

Hyaluronon As An Adjunct To Coronally Advanced Flap For The Treatment Of Gingival Recession Defects

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Abstracts: Background: The present clinical trial was undertaken to evaluate the effectiveness of hyaluronon(HA) as an adjunct in root coverage procedures. Methodology: 20 patients with millers class I and II gingival recession in a minimum of two sites were treated with HA gel combined with coronally advanced flap on the test site and sub epithelial connective tissue graft with CAF on the control sites. Results: The percentage of root coverage achieved showed no significant difference between the test and control sites at 9 months post therapy. Conclusion: HA increased the probability of achieving root coverage in Miller's class I and II recession. [Rajan P NJIRM 2015; 6(2):94-100]

Key Words: Gingival recession, Root coverage, Hyaluronic acid, Regeneration.

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Introduction: Diverse epidemiological surveys have revealed that gingival recession may affect most of the adult population. The prevalence of gingival recession ranges from 20% to 100% in adults.^{1,2} It is frequently associated with esthetic concerns, hypersensitivity, pain, difficult oral hygiene, root caries and attachment loss.^{3,4} International literature has thoroughly documented that Miller's class I and II recessions can be predictably covered by various periodontal plastic surgical procedures, including pedicle flaps and guided tissue regeneration.^{5,6}

Cochrane review by Chambrone et al have concluded that connective tissue graft (CTG) with coronally advanced flap (CAF) provide a mean root coverage of (84.0% to 95.1%).⁷ Various adjunctive agents have been applied to promote healing and further enhance clinical outcomes which include enamel matrix proteins, recombinant human genome factors and platelet rich plasma and platelet rich fibrin.^{8,9,10,11.}

Hyaluronan [HA] formerly known as hyaluronic acid is a polysaccharide of the extracellular matrix (ECM), has a multitask role, having many structural, rheological, physiological and biological functions in the body.¹² Hyaluronic acid has a high daily turnover. The hyaluronic molecules rise during early wound healing and bind to the fibrin in the clot, making the clot swell and become more porous facilitating cell migration into the matrix.¹³ As the HA becomes less viscous due to the locally

produced hyaluronidase, it not only facilitates motility and proliferation of macrophages and fibroblasts but also collagen deposition.¹⁴ The low molecular weight HA produced due to degradation by hyaluronidase in turn promotes local angiogenesis.¹⁵ HA possesses a viscosity that seems to delay the penetration of viruses and bacteria.¹⁶ High molecular weight hyaluronon stimulates osteoinduction during wound healing.¹⁷ HA is a recent addition to the local chemotherapeutic agent which has shown a number of clinical therapeutic properties.

The primary objective of the study was to evaluate the potential of HA as an adjunct to root coverage. The secondary goal was to compare the effectiveness in terms of root coverage obtained with the subepithelial connective tissue graft (SCTG). The third was to evaluate its superiority in terms of post operative complications associated with connective tissue harvesting.

Materials and methods: This was a randomised control clinical trial on treatment of gingival recession where the application of HA gel in combination with coronally advanced flap (CAF) was tried to know if HA has any potential as a root coverage material and the same was compared with SCTG with CAF.

Study population: Patients with good general health, with the age ranging between 26-42 years, including 13 females and 7 males with Miller's class

I and II gingival recession defects were selected for this split mouth study done at 40 sites from 20 patients. The following entry criteria had to be met by the patients:

- Presence of atleast two Miller's class I or class II recession, limited to upper or lower central incisors, lateral incisors, canines and 1st premolars,
 - Evidence of sufficient interdental bone radiographically.
 - Absence of pulling frenum in the keratinised tissue.
 - No history of periodontal or mucogingival surgery in the past 6 months.
- Patients with systemic disease and conditions, history of smoking or current smokers, patients with root surface restorations, patients with history of drug allergy and those who refused to sign informed consent were excluded from the study.

Pre-treatment modifications of oral hygiene habits

Following the screening examination all patients received oral hygiene instructions to modify the habits related to the etiology of the recession atleast one month before surgery.

