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ORIGINAL RESEARCH

Effect of 0.8% Hyaluronic Acid in Conventional Treatment of Moderate to Severe Chronic Periodontitis

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ABSTRACT

Objective: The aim of this study is to evaluate the effect of the subgingival application of 0.8% hyaluronic acid (HA) gel (GENGIGEL®) as an adjunct to scaling and root planing (SRP) on clinical parameters and expression of human beta defensin-2 (hBD-2) in patients with moderate to severe chronic periodontitis.

Materials and methods: In this randomized, split mouth design study, 24 participants with moderate to severe chronic periodontitis were evaluated after full mouth SRP. In the test sites 1 mL of 0.8% hyaluronan gel was applied subgingivally after SRP at baseline and 1 week post therapy. Plaque index (PI), gingival index (GI), papillary bleeding index (PBI), periodontal probing depth (PPD), and clinical attachment loss (CAL) were recorded and gingival crevicular fluid (GCF) samples were collected at baseline, after 6 and 12 weeks. Expression of human beta defensin-2 (hBD-2) was analyzed by enzyme-linked immunosorbent assay.

Results: At baseline, there were no statistical differences between test and control sites in all clinical parameters and hBD-2 expression. An improvement of PI, GI, PBI, PPD, and CAL was observed at 6 and 12 weeks ($p < 0.05$) in both groups. Clinically, it was noticed that all indices except CAL had more statistically significant reduction in test sites than control sites at 6 and 12 weeks. The hBD-2 levels were significantly higher in the test sites than in the control sites at 6 and 12 weeks.

Conclusion: The local application of 0.8% hyaluronan gel with SRP have a positive effect on periodontal health of moderate to severe chronic periodontitis patients after 6 and 12 weeks.

Clinical significance: Subgingival application of 0.8% HA gel following SRP has shown anti-inflammatory effect and has a beneficial effect on clinical parameters in moderate to severe chronic periodontitis patients.

Keywords: Adjunctive therapy, Chronic periodontitis, Hyaluronic acid, Inflammation, Scaling and root planing, Subgingival application.

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INTRODUCTION

Chronic periodontitis is an inflammatory condition of the periodontium, which induces an immune response and results in loss of supporting tissues of the teeth.[1] It may affect the general health.[2] Periodontitis is one of the biggest reasons for tooth extraction. In the population, it can be seen in children and the elderly. Sometimes a combination of mechanical and chemical treatment provides good recovery.[3] However, the final success rate of the treatment depends on the status and maintenance of oral hygiene.[4]

Primary etiology for this disease is bacterial plaque on the tooth surface that leads to marginal tissue inflammation, known as gingivitis which is a reversible condition that may develop to periodontitis when not treated. The progression rate of chronic periodontitis is slow; however, it commonly affects the adult population as compared with other age groups.[5] It is not caused by single microorganism as other diseases; it is rather caused by large number of bacteria that still are not all identifiable residing in the subgingival sulcus as dental plaque.[6]

First stage is to develop a well-designed and appropriate treatment plan that is accurate in diagnosis and that when implemented accordingly resolves the patient's periodontal infection.[7] Periodontal diagnosis is determined after careful evaluation of the clinical signs and symptoms and gathering of medical and dental histories, in addition to the findings from oral examination, radiographs, blood tests, and biopsies.[8] Clinical attachment level is the most valid method in assessing the treatment outcome.[9] The effective methods of treating periodontitis are SRP.[6] Although pocket debridement destroys components of the subgingival microflora related to periodontitis, periodontal pathogens may return to baseline levels within days or months.[10,11] The pathogens return to levels of pretreatment generally in approximately 9 to 11 weeks, but it may differ among different individuals.[12] Effective therapy of SRP reduces the gingival inflammation and PDs among the chronic periodontal disease patients.[13] Gontiya and Galgali[14] indicated that SRP is technically demanding and is not always efficient in eradicating all periodontal pathogens and in lessening inflammation. Therefore, subgingival application of chemotherapeutic agents may be used as an adjunct to nonsurgical therapy.

