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A NOVEL BIOMATERIAL C-PRF FOR POCKET REDUCTION- A SPLIT MOUTH RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Background : This split mouth randomized clinical trial aimed to assess the clinical efficacy of C-PRF as an adjunct to scaling and root planning (SRP) in stage III with grade A/B periodontitis. Platelet concentrates, of which the last is concentrated-platelet rich fibrin (C-PRF) have a tenfold increase in the platelets in the buffy coat, eventually degranulating to a higher growth factor release rate. So the novel biomaterial obtained from blood will be used in enhancing the effectiveness of SRP through its adjunctive use in treating periodontal disease. **Methods:** A total of 96 sites from 48 patients were included in the study. Baseline clinical parameters were recorded, followed by a complete full-mouth SRP. The test and control sites were randomly selected, and intra-pocket application of C-PRF was carried out at the test site and the parameters were reassessed after 6 weeks with $P < 0.05$. **Results:** The test group had a mean reduction in pocket probing depth, relative attachment level, plaque Index, gingival index and modified sulcular bleeding index in comparison to the control group at 6 weeks. From baseline to 6 weeks, improvement in the parameters was seen in the test group in comparison to the control group. **Conclusion:** This study emphasizes that C-PRF can be one of the promising modalities for practicing clinicians in treating periodontal pockets with evident reduction in inflammation.

KEYWORDS: Periodontal Disease, Scaling and root planning, Bleeding.

INTRODUCTION

A chronic multifactorial inflammatory condition called periodontitis is connected with dysbiotic plaque biofilm and is featured by the gradually developing degeneration of the tooth-shielding apparatus with pocket formation, recession or mobility.[1] Eliminating the inflammatory lesion in the attachment apparatus is the primary goal of periodontal therapy, which also aids in the resolution of inflammation and prevents the loss of attachment apparatus.[2] Inflammation, fibroblastic granulation, matrix production, and remodeling are all components of the intricate, methodical process of periodontal healing.[3] It has been established that platelets one of the key component play a crucial role in controlling the hemostasis phase by obliterating blood vessels and promoting fibrin clot formation. Important biomolecules, namely proteins of platelets, certain growth factors, including platelet-derived growth factor (PDGF), adhesion molecules, cytokines, chemokines, and angiogenic factors, can activate cells involved in wound healing, such as fibroblasts, neutrophils, macrophages, and mesenchymal stem cells (MSCs). These released by activated platelets and other cells, can promote the proliferation and activation of cells eventually leading to wound healing.[4]

Platelet concentrates of which Platelet Rich Fibrin (PRF) is a second-generation platelet concentrate that is sourced from autologous blood. This congregates all the components in a blood sample in a small fraction as a single fibrin membrane useful for healing.[5] Although there is sufficient literature regarding the usage of various derivatives of PRF in various procedures in the oral cavity, it's been said that about 2-3 time increase in platelet and 1.5 times Leukocytes are seen in liquid formulation of injectable PRF.[4]

The most recent of the PRF is concentrated-PRF (C-PRF), which is located in 0.5 mL of buffy coat placed right above the red blood cell and is known to exhibit increased platelet and leukocyte concentration and higher growth factor release rate. This can be accomplished by adhering to high centrifugation protocols. With the incorporation of leukocytes in a three dimensional scaffold, the property changes such as substantivity, malleability and disintegration can be altered.[7]

With the studies showing an exponential increase in the cell in-vitro studies, C-PRF has the potential to show an anticipated improvement in clinical application for regenerative procedures.[7] Further, the studies have shown C-PRF has increased in the growth factor release rate by tenfold, which is necessary for wound healing and resolution of the inflammation.[8] Thus it can be hypothesized that C-PRF could help in pocket depth downturn and in clinical attachment level gain. However, clinical studies reported till date have not investigated the potency of C-PRF as an adjunct to scaling and root planning (SRP). Planning a clinical study with the above knowledge, this split mouth randomized clinical trial aims to probe into the effectiveness of C-PRF as an adjunct to SRP and marked reduction in inflammation in treating periodontal pockets.