The study protocol was reviewed and approved by the institutional review board [IRB] of St. Joseph's dental college Eluru, A.P. India and an informed consent was obtained from all patients who entered into the study. In obtaining the informed consent and in the conduct of the study, the principle outlined in the declaration of Helsinki in the year 1975, as revised in 2000 on experimentation involving human subjects was adhered to. Full mouth prophylaxis was done in all the subjects before the scheduled surgical procedure. Treatment of the recession defects were scheduled only after the clinician was satisfied with the patient's plaque control.

A single examiner performed the clinical measurements at baseline and at 1,3 and 9 months follow up. The following clinical measurements were recorded at the experimental teeth at baseline and at 1,3 and 9 months follow up using UNC-15 periodontal probe.

Recession depth (RD), recession width (RW), width of keratinised gingiva (KG), probing depth (PD), clinical attachment loss (CAL), gingival index (GI) and plaque index (PI) were the other parameters recorded. Acrylic stents were fabricated and a reference point was carved on the stent to allow a reproducible periodontal probe positioning to record the measurements pre and post surgically.

The selected sites were randomly assigned to the test(HA+CAF) or control(SCTG+HA) group. In the test site intra-sulcular incision was made using no 15 blade on the labial aspect of the involved tooth after adequate anesthesia, this incision was extended horizontally to the adjacent papilla. Oblique releasing incisions were placed at the mesial and distal ends of the horizontal incisions[Fig-1]. Full thickness flap was raised extending upto the mucogingival junction after which partial thickness dissection was carried out [Fig-2]. The papilla adjacent to the involved teeth were de-epithelized. Using a sterile instrument hyaluronon gel was applied onto the root surface [Fig-3]. The flap was coronally advanced and was adapted to cover the CEJ and sutured [Fig-4]. The mesial and distal extension of the flaps were secured by placing sutures in the interdental areas as described by Allen and Miller.¹⁸ Suturing of the oblique releasing incisions were performed using 4-0 non resorbable sutures.

FIG 1: Incision on the test site.



FIG 2: Flap reflection on the test site



FIG 3: Hyaluronon gel applied on the denuded root surface.



FIG 4: Flap coronally advanced and sutured on the test site.



In the control group after the recipient site was prepared, SCTG was harvested by the technique described by Edel.¹⁹ The harvested SCTG was positioned on the denuded root surface and stabilised using resorbable sutures. The flaps were coronally advanced and sutured covering the graft

completely. The donor and the recipient sites were covered with Coe-pak. Patients were advised cold compression externally to minimise post operative swelling. All patients were advised analgesics and antibiotics.

POST SURGICAL CARE: Patients were instructed not to brush the teeth in the treated area but to use Chlorhexidine solution (0.2%) to rinse twice daily for 1 minute. 10-14 days following surgical treatment sutures were removed. Plaque control was maintained by chlorhexidine rinsing for an additional two week period. In the following visit patients were instructed in mechanical tooth cleaning of the treated area using soft brush.

Statistical Methods: Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group. Significance is assessed at 5 % level of significance.

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0 was used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Results: Mean GI and PI reduced significantly when baseline values were compared to 1, 3 and 9 months post therapy (p<0.001). [Table 1 and 2].

Table 1: Gingival index: Evaluation in months.

Gingival index	Min-Max	Mean ± SD	P value
Baseline	0.30-0.50	0.42 ± 0.07	-
1 month	0.21-0.44	0.37 ± 0.06	<0.001**
3 months	0.21-0.44	0.31 ± 0.06	<0.001**
9 months	0.20-0.41	0.26 ± 0.05	<0.001**

Table 2: Plaque index: Evaluation in months.

Plaque index	Min-Max	Mean ± SD	P value
Baseline	0.30-0.50	0.44 ± 0.06	-
1 month	0.30-0.50	0.36 ± 0.06	<0.001**

3 months	0.21-0.50	0.33 ± 0.07	<0.001**
9 months	0.21-0.32	0.25 ± 0.04	<0.001**

RD reduced significantly in both the groups at 9 months post therapy. Intergroup comparison showed a moderately significant difference between the groups at 1 month (P<0.28), however there was no significant difference between the groups at 3 and 9 months. [Table 3]

Table 3: Recession depth: Mean and standard deviation of the test and control groups.

