



Use of Injectable Hyaluronic Acid Gel and Injectable Platelet-rich Fibrin in the Treatment of Gingival Black Triangles: A Randomized Clinical Trial

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ABSTRACT

Purpose: The current study aimed to compare the efficacy of injectable hyaluronic acid gel and injectable platelet rich fibrin, either alone or combined; for the reconstruction of defective interdental papilla using clinical approaches.

Subjects and Methods: A total sample size of eighteen class I and class II deficient interdental papillae sites were randomly divided in 3 groups. Group 1 included 6 sites that were treated by 0.2ml hyaluronic acid gel injection. Group 2 included 6 sites that were treated by injectable platelet-rich fibrin (i-PRF). Group 3 included 6 sites that were treated by a combination of 0.2ml hyaluronic acid gel injection and iPRF. Each deficient papilla received 3 injections; at 0, 3 and 6 weeks intervals. The height of black triangles was recorded at baseline before injection procedure, at each injection interval and during follow ups. **Results:** A statistically significant difference in all three groups was found in the mean of the black triangle height records between the five time intervals, mainly between baseline and 6 months follow up. **Conclusion:** Hyaluronic acid injections and injectable platelet rich fibrin can be an alternative non-invasive treatment method for gingival black triangles.

INTRODUCTION

One of the most esthetically challenging tasks in periodontology, is the reconstruction of interdental papilla. Interdental papilla is the triangular shaped soft tissue that fill the gaps between teeth. Not only does the interdental papilla serve as an esthetic purpose, but it provides functional actions as well. Deficiency in interdental papillae creates

KEYWORDS

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problems with food impaction and pronunciations. Interdental papillae play a major role in protecting the periodontal tissue by blocking food impaction especially in the anterior zone ⁽¹⁾.

The interdental papilla is one of the most essential features that clinicians must consider especially in terms of esthetics. Interproximal spacing, buccal mucosa thickness, and the extent of contact areas are from the many factors that may affect interdental papilla loss. Absence of sufficient papilla will lead to an undesirable black triangle formation. Gingival black triangles may result from several factors, including age, crown form and periodontal disease ⁽²⁾.

As compared to other parts of the gingiva, interdental papilla is characterized for its distinct cellular properties that led to the advancement of different surgical and non-surgical approaches to restore lost papillae. Challenging surgical approaches have been done over the years for the reconstruction of the lost papilla as by coronally positioning and sub-epithelial connective tissue graft have been attempted. Almost all surgical procedures had unpredictable results due to the inadequate blood supply and minute papillary space aspect. Also anatomical considerations make papillary reconstruction more effortful ⁽³⁾.

The interdental papilla has been repaired using a variety of non-surgical methods in recent years. Prosthetic, restorative, and orthodontic operations are among these approaches. Hyaluronic acid (HA) based dermal fillers were utilized as a biocompatible gel to try to restore volume and shape in areas that had lost collagen or fat. HA forms a critical component of connective tissue. It promotes tissue healing qualities by contributing to tissue hydrodynamics, cell migration, and proliferation. Its biochemical capabilities demonstrate that it gives tissues flexibility and stability while also assisting in tissue regeneration. HA has been shown to control biological processes such skin restoration, anti-inflammatory activity, wound healing and tissue regeneration ⁽⁴⁾.

PRF is currently widely used for a variety of periodontal regeneration operations, including papilla repair, with promising outcomes. PRF has antibacterial capabilities and is autologous. It is also easy to make in a short amount of time and requires minimum biochemical handling ⁽⁵⁾. Injectable platelet-rich fibrin (iPRF) is comparable to PRF in qualities and is also accessible in an injectable version. The slow pace of blood centrifugation is thought to be one of the key advantages of i-PRF over other platelet concentrates. Platelets, leukocytes and growth factors may be released more often as the number of cells increases. I-PRF generates a minute clot that functions as a dynamic hydrogel with cells potentially releasing more growth factors. It serves as a source of slow-releasing growth factors that aid in the remodeling of the interdental papilla ⁽⁶⁾.

SUBJECTS AND METHODS

Patient's selection:

A randomized study was carried out on eighteen deficient interdental papilla sites and divided into three groups comprising six papillary sites in each group. Patients were selected from the outpatient clinic of Oral medicine, Periodontology, Diagnosis and Radiology department, faculty of Dental Medicine, Al-Azhar University for Girls. Prior to any procedure, all subjects were informed about the nature, and benefits of their participation in the study. Written informed consent from patients willing to participate in the study was signed. Approval number **REC- ME-22-10** from the Research Ethic Committee of the Faculty of Dental Medicine for Girls, Al-Azhar University, was received.

