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Effect of Diode Laser Photobiomodulation on Platelet-Rich Fibrin Architecture in Healthy and Diabetic Individuals with Periodontitis: An In Vitro Histomorphometric Study

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ABSTRACT: Background

Platelet-rich fibrin (PRF) is used in periodontal regeneration because its fibrin matrix carries platelets, leukocytes and biologically active mediators involved in repair. Diabetes mellitus can modify fibrin formation and platelet function, which may affect the structure and regenerative properties of PRF. Photobiomodulation (PBM) has been reported to influence cellular activity and tissue repair, but its effect on the microscopic structure of PRF obtained from patients with diabetes and periodontitis is not well established. This study compared the histomorphometric features of PRF from systemically healthy and diabetic individuals with periodontitis before and after diode laser PBM.

Methods

Twenty-four PRF samples were studied: 12 from systemically healthy individuals with periodontitis and 12 from individuals with periodontitis and diabetes mellitus. The samples were assigned to four groups: healthy control, healthy + PBM, diabetic control, and diabetic + PBM. PRF was prepared using Choukroun's protocol. The treatment

groups were exposed to a 660 nm diode laser at 0.1 W for 60 seconds in continuous, non-contact mode. Fibrin network density, platelet entrapment, leukocyte distribution, and fibrin fiber thickness were assessed.

Results

In the healthy samples, PBM was associated with a denser fibrin network and moderate platelet and leukocyte distribution compared with untreated samples. Diabetic control samples showed a compact fibrin arrangement with relatively sparse cellular entrapment. After PBM, the diabetic samples showed better fibrin organization together with greater platelet and leukocyte distribution.

Conclusion

The PRF samples exposed to diode laser PBM showed changes in fibrin organization and cellular entrapment in both study populations, with a greater increase in measured fibrin fiber thickness in the diabetic PBM group.

1. INTRODUCTION

Periodontitis is accompanied by chronic inflammation, which can influence platelet activity and the distribution of leukocytes within the PRF matrix. When diabetes is present along with periodontal inflammation, these effects may further modify the organization of the fibrin scaffold and its healing potential [1].

Platelet-rich fibrin (PRF), introduced by Choukroun and colleagues as a second-generation platelet concentrate, serves as an autologous fibrin matrix that can support wound repair and tissue regeneration [2]. The matrix contains platelets, leukocytes, cytokines and growth factors, all of which were involved in angiogenesis, cell migration and proliferation [3]. For these reasons, PRF is employed in two distinct fields of regenerative dentistry, including periodontology and implant therapy.

The properties of PRF are not independent of the systemic condition of the donor. In diabetes mellitus, persistent hyperglycaemia and the formation of advanced glycation end products can alter fibrinogen and fibrin formation. Earlier work has described changes in fibrin structure in people with diabetes, including altered fiber organization, greater compactness and reduced porosity, with possible effects on cell migration [4]. Such changes may influence the regenerative behavior of PRF prepared from diabetic patients.

Photobiomodulation (PBM) uses low-level light to produce biological effects in cells and tissues. Reported effects include changes in cellular metabolism, ATP production and inflammatory signaling, with possible benefits for tissue repair and fibrin organization [5]. However, information on the histomorphometric response of PRF from diabetic individuals with periodontitis to diode laser PBM is limited.

The present study was therefore undertaken to compare the histomorphometric features of PRF from healthy and diabetic individuals with periodontitis, with and without diode laser PBM.

1.1 Aim

To compare the histomorphometric characteristics of PRF obtained from healthy and diabetic individuals with periodontitis, before and after diode laser photobiomodulation.

1.2 Objectives

The study assessed fibrin network density, platelet entrapment and leukocyte distribution in PRF obtained from systemically healthy and diabetic individuals with periodontitis. Fibrin fiber thickness was also compared between untreated and PBM-exposed samples to examine the effect of diode laser PBM on PRF microarchitecture.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This in vitro histomorphometric study was carried out in the Department of Periodontology and Implantology, Sri Ramachandra Dental College and Hospital, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, Tamil Nadu, India. The study was completed over a period of two months.

