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CLINICAL RESEARCH:

Effectiveness of Injectable Platelet Rich Fibrin, Oxygen Releasing Gel and Hyaluronic Acid Gel as Local Drug Delivery Agents in the Treatment of Chronic Periodontitis: A Clinico-Microbiologic Study

Eficacia de la fibrina rica en plaquetas inyectable, gel liberador de oxígeno y gel de ácido hialurónico como agentes de liberación local de fármacos en el tratamiento de la periodontitis crónica: estudio clínico-microbiológico

Mansi Bansal[1] https://orcid.org/0000-0001-6930-7439
Manish Khatri[1] https://orcid.org/0000-0002-7954-9260
Deepali Mathur[1] https://orcid.org/0009-0005-7519-4748
Pratiksha Tripathi[1] https://orcid.org/0000-0002-0195-5541
Nisha Parveen[1] https://orcid.org/0000-0002-7210-7339
Nutan Tyagi[2] https://orcid.org/0000-0002-7249-181X

[1]Department of Periodontology, Institute of Dental Studies & Technologies, Kadrabad, Modinagar, India.
[2]Department of Oral Pathology and Microbiology, Institute of Dental Studies & Technologies, Kadrabad, Modinagar, India.

Correspondence to: Dr. Manish Khatri - drmanishkhatri@yahoo.com

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ABSTRACT: This study clinically and microbiologically evaluated the efficacy of liquid i-PRF, oxygen-releasing gel, and hyaluronic acid (HA) gel as local drug delivery (LDD) agents adjunctive to scaling and root planing (SRP) for treating periodontal pockets. A split-mouth randomized controlled trial involved 12 patients with 80 periodontal pockets (3-6 mm depth). Pockets were randomly assigned to four groups: Control (SRP alone), Experimental Group I (i-PRF + SRP), Group II (oxygen-releasing gel + SRP), and Group III (HA gel + SRP). Plaque samples were analyzed for spirochetes using dark-field microscopy at baseline, 1- and 3-months post-treatment. Other primary outcome assessed were Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), and Gingival Recession (GR)—were measured using a UNC-15 probe. The statistical tests used were the Bonferroni test for intragroup comparison, the paired t test, the repeated measure analysis of variance test (ANOVA), and the unpaired t test for intergroup comparison between different groups. Intragroup analysis showed significant improvements in PI, GI, PPD, CAL, and GR across all groups ($p<0.05$). Intergroup comparisons revealed no significant differences. Microbiologically, i-PRF significantly reduced spirochetes ($p<0.05$), while oxygen-releasing gel showed superior clinical outcomes ($p<0.05$). All LDD

agents have shown significant therapeutic potential. Oxygen-releasing gel excelled clinically, while i-PRF was microbiologically superior, indicating its therapeutic potential for periodontal pocket treatment.

KEY WORDS: Dental scaling; Hyaluronic acid; Periodontal pocket; Periodontitis, Platelet-rich fibrin; Root planning.

RESUMEN: Este estudio evaluó clínica y microbiológicamente la eficacia del i-PRF líquido, el gel liberador de oxígeno y el gel de ácido hialurónico (HA) como agentes de liberación local de fármacos (LDD) coadyuvantes al raspado y alisado radicular (SRP) para el tratamiento de bolsas periodontales. Se realizó un ensayo clínico aleatorizado de boca dividida, en 12 pacientes con 80 bolsas periodontales (profundidad de 3-6 mm). Las bolsas se asignaron aleatoriamente a cuatro grupos: control (solo SRP), grupo experimental I (i-PRF + SRP), grupo II (gel liberador de oxígeno + SRP) y grupo III (gel de HA + SRP). Las muestras de placa se analizaron para espiroquetas mediante microscopía de campo oscuro al inicio, y a 1 y 3 meses posteriores al tratamiento. Otros indicadores primarios evaluados fueron el índice de placa (PI), índice gingival (GI), profundidad de sondaje (PPD), nivel de inserción clínica (CAL) y recesión gingival (GR), medidos con una sonda UNC-15. Las pruebas estadísticas empleadas incluyeron la prueba de Bonferroni para comparaciones intragrupo, la prueba t pareada, el análisis de varianza de medidas repetidas (ANOVA) y la prueba t no pareada para comparaciones intergrupales. El análisis intragrupo mostró mejorías significativas en PI, GI, PPD, CAL y GR en todos los grupos ($p<0.05$). Las comparaciones intergrupales no revelaron diferencias significativas. Desde el punto de vista microbiológico, el i-PRF redujo significativamente las espiroquetas ($p < 0.05$), mientras que el gel liberador de oxígeno mostró resultados clínicos superiores ($p<0.05$). Todos los agentes de liberación local de fármacos demostraron un potencial terapéutico significativo. El gel liberador de oxígeno destacó clínicamente, mientras que el i-PRF fue superior desde el punto de vista antimicrobiano, lo que indica su potencial terapéutico para el tratamiento de bolsas periodontales.