A study conducted by Remien[15] revealed that the local delivery antimicrobial agents (LDAs) are effective in treating periodontitis. The benefits of LDAs are direct application to the site of active disease, improvement of patient compliance, and avoidance of systemic complications.[16] The transient benefits of utilizing LDAs tend to offer some merit among the patients, who are not able to approach for surgical treatments modalities.[15] Hyaluronic acid, which is an extracellular constituent of the connective tissue, was recently introduced as a local chemotherapeutic agent and has exhibited numerous clinical therapeutic properties.[14]

Hyaluronan, also known as HA, has been identified in all periodontal tissues, such as gingiva and periodontal ligament.[17] Hyaluronic acid has been studied as a marker of inflammation and is a significant factor in the growth, development, and repair of tissues.[18] Hyaluronic acid has essential biological and physiological functions. Beneficial wound healing properties on the effects of exogenous hyaluronan were confirmed in several studies.[19] Hyaluronic acid can influence and enhance tissue regenerative procedure, owing to its capability to preserve large amounts of water.[20] It was recommended that wound healing will be accelerated in the bone matrix due to stimulation of angiogenesis by HA.[21] High molecular weight hyaluronan has shown to stimulate osteoinduction during wound healing.[22] It is directly or indirectly associated with various cell functions, such as recognition, locomotion, and cell proliferation, which will provide tissue healing properties.[19]

In the same context, HA is a fundamental element of periodontal ligament matrix and performs essential roles in cell migration, adhesion, and differentiation. The large size and negative charge of HA facilitates the absorption of large amount of hydration water to exert significant pressure on the surrounding tissue. This results in the production of enlarged extracellular space.[23]

Antimicrobial peptides (AMPs) are expressed in response to bacterial toxins or oral bacteria, making them suitable biomarkers for the diagnosis of periodontal disease.[24,25] Human beta-defensins are small cationic AMPs produced mainly by epithelial cells[26] and secreted in biological fluids, including urine, bronchial fluids, nasal secretions, saliva, and GCF. There is limited information on the association between periodontal disease status and salivary AMP concentrations.[27]

Only few studies have examined the effect of hyaluronan on gingival health. Less is known about the effect of subgingival administration of hyaluronan on periodontitis.[28] Therefore, the aim of the study is to assess the impact of subgingival application of 0.8% HA gel among moderate to severe chronic periodontitis patients as an adjunct to SRP as indicated by expression of hBD-2 in GCF.

The research question: Does the application of 0.8% HA with SRP have a better result as compared to conventional SRP in patients with moderate to severe chronic periodontitis?

MATERIALS AND METHODS

The study has incorporated a randomized spilt mouth design by recruiting 24 patients, among which 14 were females and 10 were males (age ranging from 24 to 57 years). These patients were diagnosed with moderate to severe chronic periodontitis as classified by the 1999 International Workshop for classification of Periodontal Diseases and Conditions of the American Academy of Periodontology. The study was clinically registered at ClinicalTrials.gov and was approved by Institutional Review Board of the Riyadh Elm University, with approval number RC/IRB/2016/478. Patients were informed about the study and their consent was obtained in a prescribed form to take part prior to the commencement of the study.

Inclusion and Exclusion Criteria

Inclusion criteria are those systemically healthy patients who had at least 20 teeth with moderate to severe chronic periodontitis with a probing depth of more than 5 mm in at least four sites in different quadrants. Patients who are caries free, who exhibited no known allergies,

Fig. 1: Gengigel syringes containing 0.8% hyaluronic acid

and those who had the ability to attend the hospital at regular intervals were included (Fig. 1). However, the patients who were pregnant, hypertensive, smokers, nursing, suffered from chronic disease (diabetes mellitus), and exhibited allergies were not included in this study. The patients taking antibiotics or receiving periodontal therapy for the last 6 months were excluded from the study. The included patients were free from dental caries, and were not undergoing any orthodontic treatment or using any supplement and mouthwashes (Fig. 2).

