






## Original Research Article

# Coronally advanced flap with and without hyaluronic acid (HYALOSS) for the treatment of gingival recession — a randomized clinical trial

Apoorva Saxena<sup>1,\*</sup> , Prashant Bhusari<sup>2</sup> , Abhilasha Singh<sup>2</sup> , Ravleen Nagi<sup>3</sup> ,  
Shailendra Singh Chaturvedi<sup>2</sup> 

<sup>1</sup> Department of Periodontology, Maitri College of Dentistry and Research Centre, Durg, Chhattisgarh, India

<sup>2</sup> Periodontist and Implantologist, Dr Bhusari's Dental Clinic, Nagpur, Maharashtra, India

<sup>3</sup> Department of Oral Medicine and Radiology, Saveetha Dental College, Chennai, Tamil Nadu, India

(Received: 15 March 2022, accepted: 14 November 2022)

### Keywords:

Coronally advanced flap / gingival recession / hyaluronic acid / root coverage / regeneration

**Abstract – Objectives:** To compare and evaluate the surgical outcome of gingival recession treatment using CAF alone or in conjunction with hyaluronic acid (HA) on Miller's Class I and class II gingival recession defects. **Materials and methods:** After randomisation, the control group (15 patients) received CAF alone and the test group (15 patients) received HA as an adjunct to CAF technique for an isolated gingival recession accompanied by an adequate width of keratinized gingiva on maxillary canines and premolars. All patients were evaluated at 7, 15, 30, 60 and 90 days interval post-operatively. Data obtained was subjected to statistical analysis and  $p$  value  $<0.05$  was considered as statistically significant. **Results:** At 90 days interval statistically highly significant root coverage was obtained with mean gain of 66.1% in control group and 86.6% in test group. Statistically significant reduction in depth of gingival recession and gain in clinical attachment level was found for control and the test groups, and intergroup comparison showed statistically significant differences for root coverage and clinical attachment level between the groups ( $p=0.000$ ), however no significant differences were found for width of attached gingiva, keratinized gingiva and gingival thickness between the groups at 90 days ( $p > 0.05$ ). **Conclusion:** HA has proven to be beneficial in the field of regenerative therapy. Our analysis suggested that HA can be used as a promising adjunct with CAF for root coverage, however further studies are required to imply the results on larger population.

## Introduction

The ultimate goal of dental and periodontal care is to maintain the health, comfort, function, and aesthetics of the natural dentition. Gingival recession is most common periodontal condition characterized by root exposure as a result of displacement of gingival margin apical to cemento enamel junction and has become aesthetic concern due increase in clinical crown height. Incidence of GR has been found to increase with age, with prevalence rate  $>88\%$  in individuals above 65 years of age [1]. Patients are usually asymptomatic but may experience pain due to exposed root surfaces and dentinal hypersensitivity. In addition, it is associated with root caries, cervical abrasion, and greater dental plaque accumulation. Early treatment of gingival recession is necessary to prevent progressive marginal tissue recession that may adversely affect prognosis of concerned tooth [2].

Periodontal plastic surgery procedures such as free gingival grafts, pedicle flaps, (laterally or coronally displaced) and subepithelial connective tissue grafts (SCTG) have long been used to treat gingival recession defects. Reports have shown conflicting success rates of these procedures and long term stability of root coverage is still questionable [3]. Connective tissue (CT) grafts have been demonstrated to be the most predictable surgical procedure for obtaining complete root coverage and is currently considered as the "gold standard" treatment for gingival recession [4,5]. However, CT grafts require a second surgical site to harvest the tissue and therefore are associated with undesirable side effects such as postsurgical pain, discomfort and potential postoperative bleeding. Coronally advanced flap (CAF) is a modality of root coverage surgery that does not involve a palatal donor site and has an advantage over palatal grafts by reducing several postoperative complications [6]. However, CAF does not always result in the regeneration of attachment apparatus such as cementum, periodontal ligament (PDL), and alveolar bone, which are major risk factors in the recurrence of gingival recession. Therefore, modifications to the

\* Correspondence: [apoorvasaxena222@gmail.com](mailto:apoorvasaxena222@gmail.com)

CAF technique are required, with success and predictability rates similar or superior to the CT graft. Several regenerative materials such as guided tissue regeneration membranes (GTR) [7], enamel matrix proteins derivatives (EMD) [8], acellular dermal matrix, alloderm (ADM) [9], platelet rich fibrin (PRF) and living tissue-engineered human fibroblast derived dermal substitute [10], have been used with CAF in the treatment of gingival recession.

Hyaluronic acid (HA) also known as hyaluronan or hyaluronate is relatively a new biomaterial that has got the potential to augment the results of periodontal therapy. It is a linear polysaccharide found in extracellular matrices of skin and is an indispensable component of intact, healthy gingiva. HA has many structural and physiological functions within tissues, including extracellular and cellular interactions, growth factor interaction, regulation of osmotic pressure and tissue lubrication, which helps in the maintenance of the structural and homeostatic integrity of tissues [11,12]. It has many other properties that makes it a potentially ideal molecule for assisting wound healing by inducing early granulation tissue formation, inhibiting inflammation, promoting epithelial turnover and connective tissue angiogenesis [12].

The purpose of the present study was to compare the clinical predictability of coronally advanced flap alone with that of coronally advanced flap+Hyaluronic acid in the treatment of Miller's Class I and II gingival recession. The level of recession coverage, increase in width of attached gingiva, gain in clinical attachment level and thickness of keratinized tissue were assessed for a follow up period of 90 days.

## Materials and methods

### Study design

A single blinded randomized clinical trial was conducted in the Department of Periodontology after approval of the study protocol by the ethics committee of the institute. Informed consent was taken from each patient after explaining the benefits of the treatment.