PALABRAS CLAVE: Raspado dental; Ácido hialurónico; Bolsa periodontal; Periodontitis; Fibrina rica en plaquetas; Alisado radicular.

INTRODUCTION

Periodontal diseases in inflammatory conditions affecting the tooth-supporting structures, caused by specific microorganisms that lead to progressive destruction of the periodontal apparatus, resulting in pocket formation, gingival recession, or both (1). The primary goal of periodontal treatment is to eliminate microbial pathogens from subgingival plaque, thereby controlling active inflammation. Scaling and root planing (SRP) is the cornerstone of non-surgical periodontal therapy (2,3). Various non-surgical and surgical approaches have been successfully employed to treat periodontal disease. Non-surgical therapies also include laser treatment and local drug delivery (LDD) agents used as adjuncts to SRP (4).

In 1982, Lindhe introduced the concept of critical probing depth (CPD), noting that CPD for SRP alone was significantly lower (2.9 mm) compared to SRP combined with the modified Widman flap (4.2 mm) (5). Surgical treatments tend to cause greater attachment loss in shallow pockets but yield better outcomes in deeper periodontal pockets. Although surgical and non-surgical therapies produce comparable long-term results, surgical interventions may lead to gingival recession, patient discomfort, and healing via long junctional epithelium. Patient's anxiety about surgical pain often reduces accep-

tance of surgical treatments leads to increase interest in LDD agents as adjuncts to SRP(6).

LDD is a promising adjunct to subgingival instrumentation introduced by Dr. Max Goodson in 1979. Common LDD systems include chlorhexidine, doxycycline, minocycline, tetracycline, and metronidazole. Recent advancements have incorporated biomimetic agents such as oxygen-releasing gels (e.g., blue®m oral gel), injectable platelet-rich fibrin (i-PRF), and hyaluronic acid (HA) gel, which have shown predictable outcomes (7).

In 2017, Miron introduced third-generation platelet concentrates, specifically liquid injectable PRF (i-PRF), using a low-speed centrifugation protocol (700 rpm for 3 minutes)(8,9). This approach minimizes cell sedimentation due to lower g-forces, promoting periodontal tissue regeneration and aiding healing in implant dentistry.

Active oxygen-releasing gels, such as blue®m oral gel, developed by Dr. Peter Blijdorp and colleagues in the Netherlands, represent a novel non-surgical therapy. Key ingredients, including lactoferrin and active oxygen, generate oxygen free radicals that oxidize membrane lipids and proteins, damage DNA, and suppress anaerobic bacteria at the base of periodontal pockets. The gel contains sodium saccharin, sodium perborate, sodium gluconate, xanthan gum, cellulose gum, water, alcohol, and glycerin (10).

Hyaluronic acid (HA), identified in 1934 by John Palmer and Karl Mayer, is a glycosaminoglycan in the extracellular matrix of connective tissue. Acting as a barrier against oral microbes, HA supports healthy gingival tissue maintenance, delays epithelial and lymphocyte proliferation, controls inflammation, and reduces gingival pocket depths (11).

While antibiotics are effective for acute infections, modern therapies such as HA and i-PRF are more effective in promoting tissue repair, reducing inflammation, and supporting healing during periodontal treatment. These agents pose a lower risk of contributing to antibiotic resistance and provide localized biological repair to maintain periodontal health.

Till date, no studies have compared the efficacy of LDD agents such as i-PRF, oxygen-releasing gels, and HA gel. This study evaluates the clinical and microbiological effectiveness of these LDD agents as adjuncts to SRP in the non-surgical management of periodontal disease.

MATERIAL AND METHODS

Study design: This was a clinico-microbiologic split mouth randomized controlled trial. Patients with periodontal pocket sites were recruited from the Out Patient Department, (OPD) of Department of Periodontology following the mentioned criteria.

Fifteen patients with 104 periodontal pockets (3-6 mm depth) were recruited, three declined due to unavailability. Hence, twelve patients with 80 pockets enrolled, providing written consent for the study (Figure 1).

Inclusion criteria: Systemically healthy patients (age ≥ 18 years) having at least one site with probing pocket depth of ≥ 3 mm to ≤ 6 mm in each of the four quadrants of the mouth.

Exclusion Criteria: Smokers, pregnant or lactating females, patients receiving any periodontal therapy in past six months, patient taking antibiotics in past 6 months, patient having clotting and hematological disorder were excluded from the study.