Methods

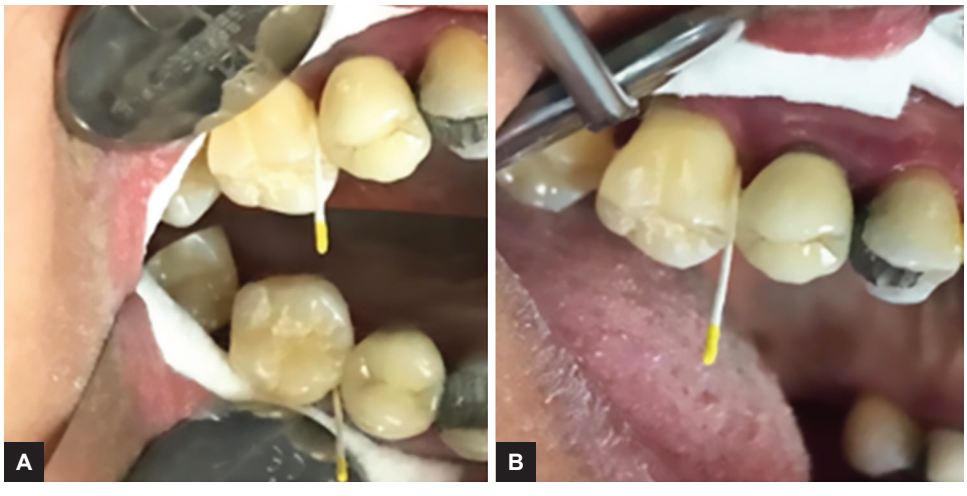
The recruited patients were divided into two quadrants through the application of spilt-mouth design. One of the quadrant was specified as control quadrant that was treated with SRP alone; however, the other quadrant (Test site) was treated with 0.8% HA along with SRP. All patients received SRP with EMS Mini Piezon ultrasonic scalers and Nordent hand scalers and Gracey curettes at baseline.

Clinical Examination

Clinical examination was performed using mouth mirror and the manual University of North Carolina probe (UNC-15) and rounded off to the nearest millimeters. During the follow-up visits of 1st week, patients received oral hygiene reinforcement; moreover, 0.8% HA was subgingivally administered at test site. All the patients were strictly directed to follow the oral hygiene program that included twice brushing using Curaprox soft toothbrush and Colgate pro-relief toothpaste and interdental flossing using Oral-B Glide dental floss twice a day. The distance from gingival margin to base of sulcus, and from the cementoenamel junction to base of the sulcus was measured at six sites for each tooth that included mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual sites at all teeth with UNC-15 probe. The PI with four gingival areas of the tooth (buccal, lingual/palatal, mesial, and distal) given a score from 0 to 3 were added and divided by four. The PI for the individual was obtained by adding the indices for the teeth and divided by the number of teeth examined. The four areas of the tooth (buccal, lingual/palatal, mesial, and distal) were scored by the sum and divided by four to get the GI for the tooth. The GI was obtained by adding the indices of the teeth and divided by the number of teeth examined. A UNC-15 probe was inserted to evaluate the papillary BI at the mesial aspect base of the papilla and then it shifted coronally to the papilla tip. It was repeated on the distal aspect of the papilla of the same tooth.

Collecting Samples

A total of 48 sites were selected from 24 patients and were randomly divided into control and experimental sites. At baseline, two GCF samples were collected for every patient at sixth and twelfth week after the periodontal therapy. The samples were pooled from two periodontal sites with a minimum PD of 5 mm within two different quadrants. Figure 2 has shown the technique of collecting



Figs 2A and B: Technique of collecting samples

samples. Before extracting the sample, the area was isolated with cotton rolls and cleaned with sterile cotton pellets supragingivally. The sterile absorbent paper was held in place for 30 seconds and then transferred to saline water with vigorous mixing. The extracted samples were stored at −70°C in the deep freezer (Labtech, Tamil Nadu, India) to identify hBD-2. The samples were centrifuged for 4 minutes at a speed of 1,600 rpm. The quantification of hBD-2 in GCF was done by an enzyme-linked immunosorbent assay (Peprotech, Rocky Hill, New Jersey, USA), according to the manufacturer's instructions at room temperature.

Statistical Analysis

All statistical analysis was done using Statistical Package for the Social Sciences version 21 (IBM Corp, Armonk NY, USA). Shapiro test was used to test the normality of data. Data were not normally distributed in PI, GI, PBI, CAL, and hBD-2. The PD data were normally distributed in each group. Nonparametric test was used to test the effect of treatment across all phases.

