjects with periodontitis.[15] In situ gel has the property to change in its physical state from sol to gel at the inserted site and does not come back due to its semisolid nature. The objective of this study was to evaluate the clinical efficacy of thermoresponsive, and mucoadhesive chitosan in situ gel in the treatment of chronic periodontitis.

2. Materials and methods

This study was a randomized, split-mouth and vehicle-controlled study. This was approved from the Institute Ethical Committee of Banaras Hindu University, Varanasi, India. Before starting the study, a written informed consent was obtained from each subject after explaining about the nature of the study. In this study, the treatment was provided by one investigator, and the all measurements were recorded by another investigator who was blinded to the treatment given.

2.1. Study design

This clinical study was performed in Faculty of Dental Sciences, IMS, BHU, Varanasi, Uttar Pradesh, India. 10 volunteer subjects with chronic periodontitis (20–50 yrs) of either sex were recruited from the outpatient department. The subjects were examined for a sign of chronic periodontal disease, based on inclusion and exclusion criteria. The inclusion criteria were as follows: (1) good general health and free from systemic disease; (2) subjects suffering from chronic periodontitis with pocket depth ≥5 mm (Figs. 1a, 2a and 3a) and having bleeding on probing (BoP) from base of the pocket; (3)

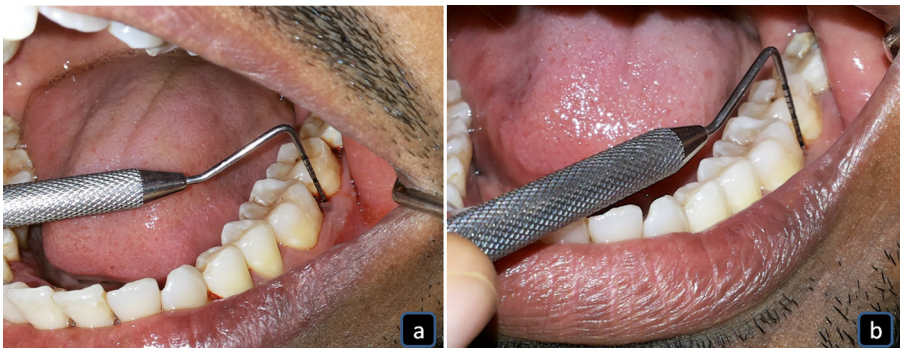


Fig. 1 – (a) Preoperative and (b) postoperative periodontal pocket depth in the scaling and root planing group.

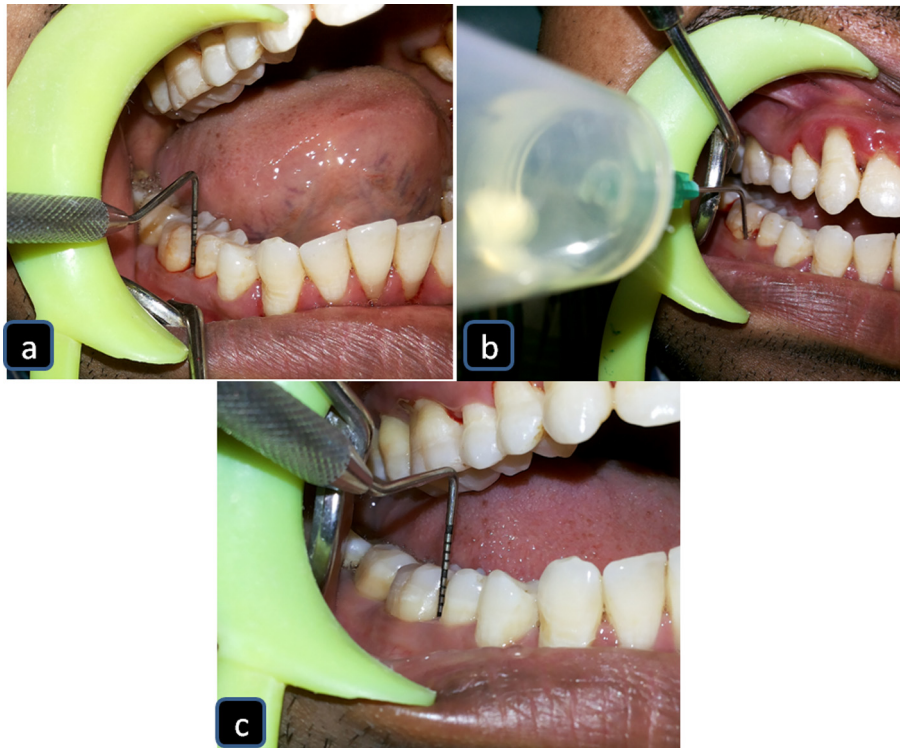


Fig. 2 – (a) Preoperative, (b) application of vehicle in situ gel in pocket with the help of syringe having 21-gauze needle, and (c) postoperative periodontal pocket depth in the vehicle in situ gel group.

no periodontal therapy in the last 6 months; and (4) no antimicrobials for 1 month prior to the study. The exclusion criteria were as follows: (1) poor oral hygiene during the treatment; (2) nonavailability of the subjects; (3) allergic history to LVF and MZ; (4) acute oral disease; (5) habits like smoking and pan chewing; and (6) pregnant or lactating women.

