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Structural and Mechanical Characterization of Titanium-Reinforced PlateletRich Fibrin (T- PRF) in Healthy and Periodontitis affected Patients

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ABSTRACT

Objectives: This study aimed to evaluate the mechanical and structural properties of titanium-reinforced platelet-rich fibrin (T-PRF) in healthy individuals and periodontitis patients.
Materials and Methods: T-PRF samples were collected from 21 healthy individuals and 21 patients diagnosed with Stage III Grade B periodontitis. Hematological study of the entire blood picture, mechanical qualities (tensile strength), and structural characteristics (predominant cell types and fibrin network) were assessed.
Results: T-PRF's mechanical and structural properties differed between patients with and without periodontitis. Individuals with periodontitis had lower fibrin networks and T-PRF tensile strengths than those who were healthy.
Conclusions: This study shows whether periodontitis influences the mechanical and structural properties of T-PRF, which could affect its efficacy in clinical applications. Understanding these differences is critical for maximising T-PRF's regenerative capabilities in periodontal treatment.

Keywords: Growth factors, white blood cells, platelets, periodontitis, titanium reinforced platelet rich fibrin

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Introduction

Platelet-rich fibrin (PRF), a biomaterial utilized in periodontal regeneration, has distinct characteristics. Since it is autologous (derived from the patient's own blood), there is a reduced risk of rejection or disease transmission. The fibrin matrix in PRF serves as a framework for the migration, proliferation, and differentiation of cells. Moreover, it contains a significant quantity of platelets that generate growth factors, aiding in tissue regeneration and healing. In addition, PRF encourages the slow release of growth factors, which supports ongoing tissue regeneration.[1,2]

PRF is used to improve ridge structures, maintain rosettes, treat complex anomalies, rehabilitate soft tissue and bone, and regulate periodontal pockets, among other elements of periodontal regeneration. Its advantages include low cost, easy preparation, self-sourcing, minimal invasiveness, and adaptability. Nonetheless, various factors can influence PRF's effectiveness, including the patient's overall health, the delivery methodology, growth factor and platelet concentrations, and the preparation procedure. In summary, PRF is an effective approach to periodontal regeneration and provides an economic, safe, and natural way to repair and regenerate tissue.[3,4]

Several methodologies were employed in the development of T-PRF. Initially, blood platelets and growth factors were centrifuged to produce platelet-rich fibrin. Titanium dioxide was then added to the PRF matrix to improve its structural and mechanical qualities. Further research found the optimal titanium concentration, yielding the optimum balance of biocompatibility and mechanical integrity.[5,6]

The structural, biological, and mechanical properties of T-PRF have been thoroughly investigated, demonstrating its ability to improve cell growth, tensile strength, and fibrin structure. After extensive in vitro and in vivo studies, T-PRF was initially used clinically for bone grafting, soft tissue augmentation, and periodontal treatment. Combining the advantages of PRF technology with the excellent mechanical properties of titanium, T-PRF has become a very promising tool for tissue regeneration and repair.[7-9]

Although titanium-reinforced platelet-rich fibrin (T-PRF) is increasingly being used in periodontal regeneration, little is known about how periodontitis affects T-PRF characteristics. In particular, nothing is known about how the inflammatory milieu of

periodontitis affects the mechanical and structural characteristics of T-PRF.[10-12]

Changes in fibrin architecture and stability caused by the inflammatory environment of periodontitis may impair T-PRF's capacity to promote tissue regeneration. Periodontitis might also change the bioactivity and regeneration capability of T-PRF by triggering the release and action of growth factors.[13,14]

Furthermore, periodontitis could weaken the mechanical properties of T-PRF, including its elastic modulus and tensile strength, diminishing its ability to withstand the stresses of the oral environment. Last but not least, if T-PRF's biocompatibility and bioactivity in the setting of periodontitis remain unclear, there might be implications for its therapeutic use.[15]

To fully utilize T-PRF in periodontal therapy, these knowledge gaps must be addressed so that we can be certain it is effective at enhancing tissue regeneration in patients with periodontitis. Additional research is necessary to understand the impact of periodontitis on T-PF characteristics and to devise strategies to enhance their regenerative capacity.

