



“EVALUATION OF I-PRF INJECTION TECHNIQUE AS AN ALTERNATIVE FOR RECONSTRUCTION OF INTERDENTAL PAPILLAE - A PILOT STUDY”

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ABSTRACT One of the most challenging and least predictable procedures in periodontal plastic surgery is the reconstruction of the lost interdental papilla. Preserving the integrity of the papillae is of utmost importance. Different variations of surgical procedures using connective tissue grafts have proved to be successful. Being less invasive in nature, non-surgical techniques such as hyaluronic acid injections have turned out to be a boon in this regard. Platelet concentrates like PRF & PRP have shown promising results when used in surgical reconstruction of the lost papilla. One of the recent advancements in PRF formulations is the injectable PRF which is a liquid concentrate encompassing higher leukocyte numbers, which resulted in higher total growth factor release, requiring slower and shorter centrifugation speeds for preparation. i-PRF was shown to be a potent inducer of cell proliferation, migration and angiogenesis, inducer of higher fibroblast migration and expression of PDGF, TGF- β , and collagen, thereby playing a vital role in tissue regeneration. With the current understanding, this study was designed to investigate the efficiency of iPRF in achieving interdental papillary enhancement. We have noted that multiple doses of this autologous formulation prove to be a non-invasive alternative for the treatment of minimal interdental papilla loss; primarily Norland & Tarnow's Class I and to an extent Class II interdental papilla loss. Further validations of this technique are required.

KEYWORDS : interdental papilla; PRF; iPRF ; growth factors ; papillary enhancement .

INTRODUCTION

'Periodontal plastic surgery' a name adopted by the World workshop in clinical periodontics, was originally proposed by Miller in 1993, included procedures like periodontal and prosthetic correction, crown lengthening, ridge augmentation, esthetic surgical correction, coverage of denuded root surface and reconstruction of interdental papillae. One of the most challenging and least predictable problems in periodontal plastic surgical procedure is the reconstruction of the lost interdental papilla, commonly known as *BLACK TRIANGLE*. The loss of papillae may cause functional, phonetic and severe esthetic problems especially in the maxillary anterior region. The etiological factors for interdental papilla loss can be due to periodontal disease, malocclusion, trauma, etc. The physiology of the papilla is more complex, that it is a biologic barrier to protect deep periodontal tissues. So it's very important to respect the papilla's integrity during routine dental practice.

Periodontists have attempted to reconstruct this lost papilla by numerous surgical methods like free gingival grafting, pedicle graft procedure¹ and use of an interposed connective tissue graft². However, these techniques are all invasive and mostly unpredictable. The subepithelial connective tissue graft have resulted in high esthetics but disadvantage of this procedure includes the need for an additional donor site and its technical difficulty. Shapiro et al has demonstrated two cases of regeneration of interdental papillae using periodic curettage of papillae³. Application of non-invasive techniques such as the use of commercially available hyaluronic acid gel injections have shown to be effective for papilla reconstruction (Mansouri S et al 2013¹², Tanwar J et al 2016¹³).

Platelet rich fibrin (PRF) is a type of platelet gel; a matrix of autologous fibrin, which has scored over platelet rich plasma by virtue of its properties, ease of preparation, and cost effectiveness. Platelet cytokines, platelet derived growth factors (PDGF)- α and (PDGF)- β , transforming growth factor beta (TGF)- β and insulin-like growth factor-1 (IGF-1) are gradually released as fibrin matrix is resorbed, aiding the process of healing. These growth factors are known to modulate and up regulate one growth factors function in the presence of second or third growth factor.²¹ Advantages of using PRF include, a) need for donor site is eliminated, b) less invasive technique, c) lessens postsurgical discomfort, d) promotes rapid soft tissue healing with less edema compared to connective tissue graft and enamel matrix derivative technique. It is easy to prepare and lacks biochemical handling of blood, which makes this preparation strictly autologous.