STUDY POPULATION AND METHODS

Experimental design

For the present split mouth randomized controlled clinical trial, the institutional ethics committee of KCDS (KCDSHEC/IP/2023/P7-V/1), Bangalore provided the ethical clearance to conduct the study. The research was conducted in accordance with the 2013 revision of the 1964 Helsinki Declaration. The clinical trial registration number for the study was NCT05958147.

Patient Screening

Screening of 48 patients who were age and gender matched (22 male and 18 female) was done and keeping the selection criteria overall 96 sites were chosen for the study. The written informed consent at the start of the investigation and willingness to come back for follow-ups were taken in the study. Systemically healthy people between the ages of 20 and 40years, moderate periodontitis (Grade II and Stage A or B, according to recent classification of periodontal disease 2017) with probing pocket depth between 4-5 mm were selected.

Inclusion criteria

1. Patient age ranging from 20-40 years
2. Contralateral sites having probing depth 5mm-7mm associated with premolar and molar teeth with no furcation involvement.
3. No systemic disease
4. Not under any medication which influence the outcome of periodontal therapy.

Exclusion criteria

1. History of any periodontal therapy within past 6 months.
2. Pregnant and lactating mothers.
3. Smokers smoking >10 cigarettes per day.
4. Patients with blood disorders or platelet count less than 1,50,000 cells/ µL.
5. Patients with any known systemic disease.
6. Non-compliant patients.

Data and statistical analysis

Sample size for the present study was estimated using G-Power software (latest –ver.3.1.9,7; Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany). Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0 Released 2013 Armonk, NY: IBM Corp., were used to perform statistical analyses. Priori analysis was done before the start of the study for sample size estimations was performed at 20% (α=0.20) with an effective size which yielded 80% power and 95% confident interval. Test of normality was done using kolmogorov-Smirnova test to find out the distribution of data. The Student paired-t test was used to analyze intra group differences. To analyze the inter group differences independent student-t test was used. For the present study significance level considered was $p<0.05$.

Assessment of clinical parameters

All the selected patients received a full mouth SRP. Clinical variables such as pocket probing depth (PPD), relative attachment level (RAL), Gingival index (GI), Plaque Index (PI), Modified Sulcular Bleeding Index (mSBI) were recorded at baseline and at 6 week as the clinical correlation of the parameters will be similar at 6 months follow up on comparison to 6 weeks as shown by Brochut PF.[16] The primary alginate impression of the patient was made so as to prepare dental cast model which was used to fabricate the custom acrylic stent to minimize the measurement bias. Two contra lateral sites were selected which were divided into test and control sites. Allocation of the test and control group was done randomly. The autologous platelet C-PRF was prepared and placed in the periodontal pocket and the other site served as a control site. The clinical parameters were reassessed after 6 weeks of the initial therapy.

Preparation protocol of C-PRF

Blood collection from the anticubital fosse was carried out using a sterile aseptic technique. About 5ml of blood was collected in a sterile glass vacutainer (BD glass vacutainer, Franklin Lakes, USA) without any anticoagulant and sealed with a lid. This was immediately centrifuged at 2700rpm for 8mins in a temperature controlled centrifugation machine (Remi CM 8 Plus) at 10^0c. Care is taken not to extend beyond 2min from the start of blood collection to the centrifugation process. After centrifugation of the blood, 0.5 to 1ml of the C-PRF which is a liquid formulation, is drawn using a 22gauge sterile needle from the buffy coat over the red blood cell layer fraction, after discarding the superficial plasma. This liquid C-PRF is allowed to clot at the room temperature for 5-8 minutes. This resulted fibrin clot were placed on PRF Xpression box (IntraLock©). Following the metal cover is slightly compressed as per the recommendation of the manufacturer. The formed clot was then standardized to 4x5mm with 2mm thickness. Contra lateral periodontal pockets in each patient were assigned based on the coin toss method. The prepared standardized C-PRF was pushed into the periodontal pocket. To prevent dislodging of the clot, a periodontal pack was placed at the site (Fig 1). The patients were advised with postoperative instructions and were instructed to report to the hospital in case of discomfort or pack dislodgement.

