

## Original Research

### Effect of injectable platelet rich fibrin (i-PRF) on thin gingival biotype: A clinical trial

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#### ABSTRACT

**Introduction and Aim:** The term gingival biotype has been used to describe the thickness of the gingiva in the faciopalatal dimension. A gingival thickness of  $\geq 2$  mm is defined as thick biotype and a gingival thickness of  $< 1.5$  mm as thin biotype. A thin gingival biotype is considered as an important predisposing factor for gingival recessions. Gingival recession being common esthetic problem encountered in routine dental practice, an attempt should be made to increase the gingival thickness in order to prevent gingival recession. A liquid injectable-platelet-rich fibrin (i-PRF) is a rich source of autologous growth factor and is recently being used in regenerative dentistry. So, the aim of this study was to evaluate the effect of i-PRF on thin gingival biotype.

**Methodology:** Forty sites from systemically healthy patients having thin gingival biotype were included in this study. i-PRF was injected at the selected site using microneedle at baseline, 1 week and 2 weeks. Plaque index (PI), gingival index (GI), gingival thickness (GT) and keratinized tissue width (KTW) were assessed before the treatment and one month and three months after the final injections.

**Results:** At baseline mean PI, GI, GT and KTW were 0.63, 0.90, 0.55mm and 5.25mm respectively. At one month and three months follow up, there was statistically significant difference seen in PI, GI, GT and KTW. Mean GT increased from 0.55mm at baseline to 1.05mm and 1.03mm at 1month and 3months respectively.

**Conclusion:** i-PRF using microneedle can be effective for increasing gingival thickness in subjects having thin gingival biotype.

#### INTRODUCTION

Marked transformation in the field of periodontics has led to the development of newer, less invasive therapeutic approaches along with improved diagnostic methods, which allows proper evaluation and management of surrounding tissues to provide best outcome of periodontal therapy. Minimally invasive treatment helps to achieve satisfactory therapeutic results with minimum trauma to tissues of any interventional process.<sup>1</sup>

The gingival biotype and different parts of the masticatory mucosa have become the subject of considerable interest in Periodontics, especially from an aesthetic and therapeutic perspective.<sup>2</sup> The size of the alveolar bone, tooth morphology, events that occur during tooth eruption and the final position of the tooth when tooth is fully erupted determine the architecture of the gingival tissue. The term 'gingival biotype' has been used to describe the thickness of gingiva in the facio-palatal dimension.<sup>2</sup> According to Ochsenbier and Ross

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(1969), gingival biotypes can be classified as scalloped and thin or flat and thick gingiva.<sup>3</sup> A gingival thickness of  $\geq 2$  mm is defined as thick biotype and a gingival thickness of  $< 1.5$  mm as thin biotype.<sup>3</sup>

In general, gingival biotype can be evaluated by direct visual assessment only, visual assessment with the aid of a periodontal probe i.e. if periodontal probe, when placed into the gingival sulcus, is visible through gingival sulcus, gingival biotype is considered as thin and when periodontal probe is not visible through gingival sulcus then it is considered as thick gingival biotype; ultrasonic devices, cone-beam computed tomography scan and direct measurements. Visual assessment methods for evaluation of gingival biotype can differentiate it as thick or thin, but direct measurement techniques provide us with actual measurement of gingival thickness.<sup>4</sup>

Thick gingival biotype is characterized by wider zone of the keratinized tissue and flat gingival contour while thin gingival biotype is characterized by thinner zone of keratinized tissue with scalloped gingival margins. Thick gingival biotype being suggestive of thick bony architecture is considered more resistant to inflammation and trauma as compared to thin gingival tissue which is suggestive of thin bony architecture and is more prone to inflammation and trauma.<sup>3</sup>

A thin gingival biotype and mucogingival conditions such as absence or reduction of keratinized tissue; and/or probing depths extending beyond the mucogingival junction are considered as important predisposing factors for gingival recession.<sup>5</sup> Gingival recession being common esthetic problem encountered in routine dental practice, an attempt should be made to increase the gingival thickness in order to prevent gingival recession. Although soft tissue grafts have been used in literature to increase the width of attached gingiva as well as for treatment of gingival recession, yet no therapy is available to prevent gingival recession by increasing the thickness of gingiva.