Allocation of study groups: Sequential randomization was carried out done in which each quadrant is randomized to treatment groups sequentially, with each new quadrant assigned

to the next available treatment group. The four quadrants of patient's mouth were as follows: Control Group and then clockwise rotation was followed to categorize remaining quadrant of patient's mouth as; Experimental Group I, Experimental Group II and Experimental Group III respectively. A well-trained periodontist other than principal investigator recorded all the clinical findings. The allocation of groups into control group, experimental group I, II and III was concealed from patient, trained periodontist and even data analyst/ statistician therefore the study was triple blinded (Figure 1).

Group Assignment: The study group received the intervention as below:

Control Group: Quadrant with sites treated with SRP alone (Figure 2. A-I).

Experimental Group I: Quadrant with sites treated with liquid i-PRF used as a LDD agent adjunctive to SRP (Figure 3. A-I).

Preparation of i-PRF: Ten ml intravenous blood (by Venu puncturing of the antecubital vein) was collected in plastic disposable tubes containing no anticoagulant and was immediately centrifuged at 700 rpm for 3 minutes using Remi R-4C centrifugal machine. With a massive proportion of leukocytes and platelets, the liquid PRF obtained as the topmost layer has more cells that not only aids in tissue regeneration but also releases chemicals that promote wound healing. The orange-colored liquid PRF was collected insulin syringe with 31G needle (9).

Experimental Group II: Quadrant with sites treated with oxygen gel used as a local drug delivery agent adjunctive to SRP (Figure 4.A-I).

Experimental Group III: Quadrant with sites treated with HA gel used as a local drug delivery agent adjunctive to SRP (Figure 5. A-I).

Sample Collection: The plaque sample was collected using a sterile curette when inserted to the maximum permissible periodontal pocket depth. The scaling stroke was applied in coronal direction and plaque was placed on sterile microscopic glass slide. The plaque sample was air-dried, fixed in alcohol solution and after mordanted it with a 0.5-1 % aqueous solution of potassium permanganate for 8-10 min, the sample was further washed in sterile water and then stained with a 2 % aqueous solution of methylene blue for 8-10 min and was again finally washed in sterile water. With this method the spirochetes were stained pinkish purple and the delicate forms stand out clearly. The stained slide was viewed under light microscope (OLYMPUS CX41) at 100X magnification under oil immersion for spirochetal analysis by a trained Oral Pathologist blinded to the treatment groups, thus eliminating bias.

Stent Fabrication: Thermoplastic stents were fabricated (upper and lower arch) for the reproducibility of clinical parameters recorded by a trained periodontist. Vertical grooves were created to define the probe penetration correctly within same vertical plane. Fixed reference point (FRP) marks for the lower limit of the vertical groove used for calculating vertical probing depth (12).

A cotton roll was used to isolate the periodontal pocket to avoid saliva contamination. After performing SRP, an insulin syringe (31G needle) was loaded with the LDD agent (i-PRF, oxygen releasing gel, and HA gel) according to the assigned group. After performing SRP, the periodontal pocket was filled with the necessary LDD agent

ensuring that the needle tip reached the depth of the pocket.

After the interventions in all the experimental groups, patients were instructed not to take any solid/liquid for the next 30 minutes.

Follow up: Patients were reinforced regarding oral hygiene protocol. No mouthwash and antibiotics were used post treatment after post-surgical intervention. For microbial analysis, dark-field microscopy was used for determining the presence of spirochetes. The microscopic analysis (primary outcome) was done by a trained Oral Pathologist blinded to the different treatment groups, thus eliminating bias.

The other primary outcomes which were assessed include Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), and Gingival Recession (GR) were calculated after 1month and 3 months post treatment.

Statistical evaluation of following collected data for comparing the treatment outcomes.