Mann–Whitney U test was used to test the differences of PI, GI, PBI, CAL, and hBD-2 in the baseline and 6 and 12 weeks between test and control groups and compare the median of clinical attachment loss between groups. Wilcoxon signed rank test was used to test the median of score of PI, GI, PBI, CAL, and hBD-2 and the effect before and after treatment for both sites (control and test). Paired sample t test was used to test the levels of PD before and after the treatment during all phases. Independent t test was used to test the difference of mean PD before and after each sites.

RESULTS

Out of the 24 patients, 2 female patients failed to revisit after 12 weeks. The results and comparison of the PI, GI, and BI before and after treatment are shown in Table 1.

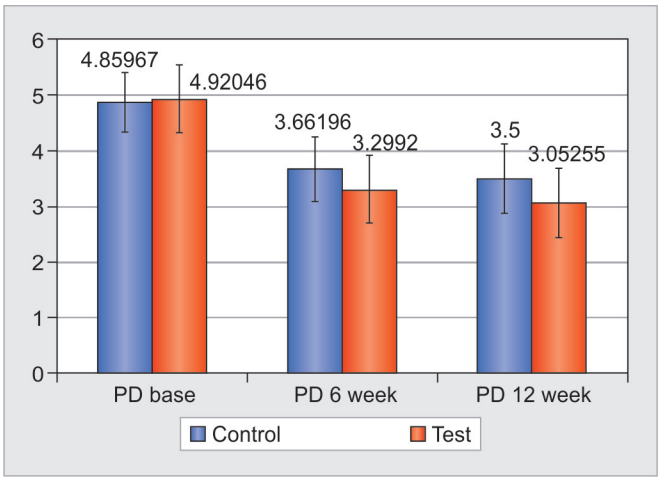
In the control site, the results of PPD showed that there was statistically significant difference between baseline and 12 weeks (t = 14.83, p-value = 0.000) and between baseline and 6 weeks (t = 17.30, p-value = 0.000) (Graph 1). There were also statistically significant differences in median of CAL between baseline and 12 weeks (Z = −4.108, p-value = 0.000) and between baseline and 6 weeks (Z = −4.28, p-value = 0.000) (Graph 2).

In the test site, the PPD levels showed that there was statistically significant difference between baseline and 12 weeks (t = 13.94, p-value = 0.000) and between baseline

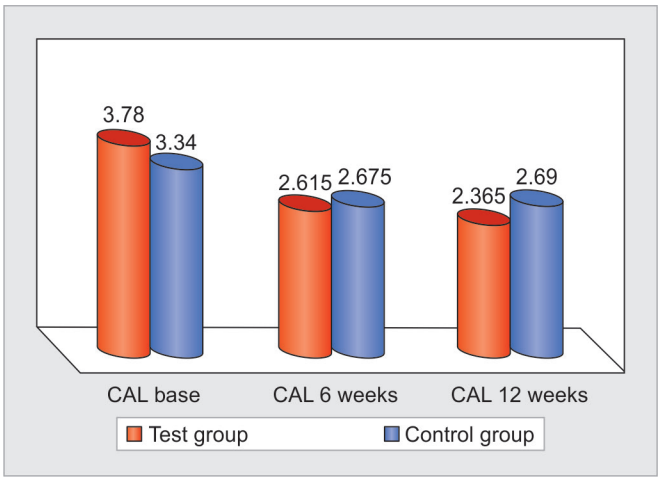
Table 1: Comparison of PI, GI, and BI before and after the treatment

		Control site		*Test site*		
		Test statistics	*p-value*	*Test statistics*	*p-value*	*Result*
PI	Baseline–6 weeks	−4.296	0.000	−4.295	0.000*	HS
	Baseline–12 weeks	−4.118	0.000	−4.118	0.000*	HS
	6–12 weeks	−1.218	0.223	−0.736	0.461	NS**
GI	Baseline–6 weeks	−4.298	0.000	−4.299	0.000*	HS
	Baseline–12 weeks	−4.119	0.000	−4.111	0.000*	HS
	6–12 weeks	−0.181	0.856	−0.260	0.795	NS
BI	Baseline–6 weeks	−4.24	0.000	−4.308	0.000	HS
	Baseline–12 weeks	−4.035	0.000	−4.043	0.000	HS
	6–12 weeks	0	>0.05	−0.200	0.841	NS