Recession depth in months	Test group	Control group	P value
Baseline	3.65 ± 1.09	3.45 ± 0.94	0.359
1 month	2.05 ± 0.69	2.45 ± 1.05	0.028*
3 months	1.50 ± 0.51	1.80 ± 0.77	0.055+
9 months	1.05 ± 0.76	1.15 ± 0.59	0.629
	P<0.001	P<0.001	

RW also reduced significantly in both the groups 9 months post therapy (p<0.001). Inter group comparison showed no significant difference. [Table 4]

Table 4: Recession width: Mean and standard deviation of the test and control groups.

Recession width in months	Test group	Control group	P value
Baseline	3.40 ± 0.68	3.35 ± 0.81	0.789
1 month	1.95 ± 0.60	2.20 ± 0.77	0.096
3 months	1.55 ± 0.51	1.75 ± 0.44	0.104
9 months	0.90 ± 0.55	1.00 ± 0.65	0.494
	P<0.001	P<0.001	

Both the groups showed a significant difference in width of KG when baseline values were compared to 9 months post therapy (P<0.001). Inter group showed no difference in width of KG at all time periods post evaluation. [Table 5]

Table 5: Width of keratinized gingiva: Mean and standard deviation of test and control groups.

Width of keratinized gingival months	Test group	Control group	P value
Baseline	2.50 ± 0.61	2.00 ± 0.65	0.014*
1 month	2.60 ± 0.60	2.85 ± 0.99	0.367

3 months	2.90 ± 0.55	3.30 ± 0.86	0.134
9 months	3.20 ± 0.95	3.30 ± 0.73	0.694
	P<0.001	P<0.001	

The mean PD reduced significantly in both the groups 9 months post therapy (P<0.002). Intergroup comparison also showed a significant difference at 3months and 9 months (p<0.002 and p<0.001) respectively [Table 6].

Table 6: Probing depth: Mean and standard deviation of test and control groups.

Probing depth in months	Test group	Control group	P value
Baseline	2.79 ± 0.63	2.30 ± 0.47	0.046*
1 month	1.80 ± 0.77	1.75 ± 0.44	0.772
3 months	1.60 ± 0.68	1.10 ± 0.31	0.002**
9 months	1.15 ± 0.75	0.50 ± 0.51	0.001**
	P<0.002	P<0.002	

Both groups showed significant gain in CAL (p<0.001). Inter group comparison also showed significant difference at 3 and 9 months. (p<0.29and p<0.001)[Table 7]

Table 7: Clinical attachment level: Mean and standard deviation of the test and control groups.

Clinical attachment level in months	Test group	Control group	P value
Baseline	6.10 ± 0.79	5.90 ± 0.85	0.214
1 month	3.15 ± 1.14	3.55 ± 0.89	0.134
3 months	2.55 ± 1.10	3.05 ± 0.83	0.029*
9 months	1.90 ± 1.07	1.10 ± 0.91	0.001**
	P<0.001	P<0.001	

The percentage of root coverage accomplished in the control group was 48.07±13.35 and 82.15±14.05 at 3 and 9 months respectively. The percentage of root coverage accomplished in the test group was 58.43±8.80 and 77.84±16.56 at 3 and 9 months respectively. The difference was found to be statistically strongly significant in both the groups(P<0.001). Intergroup comparison showed a moderately significant difference between the groups at 3months (P<0.005). However there was no significant

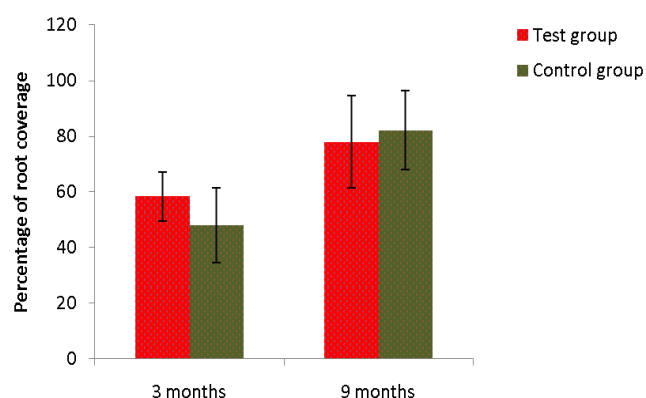
difference between the groups at 9 months. [Table 8][Graph 1].

Table 8: Percentage of root coverage in test and Control groups.