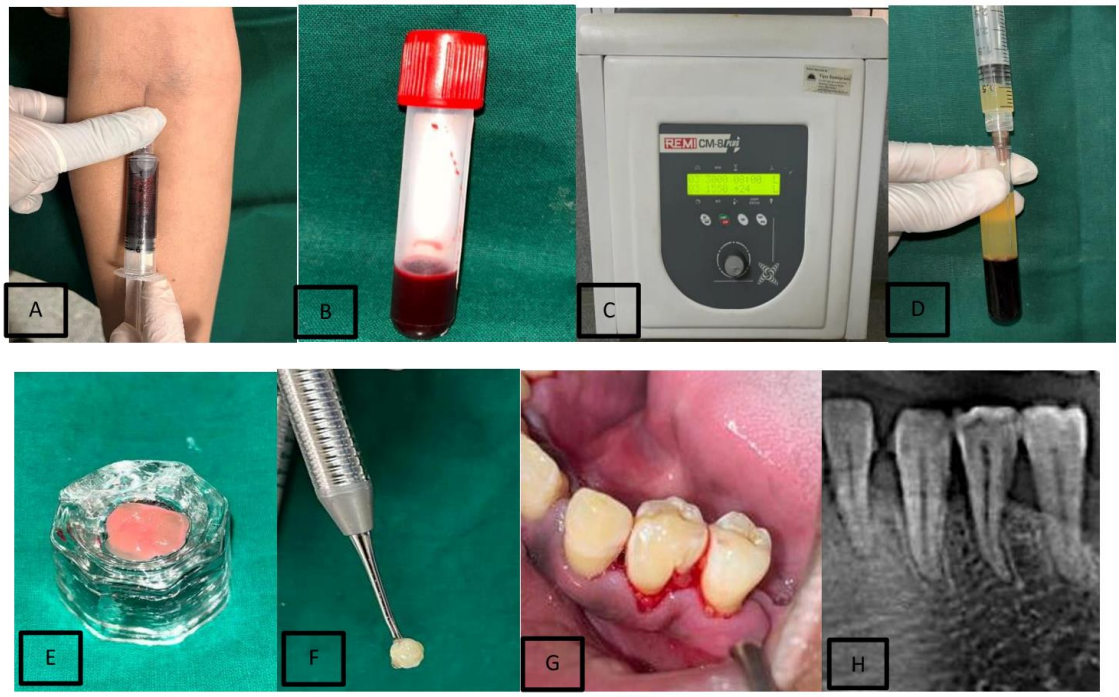

Fig. 1: Process of preparation and application of C-PRF.

A-Blood withdrwan from the antecubital vein, B-Blood is transferred to the tube, C-Centrifuge system (remi cm- 8 plus), D-Drawing 0.5ml of C-PRF from the buffy coat above the RBC layer after centrifugation, E-C-PRF is allowed to clot, F- Formed C-PRF clot, G-After C-PRF is placed into the periodontal pocket, H-Radiograph of the site of application of C-PRF.

Post-operative follow up

The patient was advised not to brush over the pack and gently clean the area so as to prevent pack dislodgement. There was no dispensing of antibiotic and mouth wash. Reinforcement of oral hygiene measures was done after 2 weeks. After 1 week the pack was removed and reassessed in 6 weeks.

RESULTS

The clinical efficacy of C-PRF as a locally delivered agent was analyzed in this study using a randomized controlled clinical study. As the data were normally distributed the pocket depth, clinical attachment loss, GI, PI and mSBI between the test (SRP+ C-PRF) and control site (SRP alone) at the baseline were analyzed using parametric t-test. The mean age of the study population was 31years and the patient were age and gender matched.

Table 1: Comparison of mean values of different study parameters b/w Baseline & follow up period in Control sites, * - Statistically Significant.