Platelet concentrations have been utilized in dentistry for over three decades as a regenerative tool capable of releasing supra-physiological doses of growth factors viz. transforming growth factor-B (TGF-B), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epidermal growth factor (EGF) and platelet-derived epidermal growth factor (PDEGF) which are responsible for inducing tissue regeneration derived from autologous sources.<sup>6,7</sup>

Over the past decade, PRF has gained enormous popularity in the dental field for the treatment of extraction sockets, gingival recessions, palatal wound closure and regeneration of periodontal defects.<sup>6</sup> In 2014, a liquid injectable-platelet-rich fibrin (I-PRF), having similar qualities of PRF was developed by modifying spin centrifugation forces, and by utilizing non-glass centrifugation tubes.<sup>8</sup> Mourao et al in 2015 gave detailed technical note on preparation of i-PRF.<sup>[9]</sup> Much like traditional PRF, i-PRF contains an increase in leucocyte number and is further able to stimulate growth factor release. Injectable PRF also has the property of bonding with the graft materials and thus facilitating the proper adaptation of the defect sites.<sup>10</sup>

In a systematic meta-analysis by Moraschini and Barboza Edos and clinical studies by Keceli et al and Gupta et al., when effect of PRF was evaluated on gingival tissues an increase in the gingival thickness was noted.<sup>11</sup> As i-PRF shares similarities with PRF in terms of tissue regeneration and minimally invasive treatment minimizes trauma to the tissues, the aim of this study was to evaluate the effect of i-PRF using microneedle on thin gingival biotype.

## MATERIALS AND METHODS

After routine oral and physical examination, forty sites with thin gingival biotype ( $< 1.5$ mm) from systemically healthy individuals between 20-40 years of age, visiting

Department of Periodontics were selected for this study. Number of sites were statistically calculated. Non-compliant patients, patients having any blood anomalies, pregnant or lactating women and smokers were excluded from the study.

Institutional Ethics Committee approved this study. Then the patients were thoroughly explained about the procedure and written informed consent was obtained.

## DATA COLLECTION

Plaque index by Silness and Loe (PI), gingival index by Loe and Silness (GI), gingival thickness (GT) and keratinized tissue width (KTW) were assessed at baseline and one month & three months after the final injections.

The methods used for evaluation of gingival thickness included visual assessment with aid of probe and direct measurement. For visual assessment, if periodontal probe, when placed into the gingival sulcus, is visible through gingival sulcus, gingival biotype is considered as thin and when periodontal probe is not visible through gingival sulcus then it is considered as thick gingival biotype. For direct measurement of gingival thickness, a reamer was inserted transgingivally into the anaesthetized selected site, stopper was used as the reference point (Fig IA) and the exact measurement was recorded with the help of digital Vernier caliper [Thermo Aerospace Stainless Steel Digital Vernier Caliper - 300mm] (Fig. IB).

Keratinized tissue width was measured from gingival margin to mucogingival junction with the help of calibrated periodontal probe (UNC 15 probe).



**Fig I a:** Direct measurement of gingival thickness using endodontic reamer

**b:** Measurement recorded on digital vernier caliper

## Preparation and injection of i-PRF:

After collection of 5 ml of peripheral blood from each subject, blood was transferred to a glass coated plastic vacutainer tube without anticoagulant. Then this tube was immediately centrifuged at 700 rpm for 3 mins [mlabs Clinical Doctor Centrifuge Machine 5000rpm.<sup>8</sup>After centrifugation, two fluid layers were formed, upper being yellow fluid layer (i-PRF) while lower layer containing red blood cells. Yellow fluid layer containing i-PRF was immediately aspirated<sup>8</sup> using microneedle (Fig II).



**Fig II:** iPRF aspirated with microneedle

The selected site was cleaned and anaesthetized with Lidocaine 10% spray (Lox 10% spray, Neon Laboratories limited, Mumbai.) and then i-PRF was

injected at the selected site into the gingival sulcus using microneedle [0.25mm (31G) x 6mm needle, BD Glide™ needle insulin syringe] till the blanching and fullness of gingiva was noted (Fig III). i-PRF was again injected at the same site after 1 week and after 2 weeks from the baseline final i-PRF injection was given.