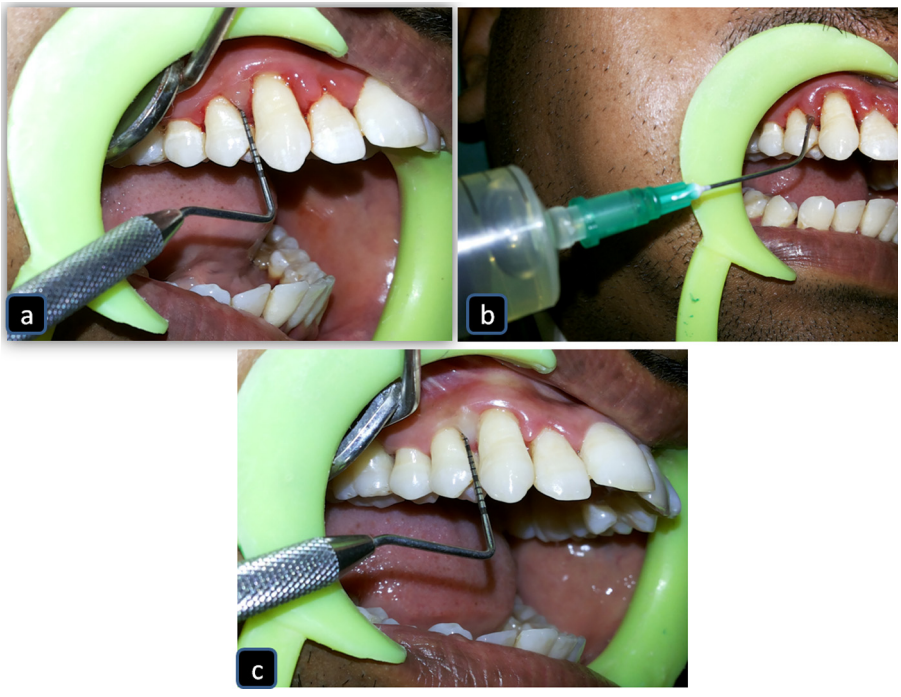


Fig. 3 – (a) Preoperative, (b) application of medicated in situ gel in pocket with the help of syringe having 21-gauze needle, and (c) postoperative periodontal pocket depth in the medicated in situ gel group.

2.2. Study groups

Three nonadjacent sites in each subject were divided into three treatment groups by lottery method of simple random sampling. These three treatment groups were as follows: (1) Group I – SRP only; (2) Group II – SRP and vehicle in situ gel (SRP + vehicle in situ gel); and (3) Group III – SRP + in situ gel containing LVF and MZ (SRP + medicated in situ gel).

2.3. Preparation of the vehicle in situ gel

Vehicle in situ gel was prepared by first dissolving the weighed amount of chitosan (1.5%, w/v) into the 0.5% (v/v) acetic acid under constant stirring to make a homogeneous solution. On the other side, 20% (w/v) poloxamer 407 gel was prepared. Prepared cold poloxamer 407 solution was mixed homogeneously with chitosan solution under constant stirring in an ice bath maintained at 4 °C.

2.4. Preparation of the medicated in situ gel

In medicated formulation, weighed amount of the drugs LVF (10%, w/v) and MZ (25%, w/v) was dispersed in the chitosan solution overnight under magnetic stirring to obtain homogeneous mixing of drugs and polymer. Further, drug containing in situ gel was prepared using a similar method as vehicle in situ gel.

2.5. Clinical parameters

Clinical parameters were plaque index (PI), gingival index (GI), BoP, probing pocket depth (PPD), clinical attachment level (CAL), and gingival recession (GR) as described in Tables 1 and 2. PI, GI, and BoP were recorded at the baseline, 15th day, 30th day, 60th day, and 90th day whereas PPD, CAL, and GR levels were recorded at baseline, 30th day, 60th day, and 90th day. PPD, CAL, and GR were measured by the UNC-15 periodontal probe. The probe was positioned at the line angle of the tooth surface and was inserted into the periodontal pocket parallel to the long axis of the tooth surface, and cementoenamel junction (CEJ) was used as fixed point for measuring the clinical parameters. Further, all the clinical parameters were recorded by a single, trained examiner.

2.6. Treatment procedure

First, the subjects were examined for the presence of pocket depth ≥5 mm and BoP from the base of the pocket. Initially, the supragingival scaling and oral hygiene instructions were given to the subjects and they were asked to come after one week. At

Table 1 – Score and criteria for plaque index,[16,17] gingival index,[18] and bleeding on probing.[19]

Plaque index (Silness and Loe)		Gingival index (Loe and Silness)		Bleeding on Probing	
Score	Criteria	Score	Criteria	The probe was carefully introduced parallel to the long axis of the tooth to the bottom of the pocket and gently moved along the pocket wall. BoP was recorded immediately or after 30 to 60 sec of insertion of the probe	
0	No plaque in the gingival area	0	Normal gingiva	Score	Criteria
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only after application of disclosing agent or by running the explorer across the tooth surface	1	Mild inflammation, slight change in color, slight edema, and no bleeding on probing	0	Absence of bleeding
2	Moderate accumulation of soft deposits within the gingival pocket and on the gingival margin and/or adjacent tooth surface that can be seen with the naked eye	2	Moderate inflammation, redness, edema and glazing, and bleeding on probing*	1	Presence of bleeding
3	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin	3	Severe inflammation, marked redness and edema, ulcerations, and tendency for spontaneous bleeding (*A periodontal probe is drawn horizontally along the soft tissue wall of the entrance to the gingival sulcus)		

Table 2 – Recording of probing pocket depth, clinical attachment level, and gingival recession.