### Inclusion criteria

Out of 50 initially screened patients, 30 sites in 30 healthy adult patients (25 to 50 years) were included based on following criteria:

- Miller Class I and II gingival recession with  $\geq 2$  mm depth.
- Presence of identifiable CEJ and adequate width of keratinized tissue apical to recession.
- Patients who gave written informed consent to participate in the study.

### Exclusion criteria

- Patients with relevant systemic condition or disease and, on drug therapy.
- Patients with presence of occlusal interferences such as mispositioned or rotated teeth.

- Habitual smokers and tobacco chewers.
- Patients with a history of previous mucogingival or periodontal surgery at an experimental site in last 6 months.
- Presence of gingival recession with caries, restoration or with pulpal pathology.

The sites of gingival recession were chosen on maxillary second premolar, first premolar, canine, lateral incisor and central incisor. GR was treated by CAF alone in control group and combination of CAF+HA (HYALLOSS-meta-advanced medical technology, Italy) in the test group.

The primary endpoint of the study was percentage of root coverage at 90 days. All patients were examined under good illumination by using mouth mirror, William's graduated periodontal probe, tweezer and pellets of cotton. The following clinical parameters (i) gingival recession depth, (ii) width of keratinized gingiva, (iii) probing depth, (iv) width of attached gingiva, (v) gingival thickness, and (vi) gain in clinical attachment level (CAL) were measured by single investigator by using cemento-enamel junction (CEJ) as a fixed point. All the patients received scaling and root planning (SRP) before surgical therapy. A thorough plaque control programme was initiated for each patient 2 weeks prior to surgery including instructions in brushing technique (Modified Stillman) and supervised tooth brushing with soft tooth brush. 1 week after initial preparation, the patients were re-examined in order to ascertain their periodontal status. The degree of gingival inflammation was monitored by Gingival Index [13], and oral hygiene effectiveness was assessed by Plaque Index [14].

### Sample size estimation

The sample size for the study was analysed using the following formula

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 * 2 * \sigma^2}{d^2}$$

where  $n$  is the minimum sample size,  $d$  is expected mean difference,  $\sigma$  pooled standard deviation (SD),  $Z_{\alpha/2}$  is 95% confidence interval,  $Z_{\beta}$  is standard normal variate power for 80% power it is 0.84.

This clinical trial was powered to detect a minimum clinically significant difference in root coverage (recession reduction) of 0.5 mm using  $\alpha = 0.05$ , a power  $(1-\beta)$  of 80%, and a hypothesized within-group sigma of 0.4 mm. Considering possible dropouts, the number of patients was increased by 15% for each arm. On the basis of these data, the minimum number of patients needed to be enrolled in this study was 30 in total, 15 for the test (CAF+HA) and 15 for the control group (CAF only).

### Randomisation, allocation concealment and blinding

Randomization was done using computer generated sequence wrapped in the sealed envelopes. The patients were operated by single surgeon with more than 10 years of experience in periodontal plastic surgery. The surgeon who was allotted to perform the surgery was given an envelope which



**Fig. 1.** Pre-surgical, surgical and post-surgical recipient site treated by CAF for Control Group, Case 1. (A) Preoperative gingival recession defect with 13. (B) Horizontal and vertical incisions performed. (C) Split-full – split thickness flap reflected. (D) CAF sutured. (E) Tin foil placed. (F) Periodontal pack placed. (G) Post-operative view at 15 days. (H) Post-operative view at 60 days. (I) Post-operative view at 90 days.

contained the information regarding the tooth on which interventional material was to be used. It was decided using coin toss by another surgeon who didn't operate or assisted during the surgery. These envelopes were opened by the surgeon right before the surgery and accordingly the patients either received test material that is CAF + Hyaloss ( $n = 15$ ) or CAF alone ( $n = 115$ ). This was a single blinded study as the investigator was unknown about the type of intervention (CAF + HA or CAF only) the patient received and only the operator and the patient were familiar with this information.

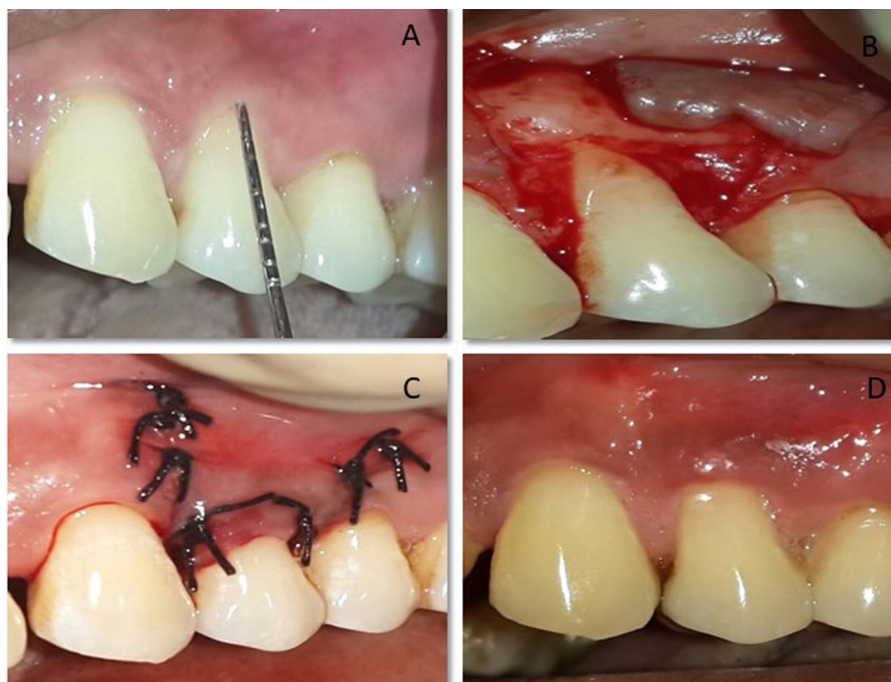
### Surgical protocol

One hour prior to surgery oral antibiotics (capsule amoxicillin 500 mg) were commenced. The area of surgery was disinfected using Povidone iodine swab and was anesthetized using 2% xylocaine infiltration with 1: 50,000 epinephrine on both the facial and lingual aspect. After local anaesthesia (LA) and before the elevation of the flap, the exposed root surfaces were planed to reduce root convexity and washed for 60 s with water spray. Intrasulcular incisions were made on the buccal aspect of the involved tooth, followed by two horizontal incisions in the interdental papillae at the level of CEJ on the mesial and distal