In the dental field, PRF has been postulated as promoters of tissue regeneration and utilized for the treatment of extraction sockets^{14,15}, gingival recessions¹², palatal wound closure¹⁵⁻¹⁷, regeneration of

periodontal defects⁸, and hyperplastic gingival tissues⁹. Arunachalam et al²² and Ahila E et al²⁴ have shown a successful surgical reconstruction of the interdental papillae using PRF membrane and when reviewed at 3 and 6 months postoperatively, it was shown to be stable in the new position.

Development of an injectable formulation of PRF (termed i-PRF) based on low-speed concept for blood centrifugation by Ghanaati et al. has been pursued with the aim of delivering to clinicians an easy to use platelet concentrate in liquid formulation which can be either utilized alone or in combination with various biomaterials¹⁶. Taking advantage of slower and shorter centrifugation speeds, a higher presence of regenerative cells with higher concentrations of growth factors can be observed when compared to other formulations of PRF. Miron et al in his in-vitro study has concluded that i-PRF demonstrated the ability to release higher concentrations of various growth factors and induced higher fibroblast migration and expression of PDGF, TGF- β , and collagen¹⁰.

The present study is conducted to analyze the benefit of iPRF injection technique for treatment of black triangles.

MATERIALS AND METHODS

The study population was selected from the outpatient department of M R Ambedkar dental college and Hospital , Bangalore, Karnataka India, belonging to both the genders. Approval from the Ethical Committee of M.R. Ambedkar Dental College and Hospital was obtained. The Patients were screened for black triangles through clinical evaluation. Measurements of the black triangles were done clinically using the periodontal probe from the tip of the papilla to the contact point of the associated teeth and keeping the interdental contact area as a reference. Inclusion criteria - systemically healthy, non smoker subjects, age range 25-55 years, at least one papillary deficient site in the upper anterior region – Class I or Class II Norland and Tarnow's classification¹⁷ (Figure 1) , and with a plaque index¹⁹ and gingival index²⁰ between zero and one, noncontributory medical history, and no consumption of drugs causing gingival hyperplasia. Vertical distance from interdental contact point to the crest of interdental bone was >5mm measured by bone sounding, Presence of a band of keratinized tissue around the test teeth was > 2mm. Patients with presence of periodontal pocket, attachment loss, malocclusion and pregnant & lactating patients were excluded from the study.

Pre-op and Post-op patient photographs were taken perpendicular to teeth of interest, under the same lighting conditions at each visit with the same digital camera. The patients were seated in an upright position, looking straight ahead. It was ensured that the same shooting positions were reproduced at each visit. The papillary enhancement

was assessed clinically at regular time intervals (at 3 weeks, 3 and 6 months) using the N & T scoring system by Norland and Tarnow.^{17,18} The actual change in black triangle space cannot be measured clinically. Therefore, the clinical term for improvement after papillary deficiency treatment was reported as a papillary enhancement rather than black space reduction. Photographically, reduction in black space and reduction in clinical measurements were suggestive of papillary enhancement.

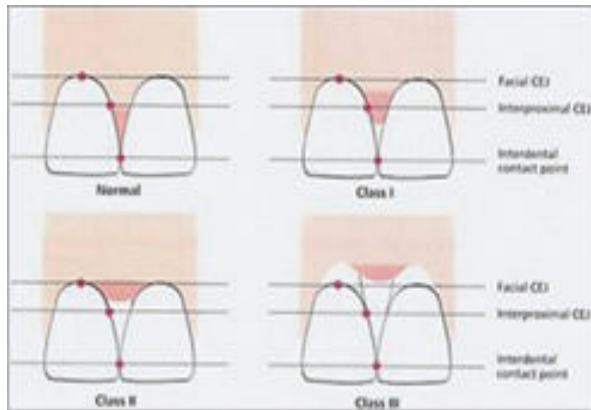


Figure 1 – Norland and Tarnow classification.

TREATMENT PROTOCOL

The treatment protocol was explained to the patient and an informed consent was obtained. After the phase one periodontal therapy, the clinical parameters were recorded. All clinical measurements were carried out by a single calibrated examiner to ensure an unbiased evaluation and rule out interexaminer variability. Injection procedure was performed by the second operator to avoid intraoperator variation.