Probing pocket depth (PPD)	Clinical attachment level (CAL)	Gingival recession (GR)
PPD was measured from the crest of the gingival margin to the base of the periodontal pocket.	CAL was measured from the cementoenamel junction (CEJ) to the base of the periodontal pocket.	The distance between the CEJ and the crest of the gingival margin was recorded as GR.

that time, baseline measurements were recorded at the selected sites (Figs. 1a, 2a and 3a) and subgingival SRP was provided with the help of hand and ultrasonic instruments up to the satisfaction of the operator. After that, Group II and Group III subjects received the vehicle in situ gel and medicated in situ gel containing LVF and MZ, respectively. In situ gel was applied in the periodontal pocket up to the gingival margin with the help of a syringe having a 21-gauze needle (Figs. 2b and 3b). The needle was carefully inserted into the periodontal pocket. The periodontal dressing (Coe-pak) was placed at the entrance of periodontal pocket for one week. The subjects were instructed to avoid the use of brush and interdental cleaning aids until the periodontal dressing was removed. The subjects were recalled at regular intervals for follow-up, i.e. 7th day, 15th day, 30th day, 60th day, and 90th day, and the clinical parameters were again measured at the scheduled time period (Figs. 1b, 2c and 3c). The oral hygiene instructions and supragingival scaling were provided if needed at the follow-up visits.

2.7. *Statistical analysis*

Mean and standard deviation (mean ± SD) were determined for each clinical parameter, i.e. PI, GI, BoP, PPD, CAL, and GR. Comparisons between preoperative and postoperative measurements were done using 't' test over time within the groups. Data were analyzed between treatment groups by using post hoc test. The level of significance for analysis was set at $P \leq 0.05$. Statistical analysis was done by SPSS version 16.0.

3. Results

All subjects were returned to follow-up. There was no pain, swelling, and redness, but only the mild discomfort felt by four subjects. The changes from the baseline value on the 15th, 30th, 60th, and 90th days for PI, GI, and BoP are summarized in Tables 3 and 5 and Fig. 4. Changes in the PI, GI, and BoP values were statistically significantly different ($P \leq 0.05$) from the baseline at determined time interval in all groups except in Group I on the 15th day for BoP. The changes on the 30th, 60th, and 90th days were also statistically significant from 15th day. These changes were insignificant ($P > 0.05$) after intergroup comparisons at all time intervals. At the end of the study, the PI was 0.7 ± 0.48, 0.4 ± 0.51, and 0.3 ± 0.48; the GI was 0.6 ± 0.51, 0.5 ± 0.52, and 0.3 ± 0.48; the BoP was 0.5 ± 0.52, 0.3 ± 0.48, and 0.2 ± 0.42 in SRP, vehicle in situ and medicated in situ gel groups, respectively.

Table 3 – Summative data of plaque index (PI), gingival index (GI), and bleeding on probing (BoP).

Clinical parameters	Groups	0 day	15th day		30th day		60th day		90th day	
		Mean ± SD	Mean ± SD	Change	Mean ± SD	Change	Mean ± SD	Change	Mean ± SD	Change
PI	SRP	2.7 ± 0.48	1.4 ± 0.51	1.3 ± 0.48*	0.3 ± 0.48	2.4 ± 0.69*,#	0.5 ± 0.52	2.2 ± 0.78*,#	0.7 ± 0.48	2.0 ± 0.81*,#
	Vehicle in situ gel	2.6 ± 0.51	1.0 ± 0.47	1.6 ± 0.51*	0.2 ± 0.42	2.4 ± 0.69*,#	0.4 ± 0.51	2.2 ± 0.63*,#	0.4 ± 0.51	2.2 ± 0.63*,#
	Medicated in situ gel	2.5 ± 0.52	0.9 ± 0.56	1.6 ± 0.51*	0.1 ± 0.31	2.4 ± 0.51*,#	0.2 ± 0.42	2.3 ± 0.48*,#	0.3 ± 0.48	2.2 ± 0.91*,#
GI	SRP	2.7 ± 0.48	1.5 ± 0.70	1.2 ± 0.42*	0.8 ± 0.42	1.9 ± 0.31*,#	0.3 ± 0.48	2.4 ± 0.84*,#	0.6 ± 0.51	2.1 ± 0.73*,#
	Vehicle in situ gel	2.6 ± 0.51	1.2 ± 0.78	1.4 ± 0.51*	0.5 ± 0.52	2.1 ± 0.56*,#	0.3 ± 0.48	2.3 ± 0.94*,#	0.5 ± 0.52	2.1 ± 0.87*,#
	Medicated in situ gel	2.5 ± 0.52	1.0 ± 0.47	1.5 ± 0.70*	0.2 ± 0.42	2.3 ± 0.48*,#	0.2 ± 0.42	2.3 ± 0.48*,#	0.3 ± 0.48	2.2 ± 0.42*,#
BoP	SRP	1.0 ± 0.00	0.7 ± 0.48	0.3 ± 0.48	0.3 ± 0.48	0.7 ± 0.48*	0.5 ± 0.52	0.5 ± 0.52*	0.5 ± 0.52	0.5 ± 0.52*
	Vehicle in situ gel	1.0 ± 0.00	0.6 ± 0.51	0.4 ± 0.51*	0.4 ± 0.51	0.6 ± 0.51*	0.3 ± 0.48	0.7 ± 0.48*	0.3 ± 0.48	0.7 ± 0.48*
	Medicated in situ gel	1.0 ± 0.00	0.5 ± 0.52	0.5 ± 0.52*	0.2 ± 0.42	0.8 ± 0.42*	0.2 ± 0.42	0.8 ± 0.42*	0.2 ± 0.42	0.8 ± 0.42*