side of the defect extending to the adjacent teeth. Two apically directed vertical releasing incisions were given at the line angles of adjacent teeth extending beyond mucogingival junction (MGJ) into the alveolar mucosa. Trapezoidal split thickness flap was elevated by sharp dissection which was then followed by full thickness flap reflection by periosteal elevator up to MGJ. Beyond MGJ the trapezoidal flap was again reflected as split thickness by sharp dissection leaving the underlying periosteum in place and extended as far as necessary to allow for flap advancement to the CEJ without tension. This necessitated a mesio-distal and apical extension of the partial thickness dissection in order to release any residual muscle tension and allow a passive coronal displacement of the flap. The vestibular epithelium of the adjacent interdental papillae was de-epithelized. The root surface was re-examined for any irregularities. Finally the flap was positioned at the level or slightly coronal to the CEJ and fixed with mattress suture. The two vertical incisions were sutured with interrupted sutures. Abundant saline irrigation was performed during the procedure. Figures 1 and 2 depict pre-surgical, surgical and post-surgical recipient site treated by CAF for Control Group.

For test group, CAF was identical to the one previously described in the control group, except for the addition of Hyaloss. After LA and root planning, the root surface was





**Fig. 2.** Pre-surgical, surgical and post-surgical recipient site treated by CAF for Control Group, Case 2. (A) Pre-operative gingival recession defect with 24. (B) Split-full – split thickness flap reflected. (C) CAF sutured. (D) Post-operative view at 90 days.

washed for 60 s with water spray and dried. HA (Hyaloss) was applied to the exposed root surfaces, starting at the base of the recession using the applicator tip. The flap was then displaced coronally without tension at the CEJ level, fully covering the recession defects. A non-resorbable, number 4–0 vicryl suture was used to secure the CAF at the CEJ level by using interrupted sutures. Both vertical incisions were closed with interrupted sutures and the operated area was covered with periodontal pack (coe-pack). No pressure was applied to the flap after suturing. Figures 3 and 4 depict pre-surgical, surgical and post-surgical recipient site treated by CAF + HYALOSS, for test group respectively.

Subjects were prescribed analgesics (Ibuprofen 400 mg BD) for 3 days, capsule amoxicillin 500 mg, BD for 5 days and were instructed to use 0.2% chlorhexidine mouthwash twice daily. All the parameters were recorded post surgically at 1st, 2nd and 3rd month. The photographs were taken pre and post-operatively to assess the change in color and contour.

### Statistical analysis

The data was entered in MS office excel spread sheet and analysed using IBM SPSS software, version 21. Descriptive statistics were expressed as mean and standard deviation (SD). Data was analysed using unpaired *t* test for intergroup statistical analysis (from baseline to 90 days in each group). Paired *t* test was applied to compare parameters for recipient teeth before and 90 days after surgery in control and test groups. *P* value of <0.05 was considered as statistically significant.

## Results

Table I shows comparative analysis of parameters at baseline between the two groups.

### Gingival recession

For control group, the mean depth of gingival recession at baseline was  $2.67 \pm 0.72$  mm which reduced to  $0.87 \pm 0.52$  mm at 90 days interval ( $p=0.000$ ) (Tab. II). For test group, the mean depth of gingival recession at baseline  $3.13 \pm 0.74$  mm reduced to  $0.47 \pm 0.64$  mm ( $p=0.000$ ) (Tabs. I and III). Intergroup comparison showed that the mean gingival root coverage in the test group was more (86.6%) as compared to the control group (66.1%) and the mean difference of  $0.87 \pm 0.06$  mm was found to be statistically significant ( $p=0.004$ ) (Tab. IV).

### Clinical attachment level

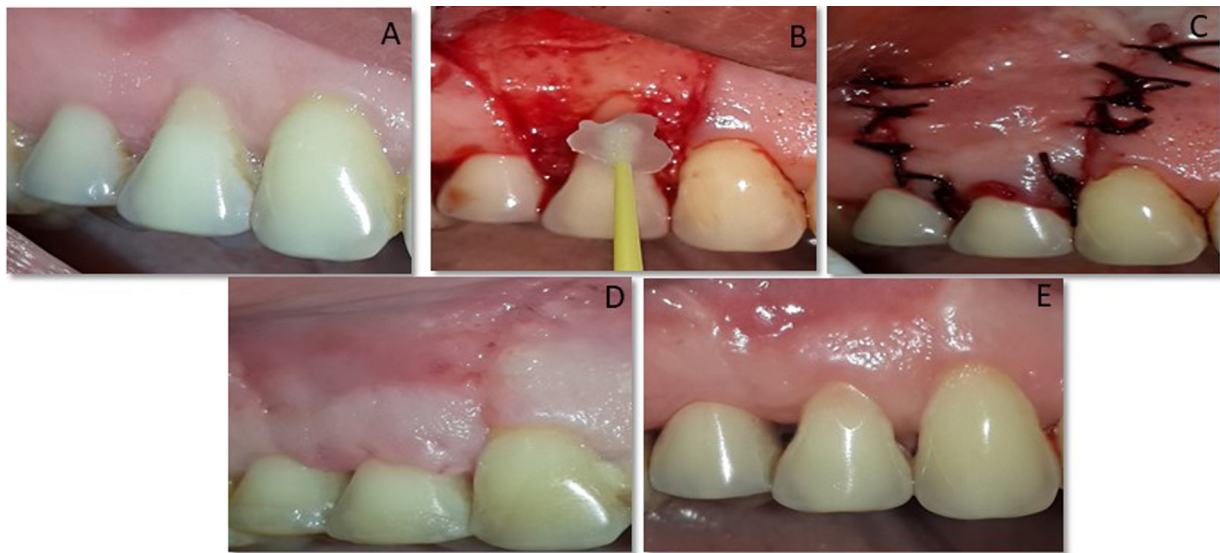
Statistically significant gain in CAL was observed for both the groups at 90 days. Mean depth of CAL for control group at baseline was  $4.53 \pm 0.83$  mm and test group was  $5.00 \pm 0.76$  mm. At 90 days, CAL reduced to  $2.60 \pm 0.83$  mm in control group and  $2.20 \pm 0.86$  mm in test group ( $p=0.000$ ) (Tabs. I–III).