2.2 Ethical Approval

The study protocol received approval from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (REF: CSP-III/26/MAY/38/162).

2.3 Sample Size

Twenty-four PRF samples were included, with six samples assigned to each of the four study groups.

Group I consisted of systemically healthy individuals with periodontitis and served as the control group. Group II included systemically healthy individuals with periodontitis whose PRF was exposed to laser PBM. Group III comprised individuals with diabetes mellitus and periodontitis and served as the diabetic control group. Group IV comprised individuals with diabetes mellitus and periodontitis whose PRF received laser PBM.

2.4 Inclusion Criteria

Participants between 20 and 65 years of age, irrespective of sex, with Stage I or Stage II periodontitis were eligible. Both systemically healthy participants and participants diagnosed with diabetes mellitus were included.

2.5 Exclusion Criteria

Participants with clotting disorders, those who had taken antiplatelet medication during the preceding three months, smokers, pregnant or lactating women, and those with systemic diseases other than diabetes mellitus were excluded.

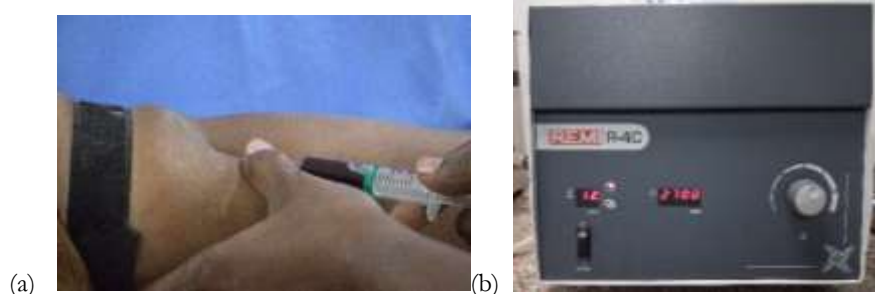
2.6 Clinical Examination

Periodontal examination was carried out in the Department of Periodontology and Implantology. Probing pocket depth, clinical attachment loss and radiographic bone loss were recorded and used for periodontal staging.

2.7 Preparation of Platelet-Rich Fibrin

For PRF preparation, venous blood was drawn from the antecubital vein into sterile 10 mL glass tubes without anticoagulant. The tubes were centrifuged immediately at 2700 rpm for 13 minutes. After centrifugation, the PRF clot located between the acellular plasma and red blood cell layers was removed with sterile tweezers and scissors.

The clot was then compressed in a PRF box to standardize its thickness and obtain the PRF membrane.



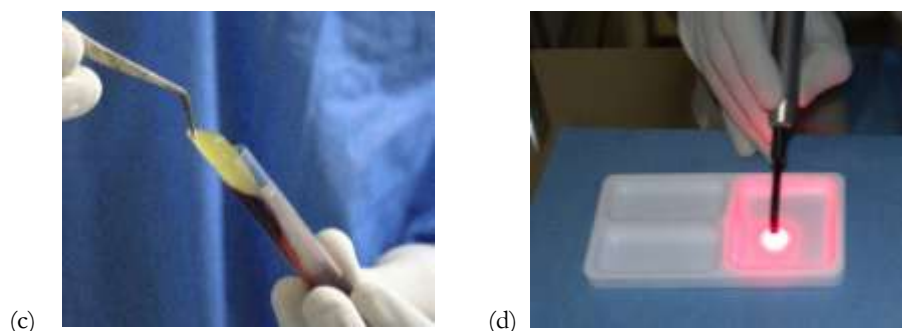


Fig 1. Preparation and photobiomodulation of platelet-rich fibrin. (a) Blood collection from the antecubital vein; (b) centrifugation at 2700 rpm; (c) retrieval of the PRF clot; (d) diode laser PBM applied to the PRF membrane.

