

Local Delivery of Hyaluronan as an Adjunct to Scaling and Root Planing in the Treatment of Chronic Periodontitis

Shyam Sunder Salavathi¹ • Srikanth Chintalapani² • Chandra Mohan Pabolu^{3*} • Arpita Paul⁴ • Ramesh Babu Mutthineni⁴ • Kiran Kumar Reddy Guntur¹

¹Senior Lecturer, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India
²Professor and Head, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India
³Professor, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India
⁴Reader, Department of Periodontics, Mamata Dental College, Khammam, Telangana, India
*Corresponding Author; E-mail: mpabolu@gmail.com

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Abstract

Background and aims. The present study was carried out to evaluate the adjunctive effect of local application of hyaluronan gel with scaling and root planing in the treatment of chronic periodontitis.

Materials and methods. Twelve patients with chronic periodontitis participated in the study with a split-mouth design. Plaque formation and bleeding on probing (BOP) were evaluated at baseline and at 1st, 4th and 12th weeks postoperatively. Probing depth (PD) and clinical attachment levels (CAL) were evaluated at baseline and at 12-week postoperative interval. 0.2 mL of 0.8% hyaluronan gel was administered subgingivally in the test sites at baseline and after 1 week.

Results. The test group exhibited a significantly lower mean plaque score and mean BOP as compared to the control group at 1st, 4th and 12th weeks ($P < 0.05$). Between the two groups, post-treatment comparison at 12th week showed lower PD value in the test group as compared to the control group and higher gain in CAL in the test group as compared to the control group. The difference between the two groups was statistically significant ($P < 0.05$).

Conclusion. Local application of hyaluronan gel in conjunction with SRP might have a beneficial effect in patients with chronic periodontitis.

Key words: Dental plaques, hyaluronan, periodontitis, root planing.

Introduction

Progressive destruction of tissues that anchor the tooth is the hallmark of periodontal disease. Periodontal disease is a polymicrobial infection that results in connective tissue destruction and eventual resorption of the alveolar bone and tooth loss.¹

Treatment of periodontitis involves both the control of oral infection and improvements of systemic health. Mechanical removal of supra- and subgingival plaque is not always effective in reducing the periodontal pocket. Scaling and root planing (SRP) does not totally eliminate the putative pathogens, thus making it necessary for repeating SRP at

regular intervals for periodontal maintenance.² Mechanical debridement performed at regular intervals will lead to secondary effects such as gingival recession, dentinal hypersensitivity and loss of enamel.

Hence, to negate the secondary effects of SRP use of systemic or controlled release local drug delivery systems have been proposed. However, systemic administration of antimicrobials in the treatment of chronic periodontal diseases has many demerits because of the equivocal evidence of benefits and potential side effects.³ Because of the concern over the emergence of widespread antibiotic resistance to systemically administered antibiotics, there has been a renewed interest in the use of controlled release, locally delivered antimicrobials placed directly in the infected periodontal site for the treatment of periodontitis.

Periodontal disease is often localized to few teeth, and use of locally delivered antimicrobial agents, either as an irrigant in the pockets or in various controlled release preparations, has been explored.⁴ A multitude of adjunctive therapies have been tried and tested to amplify the beneficial effects of SRP.

Hyaluronic acid (HA) is also known as hyaluronan or hyaluronate. It is directly or indirectly related to many cell functions like cell proliferation, recognition and locomotion, which will contribute to its tissue healing properties. Because of its unique physicochemical properties and most importantly, the non-immunogenicity of the highly purified form, hyaluronan has already found medical applications for many years. HA has many important physiological and biological functions and plays a vital role in the functioning of extracellular matrices, including those of the periodontium. The beneficial effects of local application of HA gel were noted in the treatment of plaque-induced gingivitis by Jenstch et al.⁵ and in periodontitis by Johannsen et al.⁶ However, contradictory results in periodontitis cases were observed by Xu et al.⁷ The beneficial effects of HA gel in the treatment of gingivitis offers exciting possibilities in the treatment of individual periodontally affected sites.⁶ Thus, in the present study we evaluated the adjunctive effect of local application of a hyaluronan gel (Gengigel) in association with scaling and root planing in the treatment of chronic periodontitis.