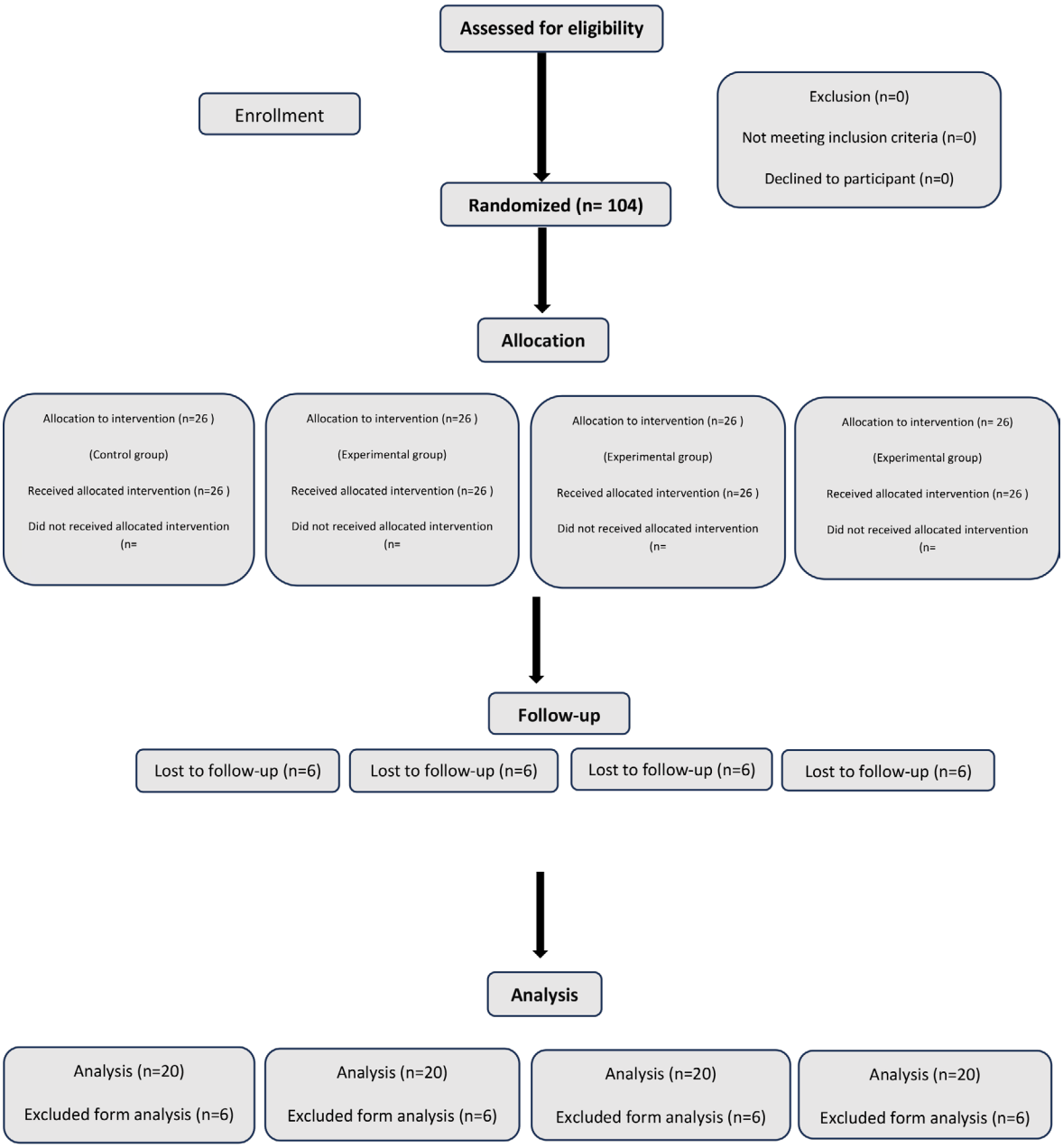

Figure 1. Allocation of study groups.

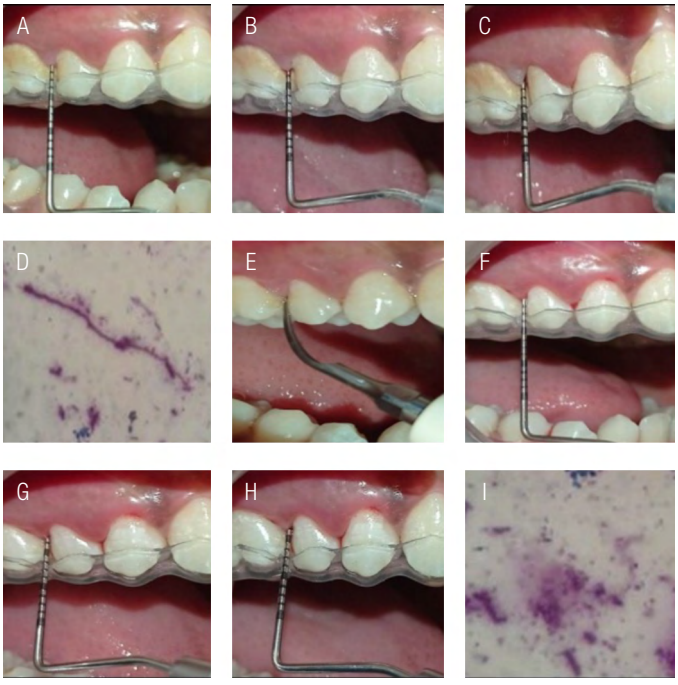

Figure 2. Control group: Scaling and root planning alone. A. FRP-GM (baseline). B. FRP-CEJ (baseline). C. FRP-BOP (baseline). D. Spirochetes analysis (baseline). E. Scaling & root planning. F. FRP-GM (3 months). G. FRP-CEJ (3 months) H. FRP-BOP (3 months). I. Spirochetes analysis (3 months).