Local anaesthesia was administered at the treatment site. iPRF was prepared as per the protocol put forth by Chokroun et al.¹ For i-PRF preparation, two plastic tubes of 5 ml of whole blood each without anticoagulant were centrifuged at 700 rpm for 3 min (60×g) at room temperature in a Centrifuge. The resulting upper liquid layer was collected using an insulin syringe as i-PRF and immediately injected 2–3 mm apical to the tip of the papilla before the liquid turned into a gel consistency. Since it is autologous, the entire obtained concentrate was injected, without any predetermined quantity. The patients were instructed not to brush on the day of treatment, resume oral hygiene the day after using a soft toothbrush at the anterior teeth and place it coronal to the gingival margin and avoid using dental floss at the sites of treatment. The patients were followed up at 3 weeks and were recalled 3 times. At the first follow-up (3 weeks later) the patients did not show any significant improvement, therefore; we decided to perform multiple shots of iPRF injections at an interval of 3 weeks, 2 more times. Follow-up at 6th month was done to assess the sustainability of the esthetic results.

STATISTICAL ANALYSIS

Means and standard errors (SE) were calculated, and data were analyzed for statistical significance and Intra group comparison was done by one way ANOVA test using SPSS software. (SPSS Software, Inc., La Jolla, CA, USA).

RESULTS

A total of 10 interdental papillae in 10 patients with a mean age of 37.5± 14.4 yrs were evaluated. Table 1 shows the demographic distribution in the study group. Of 10 interdental papillae, 8 were of females and 2 were of males. In terms of periodontal status, 9 papillae were type I and the remaining were type II and 7 were in the maxilla and remaining 3 were in the mandible. iPRF injections were uneventful. Slight tenderness at the injection site, was reported and typically lasted for the first 1–2 postoperative days.

The results of the papillary measurements are presented in Table 2 and Fig 2 and representative photographs are shown in Fig. 3-8. Differences between baseline and 3 months were statistically significant (p < 0.0001). The changes from baseline to 3 months represent an average of 41 % papillary enhancement. At 3 months, 5 sites had ≥50 % papillary enhancement, while at 6 months the results were maintained.

Table 2 shows that at the first follow up (3 weeks after the injection), 10-20% papillary enhancement with a mean of 13.38± 3.07% was observed. In the second follow up (three months later) 15-63% enhancement with a mean of 29.52± 18.72% and at the third follow up (6 months after the injection), 33-69% enhancement with a mean of 47.33± 20.20% were noted.

TABLE 1

Age & Gender distributions among study subjects			
Variables	Categories	n	%
Age Groups	25-35 yrs	4	40%
	36-45 yrs	4	40%
	46-55 yrs	1	10%
	> 55 yrs	1	10%
Sex	Males	2	20%
	Females	8	80%

TABLE 2

PAPILLARY ENHANCEMENT				
NUMBER	NUMBER	MIN PERCENTAGE	MAX PERCENTAGE	MEAN & STD DEV
THREE WEEKS	10	10	20	13.38±3.07
THREE MONTHS	10	15	63	29.52±18.72
SIX MONTHS	10	33	69	47.33±20.20

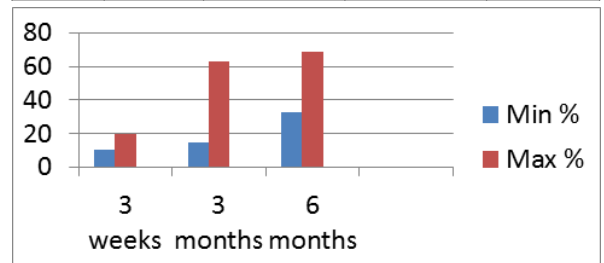


Figure 2

DISCUSSION

Esthetic problems after the loss of interdental papilla especially following periodontal surgeries have been extensively reported in literature. Several surgical techniques have been put forth in this direction but were invasive and mostly unpredictable. Jaiswal et al²² and Carnio J¹ have tried subepithelial connective tissue graft along with coronally advanced flap for reconstruction of dental papilla in patients with N & T's type I & II papillae. At 6 & 12 months follow up, they have demonstrated significant improvement. Thus, considering its traumatic and invasive nature, use of a safer and non-invasive techniques should be considered as a suitable alternative. Mansouri SS et al. had concluded that injection of hyaluronic acid gel significantly contributed to treatment of black triangle in the esthetic zone¹⁵. Promising results in this regard has been portrayed in recent times by platelet concentrates.