Materials and Methods

Study Population

Patients who reported to the Department of Periodontics, Mamata Dental College, and Khammam and were subsequently diagnosed with chronic periodon-

titis were recruited for this study. Approval of the study was obtained from the Ethics Committee of Mamata Educational Society and an informed consent was taken from all the participants before the study.

Design and Subjects

A 6-month randomized split-mouth study was designed in which a total of 24 quadrants from 12 patients were treated for 24 weeks (12+12 weeks for each group). Twenty-four quadrants from 12 patients with chronic periodontitis, selected using clinical parameters, were randomly divided into a control group - treated by scaling and root planing alone - and a test group - receiving SRP and intrasulcular application of Gengigel (Hyaluronic acid, 0.8%, GENGIGEL®: Product Literature, RICERFARMA S.R.L, Italy) (Figure 1).

Healthy male and female subjects in the 42–63-year age group had a minimum of 20 permanent teeth with at least 5 interproximal sites with pocket depths of (PD) ≥ 5 mm. Canine and premolar teeth had two interproximal sites on each tooth with pocket depths of ≥ 5 mm. The patients with no antibiotic therapy or previous periodontal treatment within the last 6 months were included in the study. Those not meeting the above criteria, patients with a history of any systemic diseases and known hypersensitivity to hyaluronic acid, female patients who were pregnant and nursing mothers, were excluded from the study.

Study Procedures

Pairs of premolar and canine teeth in the maxilla (teeth #4 through #6 versus teeth #11 through #13) or in the mandible (teeth #20 through #22 versus teeth #27 through 29) were randomized to receive the test treatment (adjunctive hyaluronan gel) or to serve as SRP controls. The jaw quadrants were treated in sequence; the control sites were always treated first to reduce potential crossover effects from the untreated test sites.

The observation interval for each jaw quadrant was 3 months. The participants were asked to make 8



Figure 1. (a) GENGIGEL (0.8% hyaluronan) pack containing prefilled bulbs and applicators; (b) insertion of prefilled bulb in the applicator.

visits – 4 visits for control sites and 4 visits for test sites in the following order: baseline, first week, fourth week and twelfth week.

At the baseline, the following assessments were recorded to the nearest mm using a 15-mm UNC probe.

1. Plaque index (PI) by Silness & Løe (1964)
2. Gingival bleeding index (GBI) by Ainamo & Bay (1975)
3. Pocket depth (PD)
4. Clinical attachment level (CAL)

All the teeth were evaluated at six sites (mesiobuccal, distobuccal, mesiolingual, distolingual, mid buccal, mid lingual). Bleeding on probing (BOP) was recorded as a positive score if bleeding occurred within 10 seconds after gentle intracrevicular probing.

PD was measured from the crest of the gingival margin to the base of the pocket using a graduated manual probe (UNC 15). CAL was recorded using a graduated manual probe (UNC 15). Any loss was calculated from the two measurements as follows:

$CAL = PD - \text{distance from free gingival margin to CEJ}$

A customized occlusal stent of 1-mm polyvinyl silicone sheet (3A MEDES Inc. KOREA) in a Biostar unit (Jaypee Instruments Corp. Kerala) was prepared for every patient and used to measure PD and CAL (Figure 2).

All the patients received SRP of the control jaw quadrants at baseline using hand instruments. PI and BOP were recorded at 1st and 4th weeks and PD and CAL were recorded at 12th weeks after treatment. Next, contra-lateral experimental jaw quadrants received the identical protocol with the addition of the subgingival administration 0.2 mL of 0.8% hyaluronan gel into all the selected sites following SRP. The hyaluronan gel was re-applied at 1st week post-treatment. No oral hygiene instructions were provided. The patients were encouraged to use their routine oral hygiene habits, similar to the control site



Figure 2. Cast with occlusal stent.

(Figure 3).

Statistical Analysis

The results were sent for statistical analysis using SPSS 17. Comparison of mean scores from baseline to follow-ups was carried out using repeated-measures ANOVA, followed by post hoc Bonferroni test. Comparisons between the test and control groups at each follow-up were carried out using Student's t-test. Comparisons of baseline with follow-ups were carried out within the groups by paired t-test.