### Keratinized gingival

For control group as well as test group, the mean width of keratinized gingiva was increased at 90 days ( $p=0.000$ ) (Tabs. I–III). However, intergroup comparison showed non-significant results between the groups ( $p=0.607$ ) (Tab. IV).



**Fig. 3.** Pre-surgical, surgical and post-surgical recipient site treated by CAF+HYALOSS, for Test Group, Case 3. (A) Pre-operative gingival recession defect with 13. (B) Horizontal and vertical incisions performed. (C) Split-Full – Split thickness flap reflected. (D) HYALOSS Gel. (E) Hyaloss applied at recipient site. (F) Tin Foil placed. (G) Vertical Incisions closed with Interrupted Sutures. (H) Periodontal Pack Placed. (I) Post-operative view at 15 days. (J) Post-operative view at 60 days. (K) Post-operative view at 90 days.



**Fig. 4.** Pre-surgical, surgical and post-surgical recipient site treated by CAF +HYALOSS, for Test Group, Case 4. (A) Pre-operative gingival recession defect with 14. (B) Hyaloss applied at recipient site. (C) Coronally advanced flap sutured over Hyaloss. (D) Post-operative view at 15 days. (E) Post-operative view at 90 days.

**Table I.** Comparative analysis of parameters at baseline between the two groups control (CAF) and the test (CAF + Hyaloss) group.

S. No.	Parameters	Group	N	Mean	SD	't' Value	p value
1.	Gingival sulcus depth	CAF (Control)	15	1.87	0.52	0.00	1.000
		CAF + Hyaloss (Test)	15	1.87	0.64		
2.	Gingival recession	CAF	15	2.67	0.72	1.74	0.092
		CAF + Hyaloss	15	3.13	0.73		
3.	Clinical attachment level	CAF	15	4.53	0.83	1.61	0.120
		CAF + Hyaloss	15	5.00	0.76		
4.	Keratinized gingival	CAF	15	3.27	0.59	0.89	0.382
		CAF + Hyaloss	15	3.47	0.64		
5.	Attached gingival	CAF	15	1.40	0.83	0.60	0.552
		CAF + Hyaloss	15	1.60	0.99		
6.	Gingival tissue thickness	CAF	15	1.46	0.45	0.33	0.745
		CAF + Hyaloss	15	1.51	0.42		

**Table II.** Shows evaluation of parameters for recipient teeth before and 90 days after surgery in control group (CAF).

S. No.	Parameters	Before Surgery (n=15)		90 days after surgery				't' value	p value
		Mean	SD	Mean	SD	Mean diff.	SD diff.		
1.	Gingival sulcus depth	1.87	0.52	1.73	0.59	0.13	0.35	1.47	0.164
2.	Gingival recession	2.67	0.72	0.87	0.52	1.80	0.78	9.00	0.000*
3.	Clinical attachment level	4.53	0.83	2.60	0.83	1.93	0.79	9.37	0.000*
4.	Keratinized gingival	3.27	0.59	4.13	0.99	0.87	0.64	5.25	0.000*
5.	Attached gingiva	1.40	0.83	2.40	1.35	1.00	0.76	5.12	0.000*
6.	Gingival tissue thickness	1.46	0.45	1.48	0.41	0.02	0.14	0.58	0.571

\*Significant p value.

**Table III.** Shows the evaluation of parameters for recipient teeth before and 90 days after surgery in test group (CAF + Hyaloss).

S. No.	Parameters	Before surgery (n = 15)		90 days after surgery				't' value	p value
		Mean	SD	Mean	SD	Mean diff.	SD diff.		
1.	Gingival sulcus depth	1.87	0.64	1.73	0.70	0.13	0.35	1.47	0.164
2.	Gingival recession	3.13	0.74	0.47	0.64	2.67	0.72	14.27	0.000*
3.	Clinical attachment level	5.00	0.76	2.20	0.86	2.80	0.86	12.58	0.000*
4.	Keratinized gingival	3.47	0.64	4.47	0.74	1.00	0.76	5.12	0.000*
5.	Attached gingiva	1.60	0.99	2.73	1.22	1.13	0.83	5.26	0.000*
6.	Gingival tissue thickness	1.51	0.42	1.60	0.42	0.09	0.21	1.63	0.125

\*Significant p value.

### Attached gingival

For both control group and test group, the mean width of attached gingiva was increased at 90 days and mean difference was statistically highly significant ( $p=0.000$ ) (Tabs. II and III). But, intergroup comparison showed that the mean difference of  $0.13 \pm 0.07$  mm between test group and control group was statistically non-significant ( $p=0.651$ ) (Tab. IV).