HS: Highly significant; NS: Nonsignificant



Graph 1: Descriptive statistics of PD of control and test sites



Graph 2: Median values of CAL

Table 2: Comparison between control and experimental sites

	Ranks					
	Site	*n*	*Mean rank*	*Sum of ranks*	*Mann–Whitney U test*	*p-value*
Baseline—hBD-2	Control	24	21.42	514.00	214	0.127
	Test	24	27.58	662.00		NS
	Total	48				
6 weeks—hBD-2	Control	24	19.42	466.00	166	0.012
	Test	24	29.58	710.00		HS
	Total	48				
12 weeks—hBD-2	Control	22	16.23	357.00	204	0.001
	Test	22	28.77	633.00		HS
	Total	44				
Baseline—plaque	Control	24	22.63	543.00	243	0.345
	Test	24	26.38	633.00		NS
	Total	48				
6 weeks—plaque	Control	24	22.94	550.50	250	0.428
	Test	24	26.06	625.50		NS
	Total	48				
12 weeks—plaque	Control	22	21.86	481.00	228	0.737
	Test	22	23.14	509.00		NS
	Total	44				
Baseline—gingival	Control	24	23.71	569.00	269	0.682
	Test	24	25.29	607.00		NS
	Total	48				
6 weeks—gingival	Control	24	32.50	780.00	96	0.000
	Test	24	16.50	396.00		HS
	Total	48				
12 weeks—gingival	Control	22	29.23	643.00	94	0.000
	Test	22	15.77	347.00		HS
	Total	44				
Baseline—bleeding	Control	24	24.52	588.50	287	0.992
	Test	24	24.48	587.50		NS
	Total	48				
6 weeks—bleeding	Control	24	30.58	734.00	142	0.002
	Test	24	18.42	442.00		HS
	Total	48				
12 weeks—bleeding	Control	22	28.55	628.00	109	0.001
	Test	22	16.45	362.00		HS
	Total	44				

HS: Highly significant; NS: Nonsignificant

and 6 weeks (t = 13.87, p-value = 0.000) in the test site (Graph 1). There were also statistically significant differences in median of CAL between baseline and 12 weeks (Z = −4.108, p-value = 0.000) and between baseline and 6 weeks (Z = −4.28, p-value = 0.000) (Graph 2).

Comparison between Control and Test Sites

The results showed that there were no significant differences at baseline for all clinical and hBD-2 expression between test and control sites (p-valu > 0.05) (Table 2). There were statistically significant differences between test and control sites at 12 weeks in GI and BI with p-values 0.000 and 0.001 respectively. For PI, there was no significant differences between test and control site in both 6 and 12 weeks with p-values > 0.05 (Table 2).

The control site had higher levels of BI than test site in 6 and 12 weeks with mean ranks 30.58 and 28.55 respectively. These values were higher than the mean ranks of the test site. There was highly statistically significant difference in mean of PPD between control and test site in 6 week (t = 2.11, p-value = 0.041) and 12 week phases (t = 2.4, p-value = 0.02) (Table 2).

The PPD in 6 weeks of the test site had lower significant level of PD as compared with control site by 0.362 with 95% confidence interval (CI) (0.0159, 0.7082), while in 12 weeks, the test site had lower significant level of PD than control site by 0.4475 with 95% CI (0.0715, 0.8234). The result of the comparison of the CAL between sites showed that there was no significant difference between control and test site in median of CAL in all durations

(baseline, 6 weeks, and 12 weeks) with p-values 0.201, 0.543, and 0.116 respectively (Table 2).

