



## COMPARATIVE EVALUATION OF THE EFFECT OF INJECTABLE PLATELET RICH FIBRIN (I-PRF) ON GINGIVAL THICKNESS IN INDIVIDUALS WITH THIN PERIODONTAL PHENOTYPE – A CLINICAL STUDY.

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### **ABSTRACT**

**Aim** – Addressing the challenges of thin gingiva through minimally invasive augmentation techniques has gained significant attention in periodontal therapy. The present study was carried out to evaluate the effect of I-PRF on gingival thickness as a minimally invasive procedure to enhance the periodontal phenotype.

**Materials and method** – The individuals were categorised into thin and thick phenotypes using visual inspection and the Probe Transparency test. A total of 18 sites in individuals with thin phenotype were selected. I-PRF was prepared using Choukrons protocol (700 rpm for 3 mins). The site with thin phenotype were injected with 0.2ml I-PRF at baseline, 1 week and 2 weeks. The clinical parameters of plaque index, gingival index, width of keratinized tissue and thickness of gingiva were evaluated at baseline, 1 month and 3 months.

**Results** - The data thus obtained was subjected to statistical analysis. The injection of I-PRF showed a significant increase in both width of keratinized tissue and thickness of gingiva at 1 month and 3 months.

**Conclusion** – The injection of platelet rich fibrin in the gingiva may help modify thin periodontal phenotype to thick. It can also help in increase of width of keratinized tissue.

**Keywords** – thin periodontal phenotype, injectable platelet rich fibrin, soft tissue augmentation

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### **INTRODUCTION**

Smile is the universal language they say and a beautiful smile is desired by one and all. A harmonious balance of the white and the pink enhances the esthetics of smile. Usually, dentists and technicians are well versed when it comes to reconstructing the white component of a smile, the teeth. However, the pink component if not restored correctly, will impair the final three dimensional esthetic outcome. The importance of pink has been gaining its pace in dentistry as the importance of soft tissues is being recognized by clinicians as well as demanded by the patients.

In addition to gingival parameters such as colour, form and contour, position of the gingival zenith and height of the interdental papilla, the assessment of gingival biotype is gaining substantial interest as one of the important biological factors in diagnosis and treatment planning to achieve long term stability of periodontal health.

Ochsenbien and Ross (1969), classified gingival biotype as scalloped and thin or flat and thick gingiva. Seibert and Lindhe 1989 considered a gingival thickness of  $\geq 2$  mm as thick biotype and a gingival thickness of  $< 1.5$  mm as thin biotype<sup>1</sup>. Another category of the periodontal phenotype are “thin-scalloped,” “thick-scalloped,” and “thick-flat” patterns<sup>2</sup>. The 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions recommended the adoption of the term “periodontal phenotype”. They described periodontal phenotype as combination of gingival phenotype (gingival thickness and width of keratinized tissue) and bone morphotype (buccal bone plate thickness). The gingival thickness is assessed by observing the periodontal probe by transparency through the gingival tissue after being inserted into the sulcus. Thus, it is assumed that the probe will be visible when gingiva is thin ( $\leq 1$  mm) and not visible in a thick gingiva<sup>3</sup>.

Thin phenotype requires special considerations during esthetic, restorative, orthodontic, prosthodontic and periodontal therapy. Patients with a thin phenotype are more vulnerable to connective tissue loss and epithelial damage, thus, they need special atraumatic tissue manipulation<sup>4</sup>. They are considered to have a higher risk of aesthetic complications after surgical or restorative treatments. Thin biotype is also less stable, and the occurrence of the marginal gingival recession is more commonly seen. Periodontal plastic surgeries are often required for gingival recession coverage. But these techniques are invasive which may lead to intense postoperative pain and donor site morbidity. Also other disadvantages include limited availability of donor tissue and increased operating time.