### Gingival tissue thickness

The gingival tissue thickness was measured by using a file with rubber stopper midway between the gingival margin and MGJ and measured with a digital vernier caliper with 0.05 mm resolution. For control group, the mean thickness of gingival tissue at baseline,  $1.48 \pm 0.45$  mm increased to  $1.58 \pm 0.41$  mm at 90 days ( $p=0.571$ ) (Tabs. I and II). Similarly, for test group the mean thickness of gingival tissue at baseline,



**Table IV.** Shows intergroup comparison of parameters at 90 days after surgery for both control (CAF) and test (CAF+ Hyaloss) group.

S. No.	Parameters	Group	N	Mean	SD	't' Value	p value
1.	Gingival sulcus depth	CAF	15	0.13	0.35	0.00	1.00
		CAF + Hyaloss	15	0.13	0.35		
2.	Gingival recession	CAF	15	1.80	0.78	3.17	0.004*
		CAF + Hyaloss	15	2.67	0.72		
3.	Clinical attachment level	CAF	15	1.93	0.79	2.86	0.008*
		CAF + Hyaloss	15	2.80	0.86		
4.	Keratinized gingival	CAF	15	0.87	0.64	0.52	0.607
		CAF + Hyaloss	15	1.00	0.76		
5.	Attached gingival	CAF	15	1.00	0.76	0.46	0.651
		CAF + Hyaloss	15	1.13	0.83		
6.	Gingival tissue thickness	CAF	15	0.02	0.14	1.04	0.309
		CAF + Hyaloss	15	0.09	0.21		

\*Significant p value.

1.51±0.42 mm increased to 1.60±0.42 mm at 90 days ( $p=0.125$ ) (Tabs. I and III). Intergroup comparison also showed non-significant results for mean gingival thickness between the groups ( $p=0.309$ ) (Tab. IV).

## Discussion

The present study compared the treatment effects on control sites (CAF alone) to that of test sites (CAF+ HA) on Miller's class I & II gingival recession. HA has shown promising results in the field of regenerative therapy. It has been used extensively in the field of dentistry for its various beneficial applications. Exogenous application of HA has been shown to have anti-edema, anti-inflammatory and good wound healing properties [15]. In periodontics it has been used to treat gingivitis, periodontitis and periodontal intrabony defects [16,17]. Considering the study design and homogeneity of groups at baseline, differences in clinical outcomes were totally attributed to the techniques employed. Plaque index and gingival index scores were the supporting measurements that were recorded which established the degree of compliance by the patient and condition of periodontal soft tissue. Plaque index score was maintained  $\leq 10\%$  throughout the study.

Both the groups showed improved clinical outcome in terms of root coverage, clinical attachment level, width of keratinized gingiva and width of attached gingiva at 90 days compared to baseline with statistically significant difference in GR (root coverage,  $p=0.004$ ) and clinical attachment level ( $p=0.000$ ) between the groups. All the grafts appeared to have been taken up successfully by the patients with no signs of infection or complications *i.e.* pain, bleeding, suppuration, gangrene, swelling or discomfort, during the period of healing. HA was well tolerated by all the patients without any allergic reaction. Clinical trials have shown that HA could be used with no drug interference. It does not need cutaneous sensitivity testing and has FDA approval [18].

In the control group the mean depth of gingival recession was reduced significantly compared to that of baseline at 90 day interval. This is in accordance with the previous studies conducted by Zingale [19], Egli *et al.* [20] and Bernimoulin *et al.* [21]. Mean percentage of root coverage obtained in the control group was 66.1% which is in accordance with the values previously reported after the use of CAF, that ranged from 56.1% to 98.8% after 6 months follow up. Three sites out of 15 in the control group achieved 100% root coverage which was comparable to the results of other studies in the literature [22,23]. In the test group, the mean depth of gingival recession at baseline was reduced significantly at 90 days interval. The mechanism of action for root coverage by HA may be explained on the basis of its anti-inflammatory effect, which may be due to its action as a scavenger by draining prostaglandin, metalloproteinases and the other bioactive molecules. It also scavenges reactive oxygen species such as superoxide radical ( $O_2^-$ ), hydroxyl radical ( $OH^\cdot$ ), thus preventing periodontal destruction [24].

HA has an anti-edematous effect which may be related to the osmotic activity. In dentistry HA has showed a positive effect on reduction of plaque and sulcus bleeding index of patients with plaque induced gingivitis [24]. In very few studies HA has been applied as an adjunct to SRP in non-surgical treatment of periodontitis [25]. Johnson *et al.* reported significant reduction of bleeding on probing and periodontal destruction after the use of subgingivally applied 0.8% HA gel immediately post SRP and after 1 week [25]. This implies that reduction in gingival inflammation and edema due to anti-inflammatory and anti-edematous properties of HA might have prevented further apical migration of gingival margin leading to GR coverage in the test group. The mean root coverage in the test group at 90 days was comparable to the results reported by Cortes *et al.* (CAF+ Acellular Dermal Matrix, ADM) [26], Pilloni *et al.* (CAF+ Enamel matrix Derivative, EMD) [27], Gupta *et al.* (CAF+ Platelet Rich Fibrin, PRF) [28], and Kumar *et al.* (CAF+Hyaluronan) [29].

In the present study, nine sites in test group achieved 100% root coverage which was favourably comparable, and mean percentage of root coverage (88.6%) in the test group was much higher than reported by Kumar *et al.* (68.33%) [29]. This might be attributed to the variability in the observation period of the studies. But the mean root coverage in our study was comparable to the results reported by Jagannathachary *et al.* (82.2% using CAF+ADM) [30], and Jaiswal *et al.* (86.3% using CAF+EMD) [31]. At 90 days the mean amount of root coverage in control group was  $1.80 \pm 0.78$  mm and in the test group was  $2.67 \pm 0.72$  mm, with statistically significant difference between the groups ( $p = 0.004$ ). The difference noted between the groups could be further explored and explained by analysing the soft tissue changes during the early healing phases. Both the procedures were performed with a clear goal of providing complete root coverage of treated roots. To reach this objective the best clinical skills was applied in trying to obtain a tension free pedicle flap, to position the flap margins coronal to CEJ and to provide flap stability with suturing technique.