In the test site hBD-2, there was statistically significant difference in hBD-2 in 6 weeks and baseline, between baseline and 12 weeks with p-values = 0 for both. The comparison of hBD-2 between test and control sites showed that there were no significant differences in baseline for hBD-2 between test and control sites (p-value > 0.05). Moreover, there were statistically significant differences between the test and control sites in 6 weeks in hBD-2 with p-values 0.012. The test site had higher levels of hBD-2 than control site in 6 and 12 weeks with mean ranks equal to 29.85 and 28.77. These rankings were higher than mean ranks of control site (Table 2).

DISCUSSION

The study has incorporated spilt mouth design to investigate the efficacy of subgingival application of 0.8% HA along with SRP in moderate to severe chronic periodontitis patient. At baseline, all clinical parameters were comparable with no statistical difference between test and control sites. It tends to eliminate variability between the two sites. The result showed a statistically significant reduction of PI, GI, BI, PPD, and CAL that was observed in 6 and 12 weeks in both the treatment groups.

A study conducted by Smiley et al[29] revealed that using HA does not reduce the need of SRP as a prime therapeutic measure and its clinical efficacy is well documented. It was noticed that all indices had more reduction in test sites than control sites, when comparing GI, BI, PD, and CAL between test and control sites. The reduction in the above-mentioned indices indicated that the resolution of inflammation in test sites was a direct effect of HA application.

The initiation and progression of disease is interrupted by HA as a result of antimicrobial and anti-inflammatory capacities.[23] The clinical parameters result of the present study are similar to the studies conducted by Eick et al,[30] Rajan et al,[13] and Polepalle et al.[28] All these studies demonstrated additional effects of HA in comparison to SRP alone. However, other studies did not find that HA had a beneficial effect on periodontal health. Xu et al[31] investigated the effect of a subgingival administrated 0.2% HA gel in combination with SRP, and found no clinical and microbial improvement between HA gel's adjunctive use compared with only SRP in relative to bleeding on probing and PDD. Differences in HA gel concentration, treatment protocols, disease severity, observation intervals, and measurements may variably explain the controversy in result. In addition, professionally administered HA had excluded the prospect of patient compliance affecting the results.

Many previous investigations have shown that hBD-2 expression is structured by numerous inflammatory mediators that are mainly correlated with inflammation by Vianna et al,[32] Diamond et al,[33] and Darveau et al.[34] In the present study, hBD-2 was detected both before treatment (diseased sites) and after treatment (healthy sites). Lu et al[35] observed that hBD-2 was found in gingival epithelia from periodontal healthy subjects and unresolved chronic periodontitis patients. There was no significant difference in the expression of hBD-2 between control and test sites at the baseline. The level of hBD-2 in test site was significantly higher than in the control sites at 6 weeks. Also at 12 weeks, the concentration was higher in test sites than control with high statistically significant and both were slightly less than 6 weeks readings. Another study conducted by Jaradat et al[36] revealed significant association between severity of periodontitis and decreased genomic copy numbers of hBD-2. A negative correlation between total bacterial count and hBD-2 was revealed by Wang et al.[37]

Conversely, the studies conducted by Pereira et al,[38] Liu et al,[39] and Yong et al[40] were inconsistent with the present study. This inconsistency was due to the differences in the study designs, complexity of periodontal diseases, differences in case definition, and other environmental factors, such as smoking have a strong modulating effect on hBD-2 expression in gingival tissue.[41] Based on the study results, HA's anti-inflammatory effects proved beneficial in moderate to severe chronic periodontitis therapy. The administration of HA has been successful in the wound healing and tissue repair process in many medical disciplines and in the periodontal sites.[42]

CONCLUSION

Within the limitations of the present study, it could be concluded that the local application of 0.8% HA gel with SRP has a positive effect on periodontal health of patients with moderate to severe chronic periodontitis after 6 and 12 weeks. Hyaluronic acid showed positive effects in influencing local inflammation. The expression of hBD-2 is more pronounced after subgingival application of 0.8% HA with SRP proving that hBD-2 played a crucial role in inflammatory process of periodontal diseases. Future study is needed to investigate variations in administration protocols of HA to optimize the results, work on a larger sample size for data reliability, and further understand the exact role of hBD-2 in periodontitis diseases. Subgingival application of 0.8% HA is recommended to use as an adjunct to SRP in the treatment of patients affected with chronic periodontitis.