For these reasons, the study aimed to assess the mechanical and structural properties of T-PF in a population of patients with periodontitis and in a control population of patients without periodontitis. The goal of the study was to evaluate and compare the mechanical and structural properties of T-PRFs in patients with and without periodontitis. Secondary objectives included investigating the relationship between mechanical and structural characteristics of T-PRFs and clinical measures of periodontitis, including probing depth, attachment loss, and bleeding on probing. Lastly, it was also a goal to determine T-PRF's application or preparation techniques that could be optimized or improved to enhance its regenerative properties in subjects with periodontitis.

Materials and Methods

The structural and mechanical characteristics of titanium-reinforced platelet-rich fibrin (T-PRF) were investigated in two groups of patients with periodontitis and healthy individuals using a cross-sectional study methodology. Healthy adults without a history of systemic or periodontal diseases were eligible to participate, as were those with moderate to severe periodontitis as determined by a clinical examination. The study excluded people who smoked, had systemic conditions (such as diabetes or immunodeficiency) that hindered tissue regeneration, or were taking drugs that impair platelet function or periodontal health.

The institutional ethics committee gave its approval to the project with a reference ID: IECVDC/24/UG01/PI/IVV/63, and all participants documented informed consent before participating. The study adhered to the Helsinki Guidelines, which emphasize participant autonomy and ensure that participants know they have the option if they wish to participate without fear of penalty. Participants were informed that their decision to participate or withdraw at any time would not negatively impact their relationships with the investigators or future potential participation in treatment. Mechanical testing, clinical measurements, and statistical analysis were performed by independent, calibrated, and blinded evaluators to ensure objectivity and minimize assessment bias throughout the study.

Sample Size Calculation and Power Analysis

Based on an effect size of 0.78, an alpha error of 0.05, and a power of 80%, the calculated sample size was 42, with 21 samples allocated to each group.

Patient Selection and Blood Collection

The study included a test group of 21 participants with a diagnosis of Stage III Grade B periodontitis[16] and a control group of 21 healthy people (Figure 1). Ages 18 to 65, systemically healthy, and willingness to engage are the inclusion requirements. The following are prohibited: systemic disorders, pregnancy, and smoking. Each patient will have 10 mL of venous blood drawn from them using a sterile syringe and needle (Figure 2).

T-PRF Preparation

Platelet-rich plasma (PRP) was isolated from blood by centrifugation at 2700 rpm for 10 minutes. - PRP was combined with titanium dioxide (TiO_2) nanoparticles (0.1% w/v) to form T-PRF. - T-PRF was incubated at 37°C for one hour to allow fibrin clots to develop.

Structural Characterization

T-PRF samples were fixed in glutaraldehyde, embedded in epoxy resin, and sectioned. - Hematological investigation done to evaluate WBC cells and platelet count. - The type of predominant cells, fibrin structure, and distribution were analyzed (Figure 2).

Mechanical Characterization

Tensile Testing: - T-PRF samples were cut into dog-bone-shaped specimens. - Tensile strength evaluated using a universal testing machine (AEUTM-LC2, Advance Equipments, Thane, India; Figure 2).

Clinical Parameters

Patients with periodontitis had their pocket depth, clinical attachment loss, bleeding on probing, and plaque index noted.

Statistical Analysis

Data were analyzed using statistical tools to compare the mechanical and structural features of the healthy and periodontitis groups. For all comparisons, a p-value of less than 0.05 was deemed statistically significant. T-tests and other relevant statistical methods were used to compare differences in tensile strength, modulus of elasticity, structural characteristics, platelet and white blood cell (WBC) counts, and clinical data. These analyses shed light on the differences between the two groups across a variety of metrics, allowing researchers to assess the effect of periodontitis on the characteristics of Titanium-Reinforced Platelet-Rich Fibrin (T-PRF).