Ballini *et al.* studied biological properties of HA that was seen to promote periodontal regeneration through space preservation and surface protection. It is said that the success of the root coverage procedure depends on the intimacy between the recipient bed and the covering tissues. A thin and stable clot assures better healing. HA promotes wound healing by cell migration and proliferation, it facilitates white blood cell infiltration and improves tissue hydration [32]. This could contribute to better stability of root coverage procedure that is seen with the use of HA.

This study showed statistically significant ( $p = 0.000$ ) increase in the width of keratinized gingiva in both the groups could be due to influence of the granulation tissue derived from the periodontal ligament or the tendency of the MG line to regain its original position. The exact role of HA in stimulating the overlying mucosa to become keratinized is still not clear. However, it may be correlated with its role in keratinocyte proliferation [33]. HA is preferred over GTR membrane as the use of GTR is technique sensitive, moreover there is instability of GTR membrane under the pedicle flap, and bacterial contamination of membrane leads to compromised results. Use of non-resorbable membrane may cause perforation of overlying soft tissue and second surgical procedure [34]. At 90 days the mean increase in width of attached gingiva in control group was  $1.00 \pm 0.76$  mm and in the test group was  $1.13 \pm 0.83$  mm. The mean difference of  $0.13 \pm 0.07$  mm was found to be statistically non-significant. The increase in the width of attached gingiva in the test group (1.13 mm) was in accordance with the results reported by Kuis *et al.* [35], and Jagannathachary *et al.* [30].

At 90 days, the mean gain in CAL in control group was  $1.93 \pm 0.79$  mm and in the test group was  $2.80 \pm 0.86$  mm. The mean difference of  $0.87 \pm 0.07$  mm was found to be statistically highly significant ( $p = 0.000$ ). Kumar *et al.* reported 2.3 mm of gain in CAL in the test group after the use of HA + CAF; but did not find any significant difference between the groups (CAF and CAF+HA) [29]. The increase in CAL in the test group could be

attributed to embryologic and wound healing properties of HA including the facilitation of cell migration and differentiation during tissue formation and repair [36]. HA shares bone induction characteristics with osteogenic substrates such as calcitonin gene related peptide (CgRPa) and bone morphogenic protein [37,38]. Recent studies demonstrate that HA aids in the repair process of both soft and hard tissue (bone) [39]. During healing in the later granulation stage, HA synthesis stops and the existing HA depolymerized by host enzymes hyaluronidase, results in the formation of low molecular compounds and alteration of granulation tissue composition. This indicates that low molecular hyaluronic fragments formed after subsequent hyaluronidase activity promotes angiogenesis in the lesion that provides the necessary components required for rapid regeneration [39]. As per the existing literature few clinical studies have assessed thickness of gingival tissue with the use of HA and results showed significant increase in the thickness of gingiva [30]. Similarly, in this study at 90 days, the mean increase in the thickness of gingiva in the control group was  $0.02 \pm 0.14$  mm and in the test group was  $0.09 \pm 0.21$  mm but the mean difference of  $0.07 \pm 0.7$  mm was found to be statistically non-significant.

HA has been found to be associated to the collagen molecules providing extracellular matrix elasticity, resistance and lubrication, and has an important function in areas with rapid cell proliferation, since it facilitates cell displacement. It is also a key component of chronic wound during the series of stages associated with the wound healing process in both mineralized and non-mineralized tissues. Though HA is mostly advocated in dentistry for topical application some recent studies were done on hyaluronic acid in biomaterials assisting regeneration [40]. Vanden Bogaerde demonstrated the osteoinductive effect of esterified low molecular weight HA preparation in the treatment of infrabony defects [41]. It was used as a co-adjunct with autogenous bone for grafting procedure. At 9 months from procedure, the dental parameters were virtually stable with a mean CAL gain of 2.6 mm. Radiographic evolution after 24 months showed defect fill. In another randomised controlled trial, Ni Jing *et al.* injected the HA gel into the base of the deficient papilla, and it was found that the height of the gingival papilla increased to 0.311, 0.45, and 0.4 mm from baseline at 3, 6, and 12 months, respectively, after treatment ( $p < 0.05$ ) [42]. Shirakata *et al.* conducted an experimental study on dogs in which they found successful periodontal regeneration of gingival recession defects after treatment with CAF+HA. Statistically significant differences were observed in CAL ( $p < 0.05$ ), width of gingival recession ( $p < 0.01$ ), in formation of cementum and connective tissue attachment ( $p < 0.05$ ) at 10 weeks in HA+CAF group [43]. From these observations we can assume some amount of true regeneration to take place under CAF with connective tissue attachment [41].

The histologic evaluations of the attachment obtained with laterally positioned flap, CAF, free gingival graft and connective tissue graft for the purpose of root coverage were most often reported in case reports [36,37]. Results of the study by



Shirakata *et al.* on dogs concluded that use of cross-linked hyaluronic acid with or without a collagen matrix has a potential to promote periodontal wound healing in Class III furcation defects [44]. Some reports showed long junctional epithelium, some connective tissue attachment while others showed small amount of regeneration. Often many reports showed more than one mode of attachment [36,37]. In the present study the tissue was firmly attached to the tooth in all the patients, and showed resistance to probing. The mean sulcus depth at 90 days in control group was  $1.40 \pm 0.51$  and in test group was  $1.20 \pm 0.41$  mm with no bleeding on probing. This type of attachment couldn't be determined without human histology. Moreover, it requires removal of successfully treated tooth. This study showed promising results for HA in promoting periodontal regeneration but more clinical trials on larger population should be carried out to validate our results and long term analysis is necessary to see the stability of the outcomes.