Parameters	Time	N	Mean	SD	Mean Diff	p-value
PI	Baseline	48	1.377	0.031	0.29	0.07
	Follow-up	48	1.083	0.127		
GI	Baseline	48	1.550	0.409	0.32	0.04*
	Follow-up	48	1.230	0.364		
SBI	Baseline	48	0.777	0.195	0.20	0.18
	Follow-up	48	0.573	0.046		
PPD	Baseline	48	6.33	0.58	0.66	0.18
	Follow-up	48	5.67	0.58		
RAL	Baseline	48	8.63	0.58	2.26	0.18
	Follow-up	48	6.37	0.58		

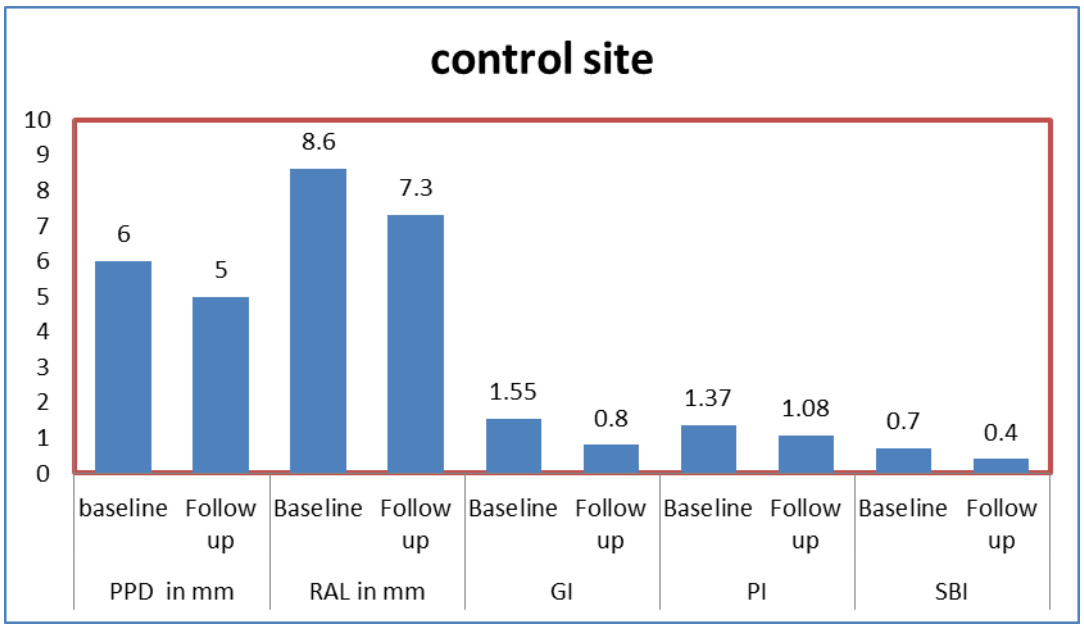

Fig 2: Comparison of mean values of different study parameters.

B/w Baseline & follow-up period in Control sites

The results of baseline parameters (Table 1 and Fig 2) are at baseline no statistical significance was observed between test and control sites. The baseline average PPD and RAL was 6.00mm for control and 6.33 for test site. After 6 weeks following intervention, the pocket depth decreased to 4.67 in control site and 3.33 in test site (Table 2 and Fig 3). Statistically significant ($p<0.05$) results were seen with respect to pocket depth reduction in both test and control sites.

Table 2: Comparison of mean values of different study parameters b/w Control & Follow-up period in Test sites

*** - Statistically Significant.**

Parameters	Sites	N	Mean	SD	Mean Diff	p-value
PI	Control	48	1.083	0.127	0.35	0.01*
	Test	48	0.730	0.072		
GI	Control	48	1.230	0.364	0.46	0.04*
	Test	48	0.767	0.058		
SBI	Control	48	0.573	0.046	0.20	0.006*
	Test	48	0.373	0.046		
PPD	Control	48	6.31	0.58	1.85	0.04*
	Test	48	4.67	0.58		
RAL	Control	48	8.67	0.58	2.34	0.04*
	Test	48	6.33	0.58		