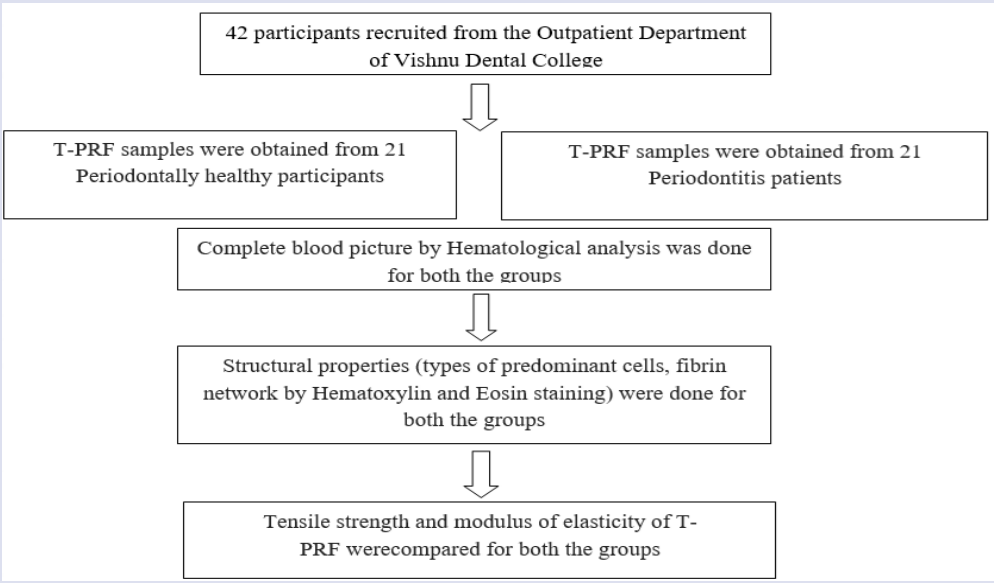

Figure 1. Flow chart of the study design.

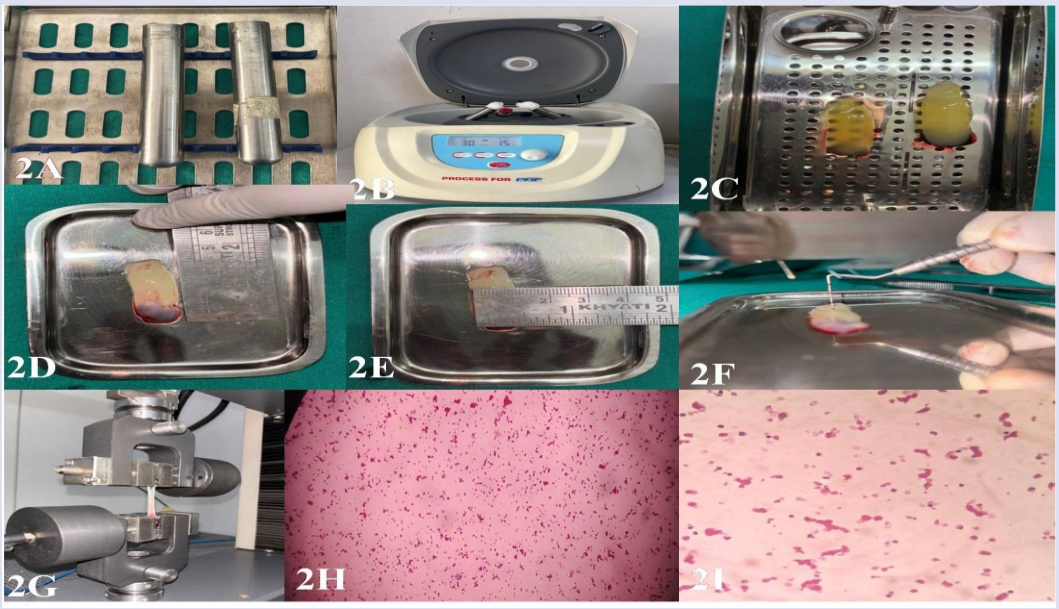

Figure 2. A) Titanium tubes, B) Centrifugation process machine, C) T-PRF Membranes prepared, D) Length of T-PRF Membranes, E) Width of T-PRF Membranes, F) Thickness of T-PRF Membranes, G) Universal testing machine, H) Microscopic picture of healthy group smear of T-PRF membrane, I) Microscopic picture of periodontitis group smear of T-PRF membrane picture.