PRP and PRF, both of which utilizes supra-physiological doses of autologous growth factors derived from the patient's own blood, is further capable of speeding up tissue regeneration. Sharma P et al²³ and Ahila et al²⁴ have reported statistically significant enhancement of interdental papilla, with a novel surgical technique using PRF membrane when reevaluated at 3 and 6 months postoperatively. Despite their widespread use, concerns have been expressed regarding the use of anti-coagulants in PRP which was initially added to centrifugation protocols in order to maintain the liquid consistency of PRP to facilitate biomaterial mixing. On the other hand, initial PRF formulations lacked a liquid concentrate of proteins, as the standardized PRF membrane contains the majority of its growth factor concentration encapsulated within its fibrin matrix. For these reasons, major development and advancements were recently made with the aim of developing a liquid formulation of PRF which does not contain any anti-coagulants or fibrin matrix. These advancements were made possible due to the recent findings by Ghanaati et al. who introduced the low-speed concept for blood centrifugation whereby lower centrifugation speeds were shown to contain higher numbers of cells including leukocytes prior to the formation of a fibrin clot¹⁶.

Leukocytes are immune cells having vast importance in tissue regeneration by directing and recruiting various cell types during the wound healing process. It was recently hypothesized that by reducing centrifugation G-force, a total increase in leukocyte numbers would remain in the top third layer of platelet concentrate tubes where PRP and PRF are collected. The added number of cells contained within this fibrin matrix was further shown to release higher total growth factor release of PDGF, TGF- β 1, VEGF, EGF, and IGF when compared to control L-PRF. i-PRF was shown to be capable of influencing cell activity of gingival fibroblasts. i-PRF formed a small clot likely as a result of fibrin components that acted as a dynamic gel with cells likely contained within its hydrogel. It is therefore hypothesized that even following 10 days, an additional release of growth factors could still be expected from i-PRF^[1,10].

To date, no investigative study has been conducted using this material for treatment of interdental papilla loss. Based on the available literature, it can replace conventional invasive methods for papilla reconstruction after gaining approval through further assessments. It should be noted that iPRF is a potent inducer of cell proliferation, migration and angiogenesis and plays an important role in tissue regeneration. Minimal or no side effects can be expected due the fact that it is autologous, hence multiples injections of iPRF done here did not pose any threat to the patient. The present study assessed the clinical outcomes of using an injectable iPRF gel for interdental papillary enhancement in anterior teeth. The results indicate that papillary enhancement can be obtained (highest achieved being 63%), although there is great variability in outcomes and complete fill of the lost papillary area is uncommon. As this study highlights that a single injection may not suffice, hence multiple injections of iPRF (3 or maybe more) maybe required to achieve the desired result. These results need to be validated with further longitudinal studies with larger sample sizes. The present study is the first one to document outcomes following iPRF gel injection for interdental papillary loss. Nevertheless it is imperative to note that this technique may prove to be helpful only in cases of minimal loss of inter-dental papillae (N & T's Class I and to an extent class II) and not beyond.



Fig 3 – Prepared iPRF gel



Fig 4 – i-PRF collected with insulin syringe



Fig 5 – Preoperative



Fig 6 – Clinical measurement



Fig 7 – iPRF injection 2–3 mm apical to tip of papilla



Fig 8 – Postoperative

CONCLUSION

This study paves way to a promising technique for regeneration of interdental papilla using a nonsurgical approach. Being nontoxic and nonimmunoreactive and encompassing the property of modulating periodontal fibroblasts, iPRF can achieve papillary enhancement to an extent. This approach limits the use of surgical procedures, thereby keeping a check on patient morbidity and compliance. For generalization of these results, further randomized controlled clinical trials are needed with higher sample size depending upon the size of the black triangle.

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