CLINICAL SIGNIFICANCE

Subgingival application of 0.8% HA gel following SRP has shown anti-inflammatory effect and has a beneficial

effect on clinical periodontal parameter in moderate to severe chronic periodontitis patients.

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REFERENCES

1. Haffajee AD, Socransky SS. Microbiological and etiologic agents of destructive periodontal diseases. Periodontol 2000 1994 Jun;5:78-111.
2. Williams RC, Barnett AH, Claffey N, Davis M, Gadsby R, Kellett M, Lip GY, Thackray S. The potential impact of periodontal disease on general health: a consensus view. Curr Med Res Opin 2008 Jun;24(6):1635-1643.
3. Slots J, Ting M. Systemic antibiotics in the treatment of periodontal disease. Periodontol 2000 2002 Jan;28(1):106-176.
4. Sukumar S, Drízhal I. Hyaluronic acid and periodontitis. Acta Medica (Hradec Kralove) 2007;50(4):225-228.
5. Shaddox LM, Walker CB. Treating chronic periodontitis: current status, challenges, and future directions. Clin Cosmet Investig Dent 2010 Aug;2:79-91.
6. Apatzidou DA, Kinane DF. Nonsurgical mechanical treatment strategies for periodontal disease. Dent Clin 2010 Jan;54(1):1-12.
7. Armitage GC. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000 2004 Feb;34(1):9-21.
8. Highfield J. Diagnosis and classification of periodontal disease. Aust Dent J 2009 Sep;54(Suppl 1):S11-S26.
9. Deepa R, Parkash S. Accuracy of probing attachment levels using a new computerized cemento enamel junction probe. J Indian Soc Periodontol 2012 Jan;16(1):74-79.
10. Slots J. The search for effective, safe and affordable periodontal therapy. Periodontol 2000 2002 Jan;28(1):9-11.
11. van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. J Clin Periodontol 2005 Aug;32(8):893-898.
12. Ranney RR. Diagnosis of periodontal diseases. Adv Dent Res 1991 Dec;5(1):21-36.
13. Rajan P, Baramappa R, Rao NM, Pavaluri AK, Rahaman SM. Hyaluronic acid as an adjunct to scaling and root planing in chronic periodontitis. A randomized clinical trial. J Clin Diagn Res 2014 Dec;8(12):ZC11-ZC14.
14. Gontiya G, Galgali SR. Effect of hyaluronan on periodontitis: a clinical and histological study. J Indian Soc Periodontol 2012 Apr;16(2):184-192.
15. Remien J. Thoughtful use of local delivery antimicrobial agents in periodontitis. Tulsa (OK): Dentistry iQ; 2015.
16. Newman MG, Kornman KS, Doherty FM. A 6-month multicenter evaluation of adjunctive tetracycline fiber therapy used in conjunction with scaling and root planing in maintenance patients: clinical results. J Periodontol 1994 Jul;65(7):685-691.
17. Embery G, Waddington RJ, Hall RC, Last KS. Connective tissue elements as diagnostic aids in periodontology. Periodontol 2000 2000 Oct;24:193-214.
18. Jentsch H, Pomowski R, Kundt G, Gocke R. Treatment of gingivitis with hyalurona. J Clin Periodontol 2003 Feb;30(2):159-164.
19. Chen WY, Abatangelo G. Functions of hyaluronan in wound repair. Wound Repair Regen 1999 Mar-Apr;7(2):79-89.
20. Nakamura M, Hikida M, Nakano T, Ito S, Hamano T, Kinoshita S. Characterization of water retentive properties of hyaluronan. Cornea 1993 Sep;12(5):433-436.
21. West DC, Kumar S. Hyaluronan and angiogenesis. In: Evered D, Whelan J, editors. The biology of hyaluronan: Ciba foundation Symposium, 143. Chichester: John Wiley & Sons; 1989. pp. 187-207.
22. Sasaki T, Watanabe C. Stimulation of osteoinduction in bone wound healing by high-molecular hyaluronic acid. Bone 1995 Jan;16(1):9-15.
23. Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. Bacteriostatic effects of hyaluronic acid. J Periodontol 1999 Apr;70(4):370-374.
24. Gorr SU. Antimicrobial peptides of the oral cavity. Periodontol 2000 2009 Oct;51(1):152-180.
25. Gorr SU, Abdolhosseini M. Antimicrobial peptides and periodontal disease. J Clin Periodontol 2011 Mar;38(Suppl 11):126-141.
26. Dale BA, Krisanaprakornkit S. Defensin antimicrobial peptides in the oral cavity. J Oral Pathol Med 2001 Jul;30(6):321-327.
27. Güncü GN, Yilmaz D, Könönen E, Gürsoy UK. Salivary antimicrobial peptides in early detection of periodontitis. Front Cell Infect Microbiol 2015 Dec;5:99.
28. 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 Soc Periodontol 2015 Jan-Feb;19(1):37-42.
29. Smiley CJ, Tracy SL, Abt E, Michalowicz BS, John MT, Gunsolley J, Cobb CM, Rossmann J, Harrel SK, Forrest JL, et al. Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. J Am Dent Assoc 2015;146(7):508-524.
30. Eick S, Renatus 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 Jul;84(7):941-949.
31. Xu Y, Höfling K, Fimmers R, Frentzen M, Jervøe-Storm PM. Clinical and microbiological effects of topical subgingival application of hyaluronic acid gel adjunctive to scaling and root planing in the treatment of chronic periodontitis. J Periodontol 2004 Aug;75(8):1114-1118.
32. Vianna ME, Conrads G, Gomes BP, Horz HP. T-RFLP-based mcrA gene analysis of methanogenic archaea in association with oral infections and evidence of a novel Methanobrevibacter phylotype. Oral Microbiol Immunol 2009 Oct;24(5):417-422.
33. Diamond DL, Kimball JR, Krisanaprakornkit S, Ganz T, Dale BA. Detection of β-defensins secreted by human oral epithelial cells. J Immunol Methods 2001 Oct;256(1-2):65-76.
34. Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. Periodontol 2000 1997 Jun;14(1):12-32.
35. Lu Q, Jin L, Darveau RP, Samaranayake LP. Expression of human β-defensins-1 and-2 peptides in unresolved chronic periodontitis. J Periodontal Res 2004 Aug;39(4):221-227.
36. Jaradat SW, Hoder-Przyrembel C, Cubillos S, Krieg N, Lehmann K, Piehler S, Sigusch BW, Norgauer J. Beta-defensin-2 genomic copy number variation and chronic periodontitis. J Dent Res 2013 Nov;92(11):1035-1040.