Results

Statistically significant differences were seen in all the assessed parameters between the study and control groups.

Plaque Scores

At baseline, the mean plaque values were 2.28 ± 0.23 and 2.45 ± 0.24 in the test and control groups, respectively, with no statistically significant difference ($P = 0.168$). The mean plaque scores for the test group at 1st, 4th and 12th week post-treatment were 1.58 ± 0.15 , 0.93 ± 0.43 and 0.60 ± 0.43 , respectively, with 1.83 ± 0.31 , 1.29 ± 0.34 and 0.94 ± 0.38 in the control group at the same intervals, respectively. Between the two groups, the test group exhibited a significantly lower mean plaque scores at the same intervals ($P = 0.02$, $P = 0.02$ and $P = 0.011$, respectively). Within each group, there was a significant difference in the mean plaque scores from baseline to follow-ups (12 weeks) in both the test and control groups ($P < 0.001$ and $P < 0.001$). In both the test and control groups, post hoc analysis showed that the mean plaque scores were significantly higher at baseline followed by 1st, 4th and 12th weeks, suggesting decreases in the plaque scores with each recall visit (Table 1).

Bleeding on Probing (BOP)

At baseline, the mean BOP sites were $95.64 \pm 6.15\%$ and $98.47 \pm 2.47\%$ in the test and control groups, re-



Figure 3. A probing depth of 6 mm at baseline (a); local delivery of Gengigel in the intrasulcular area after scaling and root planing (b); post-treatment probing depth of 4 mm at 12th week (c).

Table 1. Comparative plaque scores for the test and control groups at baseline and 1st, 4th and 12th weeks

Time interval	n	Test		Control		P-value
		Mean	SD	Mean	SD	
Baseline	12	2.28	0.23	2.45	0.24	0.168
1st week	12	1.58	0.15	1.83	0.31	0.02*
4th week	12	0.93	0.43	1.29	0.34	0.02*
12th week	12	0.60	0.43	0.94	0.38	0.011*
P-value (intra-group)		<0.001*		<0.001*		
Post hoc test		1>2>3>4		1>2>3>4		

spectively, with no statistically significant differences ($P > 0.001$). The mean BOP sites for the test group at 1st, 4th and 12th weeks were $59.69 \pm 9.81\%$, $43.87 \pm 8.50\%$ and $28.45 \pm 5.8\%$, respectively. In the control group, the mean sites with BOP at 1st, 4th and 12th weeks were $69.40 \pm 15.92\%$, $51.02 \pm 10.07\%$ and $38.32 \pm 8.90\%$, respectively. Within each group, there were significant mean differences in the mean BOP values from baseline to follow-up (12 weeks) in both the test and control groups ($P < 0.001$ and $P < 0.001$). In both the test and control groups, post hoc analysis showed that the mean BOP values were significantly higher at for baseline followed by the 1st, 4th and 12th weeks; in other words, the BOP values decreased with each recall visit. Between the two groups, comparisons between the 1st, 4th and 12th weeks showed that the mean BOP values in test group were lower for each time interval as compared to the control group and the difference was statistically significant ($P < 0.05$) (Table 2).

Probing Pocket Depth (PD)

The mean PD values at baseline for the test and control groups were 7.33 and 6.92 mm, respectively, whereas at 12-week interval, the means were 4.5 and 5.25 mm, respectively.

At baseline, the mean PD values in the test and control groups were 7.33 ± 0.98 and 6.92 ± 0.90 mm, with no significant difference ($P > 0.001$). Within each group, there was a significant decrease in the mean PD value from baseline to 12th week in both groups ($P < 0.001$ and $P < 0.001$). Between the two

groups, comparison at 12th week showed that PD value in test group was 4.5 ± 0.80 mm as compared to 5.25 ± 0.87 mm in the control group and the difference was significant ($P < 0.05$) (Table 3).

Clinical Attachment Level (CAL)

The mean CAL at baseline for the test and control groups was 7.58 and 7.67 mm, respectively, whereas at 12th week, the mean CAL values were 4.67 and 6 mm, respectively.