* The statistically significant ($P \leq 0.05$) on the 15th, 30th, 60th, and 90th days from the baseline data.
\# The statistically significant ($P \leq 0.05$) on the 30th, 60th, and 90th days from the 15th day.
SRP – scaling and root planing.

Table 4 – Summative data of probing pocket depth (PPD), clinical attachment level (CAL), and gingival recession (GR).

Clinical Parameters	Groups	0 day	30th day		60th day		90th day	
		Mean ± SD	Mean ± SD	Change	Mean ± SD	Change	Mean ± SD	Change
PPD	SRP	6.0 ± 1.05	4.2 ± 0.91	1.8 ± 0.63*	3.5 ± 0.97	2.5 ± 0.52*,#	3.5 ± 0.97	2.5 ± 0.52*,#
	Vehicle in situ gel	5.9 ± 0.73	3.7 ± 0.67	2.2 ± 0.42*	2.8 ± 0.63	3.1 ± 0.31*,#	2.5 ± 0.70	3.4 ± 0.69*,#
	Medicated in situ gel	7.1 ± 0.73	4.3 ± 0.67	2.8 ± 0.42*	3.2 ± 0.63	3.9 ± 0.31*,#	2.5 ± 0.70	4.6 ± 0.51*,#,$
CAL	SRP	6.1 ± 0.56	5.4 ± 0.51	0.7 ± 0.48*	4.9 ± 0.56	1.2 ± 0.42*,#	4.9 ± 0.56	1.2 ± 0.42*,#
	Vehicle in situ gel	7.0 ± 0.81	5.7 ± 0.82	1.3 ± 0.67*	4.7 ± 0.67	2.3 ± 0.67*,#	4.4 ± 0.69	2.6 ± 0.84*,#
	Medicated in situ gel	7.2 ± 0.63	5.2 ± 0.78	2.0 ± 0.47*	4.1 ± 0.73	3.1 ± 0.31*,#	3.4 ± 0.51	3.8 ± 0.42*,#,$
GR	SRP	0.3 ± 0.48	1.2 ± 0.78	0.9 ± 0.56*	1.4 ± 0.84	1.1 ± 0.73*	1.4 ± 0.84	1.1 ± 0.73*
	Vehicle in situ gel	1.2 ± 0.78	2.0 ± 0.94	0.8 ± 0.42*	2.1 ± 0.99	0.9 ± 0.31*	2.2 ± 0.78	1.0 ± 0.00
	Medicated in situ gel	0.3 ± 0.48	0.8 ± 0.78	0.5 ± 0.52*	0.8 ± 0.78	0.5 ± 0.52*	0.8 ± 0.78	0.5 ± 0.52*

* The statistically significant ($P \leq 0.05$) on the 30th, 60th, and 90th days from the baseline data.
\# The statistically significant ($P \leq 0.05$) on the 60th and 90th days from the 30th day.
$ The statistically significant ($P \leq 0.05$) on the 90th day from the 60th day.
SRP – scaling and root planing.

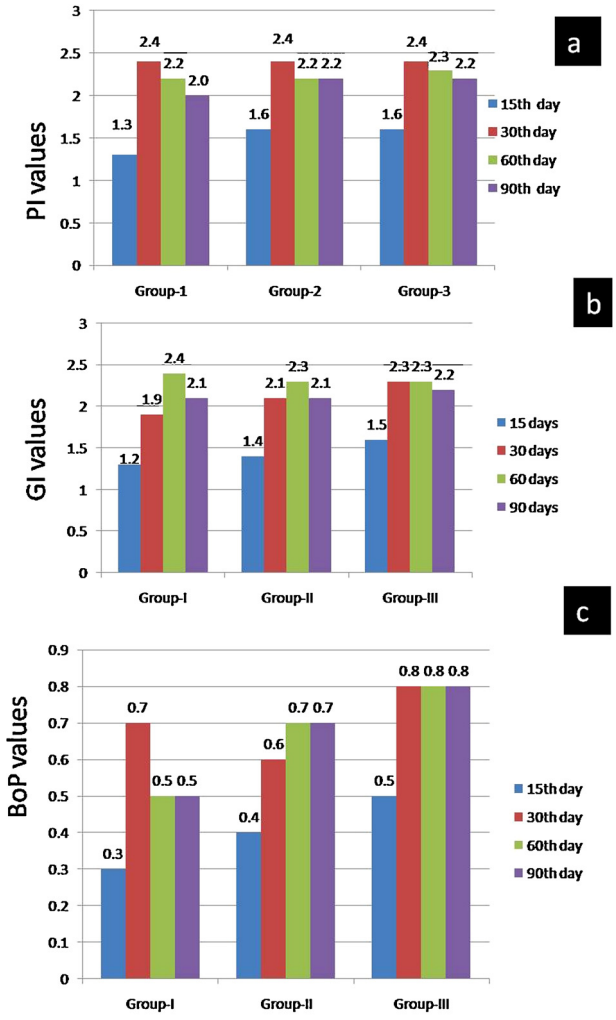


Fig. 4 – The changes in plaque index, gingival index, and bleeding on probing in all treatment groups.