**Fig III:** iPRF injected into the gingival sulcus

### STATISTICAL ANALYSIS

The data for plaque index, gingival index, gingival thickness and keratinized tissue width for the total subjects was represented as Mean and Standard deviation. The evaluation of variation in the clinical parameters from baseline to 1month and 3months was done by one-way ANOVA test and p-value  $\leq 0.05$  was considered statistically significant.

### RESULTS

The results for the mean plaque index, gingival index, gingival thickness and keratinized tissue width at baseline, 1 month and 3 months are shown in Table: 1. During this study, no adverse reactions were noted and none of the participant exited from the study.

**Table 1:** Comparison of mean plaque index, gingival index, gingival thickness and keratinized tissue width at baseline, 1 month and 3 months

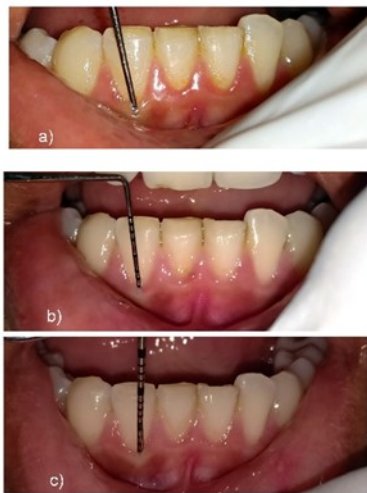
Interval	N	Mean	Std. Deviation	F value	p value
Baseline PI	40	0.63	0.628	3.904	0.036*
1 Month PI	40	0.40	0.591		
3 months PI	40	0.37	0.490		
Baseline GI	40	0.90	0.632	19.349	0.001*
1 Month GI	40	0.25	0.439		
3 months GI	40	0.22	0.530		
Baseline GT	40	0.55	0.504	27.568	0.001*
1 Month GT	40	1.05	0.316		
3 months GT	40	1.03	0.423		
Baseline KTW	40	5.25	1.354	3.793	0.027*
1 Month KTW	40	5.38	1.372		
3 months KTW	40	5.42	1.448		

**Plaque index and Gingival index:** Mean plaque index at baseline, 1 month and 3 months was 0.63, 0.40, 0.37 respectively while mean gingival index at baseline, 1

month and 3 months was 0.90, 0.25 and 0.22 respectively. Both the indices showed statistically

significant difference ( $p \leq 0.05$ ) at baseline, 1 month and 3 months examination.

**Gingival thickness and keratinized tissue width:** Mean gingival thickness at baseline, 1 month and 3 months was 0.55mm, 1.05mm and 1.03mm (Fig IV) respectively while mean keratinized tissue width at baseline, 1 month and 3 months was 5.25mm, 5.38mm and 5.42mm respectively. Both these parameters also showed statistically significant difference ( $p \leq 0.05$ ) at baseline, 1 month and 3 months examination.



**Fig IV:** Gingival thickness at a) baseline, b) 1 month and c) 3 months

## DISCUSSION

Aesthetics, along with combined effect of form and function strongly influence the modern dental practice. To achieve a successful aesthetic outcome in periodontal therapy, dental implants, and restorative procedures, a thorough understanding of how tissue responds to therapy is of critical importance.<sup>2</sup> Evidences suggest that in patients with thin biotypes the frequency of gingival recession is high<sup>3</sup> following regenerative periodontal therapy, implant placement<sup>3</sup> and restorative treatment. Thick gingival tissues are more resistant to mucosal

recession or mechanical irritation.<sup>3</sup> Andereg et al measured the thickness of mucoperiosteal flap to evaluate whether the thickness of tissue used to cover guided tissue membrane influences postsurgery recession, they found that there was less post treatment gingival recession for tissue thickness  $>1$ mm than tissue thickness  $< \text{or} = 1$ mm.<sup>12</sup> Also, Baldi et al found better clinical outcome of coronally advanced flap for root coverage in thick gingival tissue as compared to thin gingival tissue.<sup>13</sup>