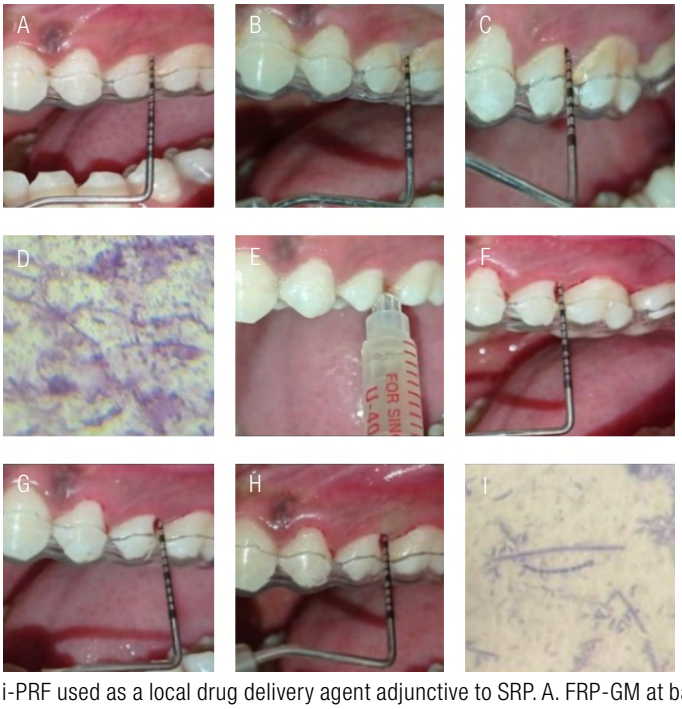

Figure 3. Experimental Group I: i-PRF used as a local drug delivery agent adjunctive to SRP. A. FRP-GM at baseline. B. FRP-CEJ at baseline. C. FRP-BOP at baseline. D. Spirochetes analysis at baseline. E. Scaling & root planning. F. FRP-GM at 3 months. G. FRP-CEJ at 3 months. H. FRP-BOP at 3 months. I. Spirochetes analysis at 3 months.

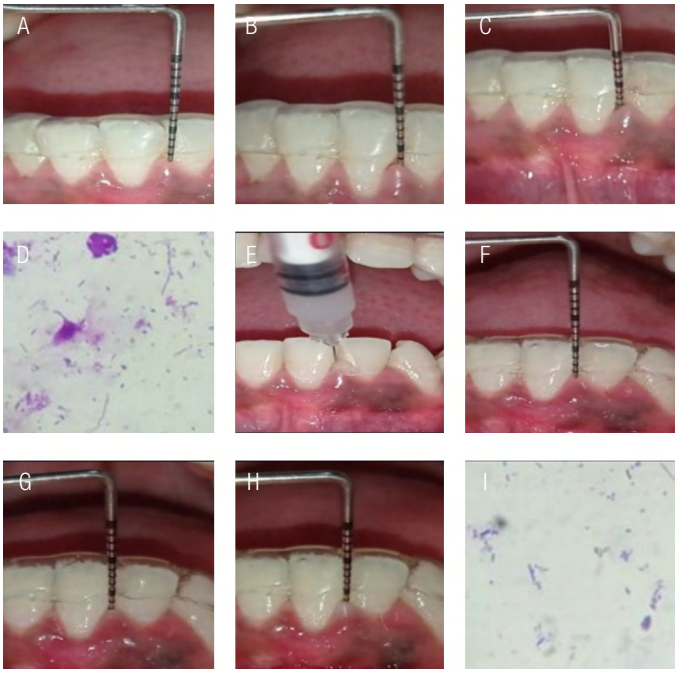

Figure 4. Experimental group II: Oxygen releasing gel used as a local drug delivery agent adjunctive to SRP. A. FRP-GM. B. FRP-CEJ. C. FRP-BOP D. Spirochetes analysis (a to d- baseline). E. Scaling & root planning. F. FRP-GM (3 months). G. FRP-CEJ (3 months). H. FRP-BOP (3 months). I. Spirochetes analysis at 3 months.