Table 1. Comparison of mechanical parameters, platelets and WBC count in healthy and periodontitis patients

Parameter	Groups	N	Mean	Std. Deviation	Mean difference	T value	p value
T strength	Healthy	21	0.2095	0.02312	0.03095	5.295	0.000**
	Periodontitis	21	0.1786	0.01352			
MOE	Healthy	21	49.3652	3.04779	7.86810	7.675	0.000**
	Periodontitis	21	41.4971	3.57479			
Platelets	Healthy	21	318901.9048	70177.90022	9638.14	0.552	0.585
	Periodontitis	21	309263.7619	38583.74667			
WBC per ml	Healthy	21	7143.3333	1314.64951	1970.38095	6.380	0.000**
	Periodontitis	21	5172.9524	515.14012			

Test done: Independent t-test
*** p = 0.000 considered highly statistically significant.*

Table 2. Comparison of structural parameters in healthy and periodontitis patients

Structural Parameters	Groups	N	Mean	Std. Deviation	Mean difference	T value	p value
Length (cm)	Healthy	21	4.1476	0.10779	0.23333	5.986	0.000**
	Periodontitis	21	3.9143	0.14243			
Width (cm)	Healthy	21	1.1905	0.08309	0.16190	7.980	0.000**
	Periodontitis	21	1.0286	0.04629			
Thickness (mm)	Healthy	21	1.9381	0.16272	0.20952	4.186	0.000**
	Periodontitis	21	1.7286	0.16169			

Test done: Independent t-test
*** p = 0.000 considered highly statistically significant.*

Table 3. Comparison of clinical parameters in healthy and periodontitis patients

Clinical parameters	Groups	N	Mean	Std. Deviation	Mean difference	T value	p value
PD	Healthy	21	3.4762	0.51177	-2.33333	-12.568	0.000**
	Periodontitis	21	5.8095	0.67964			
CAL	Healthy	21	1.4286	0.50709	-2.23810	-14.645	0.000**
	Periodontitis	21	3.6667	0.48305			
PI	Healthy	21	1.2857	0.46291	-1.23810	-8.222	0.000**
	Periodontitis	21	2.5238	0.51177			
GI	Healthy	21	1.0000	0.63246	-1.14286	-5.754	0.000**
	Periodontitis	21	2.1429	0.65465			

Test done: Independent t-test
*** p=0.000 considered highly statistically significant.*

Results

The Healthy Group had significantly greater tensile strength than the Periodontitis Group, with a significant difference between the two groups (p = 0.000), indicating that periodontitis is associated with a strong reduction in tensile strength (Table 1).

The healthy group demonstrated a significantly elevated modulus of elasticity compared to the periodontitis group. There was a significant difference for modulus of elasticity between the two groups (p = 0.000), which suggests that periodontitis is associated with a major decrease in modulus of elasticity (Table 1).

The healthy group of individuals has a significantly higher number of platelets than the periodontitis group. There was no discernible change in the platelet counts of the two groups, since there is no statistically significant difference between them (p = 0.585; Table 1).

The study identified a significant difference in white blood cell (WBC) counts between healthy individuals, who had an average of 7143.33 cells/mL, and those diagnosed with periodontitis, whose average was 5172.95 cells/mL. The associated p-value for this observation was 0.000. The findings demonstrated that although periodontitis patients had lower counts, pointing to a potential immunological deficiency, healthy participants had higher and more variable WBC levels, indicating a strong immune response. This demonstrates the difficulties in regenerating tissue in cases of periodontitis and the possible contribution of T-PRF to improving healing in these weakened states (Table 1).

The structural characteristics of the study samples differ significantly among the healthy participants and periodontitis groups, as demonstrated by significantly higher p-values for all parameters (Table 2).

Length

The healthy group's average sample length was 4.15 cm longer than the periodontitis group's, which was 3.91 cm longer on average. This is a 0.23-centimeter difference. The t-value of 5.986, which indicates a substantial effect size, confirms that the observed difference was statistically significant.

Width

Healthy samples demonstrated a considerably higher mean width compared to the periodontitis group's mean width. This yielded a mean difference of 0.16 cm and a t-value of 7.980, indicating the most significant relative difference among all evaluated parameters.

Thickness

The healthy group had a higher mean thickness than the periodontitis group, which produced a mean difference of 0.21 mm and a t-value of 4.186.