Recently, another treatment modality for enhancing periodontal phenotype is becoming popular which is the use of autologous blood derived platelet concentrates called as Platelet Rich Fibrin (PRF). In 2014, injectable platelet-rich fibrin (I-PRF) was developed by modifying spin centrifugation force<sup>5</sup>. I-PRF can provide a significant advantage for the regeneration process, as it is rich in platelets, leucocytes and growth factors. I-PRF clots and turns into gel form after approximately 10–15 min and preserves its content in the tissue for sustained release<sup>6</sup>. I-PRF shares similarities with PRF in terms of tissue regeneration. It uses growth factors to promote periodontal regeneration is an important strategy for the reestablishment of structure and function<sup>7</sup>.

Currently in literature there are very few treatment options known for prevention of gingival recession and other clinical/surgical complications of thin phenotype. By formulating a protocol to increase the thickness of gingiva at prophylactic level, invasive surgical procedures and foreseen drawbacks of thin phenotype may be avoided. Injection of I-PRF in gingival sulcus is a minimally invasive procedure which is cost effective with no side effects and minimum pain.

## **MATERIAL AND METHOD**

The study was conducted at Department of Periodontology. The research proposal was approved by the Institutional Ethics Committee (IEC). Individuals visiting the Outpatient Department of Periodontology were carefully selected based on the inclusion and exclusion criteria.

### **Inclusion criteria:**

1. Systemically healthy individuals.
2. Age group 18 years and above.
3. Individuals with  $< 1.5$ mm gingival thickness.

4. Patients who had completed Phase I therapy.

**Exclusion criteria:**

1. Individuals with >2mm gingival thickness.
2. Individuals with gingival recession.
3. Individuals with gingivitis and periodontitis.
4. Pregnant and lactating females.
5. Individuals with history of smoking and alcoholism.
6. Individuals under medications which are known to cause gingival enlargement.
7. Individuals with heavy melanin pigmentation.

Individuals fulfilling the inclusion criteria and willing to be a part were included in this study. The need of the study was explained to them in their vernacular language. They were provided with participant information sheet to explain the study design including the benefits and risks of the study. An informed written consent was obtained.

**Study Protocol**

Visual examination - A dense and fibrous appearing gingiva was categorized as thick phenotype whereas delicate and translucent appearing gingiva was categorized as thin phenotype.

The phenotype was further confirmed using Probe Transparency Method (TRAN). A stainless steel periodontal probe (University of North Carolina / UNC-15) was introduced into the gingival sulcus. When the probe was visible through the gingiva the phenotype was categorized as thin and when the probe was not visible through the gingiva it was categorized as thick phenotype (Fig 1).



Fig 1: - Thick Phenotype and Thin Phenotype  
Probe Transparency Method (TRAN)

A total of 18 sites in individuals with thin phenotype confirmed by visual examination and Probe Transparency Method (TRAN) were included in the study.

Clinical parameters which were recorded in the study participants included:-

1. Turesky, Gilmore, Glickman modification of Quigley-Hein Plaque Index
2. Silness and Loe Gingival Index
3. Thickness of gingiva

#### 4. Width of keratinized tissue

All the clinical parameters were recorded at baseline, 1 month and 3 months. 0.2 ml of I-PRF fluid was injected in the gingival sulcus at each site with thin periodontal phenotype.

#### **Thickness of gingiva and width of keratinized tissue**

A reamer was inserted transgingivally into the anaesthetized selected site, stopper was used as the reference point (Fig 2a) and the exact measurement was recorded with the help of digital Vernier caliper (Fig 2b). Keratinized tissue width was measured from gingival margin to mucogingival junction with the help of acrylic stent for reproducibility of measurements and calibrated periodontal probe (UNC 15 probe) (Fig 2c).



Fig 2a: -Measurement of thickness of gingiva using endodontic reamer



Fig 2b: -Measurement with Vernier callipers



Fig 2c: - Measurement of width of keratinized tissue using acrylic stent

#### **Preparation and injection of I-PRF**

20 ml of peripheral venous blood was collected from the median cubital vein after thorough asepsis of the antecubital fossa using surgical spirit. 2 sterile glass coated plastic vacutainer tubes (Choukroun original I-PRF tubes) without anticoagulant were used. 10 ml blood was withdrawn using butterfly cannula (BC 12) into each tube (Fig 3). The tubes were placed such that their weights were counter balanced and then immediately centrifuged in the Choukroun PRF Duo Quattro System (Process for PRF, Nice, France) at 700 rpm for 3 mins (Fig 4). After the centrifugation cycle was complete, the tubes were removed from the centrifuge and placed on PRF tube stand (Fig 5).