The following inclusion criteria were used to identify all patients: Patients aging from 20 up to 50 years old, highly motivated patients with no missing teeth in the anterior region having deficient papilla class I and II according to Nordland and Tarnow ⁽⁷⁾, Papillary marginal gingival index equal to zero and no open contacts between the affected teeth. Asymptomatic free patients from any systemic

disease. Excluded patients; smokers, pregnant or breastfeeding females, patients who take any medications known to cause gingival enlargement, gingival fillers previously done, teeth with caries, proximal restorations, fixed or orthodontic appliances and finally those who had a periodontal surgeries six months prior to selection.

Sample size calculation:

According to Cohen (1988), a total sample size of 18 samples is required to detect an effect size of 0.9⁽⁸⁾, with a power ($1-\beta=0.80$) of 80% at a significance probability level of $p \leq 0.05$ (f: is the effect size=0.9; $\alpha=0.05$; $1-\beta=0.80$) and each group represented by a minimum of 6 samples.

Outcome Measurements:

1-Patient satisfaction

Assessment of patient satisfaction was done prior to baseline injections and at the end of the 6 months follow up period, by distributing a visual analog scale (VAS) questionnaire of 10 cm length⁽⁹⁾ (0= unsatisfied, 10= very satisfied) regarding satisfaction with current dental esthetics and previous esthetic. A comparison between pre and post injection appearances was done by showing clinical photographs taken before the injection procedure to each patient⁽¹⁰⁾.

2- Black triangle height

Clinical measurements of the black triangle height were done by measuring the distance between the contact point and deficient papilla tip. This was done with the aid of a UNC-15 periodontal probe and a modified custom-made occlusal stent for standardized and accurate probe positioning at each time of measurement⁽¹⁰⁾ at weeks 0, 3, 6 and after 3, 6 months. To avoid angulation, the interdental section of the stent was fully trimmed, and the probe was inserted parallel to the tooth's long axis, touching the tip of the papillae immediately buccal to the contact point⁽¹¹⁾.

Clinical images of the teeth of interest were taken perpendicular to them, then transferred to a computer and processed utilizing (Imagej)[®] analysis software and calibrated by pixel size (<https://imagej.nih.gov/ij>). To set the scale of imageJ, pixel values on a clinical photograph of UNC-15 probe length of 15mm were measured and used as a reference object for converting the values expressed in pixels to length in (mm). The black triangle portion to be measured was demarcated on the image and converted automatically to millimeters according to the set scale. Calculation of the black triangle height was done and papillae fill were compared at 0, 3, 6 weeks, 3 and 6 months as shown in (Fig. 1).

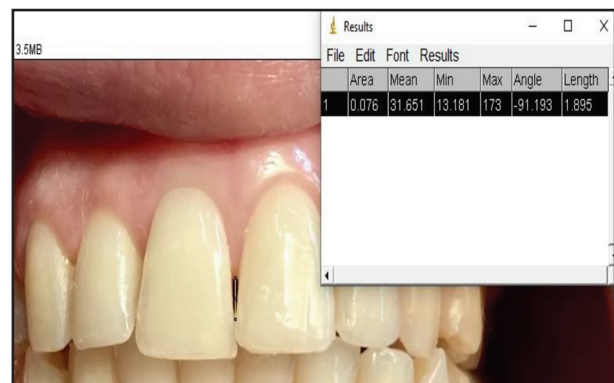


Figure (1) Black triangle height measured using imageJ software program

Injectable PRF preparation protocol and procedure⁽¹²⁾

1. Injectable PRF was prepared by collecting 3ml of serum samples under aseptic conditions before being centrifuged for 3 minutes at 700 rpm in a glass tube without any additives.
2. The top layer was formed of I-PRF, which was immediately collected with a 25-gauge needle into a 2ml syringe and injected at 0 (baseline), 3, and 6 week intervals.
3. I-PRF was then injected 2-3 mm below the interdental papillary tip by inserting the needle at a 45 degree angle.
4. While injecting the iPRF, the needle slant was pointed upward to guarantee that the bevel was up.

- 5- After the injection, the area was gently massaged for one minute. Patients were asked not to perform mechanical plaque management in the area for a day after each treatment session.

Hyaluronic acid protocol and procedure

- 1- Cross-linked hyaluronic acid gel (MonaLisa, Genoss®. Co. South Korea. Approval number: 2016-I10-29-1751) composed of particle size 200 microns was used. It is supplied with its 1ml volume syringe of 30 gauge needle.
- 2- Hyaluronic acid gel was injected in the papillae at 0 (baseline) and repeated after 3 and 6 weeks.

Statistical analysis:

The results were analyzed statistically using one-way ANOVA to compare normal distributed data, followed by a Post Hoc test (Tukey) for multiple comparisons across groups. Kruskal-Wallis was used to analyze non-parametric data, followed by the Mann-Whitney Test for pairwise comparisons between groups. P-values of 0.05 or less were considered statistically significant (at 95%), while P-values lower than 0.001 were considered highly statistically significant (99% significance level). The Shapiro Wilk test was performed to indicate whether the data was normal. The statistical software SPSS was used to analyze the data (version 23, IBM Co. USA).