The changes from the baseline value on the 30th, 60th, and 90th days for PPD, CAL, and GR are summarized in Tables 4 and 5 and Fig. 5. On intragroup comparison, the PPD and CAL values were statistically significantly ($P \leq 0.05$) different from the baseline on the 30th, 60th, and 90th days in all groups. The changes on the 60th and 90th days were also statistically significant ($P \leq 0.05$) from 30th day. On intergroup comparison, the changes in PPD and CAL values were statistically significant ($P \leq 0.05$) at all time intervals except on the 30th day for PPD value in SRP group. At the end of the study, the reduction in the PPD was 2.5 ± 0.52 mm, 3.4 ± 0.69 mm, and 4.6 ± 0.51 mm and the gain in the CAL was 1.2 ± 0.42 mm, 2.6 ± 0.84 mm, and 3.8 ± 0.42 mm in SRP, vehicle in situ, and medicated in situ gel groups, respectively. GR value was statistically significantly ($P \leq 0.05$) changed from the baseline on the 30th, 60th, and 90th days in all groups excluding in Group II on the 90th day. On intergroup comparison, the changes in medicated in situ gel group were significant from SRP and vehicle in situ groups on the 90th day and only from SRP group on the 60th day. On the 90th day, the changes in the GR value were 1.1 ± 0.73 mm, 1.0 ± 0.00 mm, and 0.5 ± 0.52 mm in SRP, vehicle in situ, and medicated in situ gel groups, respectively.

4. Discussion

A total of 30 periodontal nonadjacent sites having ≥5 mm pocket depth in 10 systemically healthy subjects were equally and randomly divided (i.e. 3 sites in each subject) and all treatment groups were provided to each subject. Split-mouth study was designed to exclude the effects of microbes and host-dependent variables on the clinical outcomes. In this study, CEJ was used as fixed point for measuring the clinical parameters which has been used by various investigators. This method is easy, simple, and involves no special equipment. Custom-made plastic occlusal stent has also been used in various study

Table 5 – 'P' value after comparison between the treatment groups.

Clinical parameters	Groups	15th day	30th day	60th day	90th day
Plaque index	SRP vs vehicle in situ gel	0.19	1.0	1.0	0.58
	SRP vs medicated in situ gel	0.19	1.0	0.73	0.58
	Vehicle vs medicated in situ gel	1.0	1.0	0.73	1.0
Gingival index	SRP vs vehicle in situ gel	0.43	0.34	0.77	1.0
	SRP vs medicated in situ gel	0.24	0.06	0.77	0.75
	Vehicle vs medicated in situ gel	0.69	0.34	1.0	0.75
Bleeding on probing	SRP vs vehicle in situ gel	0.66	0.64	0.35	0.35
	SRP vs medicated in situ gel	0.38	0.64	0.17	0.17
	Vehicle vs medicated in situ gel	0.66	0.35	0.64	0.64
Probing pocket depth	SRP vs vehicle in situ gel	–	0.08	0.002	0.002
	SRP vs medicated in situ gel	–	0.000	0.000	0.000
	Vehicle vs medicated in situ gel	–	0.01	0.000	0.000
Clinical attachment level	SRP vs vehicle in situ gel	–	0.02	0.000	0.000
	SRP vs medicated in situ gel	–	0.000	0.000	0.000
	Vehicle vs medicated in situ gel	–	0.008	0.001	0.000
Gingival recession	SRP vs vehicle in situ gel	–	0.66	0.42	0.67
	SRP vs medicated in situ gel	–	0.09	0.02	0.01
	Vehicle vs medicated in situ gel	–	0.19	0.11	0.04

SRP – scaling and root planing.