37. Wang P, Duan D, Zhou X, Li X, Yang J, Deng M, Xu Y. Relationship between expression of human gingival beta-defensins and levels of periodontopathogens in subgingival plaque. J Periodontal Res 2015 Feb;50(1):113-122.
38. Pereira AL, Franco GC, Cortelli SC, Aquino DR, Costa FO, Raslan SA, Cortelli JR. Influence of periodontal status and periodontopathogens on levels of oral human β-defensin-2 in saliva. J Periodontol 2013 Oct;84(10):1445-1453.
39. Liu J, Chen J, Du X, Hu L, Chen L. The expression of hBDs in the gingival tissue and keratinocytes from healthy subjects and periodontitis patients. Arch Oral Biol 2014 Feb;59(2): 193-198.
40. Yong X, Chen Y, Tao R, Zeng Q, Liu Z, Jiang L, Ye L, Lin X. Periodontopathogens and human β-defensin-2 expression in gingival crevicular fluid from patients with periodontal disease in Guangxi, China. J Periodontal Res 2015 Jun;50(3): 403-410.
41. Wolgin M, Liodakis S, Pries AR, Zakrzewicz A, Kielbassa AM. HBD-1 and hBD-2 expression in HaCaT keratinocytes stimulated with nicotine. Arch Oral Biol 2012 Jun;57(6):814-819.
42. Casale M, Moffa A, Vella P, Sabatino L, Capuano F, Salvinelli B, Lopez MA, Carinci F, Salvinelli F. Hyaluronic acid: perspectives in dentistry. A systematic review. Int J Immunopathol Pharmacol 2016 Dec;29(4):572-582.