2.8 Laser Photobiomodulation Protocol

The PRF membranes in Groups II and IV were exposed directly to diode laser PBM.

Laser Parameters

1.	Type of laser	Diode laser
2.	Manufacturer	Dentsply Sinora
3.	Wavelength	660 nm
4.	Duty cycle	CW
5.	Power	0.1W
6.	Exposure time	60 seconds
7.	Mode	Continuous wave, non-contact mode

Histological processing and microscopy

After treatment, the PRF samples were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Sections were stained with haematoxylin and eosin and examined microscopically at 10× magnification.

Table I. Histomorphometric evaluation criteria

Parameter	Score 1	Score 2	Score 3
Fibrin Network Density	Loose network	Moderate density	Dense compact network
Platelet Entrapment	Sparse	Moderate	Dense clusters
Leukocyte Distribution	Few cells	Moderate distribution	Dense aggregation
Fibrin Fiber Thickness	Thin fibers	Moderately thick fibers	Thick, densely packed fibers

Histomorphometric measurements were obtained using ImageJ software.

2.9 Statistical Analysis

The data were entered into Microsoft Excel and analyzed with IBM SPSS Statistics version 26.0. Results were summarized as mean and standard deviation. Differences between the four groups were assessed using one-way ANOVA, followed by Tukey's post-hoc test. A p-value below 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Twenty-four PRF samples were evaluated. Differences in fibrin organization and cellular entrapment were observed between the PBM-treated and control samples.

Healthy Individuals with Periodontitis

The untreated healthy samples showed sparse platelet entrapment, few leukocytes and a relatively loose fibrin network. In the corresponding PBM-treated samples, platelet and leukocyte distribution was moderate and the fibrin network appeared more compact.

Mean fibrin fiber thickness was $0.52 \pm 0.13 \mu\text{m}$ in the Healthy Control group and $3.23 \pm 0.25 \mu\text{m}$ in the Healthy + PBM group.

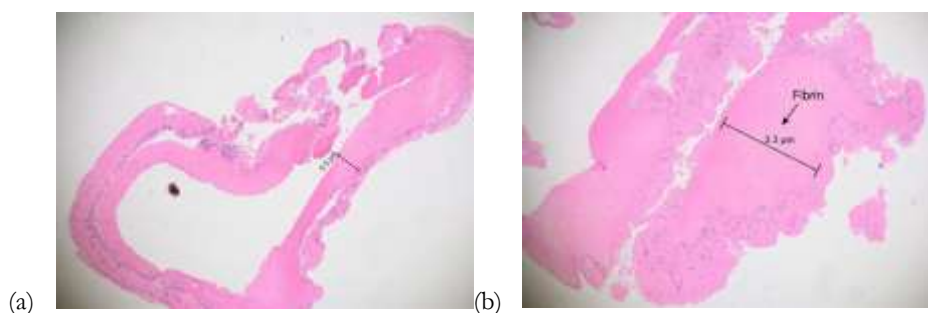


Fig 2. Microscopic appearance of PRF architecture in the healthy groups. (a) Healthy control sample showing a loose fibrin network with sparse cellular entrapment; (b) healthy PBM-treated sample showing a denser, compact fibrin network.

Table II: Histomorphometric characteristics of PRF in healthy individuals

Parameter	Healthy Control	Healthy + PBM
Platelet Entrapment	Sparse	Moderate
Leukocyte Distribution	Few cells	Moderate distribution
Fibrin Network Density	Loose network	Dense compact network

Diabetic Individuals with Periodontitis

The diabetic control samples showed sparse platelet entrapment, few leukocytes and a predominantly compact fibrin arrangement. In the diabetic PBM group, platelet and leukocyte distribution was moderate and the fibrin arrangement appeared better organized.

Mean fibrin fiber thickness was $3.35 \pm 0.36 \mu\text{m}$ in the Diabetic Control group and $7.78 \pm 0.50 \mu\text{m}$ in the Diabetic + PBM group.