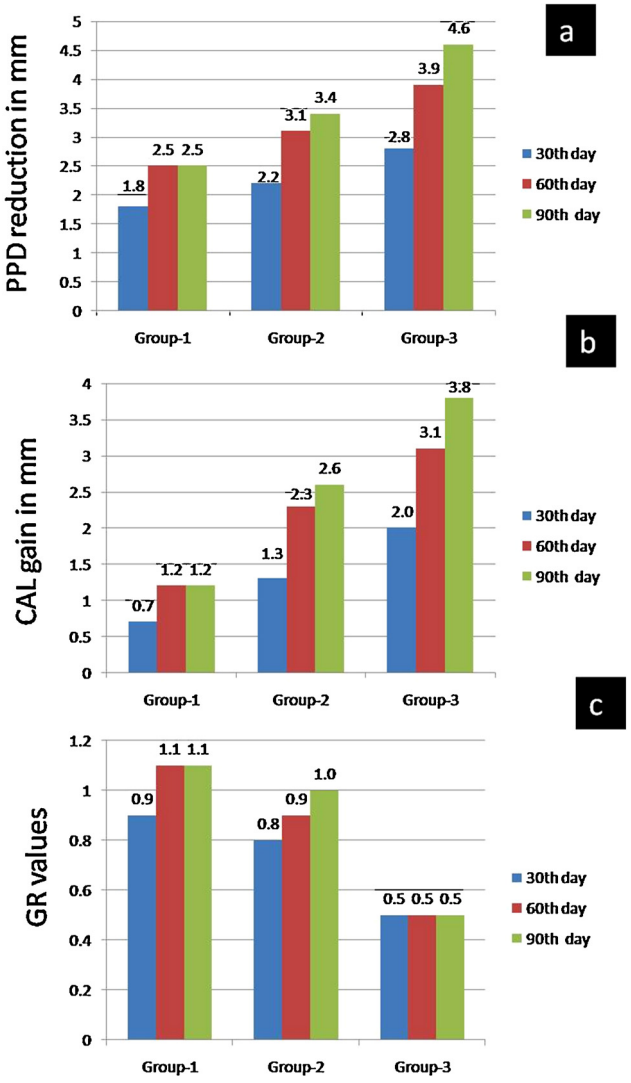


Fig. 5 – The changes in probing pocket depth, clinical attachment level, and gingival recession in all treatment groups.

to ensure the angulation of the probe for reproducibility of the examinations; however, changes in tooth mobility, loss or breakage of the appliance, and tooth extraction can alter the subsequent readings. Instead of that, CEJ must still be explored to ensure that the stent is properly seated on the tooth when used for replicate measurements.[20–22]

All subjects were returned to follow-up period at predetermined time without any problem like redness, allergy, pain, staining of teeth, and nausea, except mild discomfort that was felt by few subjects at the time of placement of in situ gel in the periodontal pockets. In situ gel was applied in the periodontal pocket in the sol form with syringe; therefore, in situ gel is easy to apply and can reach into the deepest portion of the pocket. Initially, in situ gel was stored at 4 °C, and during insertion into the periodontal pocket, the temperature of the in situ gel was maintained at around 25 °C so that it become solid in the periodontal pocket within few seconds and cannot be displaced from the pocket. To avoid the displacement of pocket, periodontal dressing was also applied. In situ gel was thermoreversible in nature due to the presence of poloxamer 407, which is a triblock copolymer consisting of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide). At low temperature, in situ gel was in the form of sol due to its hydrophilic nature. As the temperature rises, the hydrophobic domain predominates as a result of dehydration of the polymer and results in the gel formation.[23]

In the present study, the effect of in situ gel was examined on the clinical parameters such as PI, GI, BoP, PPD, CAL, and GR. On the 90th day, PI was 0.7 ± 0.48, 0.4 ± 0.51, and 0.3 ± 0.48 for SRP, vehicle, and medicated in situ gel groups, respectively that was significantly reduced from the baseline value and on 15th day without significant difference between the groups. The lowest value of PI was in the medicated in situ gel group. The PI was reduced to the maximum on the 30th day, and thereafter it slightly increased. The reason behind this observation was the poor maintenance of oral hygiene. Therefore, oral prophylaxis was provided on the 60th day. The GI value was 0.6 ± 0.051, 0.5 ± 0.52, and 0.3 ± 0.48 for SRP, vehicle, and medicated in situ gel groups. The reduction in GI values was statistically significant from the baseline data and the 15th day at the end of the study without significant different from each other. Medicated in situ gel group showed the maximum reduction in GI. On the 90th day, the GI was slightly increased from the 60th day. This finding could be attributed to the accumulation of supragingival plaque on teeth and results in the changes in the clinical features of gingiva. BoP was 0.5 ± 0.52, 0.3 ± 0.48, and 0.2 ± 0.42 for SRP, vehicle, and medicated in situ gel groups. At the end of the study, BoP was statistically significantly changed from the baseline in all groups, except in SRP group on the 15th day without significant difference between each other. The maximum reduction in BoP was found in medicated in situ gel group. In SRP group, BoP was increased after the 30th day in comparison to vehicle and medicated in situ gel groups. This finding could be attributed to the presence of chitosan in both in situ gels and medicine in the medicated in situ gel. Ikinci et al.[24] found that chitosan has antimicrobial activity against the *Porphyromonas gingivalis* microorganism that is one of the causative agents in the progression of periodontal tissue destruction in chronic periodontitis.

The results of the study showed that the PPD reduction was 2.5 ± 0.52 mm, 3.4 ± 0.69 mm, and 4.6 ± 0.51 mm and gain in CAL was 1.2 ± 0.42 mm, 2.6 ± 0.84 mm, and 3.8 ± 0.42 mm for SRP, vehicle, and medicated in situ gel groups, respectively. PPD reduction and gain in CAL was statistically significant from the baseline and 30th day. In medicated in situ gel group, the PPD and CAL values on the 90th day were also significant from the values on the 60th day. The PPD reduction and CAL gain were also statistically significant between the groups. On the 90th day, the changes in GR were 1.1 ± 0.73 mm, 1.0 ± 0.00 mm, and 0.5 ± 0.52 mm in SRP, vehicle, and medicated in situ gel groups, respectively. GR value was statistically significantly changed from the baseline on the 30th, 60th, and 90th days in all groups excluding in vehicle in situ gel group on the 90th day where the correlation and 't' cannot be computed because the standard error of the difference was 0. In this study, the SRP group has maximum change in the GR because of the highest score of GI at the beginning of the study. The high score of GI corresponds to more GR. On the other hand,

PPD has been reduced due to recession in gingival margin, reduction in the inflammation at the base of the pocket, as well as gain in CAL.