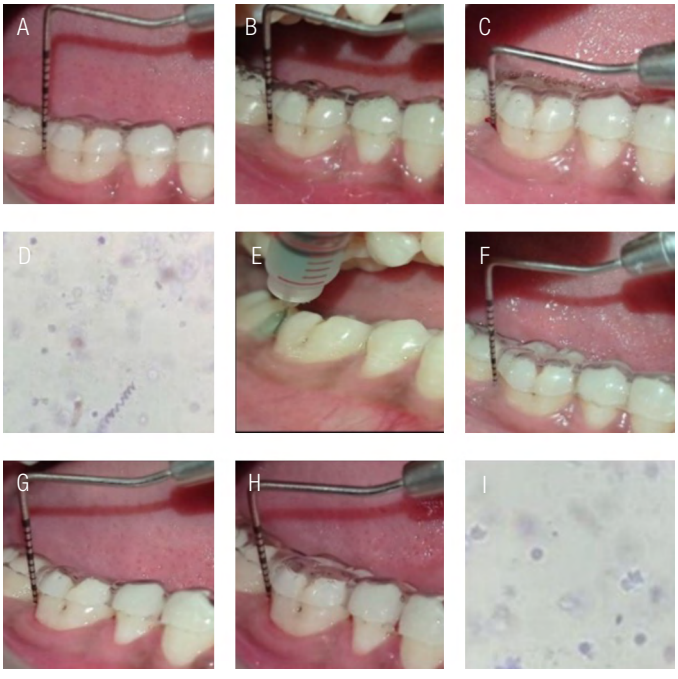

Figure 5. Experimental III: Hyaluronic acid (HA) gel used as a local drug delivery agent adjunctive to SRP. A. FRP-GM. B. FRP-CEJ. C. FRP-BOP. D. Spirochetes analysis (a to d- baseline). E. Scaling & root planning. F. FRP-GM (3 months). G. FRP-CEJ (3 months). H. FRP-BOP (3 months). I. Spirochetes analysis (3 months).

STATISTICAL EVALUATION

A statistical analysis was conducted on the preoperative data at baseline, one month, and three months post-surgery. The statistical analysis software, SPSS (Statistical Package for Social Sciences), version 26.0, was supplied by the IBM company, which is based in New York, USA. The statistical tests used were the Bonferroni test for intragroup comparison, the paired t test, the repeated measure analysis of variance test, and the unpaired t test for intergroup comparison between different groups.

RESULT

Twelve systematically healthy individuals with eighty periodontal pockets were equally distributed in both genders (50% sites in 7 females and 50% sites in 5 males). For the selected study population, 44.0±17.2 years was the mean age for males and 34.1±12.3 years for females.

On intragroup comparison, statistically significant reduction was observed from baseline to 3 months in the control group and all the experimental groups for clinical as well as microbial analysis (Table 1). No complication was experienced in the surgical sites and the healing was uneventful.

Clinically, on intergroup analysis, gain in clinical attachment level and better reduction in probing depth was observed in Experimental Group II (blue®m gel as an adjunct to SRP) followed by other experimental and control group (Table 2).

Microbiologically, on intergroup comparison, statistically significant reduction in number of spirochetes was observed within all the three experimental groups when compared to Control group at 1 month post treatment. However, at 3-month post treatment, only Experimental Group I (i-PRF as an adjunct to SRP) showed statistically significant reduced number of spirochetes.

Table 1. Intragroup comparison of clinical parameters (mean plaque index, gingival index, probing pocket depth, clinical attachment level and recession) and microbial analysis (spirochete count) at different time intervals.

		P I	G I	PD	CAL	R	S.C
Group	**Time**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**	**Mean±S.D**
Control group	Baseline	1.99±0.24	1.99±0.54	5.35±0.49	3.85±1.46	-1.35±1.75	41.85±15.96
	1 month	0.75±0.16	0.74±0.18	4.10±0.91	2.70±1.34	-1.30±1.78	24.15±11.81
	3 months	0.46±0.13	0.52±0.11	3.10±0.85	1.85±1.42	-1.05±1.91	10.05±3.82
*p-value		0.033*	0.001*	0.001*	0.001*	0.108	0.0001*
Experimental group I	Baseline	1.97±0.44	1.99±0.30	5.40±0.59	3.85±1.23	-1.30±1.17	36.60±17.74
	1 month	0.74±0.16	0.79±0.16	4.05±0.99	2.80±0.77	-1.10±1.29	13.10±4.66
	3 months	0.42±0.13	0.48±0.14	3.10±0.85	2.00±0.97	-1.05±1.32	7.45±2.86
*p-value		0.001*	0.001*	0.001*	0.001*	0.203	0.001*
Experimental group II	Baseline	2.04±0.38	2.00±0.34	5.40±0.82	4.10±1.33	-1.25±1.58	41.40±13.26
	1 month	0.74±0.12	0.74±0.17	3.95±1.05	2.70±1.03	-1.15±1.66	13.50±3.98
	3 months	0.43±0.16	0.44±0.16	3.00±0.79	2.00±1.03	-1.05±1.50	8.50±2.19
*p-value		0.001*	0.001*	0.001*	0.001*	0.152	0.001*
Experimental group III	Baseline	2.07±0.24	2.01±0.58	5.05±0.51	3.85±1.18	-1.20±1.44	42.65±13.56
	1 month	0.78±0.12	0.71±0.20	3.80±0.77	2.65±1.14	-1.10±1.55	14.40±5.32
	3 months	0.46±0.15	0.46±0.16	2.90±0.72	1.60±1.46	-1.10±1.48	8.05±2.59
*p-value		0.001*	0.001*	0.001*	0.001*	0.270	0.001*

*p-value significant at $p<0.05$ (one way ANOVA)
PI-plaque index; GI-gingival index; PD-probing depth; CAL- clinical attachment level; R-recession and for microbial analysis, S.C-spirochete count.