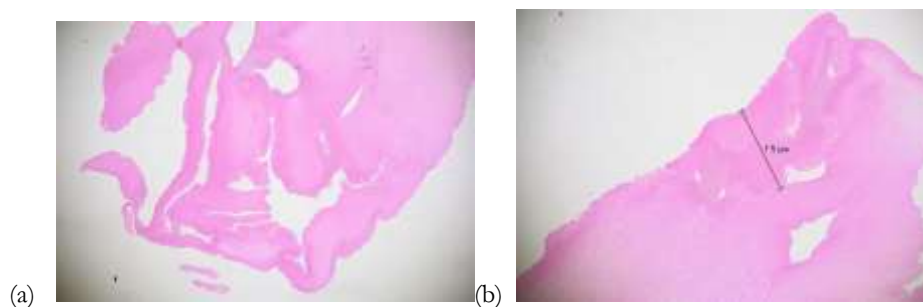


Fig 3. Microscopic appearance of PRF architecture in the diabetic groups. (a) Diabetic control sample showing altered fibrin architecture with sparse cellular entrapment; (b) diabetic PBM-treated sample showing improved fibrin organization and greater fibrin fiber thickness.

Table III: Histomorphometric characteristics of PRF in diabetic individuals

Parameter	Diabetic Control	Diabetic + PBM
Platelet Entrapment	Sparse	Moderate
Leukocyte Distribution	Few cells	Moderate distribution
Fibrin Network Density	Dense compact network / Moderate density	Moderate density / Dense compact network

Table IV: Quantitative comparison of fibrin fiber thickness among study groups

Group	Mean $\pm$ SD (μm)
Healthy Control	0.52 ± 0.13
Healthy + PBM	3.23 ± 0.25
Diabetic Control	3.35 ± 0.36
Diabetic + PBM	7.78 ± 0.50

3.1 Intergroup Comparison

One-way ANOVA showed a statistically significant difference in fibrin fiber thickness among the four groups ($F = 467.02$, $p < 0.001$). The largest mean value was recorded in the Diabetic + PBM group and the smallest in the Healthy Control group. In both health-status groups, the PBM-treated samples had greater mean fibrin fiber thickness than their respective controls.

3.2 Overall Findings

Across the study groups, PBM-treated samples showed greater platelet entrapment, leukocyte distribution and fibrin organization than the corresponding controls. The difference in mean fibrin fiber thickness was larger in the diabetic samples (3.35 to $7.78 \mu\text{m}$) than in the healthy samples (0.52 to $3.23 \mu\text{m}$).

Table V: ANOVA Table

Parameter	F value	p value
Fibrin fiber thickness	467.02	<0.001*

*Statistically significant.

3.3 Discussion

PRF provides a fibrin scaffold that contains platelets, leukocytes and a wide range of growth factors and cytokines that are involved in tissue repair [6]. Its biological behaviour is influenced by the arrangement of the fibrin network, the number and distribution of entrapped cells, and the thickness of the fibrin fibers [7]. Conditions that modify clot formation may therefore change the characteristics of the resulting PRF as well. In the present study, the effect of diode laser PBM on PRF architecture was examined using samples obtained from healthy and diabetic individuals with periodontitis.

The microscopic findings differed between the untreated and PBM-treated samples in both groups. In the healthy controls, the fibrin network was relatively loose, with sparse platelets and few leukocytes. After PBM exposure, the network appeared more compact and cellular entrapment was greater. Mean fibrin fiber thickness also increased from $0.52 \pm 0.13 \mu\text{m}$ to $3.23 \pm 0.25 \mu\text{m}$. These observations indicate a structural response of the PRF membrane to the laser exposure, although the present study cannot establish the biological mechanism responsible for this change.