RESULTS

A total number of 18 deficient papilla sites were included in the study to evaluate their clinical parameters. Satisfaction score (VAS) and the black triangle height measurement was assessed before treatment, after each injection interval (1, 3, 6 weeks) and 3, 6 months follow up.

1. Satisfaction score (VAS):

Regarding hyaluronic acid group, the mean was recorded at baseline (3.8 ± 0.8) and increased after 6 months to (9.1 ± 0.9) achieving the highest satisfaction, that resulted in considerable improvement in interdental papillary defect as shown in (Fig. 2). Furthermore, iPRF group mean score was (3.7 ± 0.5) at baseline that increased after 6 months of follow up to (7.8 ± 0.8) achieving the lowest satisfaction. For HA+iPRF Mix group, the mean of satisfaction score at baseline was (3.7 ± 0.5) increased after 6 months of follow up to (7.8 ± 0.8). For all groups, the satisfaction of patients after 6 months of follow-up was significantly higher than before starting the treatment as shown in (Fig. 3).

2. The Black Triangle Height:

Between the three groups, the mean black triangle height varied significantly as shown in (Fig. 4). The overall p value for inter-group comparison was statistically significant ($P > 0.05$), showing that the mean of black triangle height between the three groups significantly differed statistically.



Figure (2) A case of interdental papilla fill. (A) At baseline. (B) After 6 months follow up.

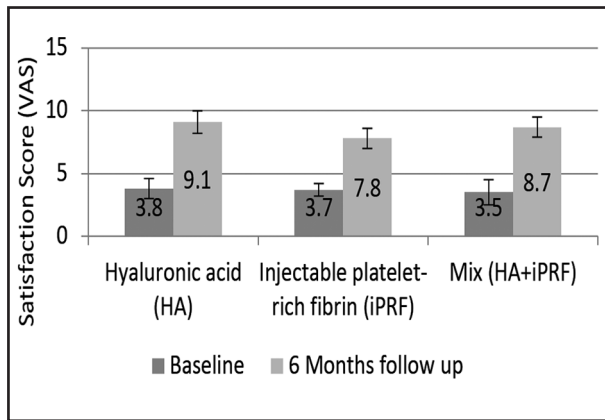


Figure (3) Bar chart shows the mean and SD of satisfaction Score (VAS) distribution between the three groups at different time intervals.

The percentage of change seen in (table 1), the HA group had the highest percentage change (92.35 percent) after 6 months, and there were statistically significant variances in percentage change between HA and HA+iPRF Mix groups, as well as between HA and iPRF groups, while between iPRF and HA+iPRF Mix groups, there wasn't a significant difference. The total p value was very significant at the 0.01 level ($P > 0.01$ & confidence 99%), indicating a highly significant difference in percentage change between the three groups, primarily between HA (the highest %) and HA+iPRF Mix (the lowest %).

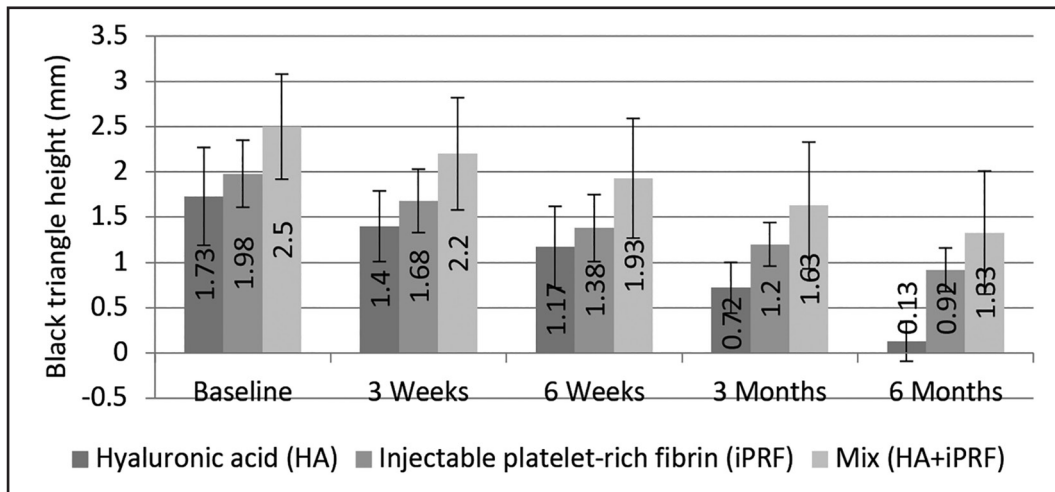


Figure (4) Bar chart shows the mean and SD of the black triangle height at different time intervals for the three groups at different time intervals.