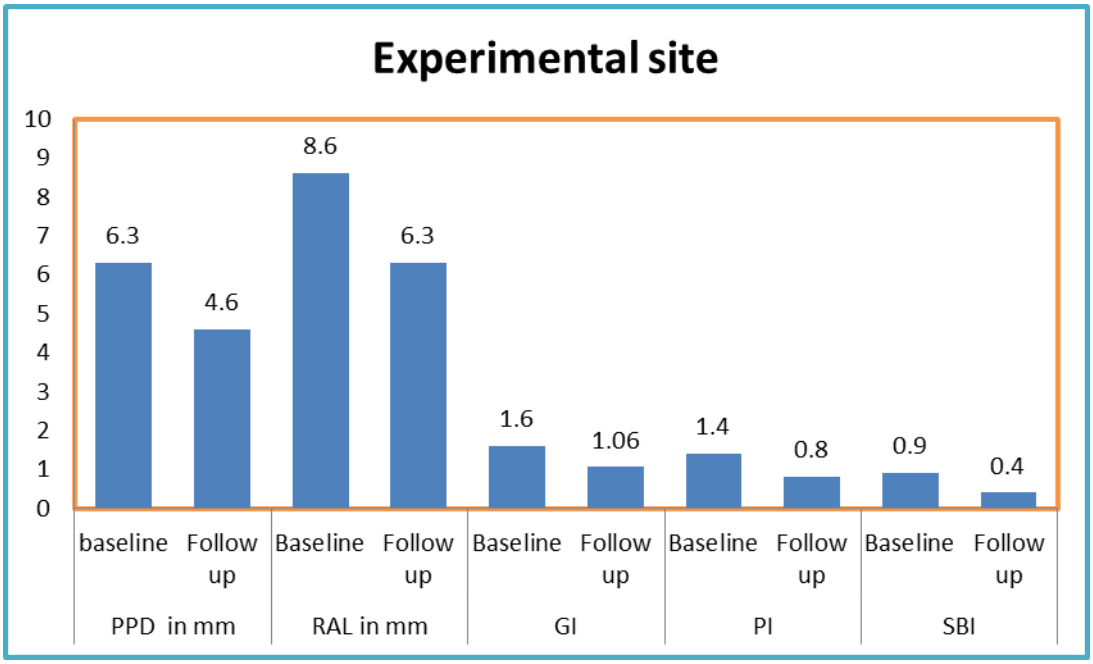

Fig 3: Comparison of mean values of different study parameters.

B/w Baseline & follow-up period in Test sites

The PI at the baseline 1.377 and 1.400 at the control and test site respectively. After 6 weeks following intervention PI decreased to 1.083 in control site and 0.730 in test site which was statistically significant.

The GI at the baseline 1.550 in control site and 1.600 in test site which decreased to 1.230 and 0.767 in control and test sites respectively following 6 weeks of intervention which were statistically significant.

The mSBI at the baseline 0.777 and 0.900 in control and test sites respectively after 6 weeks of following intervention mSBI decreased to 0.573 in control site which is not statistically significant and 0.373 at test site which was statistically significant.

Comparison of mean values of different study parameters between control and test sites showed a statistically significant result in all parameters. The PI score of 0.35, GI score of 0.46, SBI score of 0.20, PPD of 1.85 and RAL of 2.34 were seen between control and test site (Table 2 and Fig 3).