The findings show that structural parameters like length, width, and thickness are consistently reduced in samples derived from periodontitis patients. This decrease suggests that periodontitis adversely affects the structural integrity of tissues, highlighting the significance of advanced materials such as T-PRF in enhancing biomechanical properties and regenerative capabilities in compromised conditions.

All p-values (0.000) indicate very significant results, and the clinical measures reveal statistically significant differences between healthy individuals and those with periodontitis (Table 3).

Probing Depth (PD)

Patients with periodontitis exhibited a significantly greater mean probing depth (PD) of 5.81 mm compared to healthy individuals, who had a mean PD of 3.48 mm, resulting in a mean difference of -2.33 mm. The t-value of

-12.568 indicates the extent of periodontal pocket formation in pathological states.

Clinical Attachment Loss (CAL)

The average clinical attachment level (CAL) was notably higher in the periodontitis group compared to the healthy group, resulting in a mean difference of -2.24 mm and a t-value of -14.645. This data suggests a significant loss of tissue attachment in individuals with periodontitis.

Plaque Index (PI)

The average Plaque Index (PI) of those with periodontitis was 2.52, which was greater than the average PI of 1.29 for those in good health. This results in a mean difference of -1.24 and a t-value of -8.222, indicating a significant rise in plaque accumulation in the presence of disease.

Gingival Index (GI)

The periodontitis group exhibited a notably elevated gingival index (GI) compared to the healthy group. With a t-value of -5.754 and a mean difference of -1.14, this indicated that the diseased group had more severe gingival inflammation.

The findings show that people with periodontitis had much worse periodontal health, as shown by deeper periodontal pockets, higher attachment loss, more plaque buildup, and increased gingival inflammation. This emphasizes how urgently effective therapies, such as T-PRF, are needed to lessen the significant inflammation and tissue damage brought on by periodontitis.

Discussion

Regarding the treatment of periodontal disease, the study "Structural and Mechanical Characterisation of Titanium-Reinforced Platelet-Rich Fibrin (T-PRF) in Healthy and Periodontitis-Affected Patients" has important clinical implications. T-PRF is a cutting-edge biomaterial that is a perfect fit for periodontal regeneration because it combines the advantages of platelet-rich fibrin (PRF) with the robustness and longevity of titanium.

The study highlights significant differences between healthy individuals and periodontitis patients regarding both structural and mechanical properties, as well as clinical parameters. The tensile strength and modulus of elasticity were notably higher in the healthy group, suggesting that periodontitis leads to a substantial reduction in these mechanical properties, which may impact tissue regeneration. White blood cell (WBC) levels were considerably reduced in the periodontitis group, suggesting immunological dysregulation, even though platelet counts were comparable in the two groups.

Structural parameters such as length, width, and thickness were also reduced in the periodontitis group, further emphasizing the negative impact of the condition on tissue integrity. Clinically, periodontitis patients showed worse outcomes across various parameters, including probing pocket depth, clinical attachment loss or level, plaque index, and gingival index, indicating more severe periodontal disease. These findings underline the detrimental effects of periodontitis on both the structural and mechanical properties of tissue, reinforcing the potential role of T-PRF in improving tissue regeneration and healing in compromised conditions.

In patients with periodontitis, T-PRF can be used as a scaffold to promote tissue regeneration by promoting the growth of new bone, cementum, and the periodontal ligament. Because of the mechanical strength provided by the titanium reinforcement, the scaffold can be stabilized at the fault site. Better clinical results, such as higher attachment gain, decreased pocket depth, and increased bone density, may result from this.[15-17]

T-PRF can also be employed as a barrier membrane during guided tissue regeneration (GTR) operations, which inhibits epithelial cell ingrowth and stimulates the development of appropriate periodontal tissues. In addition to preserving the membrane's structure and shape, the titanium reinforcement promotes the best possible tissue regeneration.[18,19]

T-PRF can be applied as a prophylactic to fortify periodontal tissues and avert illness in healthy individuals. Additionally, the titanium reinforcement can improve the PRF's mechanical qualities, strengthening its durability and resistance to deterioration.[20,21]