Fig 3: -Blood withdrawal using



Fig 4: -I-PRF tubes placed in the Choukrons process for PRF centrifuge and centrifuged at 700 rpm for 3 mins

Two fluid layers were visible, upper being yellow fluid layer I-PRF (containing fibrin network with leukocytes, platelets and growth factors) while lower layer being red (containing red blood cells). A sterile insulin syringe of 30 gauge was used to aspirate the yellow fluid layer containing I-PRF (Fig 6).

The selected test sites were anaesthetized with 10% lidocaine hydrochloride spray. A 0.2 ml



Fig 5: -Two fluid layers formed; yellow fluid layer I-PRF, red fluid of RBCs



Fig 6: -I-PRF fluid aspiration using insulin syringe



Fig 7: -Injection of 0.2 ml I-PRF into the thin gingiva per tooth

of I-PRF fluid was injected into the gingiva through gingival sulcus using the insulin syringe (Fig 7). The same procedure was repeated at same sites after 1 week and after 2 weeks from the baseline.

## RESULTS

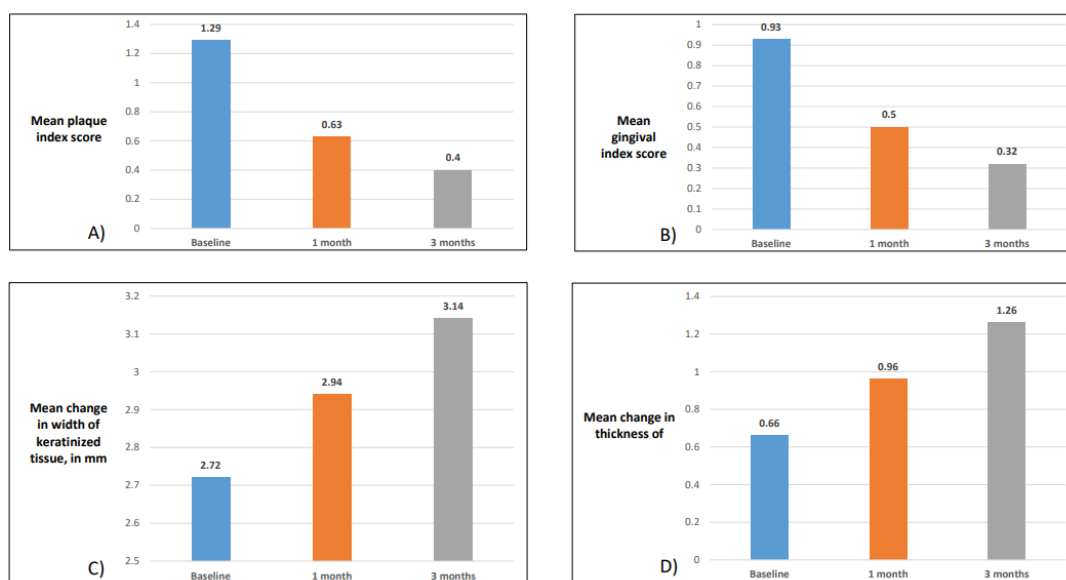
This study was carried to evaluate and compare the effect of injectable platelet rich fibrin (I-PRF) on gingival thickness in individuals with thin periodontal phenotype. The results for the mean plaque index, gingival index, gingival thickness and keratinized tissue width at baseline, 1 month and 3 months are shown in Table 1.

Plaque Index and Gingival Index - Mean gingival index was 0.93, 0.5, 0.32 and mean plaque index was 1.29, 0.63, 0.4 at baseline, 1 month and 3 months respectively (Graph A & B). The result show that there is a statistically insignificant difference ( $p \leq 0.05$ ) at baseline, 1 month and 3 months examination.