Lang & Loe demonstrated that although tooth surfaces may be kept free of clinically detectable plaque, areas with  $<2$  mm of keratinized gingiva tended to remain inflamed.<sup>14</sup> When relationship of dental implants with mucosal thickness was considered, Linkevicius et al (2009) observed an increased peri-implant bone loss in areas where tissue was thinner than 2mm.<sup>15</sup> Wennstrom and Derks (2012) also reported that mucosal thickness has a major influence on the degree of early peri-implant bone loss.<sup>15</sup> As thin gingival tissue and reduced keratinized tissue width leads to gingival recession as well as influence the outcome of implant therapy, there is a need to convert a thin gingival tissue to a thick gingival biotype in order to prevent gingival recession and to have predictable outcome of procedure.

Although initially in liquid phase, macroscopic findings demonstrate that i-PRF initiates a fibrin polymerization process by acquiring a gel phase over time.<sup>8</sup> i-PRF forms a dynamic fibrin gel, rich in platelets and leukocytes, acting as a scaffold for wound healing.<sup>8</sup> The three-dimensional fibrin matrix also plays a key role in tissue repair although most studies have primarily focused on the effect of growth factors. Fibrin acts as a scaffolding biological material for agglomeration of adherent cells at the site of tissue healing.<sup>16-18</sup> Additionally, fibrin is a carrier of growth factors in a well-controlled release system that sustains proper bioactivity over the healing period.<sup>19,20</sup>

The fibrin network structure and leukocyte concentration in L- and i-PRF are known to influence growth factor release from platelet derived concentrates.<sup>8</sup> Studies revealed a high release rate of PDGF and VEGF for 8 hrs and 24 hrs respectively, followed by a gradual decrease with time.<sup>[21]</sup> VEGF is a potent angiogenic factor and PDGF acts as a chemoattractant for cells of mesenchymal origin including fibroblasts providing a role in periodontal regeneration.<sup>22</sup>

Richard J. Miron et al in 2017 compared standard PRP and i-PRF for growth factor, fibroblast biocompatibility; fibroblast migration and proliferation expression of PDGF, TGF- $\beta$ , and collagen 1, found that i-PRF demonstrated the ability to release higher concentrations of various growth factors and induced higher fibroblast migration and expression of PDGF, TGF- $\beta$ , and collagen1.<sup>6</sup>

In our study, a significant increase in gingival thickness and keratinized tissue width was observed at all periods (1, 3 months) compared to baseline i.e.  $p = 0.001$  for gingival thickness and  $p = 0.027$  for keratinized tissue width respectively. These outcomes are in agreement with previous study by Ozsagir Z.B. et al in 2018 who randomly treated patients with thin gingiva. They injected i-PRF on one side with 30 gauge needle while on other side with 24 gauge needle and observed increase in gingival thickness within both 24 gauge and 30 gauge needle groups. But statistically significant increase in KTW was only seen in 30 gauge needle group.<sup>23</sup>

Padma et al showed an increase in KTW in subjects who were treated with PRF along with coronally advanced flap<sup>24</sup> while Woodyard et al noted an increase in gingival thickness with coronally positioned flap plus an acellular dermal matrix.<sup>25</sup> Although both of these studies showed an increased gingival thickness and keratinized tissue width, the treatment modality used was invasive in nature. In our study we have obtained a significant

increase in gingival thickness and keratinized tissue width using a minimally invasive procedure which resulted in better patient comfort and less tissue trauma.

Also, Tuttle D et al in 2018 demonstrated a new biological approach involving blood derivatives A-PRF and i-PRF combined with a minimally invasive surgical approach (Gum drop technique) for root recession elimination which showed faster healing without the need for a donor site to provide connective tissue.<sup>26</sup>

## CONCLUSION

Various approaches are available in literature for treatment of gingival recession. But very few treatment options are available for prevention of gingival recession. Also, the treatment options which are available for correction of gingival recession mostly involves invasive surgical procedures. As thin gingival biotype is more prone to gingival recession, we can prevent gingival recession by increasing the gingival thickness.

Therefore, within the limitations of this study viz., small sample size, no control group, short follow up period, it can be concluded that injection of i-PRF with microneedle, a minimally invasive procedure, may be beneficial for increasing the gingival thickness and could also play a role to increase the keratinized tissue width.

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