Table 2. Intergroup comparison of Clinical parameters (mean plaque index, gingival index, probing pocket depth, clinical attachment level and recession) and microbial analysis (spirochete count) at different time intervals.

Parameters	Baseline (Mean ± Standard Deviation)				
	Control	Experimental I	Experimental II	Experimental III	p value
P I	1.99±0.24	1.97±0.44	2.04±0.38	2.07±0.24	0.786
G I	1.99±0.54	1.99±0.30	2.00 ± 0.34	2.01 ± 0.58	0.082
PD	5.35 ± 0.49	5.40 ± 0.59	5.40 ± 0.82	5.05 ± 0.51	0.567
CAL	3.85 ± 1.46	3.85± 1.23	4.10±1.33	3.85±1.18	0.312
R	-1.35±1.75	-1.30±1.17	-1.25±1.58	-1.2±1.44	0.990
S.C	41.85±15.96	36.6±17.74	41.40±13.26	42.65±13.56	0.590
	1 Month (Mean ± Standard Deviation)				
P I	0.75±0.16	0.74±0.16	0.74±0.12	0.78±0.12	0.806
G I	0.74±0.18	1.99±0.30	0.74±0.17	0.71±0.20	0.025
PD	4.10±0.91	4.05±0.99	3.95±1.05	3.8±0.77	0.350
CAL	2.70 ±1.34	2.8 ±0.77	2.70 ±1.03	2.65 ±1.14	0.079
R	-1.30 ±1.78	-1.10 ± 1.29	-1.15 ±1.66	-1.10±1.55	0.975
S.C	24.15±11.81	13.10 ±4.66	13.50 ± 3.98	14.40±5.32	0.001*
	3 Month (Mean ± Standard Deviation)				
P I	0.46±0.13	0.42±0.13	0.43±0.16	0.46±0.15	0.685
G I	0.52±0.11	0.48±0.14	0.44 ±0.16	0.46±0.16	0.021
PD	3.10±0.85	3.10±0.85	3.00±0.79	2.90±0.72	0.183
CAL	1.85±1.42	2.00±0.97	2.00±1.03	1.60 ±1.46	0.712
R	-1.05 ±1.91	-1.05 ±1.32	-1.05±1.50	-1.10 ±1.48	0.999
S.C	10.05±3.82	7.45±2.86	8.50±2.19	8.05±2.59	0.041*

PI-plaque index; GI-gingival index; PD-probing depth; CAL- clinical attachment level; R-recession and for microbial analysis, S.C-spirochete count).

DISCUSSION

Non-surgical (scaling and root planing, SRP) and surgical (gingivectomy and flap surgery) periodontal therapies are commonly employed to halt disease progression and prevent tooth loss due to periodontitis. However, conventional treatments may not fully debride periodontal pockets, prompting research into local drug delivery (LDD) agents as adjuncts to SRP (11).

Biomimetic agents mimic natural biological structures and processes. In this study, novel LDD agents, including injectable platelet-rich fibrin (i-PRF), blue®m oxygen-releasing gel, and hyaluronic acid (HA) gel, were evaluated for their efficacy as adjuncts to SRP.

Microbial analysis targeted elevated concentrations of red complex bacteria in periodontal pockets, which are critical to periodontal tissue

destruction. Spirochetes, predominant in subgingival plaque, thrive in anaerobic conditions with abundant nutrients. These protected niches, minimally affected by saliva or masticatory forces, promote spirochete proliferation, exacerbating periodontal disease progression.

Given the limited long-term data on LDD agents combined with SRP, this study assessed the clinical and microbiological outcomes of i-PRF, oxygen-releasing gel, and HA gel as adjunctive therapies.

Site randomization was performed using an initial lottery draw. A trained periodontist and oral pathologist, distinct from the principal investigator, recorded clinical findings and spirochete counts. Group allocation (control and experimental groups I, II, and III) was concealed from patients, the periodontist, the oral pathologist, and the data analyst/statistician, ensuring a triple-blinded study design.

To ensure reliable and reproducible clinical measurements, Laxman V.K. and Khatri M. *et al.*(12) emphasized the importance of a stable fixed reference point. Accordingly, customized stents were used in this study to provide a consistent reference for measuring probing depth (PD), clinical attachment level (CAL), and recession depth before and after treatment.