Clinical Parameters	Mean	Standard deviation	P value (Repeated measures ANOVA)
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<b>Baseline PI</b>	1.29	0.18	0.089
<b>1 month PI</b>	0.63	0.29	
<b>3 months PI</b>	0.40	0.26	
<b>Baseline GI</b>	0.93	0.40	0.415
<b>1 month GI</b>	0.50	0.17	
<b>3 months GI</b>	0.32	0.17	
<b>Baseline KTW</b>	2.72	0.67	<0.001*
<b>1 month KTW</b>	2.94	0.62	
<b>3 months KTW</b>	3.14	0.59	
<b>Baseline GT</b>	0.66	0.14	<0.001*
<b>1 month GT</b>	0.96	0.11	
<b>3 months GT</b>	1.26	0.10	

**Table no. 1 - Comparison of mean plaque index, gingival index, gingival thickness and keratinized tissue width at baseline, 1 month and 3 months**



Graph – A) Plaque index of the study participants over 3 months, B) Gingival index of the study participants over 3 months C) Changes in the width of keratinized tissue over 3 months, D) Changes in the thickness of gingiva over 3 months

Width of keratinized tissue and thickness of gingiva - Mean width of keratinized tissue at baseline was 2.72, 2.94, 3.14 and mean thickness of gingiva at baseline was 0.66, 0.96, 1.26 at baseline, 1 month and 3 months respectively (Graph C & D). The result show that there is a statistically significant difference ( $p \leq 0.05$ ) at baseline, 1 month and 3 months examination.

## **DISCUSSION**

Gingival esthetics play a monumental role in creating a perfect smile. Uneven gingival contours, loss of interdental papillae, exposed root surfaces resulting from receding gums pose severe challenges. In addition to gingival parameters such as, colour, form and contour, position of the gingival zenith and height of the interdental papilla, periodontal phenotype (thickness of gingiva and width of keratinized tissue) is gaining substantial interest as one of

the important biological factors in diagnosis and treatment planning to achieve long term stability of periodontal health. Three different periodontal phenotypes have been reported in literature viz. thick, moderate and thin gingival phenotype<sup>8</sup>.

Each phenotype reacts differently to physical, chemical or bacterial damage, and also to periodontal, prosthetic and restorative procedures. Thick tissues can withstand trauma and exhibit less clinical inflammation. A thin biotype however, is less resistant to gingival recession when it is subjected to inflammatory and surgical insults<sup>9</sup>. A pre-existing thin gingival biotype can impede ideal esthetic result of many therapies.

Gingival biotype is genetically predetermined and site specific<sup>4</sup>. Variations in gingival thickness depending on age and gender, race and ethnicity<sup>10</sup> have been reported in literature. Venkatesh PML 2019<sup>11</sup> evaluated the influence of race and ethnicity on gingival thickness in Dravidian population of southern India and the Indo Aryan (e.g. Mongoloid population) population of northern India. A statistically significant difference in mean gingival thickness between the Dravidian (maxilla: 1.73±0.09 mm; mandible: 1.40±0.15 mm) and Mongoloid (maxilla: 1.88±0.15 mm; mandible: 1.60±0.18 mm) population was reported.

Thick phenotype is characterized by thick heavy periodontium, gingival margin, usually placed coronal to CEJ, wide zones of keratinized gingiva, flat gingival contour, and thick flat osseous contour. They are resistant to gingival recession and mucosal irritation. 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>12</sup>.

In the event of inflammation or any other type of insult, in thin biotype, we see more inflammatory changes and recession of gingiva<sup>13</sup> and are vulnerable to connective tissue loss and epithelial damage. Mamta Singh et al<sup>14</sup> 2016 evaluated correlation between gingival biotype and occurrence of gingival recession. They concluded that patients with thin biotype are susceptible to higher incidence of gingival recession. Linkevicius et al (2009) observed an increased peri-implant bone loss in areas where tissue was thinner than 2mm<sup>15</sup>.

The thickness of gingival tissues may be a primary determinant of the effectiveness of various periodontal surgical procedures like guided tissue regeneration<sup>16</sup> and crestal bone preservation around implant<sup>17</sup>. It is suggested that a thick flap is associated with more predictable prognosis.<sup>18</sup> Thus, there is a need to convert a thin gingival tissue to a thick one in order to prevent the detrimental effects and to have predictable outcome of procedures.