Table (1) Comparing the percentage change (%) of the black triangle height for the three groups at different time intervals.

	3 Weeks	6 Weeks	3 Months	6 Months
HA	17.65% ^A	31.18% ^A	57.65% ^A	92.35% ^A
iPRF	15.15% ^A	30.30% ^A	39.39% ^B	53.54% ^B
HA+iPRF	12.00% ^A	22.80% ^A	34.80% ^B	46.80% ^B
P-value**	0.321 ^{NS}	0.067 ^{NS}	0.000 ^{HS}	0.000 ^{HS}

-HS =highly significant (p-value ≤ 0.001)

-NS =Non-significant (p-value >0.05)

** Overall p value for Inter group comparison

DISCUSSION

The reconstruction of the interdental papilla is one of the most complex periodontal treatments. The loss of papilla can lead to deformities so-called "black triangle disease". Not only does it presume a major aesthetic importance, but it also plays a significant role in the protection of underlying periodontal tissues. Its complex anatomy renders the papilla sensitive to recession, which makes papillary enhancement a very challenging periodontal procedure ⁽⁶⁾.

Hyaluronic acid (HA) is a tissue volumizing agent that promotes healing qualities by assisting tissue hydrodynamics, cell migration, and proliferation. HA has been found to be a promising therapy option for interdental papilla defects in numerous studies showing complete interdental papilla reconstruction ⁽¹³⁾. In the present study, HA was injected for the repair of missing interproximal tissue that resulted in considerable modifications. This may be attributed to its biochemical capabilities that gives tissues flexibility, stability and assists in tissue regeneration ⁽¹⁴⁾.

A novel approach for the regeneration of interdental papilla is the use of injectable platelet rich fibrin (iPRF) that has been found to have more regenerative cells and higher concentration of growth factors than PRF due to its shorter centrifugation time. This was found to aid in tissue regeneration and thus may be used for increasing interdental papillary height with excellent effects ⁽¹⁵⁾. I-PRF has several advantages over hyaluronic acid. It is autologous reducing the risk of immune inflammation reaction, ease of availability, preparation and use. In addition it is cost effective. Also, it is an alternative option for patients who are allergic to hyaluronic acid ⁽¹⁶⁾.

Regarding patient satisfaction, the results of the current study showed that for all 3 groups, the satisfaction of patients after 6 months of follow-up was significantly higher than before starting the treatment.

In line with our results for HA group (1), a recent study performed in 2020 ⁽¹⁷⁾ showed that most of the study subjects were very satisfied with the HA injection results esthetic wise even in cases where papilla fill was not completely achieved. Furthermore, the satisfaction rate had additional motivating effect on the patients' willingness to take extra good care of their periodontal health.

In terms of black triangle height, the results of the current study showed a percentage change in HA group (1) achieving the highest percentage change (92.35%), iPRF group (2) showed (53.54%), while the mix group (3) has the lowest change (46.80%). This is attributed to the superior properties of hyaluronic acid that forms a key component of connective tissue and assists tissue regeneration ⁽¹⁷⁾.

In a study performed in 2020 ⁽¹¹⁾, HA injections resulted in a statistically significant decrease in black triangle height over the course of 6 months, with around 8%, 39%, and 29% reductions at 3 weeks, 3 months, and 6 months, respectively. Those findings are consistent with the finding of our present study concerning HA group (1).

In contrast to our findings, a study conducted in 2017 ⁽¹⁸⁾ found no significant differences at baseline, 3 or 6 months after HA injection ($p=0.734$). Such discrepancies in results could be due to changes in the substance and concentration employed, technique and site of injection and the apparent size and type of pre-treatment papillary loss.

In coordination with our present study regarding iPRF group (2), a study conducted in 2017 ⁽¹⁹⁾, PRF was used in the anterior aesthetic region to reconstruct the interdental papilla, observing excellent fill in the interdental region at 3 and 6 months postoperatively, suggesting that PRF may be a solution for interdental papilla augmentation.

In contrast with our clinical findings, a recent study performed in 2021 ⁽¹⁶⁾ where iPRF was compared with HA injections for reconstruction of black triangles indicated that iPRF showed equally

significant results as HA at 1 and 3 months' time interval. This differed from our results that showed a remarkable variance between HA group 1 and the 2 other groups with the highest difference in change in the HA group 1.

CONCLUSION

For interdental papilla reconstruction, the application of hyaluronic acid injections and injectable platelet rich fibrin was considerably effective and can be an alternative non-invasive treatment method for gingival black triangles which responds to the growing esthetics demands of patients.

RECOMMENDATIONS

Further studies of using hyaluronic acid and injectable platelet-rich fibrin for the regeneration of inter-dental papillary defects is needed.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

FUNDING

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