At baseline, the mean CAL values were 7.33 ± 0.98 and 6.92 ± 0.90 mm in the test and control groups, respectively, with no statistically significant difference ($P > 0.001$). Within each group, there was a significant decrease in the mean CAL from baseline to 12th week in both groups ($P < 0.001$ and $P < 0.001$). Between the two groups, comparison at 12th week showed that CAL values in the test and control groups were 4.5 ± 0.80 and 5.25 ± 0.87 mm, respectively, with a significant difference between the two groups ($P < 0.05$). In other words, the gain in CAL in the test group was higher than that in the control group (Table 4).

Discussion

Periodontitis is considered a bacteria-induced inflammatory destruction of periodontal tissues and alveolar bone.¹ Clinical characteristics of periodontitis include loss of periodontal attachment and resorption of alveolar bone, resulting in formation of pathological pockets and/or gingival recession. Suc-

Table 2. Comparative BOP values (as percentages) for the test and control groups at baseline and 1st, 4th and 12th weeks

Time interval	N	Test		Control		P-value
		Mean (%)	SD	Mean (%)	SD	
Baseline	12	95.64	6.15	98.47	2.47	0.112
1st week	12	59.69	9.81	69.40	15.92	0.059
4th week	12	43.87	8.50	51.02	10.07	0.016
12th week	12	28.45	5.82	38.32	8.90	0.002
P-value (intra-group)		<0.001*		<0.001*		
Post hoc test		1>2>3>4		1>2>3>4		

successful periodontal therapy is dependent on anti-infective procedures aiming at effectively controlling pathogenic organisms found in dental plaque biofilm.⁸

Topical delivery of therapeutic agents into the sulcus has the advantage of bringing a high concentration of the drug where needed without exposing the whole body. The side effects of systemic antibiotic therapy and the possible failing compliance of the patient can be minimized by using locally applied antibiotics. Therefore, a positive influence on the subgingival biofilm may be accomplished with high concentrations of antibiotics by local delivery of the antimicrobial drug.⁷

However, not all patients respond well to non-surgical periodontal therapy nor are they able to maintain a stable periodontium over extended periods of time following successful treatment.⁹ Although mechanical treatment is effective in treating most of the periodontal diseases, it can control the disease with minimal side effects and should be the first choice for periodontal treatments. Not all periodontal diseases and not all periodontal disease sites respond well to this mechanical treatment. The host modulatory therapies were considered as an adjunct to the mechanical treatment for such a scenario.¹⁰

Hyaluronan gel is tasteless, odorless and colorless. It is easy to apply, does not stain teeth and is not inactivated by sodium lauryl sulphate. It has no known adverse patient reactions or drug interactions. As hyaluronan is presented in gel form, it can be economically and easily delivered to all areas undergoing therapy. When used in combination with non-surgical periodontal therapy, a more effective outcome was achieved.¹¹ Since no established protocol and regimen for local application of 0.8% HA gel is available, we prepared a treatment protocol, i.e. subgingival application of 0.2 mL of 0.8% hyaluronan gel at baseline and at 1st week, which conformed to routine clinical practice and to reasonable assump-

tions about HA as an active agent.

The present study demonstrated a positive effect of subgingival hyaluronan application on dental plaque formation; the test group showed a significant lower mean plaque score as compared to the control group at 1st, 4th and 12th weeks. This is in accordance with studies conducted by Eick et al,¹² Pilloni et al,¹³ Polepalle et al,¹⁴ and Sapna et al,¹⁵ who showed reductions in PI scores in patients with topical and intrasulcular hyaluronan gel application. Jentsch et al⁵ suggested a beneficial effect of hyaluronan gel during the therapy of plaque-induced gingivitis proven by clinical and paraclinical variables. The results of the present study do not coincide with the results obtained by El Syed et al,¹⁶ Johansen et al⁶ and Pistorius et al,¹⁷ who did not find any significant difference in plaque values throughout the measurement period for which they concluded that the increase in inflammation parameters was not based on a reduction in plaque.