Previously, Akincibay et al.[25] suggested that chitosan itself as well as its combination with MZ is effective in the treatment of chronic periodontitis and reported that the mean reduction in PPD values were 0.94 mm, 1.21 mm, and 1.48 mm for SRP, chitosan gel, and chitosan + MZ groups, respectively, whereas this study showed 2.5 mm, 3.4 mm, and 4.6 mm mean PPD reduction in SRP, vehicle, and medicated in situ gel, respectively. In another study, 2% minicycline active or vehicle gel was applied three times at an interval of 2 weeks immediately after SRP in 30 patients. This study has shown that CAL was significantly gained and probing depth was more reduced but did not reach up to the significant level in minicycline group.[26] 25% MZ dental gel was applied two times at 1-week interval compared with subgingival scaling of 206 patients in multicenters. At 24 weeks, PPD and BOP were significantly reduced in both groups for both parameters ($P < 0.01$). PPD was reduced by 1.3 mm in dental gel group whereas 1.5 mm after scaling and BOP was reduced by 32% and 39% for gel and SRP group, respectively. The difference between the treatments was statistically significant but clinically insignificant.[27] In our previous study, chitosan-based periodontal film containing LVF and MZ was placed in the patient with periodontitis and compared with SRP, vehicle alone, and LVF film groups. The results revealed at the end of the study that a significant reduction in PPD, GI, BoP, and gain in CAL was observed for LVF film and LVF + MZ film groups from SRP group, and LVF + MZ film has shown more superior result as compared to LVF film without significant difference.[15] In this study, the PPD reduction and CAL gain were statistically significant between the treatment groups. In our opinion, the comparison of the clinical outcomes of the clinical studies seems to be difficult due to differences in the patient selection criteria, healing pattern, microbial pathogens, pathogenesis of the disease, study design, treatment protocols, formulated device, and observation intervals from one study and control of some of these factors is really difficult.

In this study, the results showed the better clinical outcomes in the vehicle and medicated in situ gel as compared to SRP group that might be due to the presence of chitosan polymer, which is nontoxic, biocompatible, biodegradable, antimicrobial, and mucoadhesive. Moreover, it also has wound healing and regenerative potential that helps in the reduction of pocket depth and gain in CAL. Mucoadhesivity is due to the hydrophilic nature of chitosan that enhances the substantivity of the drug in the periodontal pocket and controls the release of the drug.[13,14,28] Bioadhesion formulations are currently receiving much interest as drug delivery system for periodontal pocket and oral mucosa.[29,30]

In this study, after comparison between the vehicle and medicated in situ gels, medicated in situ gel showed better improvement in clinical outcomes. The reason behind these findings is the delivery of MZ and LVF present in the medicated in situ gel at the site of action. Thus, chitosan acts as a good vehicle for the delivery of pharmacologic agent. In this study, LVF and MZ were present in the medicated in situ gel. Both are bactericidal in nature and act synergistically. MZ has efficacy against obligate anaerobes and LVF has efficacy both aerobic gram-positive and gram-negative organisms and facultative anaerobic bacteria. LVF also modulates the immune defense mechanism by inhibiting monocytic IL-1 and TNF-α synthesis.[6–11,31] In a previous study, it has been stated that MZ combined with ciprofloxacin has been shown as powerful combination for the treatment of mixed infections.[32] Although the results have shown that PPD reduction and CAL gain in medicated in situ gel were statistically significant from the vehicle in situ gel, further clinical study on a large number of periodontal sites is needed to establish the findings of this study.

5. Conclusions

Within the limitations of the study, it can be concluded that vehicle and medicated in situ gel showed the better clinical outcomes in terms of PI, GI, BoP, PPD, CAL, and GR as compared to SRP alone. However, comparative results between these two in situ gels are also statistically significant in PPD reduction and CAL gain but it cannot be conclusive because the sample size was too small. The results suggest that chitosan is not only effective in the treatment of periodontal disease but also it acts as a good carrier to deliver the drug into periodontal pockets. At last, further clinical study as well as microbial study is recommended on a large number of samples to show the efficacy of in situ gel in periodontal sites and on periodontal microorganisms.

Conflicts of interest

The authors have none to declare.

Acknowledgment

The authors are thankful to Dr. Tej Bali Singh, Division of Biostatistics, Department of Community Medicine, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India for doing the statistical analysis.