DISCUSSION

Plaque-associated diseases known as periodontal disorders are brought on by the development and buildup of pathogenic biofilm on the surfaces of teeth and oral mucosal areas.[5] Periodontitis can manifest in a generalized form, but it typically manifests in the patient's mouth in localized regions.[1] A clear measurement of the depth of the pocket can be done after Phase I therapy. The gold standard treatment for treatment of shallow pocket is scaling and root planning using hand instrument but the healing outcome is susceptible to.[10] The initial wound healing with abundant growth factor adds on to the success of regenerative therapy.[11] The latest C-PRF has shown an enormous increase in the growth factor up to 10 times compared to all yester preparation protocol of PRF.[8] The reason being the centrifugation to a high number of centrifugation to shorter time so as to keep in liquid form.[12] Subgingival debridement has historically been supplemented with systemic antibiotics, local drug administration, host modulation, subgingival irrigation, and photodynamic treatment in an effort to increase its effectiveness.[10] However, the findings of these trials fell short of producing significant outcomes. Hence, the present randomized, split-mouth, clinical trial estimated the use of C-PRF as an adjunct to SRP for treatment of periodontal pockets. So far to our awareness, this is the first study that assesses the effect of C-PRF placed on periodontal pockets. The results of the present trial showed significant improvements in all outcome variables with use of C-PRF as an adjunct to SRP compared to SRP alone for the treatment of moderate periodontal pockets. The present study utilized a centrifugation machine, which is temperature controlled so as to keep the cell viability and with the advantage of swing out rotor it was possibly to centrifuge the blood horizontally.[13]

First described by Choukroun et al., PRF belongs to the second generation of platelet concentrates. PRF can be used to boost wound healing, bone regeneration, graft stabilization, wound sealing, and hemostasis.[5] Because the fibrin matrix is better organized, it is able to more efficiently direct stem cell migration and the healing program. The therapeutic application of PRF was enhanced by the growth factors released from PRF through in vitro studies and positive outcomes from in vivo studies.[14]

It was demonstrated in 2017 that i-PRF assisted the shaping of a stable fibrin clot, which has been demonstrated to supplement the slow and gradual release of growth factors over time.[6] However, it has been noted that i-PRF releases growth factors in smaller amounts and ratios than anticipated. That led to the development of C-PRF which is formulated by following the protocol of 2700 rpm for 8 mins.[15] According to the research, when compared to results acquired using traditional i-PRF methods, C-PRF has shown better and platelet concentrations. In comparison to PRF acquired by traditional methods, C-PRF caused an exponential increase in cells with added release of growth factor over a 10-day interval and additionally prompted a fourfold increase in migration of gingival fibroblast, gene expression of PDGF, and synthesis of type I collagen.[8]

A significant improvement in clinical parameters in both test and control sites was noticed in the current study. However, the difference between the clinical parameters between test and control groups showed significance for the test group at the end of the study period.

The reduced follow-up period and lack of histological evidence for assessing periodontal healing were the limitations of this study. However, usage of autologous C-PRF is achievable in terms of clinical outcomes, technique sensitivity, and minimally invasive procedure. Studies at large scale or multi-centre studies might strengthen the evidence of C-PRF efficacy in moderate periodontal pockets as an adjunct to mechanical therapy, thus circumventing the need for

surgery. Supplementary studies should be taken up with larger sample size and evaluating biochemical and microbiological parameters to demonstrate the added regenerative potential of C-PRF.

CONCLUSION

A successful healing and improvement in clinical parameters were seen after C-PRF placement. With the additional benefit of improving clinical parameters, C-PRF is a promising novel biomaterial. The use of the autologous blood products as one of the treatment modalities for pocket remains an inexpensive economical breakthrough option. The results of this study demonstrate that C-PRF could be a defensible adjunct to SRP. Hence the use of C-PRF, an autogenous biomaterial with ten times higher growth factor levels, is a boon to dentistry in the treatment of periodontal pockets as a local delivery agent.

Ethical approval statement: The institutional ethics committee of KCDS **(KCDSHEC/IP/2023/P7-V/1),** Bangalore provided the ethical clearance for this study.

The **clinical trial registration number** for the study was NCT05958147.

Contributory statement: Author 1 is involved in the substantial contribution towards conception, design, drafting and final approval of the manuscript. Author 2 and Author 3 are involved in the design, acquisition of data, and final approval.

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Conflict of interest statement: This study is self funded and hence conflict was not seen among the authors.

Patient consent statement: All volunteers provided their free consent to participate in this study.

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