There was significant reduction in the mean PD from baseline to 12th week post-treatment in both groups and a statistically significant PD reduction in the test group was observed as compared to the control group. This is consistent with the studies conducted by Eick et al,¹² Johansen et al,⁶ Pilloni et al,¹³ and Polepalle et al.¹⁴ The present study is in contrast to the study by El Syed et al.¹⁶ The absence of significant PD reduction may be explained by the improvement in CAL in the test sites in which the absence of an improvement in gingival recession could have meant an eventual reduction in PD. The present study is also in contrast to earlier studies by Engstrom et al¹⁸ and Xu et al,⁷ who failed to demonstrate any significant reduction in PD.

In the present study, a statistically significant reduction in CAL was seen in both groups from baseline to 12th week post-treatment and a statistically significant reduction was observed in the test group compared to the control group. This is in accordance

Table 3. Comparative probing depth level values (in mm) for the test and control groups at baseline and 12th week

Time interval	N	Test group		Control group		P-value
		Mean	SD	Mean	SD	
Baseline	12	7.33	0.98	6.92	0.90	0.175
12th week	12	4.5	0.80	5.25	0.87	0.005*
P-value (intra-group)		<0.001*		<0.001*		

Table 4. Comparative clinical attachment level values (in mm) for the test and control groups at baseline and 12th week

Time interval	N	Test group		Control group		P-value
		Mean	SD	Mean	SD	
Baseline	12	7.58	1.16	7.67	1.67	0.175
12th week	12	4.67	1.3	6	1.6	0.005*
P-value (intra-group)		<0.001*		<0.001*		

with previous studies by El Syed et al,¹⁶ Eick et al,¹² Pilloni et al¹³ and Polepalle et al,¹⁴ who reported significant improvements in CAL following hyaluronan gel application. The results of the present study are in contrast to studies conducted by Xu et al⁷ and Johannsen et al,⁶ who did not demonstrate clinical or microbiological improvements by the adjunctive use of hyaluronan gel compared to SRP alone. The absence of improved clinical improvements in these studies may indicate that the hyaluronan used were well below the optimum levels required to achieve a significant clinical improvement.¹¹

The decrease in PD and CAL in the present study might be due to the difference in concentration of Gengigel from the earlier studies. In the present study, 0.2 mL of 0.8% hyaluronan gel was applied subgingivally at baseline and after one week, a protocol was used supported by studies conducted by Amit et al,¹¹ Eick et al,¹² Pilloni et al¹³ and Polepalle et al,¹⁴ who demonstrated highly significant improvements in PD after using hyaluronan at a concentration of 0.8%. The intrasulcular application served as the better mode of application for the HA gel so that all the beneficial actions of HA were potentiated by its close contact with inflamed sulcular tissues.¹² No adverse effects were observed on clinical examination as well as reported by the patients. This can be attributed to the safety of HA gel also reported in studies by Jentsch et al,⁵ Johannsen et al⁶ and Xu et al.⁷

In the present study, clinical significance of repeated applications of HA gel in each clinical session or at each review session still needs to be investigated. Furthermore, the additional effects of application which combined 0.8% HA gel with other HA products, such as HA mouthrinse, 0.2% customer HA gel and HA containing spray, should be explored as well. The preliminary results of the current clinical trial are only based on the short-term observations, so further follow-ups are necessary to evaluate the long-term clinical values of local application of HA gel in the management of periodontitis patients.

Conclusion

The present study concluded that use of local delivery of hyaluronic acid (HA) gel as intrasulcular application in conjunction with scaling and root planing may have a beneficial effect in the treatment of chronic periodontitis. Adjunctive use of 0.8% HA gel has no side effects, but further long-term investigations are necessary to evaluate its effects in the management of chronic periodontitis and to devise a universal treatment regimen for its use in non-

surgical periodontal therapy.