## Conclusion

Hyaluronic acid is a relatively newer biomaterial, the use of which is extending in dentistry for the treatment of mucogingival problems, reconstruction of interdental papilla, furcation involvement and periodontal regeneration. Our analysis suggested that HA may be used for successful recession coverage with coronally advanced flap but further studies are required to imply the results on larger sample size.

## Conflict of interest

Authors declare no conflict of interest.

## Funding

No funding.

## Ethical approval

Ethical clearance has been obtained from ethics committee of the institute.

## Informed consent

Has been obtained from the patients after explaining the aim and procedure.

## Authors' contribution

Apoorva Saxena: Study Design, Conducting Research, Data Collection, Statistical Analysis and Manuscript Writing. Prashant Bhusari: Study Design and Concept, Manuscript Editing, Formatting and Writing. Abhilasha Singh: Statistical Analysis, Data Collection & Compilation, and Manuscript

Drafting. Ravleen Nagi: Manuscript Editing, Statistical Analysis, and Revision. Shailendra Singh Chaturvedi: Manuscript Editing, and Final Revision.

## References

- Ahrari F, Keshavarzi F, Bijani A, Jenabian N. Efficacy of Application of Plasma Rich in Growth Factors Along with the Tunnel Technique for Treatment of Gingival Recession: a Clinical Trial. *J Dent (Shiraz)* 2020; 21:275–283.
- Bellver-Fernández R, Martínez-Rodríguez AM, Gioia-Palavecino C, Caffesse RG, Peñarocha M. Surgical treatment of localized gingival recessions using coronally advanced flaps with or without subepithelial connective tissue graft. *Med Oral Patol Oral Cir Bucal* 2016;21:e222–e228.
- Barootchi S, Tavelli L, Di Gianfilippo R, Byun HY, Oh TJ, Barbato L, Cairo F, Wang HL. Long term assessment of root coverage stability using connective tissue graft with or without an epithelial collar for gingival recession treatment. A 12-year follow-up from a randomized clinical trial. *J Clin Periodontol* 2019; 46: 1124–1133.
- Harris RJ. A comparative study of root coverage obtained with guided tissue regeneration utilizing a resorbable membrane versus the connective tissue with partial thickness double pedicle graft. *J Periodontol* 1997; 68: 779–790.
- Chambrone L, Chambrone D, Pustiglioni FE, Chambrone LA, Lima LA. Can subepithelial connective tissue grafts be considered the gold standard procedure in the treatment of Miller Class I and II recession-type defects? *J Dent* 2008; 36: 659–671.
- Pini-Prato G, Baldi C, Pagliaro U, Nieri M, Saletta D, Rotundo R, Cortellini P. Coronally advanced flap procedure for root coverage. Treatment of root surface: root planning versus polishing. *J Periodontol* 1999; 70: 1064–1076.
- Pini Prato G, Tinti C, Vincenzi G, Magnani C, Cortellini P, Clauser C. Guided tissue regeneration versus mucogingival surgery in the treatment of human buccal gingival recession. *J Periodontol* 1992; 63: 919–928.
- Pilloni A, Paolantonio M, Camargo PM. Root coverage with a coronally positioned flap used in combination with enamel matrix derivative: 18-month clinical evaluation. *J Periodontol* 2006; 77: 2031–2039.
- Woodyard JG, Greenwell H, Hill M, Drisko C, Iasella JM, Scheetz J. The clinical effect of acellular dermal matrix on gingival thickness and root coverage compared to coronally positioned flap alone. *J Periodontol*. 2004; 75: 44–56.
- Wilson TG, Jr, McGuire MK, Nunn ME. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast-derived dermal substitute. II. Comparison to the subepithelial connective tissue graft: A randomized controlled feasibility study. *J Periodontol* 2005; 76: 881–999.
- Moseley R. Periodontology, Hyaluronan and its role in periodontal wound healing. *Dent Update* 2002; 29: 144–148.
- Kumar R, Srinivas M, Pai J, Suragimath G, Prasad K, Polepalle T, *et al.* Efficacy of hyaluronic acid (hyaluronan) in root coverage procedures as an adjunct to coronally advanced flap in Millers Class I recession: a clinical study. *J Indian Soc Periodontol* 2014; 18: 746–750.
- Loe H, Silness J. Periodontal disease in pregnancy. *Acta Odontol Scand* 1963; 20: 553–561.
- Silness J, Loe H. Correlation between oral hygiene & periodontal condition. *Acta Odontol Scand* 1964; 22: 121–135.