REFERENCES

1. Mealy BL. Influence of periodontal infections on systemic health. *Periodontology 2000*. 1999;21:197–209.
2. Carranza FA, Camargo PM. The periodontal pocket. In: Newman MG, Takei HH, Carranza FA, eds. In: *Clinical Periodontology* 9th ed. New Delhi; 2003:336–353.
3. Rams TE, Slots J. Antibiotics in periodontal therapy: an update. *Compend Contin Educ Dent*. 1992;13:1130.
4. Walker CB, Gordon JM, Socransky SS. Antibiotic susceptibility testing of subgingival plaque samples. *J Clin Periodontol*. 1983;10:422–432.
5. Jolkovsky DL, Ciancio SG. Chemotherapeutic agents in the treatment of periodontal diseases. In: Newman MG, Takei HH, Carranza FA, eds. In: *Clinical Periodontology* 9th ed. New Delhi; 2003:675–687.

6. Noyan U, Yilmaz S, Kuru B, Kadir T, Acar O, Buget E. A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients. *J Clin Periodontol*. 1997;24:158–165.
7. Slots J. Selection of antimicrobial agents in periodontal therapy. *J Periodontal Res*. 2002;37:389–398.
8. Lofmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis*. 2010;50(suppl 1):S16–S23.
9. Davis R, Bryson HM. Levofloxacin. A review of its antibacterial activity, pharmacokinetics and therapeutic efficacy. *Drugs*. 1994;47:677–700.
10. Ogrendik M. Levofloxacin treatment in patients with rheumatoid arthritis receiving methotrexate. *South Med J*. 2007;100:135–139.
11. Reisbeck k. Immunomodulating activity of quinolones: review. *J Chemother*. 2002;14:3–12.
12. Goodson JM. Pharmacokinetic principles controlling efficacy of oral therapy. *J Dent Res*. 1989;68:1625–1632.
13. Denkbas EB, Ottenbrite RM. Perspectives on: chitosan drug delivery systems based on their geometries. *J Bioact Compat Polym*. 2006;21:351–368.
14. Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur J Pharm Biopharm*. 2004;57:19–34.
15. Khan G, Yadav SK, Patel RR, Nath G, Bansal M, Mishra B. Development and evaluation of biodegradable chitosan films of metronidazole and levofloxacin for the management of periodontitis. *AAPS PharmSciTech*. 2015 [Epub ahead of print].
16. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135.
17. Loe H. the gingival index, the plaque index and the retention index system. *J Periodontol*. 1967;38(suppl):610–616.
18. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551.
19. Carranza FA. Clinical diagnosis. In: Newman MG, Takei HH, Carranza FA, eds. In: *Clinical Periodontology* 9th ed. New Delhi; 2003:336–353.
20. Christopher Clark D, Chin Quee T, Bergeron MJ, Chan ECS, Lautar-Lemay C, De Gruchy KD. Reliability of attachment level measurements using the cementoenamel junction and a plastic stent. *J Periodontol*. 1987;58:115.
21. Bowen JA, Mellonig JT, Gray JL, Towle HT. Comparison of decalcified freeze-dried bone allograft and porous particulate hydroxyapetite in human periodontal osseous defects. *J Periodontol*. 1989;60:647–654.
22. Schyeyer ET. A clinical comparison of a bovine derived xenograft used alone or in combination with enamel matrix derivative for the treatment of periodontal osseous defects in humans. *J Periodontol*. 2002;73:423.
23. Escobar-Chávez JJ, López-Cervantes M, Naïk A, Kalia YN, Quintanar-Guerrero D, Ganem-Quintanar A. Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations. *J Pharm Pharm Sci*. 2006;9:339–358.
24. Ikinci G, Senel S, Akincibay H, et al. Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis*. *Int J Pharm*. 2002;235:121–127.
25. Akincibay H, Senel S, Ay ZY. Application of chitosan gel in the treatment of chronic periodontitis. *J Biomed Mater Res Part B: Appl Biomater*. 2007;80:290–296.
26. Graca MA, Watts TLP, Wilson RF, Palmer RM. A randomized controlled trial of a 2% minicycline gel as an adjunct to non surgical periodontal treatment, using a design with multiple matching criteria. *J Clin Periodontol*. 1997;24:249–253.
27. Ainamo J, Lie T, Ellingsen BH, et al. Clinical responses to subgingival application of a metronidazole 25% gel compared to the effect of subgingival scaling in adult periodontitis. *J Clin Periodontol*. 1992;19:723–729.
28. Needleman IG, Martin GP, Smales FC. Characterization of bioadhesives for periodontal and oral mucosal drug delivery. *J Clin Periodontol*. 1998;25:74–82.
29. Nair MK, Chien YW. Development of anticandidal delivery systems. II. Mucoadhesive devices for prolonged drug delivery in the oral cavity. *Drug Dev Ind Pharm*. 1996;22: 243–253.
30. Bouckaert S, Schautteet H, Lefebvre RA, Remon JP, Van clooster R. Comparison of salivary miconazole concentration after administration of a bioadhesive slow release buccal tablet and an oral gel. *Eur J Clin Pharmacol*. 1992;43:137–140.
31. Kumari N, Pathak K. Dual controlled release, in situ gelling periodontal sol of metronidazole benzoate and serratiopeptidase: statistical optimization and mechanistic evaluation. *Curr Drug Deliv*. 2012;9:74–84.
32. Rams TE, Feik D, Slots J. Ciprofloxacin/metronidazole treatment of recurrent adult periodontitis. Abstract. *J Dent Res*. 1992;71:319.