References

1. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol* 2000 1994;5:78-111. doi: 10.1111/j.1600-0757.1994.tb00020.x
2. Wilson TG. Supportive periodontal treatment introduction-definition, extent of need, therapeutic objectives, frequency and efficacy. *Periodontol* 2000 1996;12:11-15. doi: 10.1111/j.1600-0757.1996.tb00074.x
3. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann Periodontol* 2003;8:115-181. doi: 10.1902/annals.2003.8.1.115
4. Suleyman AS, Yukna RA, Vastardis S, Layman D, Lallier T. Effect of locally delivered doxycycline hyclate on human fibroblast attachment to subgingival calculus. *J Periodontol* 2005;76:221-228. doi: 10.1902/jop.2005.76.2.221
5. Jentsch H, Pomowski R, Kundt G, Gocke R. Treatment of gingivitis with hyaluronan. *J Clin Periodontol* 2003;30:159-164. doi: 10.1034/j.1600-051x.2003.300203.x
6. Johannsen A, Tellefsen M, Wikesjo U, and Johannsen G. Local delivery of hyaluronan as an adjunct to scaling and root planing in the treatment of chronic periodontitis. *J Periodontol* 2009;80:1493-1497. doi: 10.1902/jop.2009.090128
7. Xu Y, Hofling K, Fimmers R, Frentzen M, Jervoe storm PM. A clinical trial of local delivery of hyaluronic acid gel as an adjunct to non-surgical treatment of chronic periodontitis. *J Periodontol* 2004;75:1114-1118. doi: 10.1902/jop.2004.75.8.1114
8. Bollen, CML, Mongardini C, Papaioannou W, Van Steengerghe D, Quirynen M. The effect of one-stage full-mouth disinfection on different intraoral niches. Clinical and microbiological observations. *J Clin Periodontol* 1998;25:55-66. doi: 10.1111/j.1600-051x.1998.tb02364.x
9. Mombelli A, Lehmann B, Tonetti M, Lang NP. Clinical response to local delivery of tetracycline in relation to overall and local periodontal conditions. *J Clin Periodontol* 1997;24:470-477. doi: 10.1111/j.1600-051x.1997.tb00214.x
10. Reddy MS, Geurs NC, Gunsolley JC. Periodontal host modulation with antiproteinase, anti-inflammatory, and bone-sparing agents: A systematic review. *Ann Periodontol* 2003;12-38. doi: 10.1902/annals.2003.8.1.12
11. Amit K, Paresch P, Robert B, Dawett B, Peter G. A comparison in post operative healing of sites receiving non surgical debridement augmented with and without a single application of hyaluronan 0.8% gel. *Preventive Dentistry* 2007;2(3):34-36.
12. Sapna N and Vandana KL. Evaluation of hyaluronan gel (Gengigel) as a topical applicant in the treatment of gingivitis. *J Invest Clin Dent* 2011;2:162-170. doi: 10.1111/j.2041-1626.2011.00064.x
13. Pilloni A, Annibali S, Dominici F, Di Paolo C, Papa M, Casini MA, et al. Evaluation of the efficacy of an hyaluronic acid-based biogel on periodontal clinical parameters. A randomized-controlled clinical pilot study. *Ann Stomatol (Roma)* 2011;2:3-9.
14. Eick S, Renuis A, Heinicke M, Pfister W, Stratul SI, Jentsch H. Hyaluronic Acid as an adjunct after scaling and root planing: A prospective randomized clinical trial. *J Periodontol* 2013;84:941-9. doi: 10.1902/jop.2012.120269
15. Polepalle T, Srinivas M, Swamy N, Aluru S, Chakrapani S, Chowdary BA. Local delivery of hyaluronan 0.8% as an adjunct to scaling and root planing in the treatment of chronic periodontitis: A clinical and microbiological study. *J Indian*

18 Salavadhi et al.

- Soc Periodontol* 2015;19:37-42. doi: 10.4103/0972-124x.145807
16. El sayed KM, Dahaba MA, Aboul- Ela S, Darhous MS. Local application of hyaluronan gel in conjunction with periodontal surgery: a randomized controlled trial. *Clin Oral Invest* 2012;16(4):1229-36. doi: 10.1007/s00784-011-0630-z
17. Pistorius A, Priv-Doz, Martin M, Willershausen B, Rockman P. The clinical application of hyaluronic acid in gingivitis therapy. *Quintessence Int* 2005;36:531-538.
18. Engstrom P, Shi XQ, Tronje G, Larsson A, Welander U, Friethiof L. The effect of hyaluronan on bone and soft tissue and immune response in wound healing. *J Periodontol* 2001;72:1192-1200. doi: 10.1902/jop.2000.72.9.1192