There are several surgical procedures to modify a thin phenotype. These include connective tissue graft<sup>19</sup> acellular dermal matrix<sup>20</sup> with placental membranes<sup>21</sup> combination of a highly cross-linked ribose porcine type I collagen membrane and a subepithelial palatal connective tissue graft<sup>22</sup> and L-PRF membranes<sup>23</sup>. These procedures have been shown to increase gingival thickness, but have several disadvantages like pain, donor site morbidity, expensiveness, technique sensitivity etc. However, very few studies have been reported in the literature to augment the thin phenotype using minimally invasive approach. The present clinical study was carried out in an attempt to develop a protocol for minimally invasive approach for augmentation of thin gingival biotype.

Platelet-rich fibrin (PRF) is the second generation platelet derivative. PRF is a physiological bio-scaffold rich in integrated platelets and leukocyte cytokines that are essential for regeneration and healing. PRF has properties of being simple, autologous, and economical

and has shown success in intrabony defects, furcation defects, gingival recession, and extraction socket management<sup>24</sup>.

Injectable platelet rich fibrin technique is simple and is prepared according to low-speed centrifugation concept (700 rpm for 3 mins). A higher presence of regenerative cells with higher concentrations of growth factors can be observed in I-PRF. Increased cellular fibroblasts and osteoblasts migration and collagen-1 synthesis can be seen. The combined effects of growth factor secretion and fibroblast recruitment reorganize this fibrin matrix in I-PRF and work synergistically to promote collagen synthesis and tissue regeneration<sup>25</sup>.

The human liquid fibrinogen in the I-PRF is slowly converted into a dynamic three dimension fibrin gel embedding platelets, leukocytes, type 1 collagen (COL1), osteocalcin (OC), and growth factors<sup>26</sup> that can act as an autologous fibrin binder (AFB) which promotes an agglomeration or coating of biomaterials to enhance wound healing<sup>27</sup> with increased vascularization<sup>28</sup>. I-PRF clots and forms a gel form after approximately 10–15 min and preserves its content in the tissue for sustained release<sup>29</sup>.

Richard J. Miron et al in 2017 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>29</sup>.

Our study aimed at increasing the gingival thickness in individuals with thin phenotype. The results showed a statistically significant increase in thickness of gingiva at 1 month and 3 months from baseline i.e  $p = <0.001$ . These outcomes are in agreement with a previous study by Ozsagir Z.B. et al in 2018 who conducted a split mouth study to evaluate the use of injectable PRF (I-PRF) along with microneedling to enhance thin biotype and the results were effective. They injected i-PRF on one side with 27 gauge needle into the apical region of the mucogingival margin in alveolar mucosa of the study area while on other side microneedling with a 30 gauge lancet needle followed by injection of I-PRF on one side with 27 gauge needle was done. They concluded that both injection of I-PRF alone and along with microneedling resulted in increase of gingival thickness<sup>30</sup>.

Dayoub S et al 2019<sup>23</sup> conducted a clinical study comparing role of platelet rich fibrin vs connective tissue graft with tunnel flap in increasing gingival thickness. The results showed a mean increase in gingival tissue thickness in both test and control groups. They concluded that PRF is a successful treatment option for modifying thin gingival biotype and could serve as an alternative to connective tissue graft.

A. Temmerman et al 2018<sup>31</sup> conducted a split mouth clinical study which aimed at evaluating the use of the leukocyte- and platelet-rich fibrin (L-PRF)<sup>32</sup> membranes in increasing the width of the keratinized tissue around implants. The total bucco-lingual width of KT was significantly increased in both groups concluding that L-PRF membranes can be used to increase the width of keratinized tissue.

These studies have shown an increase in the gingival thickness and width of keratinized tissue but they are invasive treatment options. The results of our study show that injection of I-PRF in the gingiva can increase the gingival thickness in individuals with thin periodontal phenotype. It can also increase the width of keratinized tissue. Thus, this procedure can be used to modify the thin phenotype before various surgical or dental procedures. Further studies with larger sample size and long term follow up are required to determine the efficiency of these techniques.



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