A possible explanation for a response to PBM is its interaction with cellular photoreceptors, including cytochrome C oxidase. Light exposure can influence mitochondrial activity, ATP production and downstream signaling, including pathways related to oxidative balance and tissue repair [8]. These effects have been proposed as mechanisms through which PBM can modify the cellular behaviour and the local tissue environment [9].

Diabetes is associated with impaired wound repair and changes in fibrin formation related to hyperglycaemia, oxidative stress, vascular dysfunction and advanced glycation end products accumulation [10]. These changes can affect fibrinogen and the structure of the resulting clot [11]. In the present study, diabetic control PRF showed sparse platelet entrapment, limited leukocyte distribution and a predominantly compact fibrin pattern. The mean fibrin fiber thickness was $3.35 \pm 0.36 \mu\text{m}$.

The diabetic samples also showed a clear difference after PBM exposure. Platelet and leukocyte distribution increased from sparse to moderate, and the fibrin arrangement appeared more organized. Mean fibrin fiber thickness rose from $3.35 \pm 0.36 \mu\text{m}$ in the diabetic control group to $7.78 \pm 0.50 \mu\text{m}$ in the diabetic PBM group. The increase was greater in absolute terms than that observed in the healthy samples. These findings are consistent with a structural effect of PBM on PRF prepared from diabetic participants, but they should not be interpreted as evidence that PBM reverses all diabetes-related changes in fibrin biology.

Fibrin architecture is relevant to the function of a PRF membrane because the scaffold provides a temporary matrix for cell migration and can retain biologically active molecules [12]. The greater fiber thickness seen after PBM may therefore be of biological interest. Nevertheless, the present study measured morphology only; it did not assess growth-factor release, cellular migration, angiogenesis or clinical periodontal regeneration. Any clinical implication should therefore be considered preliminary.

The present observations are broadly in line with earlier work on laser-treated PRF. **Suresh SK et al.** reported changes in the microscopic appearance of PRF following low-level laser treatment [13]. **Surmeli Baran et al. (2021)** also reported beneficial effects of photobiomodulation on leukocyte- and platelet-rich fibrin in an experimental model [14]. More recent studies by **Kohir et al. (2025)** and **Böcüoğlu et al. (2026)** have examined laser-related changes in PRF structure and the release of angiogenic growth factors [5,12]. Together, these reports provide a basis for further investigation of PBM as a method for modifying PRF characteristics.

The quantitative finding was supported by the statistical analysis. One-way ANOVA identified a significant difference in fibrin fiber thickness across the four groups ($F = 467.02$, $p < 0.001$). The highest mean thickness occurred in the diabetic PBM group

and the lowest in the healthy control group. Because the present analysis focused on fibrin fiber thickness, the statistical result should not be extended to the qualitative platelet and leukocyte observations.

Several limitations should be considered. The study was performed in vitro and included only 24 samples, with six samples in each group. The assessment was limited to microscopic structural features; growth-factor concentrations, molecular pathways, cellular migration and clinical periodontal outcomes were not examined. Further studies with larger sample sizes, more detailed microscopic techniques such as scanning electron microscopy, biochemical or molecular analyses, and clinical follow-up would help determine whether the structural changes observed here translate into a measurable regenerative benefit.

4. CONCLUSION

Within the limits of this in vitro study, diode laser PBM was associated with changes in PRF microarchitecture in both healthy and diabetic samples. PBM-treated samples showed greater platelet and leukocyte entrapment and a more compact fibrin arrangement, while mean fibrin fiber thickness increased in both groups. The increase was greatest in the diabetic PBM group. These findings provide preliminary evidence that PBM can modify the structural characteristics of PRF, but clinical studies and biological assays are needed before its effect on regenerative outcomes can be established.

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AUTHOR CONTRIBUTIONS

Arvinda Siva J, Dhanadivya. K: Conceptualization, Investigation.

Sharada T Rajan: Methodology, Laboratory Analysis.

Pratyusha Mahanta: Data Curation, Formal Analysis.

Divya Kumar, S.K. Balaji: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

Ethical approval was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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