15. Ferguson EL, Roberts JL, Moseley R, Griffiths PC, Thomas DW. Evaluation of the physical and biological properties of hyaluronan and hyaluronan fragments. *Int J Pharm* 2011; 420: 84–92.
16. Jentsch H, Pomowski R, Kundt G, Göcke R. Treatment of gingivitis with hyaluronan. *J Clin Periodontol* 2003; 30: 159–164.
17. Eliezer M, Imber JC, Sculean A, Pandis N, Teich S. Hyaluronic acid as adjunctive to non-surgical and surgical periodontal therapy: a systematic review and meta-analysis. *Clin Oral Investig* 2019; 23: 3423–3435.
18. Becker W, Gabitov I, Stepanov M, Kojs J, Smidt A, Becker M. Minimally invasive treatment for papillae deficiencies in the esthetic zone. A pilot study. *Clin Implant Dent Related Res* 2010; 12: 1–8.
19. Zingole J. Observation on free gingival autografts. *J Periodontol* 1974; 45: 748.
20. Egti U, Vollmer WH, Rateitschak KH. Follow up studies of free gingival grafts. *J Clin Periodontol* 1975; 2: 98.
21. Bernimoulin J, Lüscher B, Mühlemann HR. Coronally positioned periodontal flap. Clinical evaluation after one year. *J Clin Periodontol* 1975; 2: 1–13.
22. Hagewald S, Spahr A, Rompola E, Haller B, Heijl L, Bernimoulin JP. Comparative study of Emdogain and coronally advanced flap technique in the treatment of human gingival recessions. *J Clin Periodontol* 2002; 29: 35–41.
23. Cueva M, Boltchi F, Hallmon W, Nunn M, Rees T. A comparative study of coronally advanced flaps with and without the addition of enamel matrix derivative in the treatment of marginal tissue recession. *J Periodontol* 2004; 75: 949–956.
24. Dahiya P, Kamal R. Hyaluronic Acid: a boon in periodontal therapy. *N Am J Med Sci* 2013; 5: 309–315.
25. Johannsen A, Tellefsen M, Wikesjö U, 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.
26. Côrtes A, Martins AG, Sallum FA, Casati ZM, Sallum EA. Coronally positioned flap with or without acellular dermal matrix graft in the treatment of class I gingival recessions: a randomized controlled clinical study. *J Periodontol* 2004; 75: 1137–1144.
27. Pilloni A, Paolantonio M, Camargo PM. Root coverage with coronally positioned flap and Enamel matrix derivative. *J Periodontol* 2006; 77: 2031–2039.
28. Gupta S, Banthia R, Singh P, Banthia P, Raje S, Aggarwal N. Clinical evaluation and comparison of the efficacy of coronally advanced flap alone and in combination with platelet rich fibrin membrane in the treatment of Miller Class I and II gingival recessions. *Contemp Clin Dent [serial online]* 2015; 6: 153–160.
29. Kumar R, Srinivas M, Pai J, Suragimath G, Prasad K, Polepalle T, *et al.* Efficacy of hyaluronic acid (hyaluronan) in root coverage procedures as an adjunct to coronally advanced flap in Millers Class I recession: a clinical study. *J Indian Soc Periodontol* 2014; 18: 746–750.
30. Jagannathachary P. Alloderm in the treatment of gingival recession defects. *Contemp Clin Dentistry* 2010; 1: 73–74.
31. Jaiswal GR, Kumar R, Khatri PM, Jaiswal SG, Bhongade ML. The effectiveness of enamel matrix protein (Emdogain<sup>®</sup>) in combination with coronally advanced flap in the treatment of multiple marginal tissue recession: a clinical study. *J Indian Soc Periodontol* 2012; 16: 224–230.
32. Ballini A, Cantore S, Capodiferro S, Grassi FR. Esterified hyaluronic acid and autologous bone in the surgical correction of the infra-bone defects. *Int J Med Sci* 2009; 6: 65–71.
33. da Silva RC, Julio CJ, de Lima AFM, Tatakis DN. Root coverage using the coronally positioned flap with or without a subepithelial connective tissue graft. *J Periodontol* 2004; 75: 413–419.
34. Hagewald S, Spahr A, Rompola E, Haller B, Heijl L, Bernimoulin JP. Comparative study of Emdogain and coronally advanced flap technique in the treatment of human gingival recessions. *J Clin Periodontol* 2002; 29: 35–41.
35. Kuis D, Sciran I, Lajnert V, Snjaric D, Prpic J, Pezelj-Ribaric S, Bosnjak A. Coronally advanced flap alone or with connective tissue graft in the treatment of single gingival recession defects: a long-term randomized clinical trial. *J Periodontol* 2013; 84: 1576–1585.
36. Bertolami CN. Glycosaminoglycan interactions in early wound repair. In Hunt TK, Heppenstall RB, Pines E, *et al.*, eds. *Soft and Hard Tissue Repair, Biological and Clinical Aspects*. New York: Praeger Publishers 1984; 67–97.
37. Bernard GW, Shih C. The osteogenic stimulatory effect of neuroactive calcitonin gene-related peptide. *Peptides* 1990; 11: 625–632.
38. Wang EA, Rosen V, D'Alessandro JS. Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci* 1990; 81: 2220–2224.
39. Cortivo R, Brun P, Cardarelli, O'Regan M, Radice M, Abatangelo G. Antioxidant effects of hyaluronan and its alpha-methyl-prednisolone derivative in chondrocyte and cartilage cultures. *Semin Arth Rheum* 1996; 26: 492–501.
40. Moseley R. Periodontology, Hyaluronan and its role in periodontal wound healing. *Dent Update* 2002; 29: 144–148.
41. Vanden Bogaerde L. Treatment of infrabony periodontal defects with esterified hyaluronic acid: clinical report of 19 consecutive lesions. *Int J Periodontics Restorative Dent* 2009; 29: 315–23.
42. Ni J, Shu R, Li C. Efficacy evaluation of hyaluronic acid gel for the restoration of gingival interdental papilla defects. *J Oral Maxillofac Surg* 2019; 77: 2467–2474.
43. Shirakata Y, Imafuji T, Nakamura T, Shinohara Y, Iwata M, Setoguchi F, *et al.* Cross-linked hyaluronic acid gel with or without a collagen matrix in the treatment of class III furcation defects: a histologic and histomorphometric study in dogs. *J Clin Periodontol* 2022.
44. Shirakata Y, Nakamura T, Kawakami Y, Imafuji T, Shinohara Y, Noguchi K, *et al.* Healing of buccal gingival recessions following treatment with coronally advanced flap alone or combined with a cross-linked hyaluronic acid gel. An experimental study in dogs. *J Clin Periodontol* 2021; 48: 570–580.