% root cover- age	3 months	9 months	P value
Test group	58.43 ± 8.80	77.84 ± 16.56	<0.001**
Control group	48.07 ± 13.35	82.15 ± 14.05	<0.001**
P value	0.005**	0.177	-

CI: Confidence interval

Graph 1:Percentage of root coverage in test groups and Controls group



Discussion: The study showed a significant reduction in PD, in the test group where hyaluronon was used compared to the control at 3 as well as 9 months post therapy. The findings of our study is in accordance with Sigrun Eric et al who analysed the effect of additional application of HA gel during scaling and root planning(SRP) and reported improved probing depth measurements. The authors concluded that the reason for PD reduction could be due to the antibacterial effect of high molecular weight HA gel on periodontal pathogens.²⁰Johannesen et al from their study concluded that local drug delivery of hyaluronon in conjunction with SRP had a beneficial effect as in terms of probing depth reduction.²¹

King et al speculated but didnot come to a conclusion as to whether HA exerted direct action on cell proliferation and migration or by hydrating the environment it endured moist healing condition.²²Gengigel used in the present study

seems to be a promising method for periodontal regeneration by inducing significant reduction in probing depth and gain in clinical attachment. Significant reduction in probing depth and CAL gain was observed when esterified HA was used in infrabony defects by Bogarde et al.²³ Francesco et al found that HA provides additional benefit in terms of CAL gain, PD reduction and predictability when compared to open flap debridement in infrabony defects.²⁴

Study conducted by Pini prato et al concluded that the more coronal the level of gingival margin after suturing, the greater is the probability of complete root coverage.²⁵ Accordingly in this study the flap was coronally advanced as much as possible and sutured in both the test and control sites. Inter group comparison showed a significant difference in the percentage of root coverage accomplished at the end of 3 months [Table 8]. The increased percentage of root coverage observed in the test group could be attributable to the local angiogenesis promoted by HA.^{13,15.}

Integrity of the wound during the early healing phase rests primarily with the suture that is offered and that the tooth mucogingival flap interface is vulnerable to disruption by mechanical forces for a considerable period of time post surgery.²⁶ Maintenance of wound stability is a key factor in attaining a successful outcome in a regenerative periodontal surgery. Wound stability achieved at the surgical sites could have contributed primarily to the root coverage achieved in both the groups in this study. The percentage of root coverage accomplished at the end of 9 months in the control group was 82.15 ± 14.05 . The percentage of root coverage accomplished at the end of 9 months in the test group was 77.84 ± 16.56 . The predictable root coverage obtained in the test site in the present study could be attributed to the greater extent to the known angiogenic property of HA, its role as an hydrating agent, its property of enhancing motility of cells such as lymphocytes inflammatory and connective tissue cells by binding to the CD44 receptors on them.²⁷ A lack of statistically significant difference between the groups in terms of root coverage at 9 months post therapy does not

mean that there is an equivalence existing between the two techniques.

Within the group variability may be the reason for lack of significance observed which could have been overcome by the larger sample size. The study is ongoing with the larger number of patients to confirm the data.

SCTC has emerged as the most effective treatment modality with respect to high percentage of root coverage.^{5,28.} However it is highly technique sensitive and requires a learning curve. If palatal soft tissue is not thick enough, connective tissue harvesting techniques are not advised primarily because of the risk of flap necrosis and/or inadequacy of the graft due to presence of fatty, glandular tissue instead of connective tissue. The HA gel used in the present study offered several advantages over the autogenous graft including the elimination of a second surgical site for graft harvesting and associated morbidity, post surgical trauma, reduction in operating time, increased acceptance of the procedure by the patients and in satisfying patients esthetic demand. It would be interesting to note whether the beneficial effect of HA accomplished in the present study is predictable for longer time periods (5-10 yrs).

This study failed to demonstrate the difference in pain killer consumption and the surgical chair time between the groups

Conclusion: Though growth factors can take away most of the credit for wound healing, HA appears to have a definite facilitating role as evident by the available literature. The undesirable biocompatibility, ease of handling, extent of healing, absence of complications, proved clinical parameters of HA makes it worthy a material for future clinical research which requires a close collaboration among scientists and clinical dental researchers. To accelerate the chair-side application of HA in periodontal regenerative therapy further studies are expected with more stable and modified form of HA that can be developed by cross linking and coupling reactions.

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