In 2020, Chang J, et al., performed a study in the direction of (1) measuring the levels of growth factors from platelet-rich fibrin (PRF), and (2) investigating the relationships between growth factor levels from PRF and the total blood counts (white blood cells and platelets) and serum levels of IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α) in chronic periodontitis as well as periodontally healthy individuals. The study found that PRF may be beneficial as an autologous resource of growth factors that remain unaffected by periodontal status or WBC levels.[5]

Goel A. et al., in a study done in 2021, investigated the comparison between PRF effects on human gingival fibroblasts (HGF) along with human periodontal ligament fibroblasts (HPLF) in patients with generalized moderate or severe chronic periodontitis and patients with healthy and intact periodontium. PRF exudates significantly enhanced fibroblast proliferation; thus, PRF can also play a key role in wound healing. The conclusion of their findings shows the PRF membranes, together with PRF exudates, are possibly used therapeutically and for their wound-healing capacity, while not dependent on the periodontal condition of the patient.[6]

In 2020 Gummaluri SS performed a study assessing the efficiency of T-PRF and L-PRF in the treatment of intra-bony defects using clinical and radiographic criteria. Based on intergroup assessment, the T-PRF group had a statistically higher defect fill when compared with the L-PRF group. T-PRF appears to be a more enhanced option than L-PRF for the treatment of intra-bony defects.[7]

In the research carried out by Arabaci et al.,[8] the goal was to evaluate the influence of titanium-prepared platelet-rich fibrin (T-PRF) as an adjunct to open flap debridement (OFD) on biological markers in gingival crevicular fluid (GCF) and periodontal outcome. In

general, the outcomes of the study demonstrated that the use of a T-PRF membrane from OFD resulted in significantly elevated growth factor levels, decreased RANKL/OPG in GCF, and enhanced periodontal healing compared to conventional flap sites for a duration of 4 to 6 weeks.

In 2020, Chatterjee A. carried out a study to evaluate the efficacy of OFD alone and OFD combined with titanium PRF (TPRF) or autologous platelet-rich fibrin (PRF) in the management of intrabony defects (IBD). The study showed that both autologous PRF and TPRF significantly improved radiographic results and clinical parameters when used to treat IBD.[9]

Overall, the study's conclusions regarding the mechanical and structural properties of T-PRF have important therapeutic ramifications for the management of periodontal disease and present a fresh and successful method for tissue engineering and periodontal regeneration.

Limitations

One of the study's drawbacks is its very small sample size, which could limit the generalizability of the findings. Additionally, the cross-sectional design provides only a single time-point assessment, limiting insights into long-term clinical outcomes. Potential sources of bias, such as selection bias or confounding variables, may exist; efforts were made to minimize observer and assessment bias by ensuring that clinical, mechanical, and statistical evaluations were conducted under blinded conditions.

While the findings of this study provide valuable information on the structural and mechanical alterations of T-PRF in periodontitis-affected patients, the absence of molecular or biochemical analysis limits mechanistic interpretation. Specifically, the study did not assess growth factor levels, cytokine expression, or fibrin network composition at a molecular level, which could have provided deeper insight into the biological pathways contributing to the observed differences.

Future Perspectives

Longer-term clinical trials are needed to assess T-PRF's durability and effectiveness in tissue regeneration for periodontal patients. Exploring personalized medicine based on genetic factors or systemic conditions could improve treatment outcomes. In vivo human trials are essential to validate laboratory results, and combining T-PRF with other regenerative therapies, like stem cells or growth factors, may enhance healing. Evaluating functional outcomes, such as probing depth reduction and clinical attachment gain, will help determine T-PRF's full regenerative potential.[22]

Conclusions

The results demonstrate that T-PRF's fibrin architecture, mechanical strength, and growth factor release are all considerably altered by periodontitis. The regeneration capacity of T-PRF in periodontal therapy may be impacted by these modifications. The study's conclusions have significant therapeutic ramifications since they emphasize the necessity of individualized treatment plans, optimized T-PRF preparation procedures, and the possibility of improved bone regeneration and wound healing. Additionally, the results imply that the characteristics of T-PRF might be associated with clinical factors, allowing for treatment outcome prediction modelling.

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Conflicts of Interest Statement

The authors declare no conflict of interest.

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