In the intragroup comparison, significant changes in both gingival and plaque indices were noted across experimental groups. The control (SRP) group showed results consistent with those of Roman-Torres C.V.G. *et al*. (15), who found a significant reduction in GI, PI, and microbial parameters. Similarly, the PI and GI improvements observed for i-PRF after SRP were comparable to findings of Soni R. *et al*. (16) and Fotani S. *et al*. (17), who demonstrated that i-PRF promoted soft-tissue healing and reduced infections through the presence of immune cells (leukocytes) that target microbes. This reduction in microbial concentration helped decrease inflammation, leading to a subsequent reduction in PI, GI, and bleeding on probing values.

Oxygen releasing gel like blue® gel used with SRP are in accordance with study of Cunha E.J. *et al.* (18), Koul A. *et al*. (19), Asha A. *et al*. (20) and who suggested oxygen releasing gel products have oxygen and lactoferrin as an active ingredient and possess antiplaque and anti-gingivitis efficacy. PI, GI results of present study in which HA gel used along with SRP are similar to the study of Johannsen *et al*. (21) who observed that there was significant reduction of plaque and gingival index between baseline to 12 weeks in sites treated with HA gel.

However, intergroup analysis of present study has shown non- significant results within all the treatment groups in mean plaque and gingival index from baseline to 3 months post treatment. Clinically, Experimental Group II had a greater decline in PI and GI scores than Experimental Group III, which was followed by Experimental Group I while control group experienced the least reduction.

Gingival recession and probing depth are significant factors in determining the long-term stability of the outcomes, whereas the standard parameter of clinical attachment level (CAL) is used for evaluating the effectiveness of periodontal therapy. Reductions in probing depth could be attributed to either an improvement in CAL or an increase in recession depth. However, the present study found that gingival recession was not significantly different across any of the groups. On intra group analysis, the control group as well as all the groups have shown statistically significant reduction in mean probing depth along with gain in mean CAL, thereby making the mean recession depth of control and all the experimental groups was found to be non-significant at different time

intervals. This result of the present study for SRP alone is in accordance to Lang *et al*. (22), Lindhe *et al*. (23) and Badersten *et al*. (24).

The use of i-PRF has also been demonstrated to reduce pocket depth and increase the clinical value of CAL gain by Miron *et al*. (25) and Fontani *et al*.(17). This may be because of faster wound healing, less gingival inflammation in smaller duration and a longer-lasting decrease in periopathogenic bacteria. In contrast, Pullishery F. *et al*. (26) conducted a systematic study and found no discernible change in CAL between the i-PRF-treated group and the conventional group. These results for blue® gel group were similar to results in accordance with Niveda *et al*. (27) who compared blue M gel with Hexigel and shown that the supplementary topical oxygen therapy reduces the number of bacteria at base of pocket, which causes less periodontal deterioration and faster improvements in clinical parameters. Blue M also reduces cellular hypoxia, proliferates cell growth, promotes angiogenesis and metabolism which was also supported by Deliberator *et al.* (28) and Cunha *et al. (*18) Gain in CAL achieved in this study could be possible due to increased biocompatibility of oxygen with cellular components like cementoblasts fibroblasts and epithelial cells. This was supported by Kour A. *et al*.(19), Aggarwal R. *et al.* (29) and Koul A. e*t al*. (19) who stated that blue® M gel not only causes fast and progressive healing superficially but also to the deeper periodontal pockets leading to increased reduction in probing pocket depth (PPD) and CAL gain.

HA group may have a lower probing pocket depth because of its high molecular weight, which stabilises its structure, inhibits the growth of epithelial cells like fibroblasts and lymphocytes, which lowers inflammation, and improves the periodontal lesion in patients with chronic periodontitis. It was in accordance with Mesa et al11 who suggested that HA not only acts as a physical barrier against bacteria but has a chemotrypsin-induced binding to Treponema dentícola and other periodontal pathogens which prevents their destructive action on the periodontium.

When all clinical parameters (PD, CAL, and GR) were compared between groups, i-PRF group had a clinically better decrease in probing depth and an increase in clinical attachment level compared to experimental group II Also, Blue M gel group showed clinically better reduction in probing depth and an increase in clinical attachment level compared to i-PRF group. The least successful groups were Experimental Group III (HA gel group along with SRP) and Control Group, which simply performed SRP.

The bank of growth factors- "i-PRF" attained its success due to its autologous nature. When Serafini G. *et al*. (31) examined the effect of i-PRF on human periodontal ligament cells (PDLC), they verified the findings of Dohan *et al*. (30) and Vuckovic *et al*. (9) who proposed that this improvement in clinical parameters might be related to the soft tissue healing properties of i-PRF. It markedly reduced the inflammation brought on by lipopolysaccharides and markedly increased human PDLC activity and differentiation. Additionally, Zhou X. *et al*. (32) noted that i PRF emits a variety of pro-fibrotic and pro-angiogenic substances that can accelerate the repopulation of periodontal tissue and, consequently, gain in CAL.

The subgingival microbial flora in periodontitis includes a significant proportion of spirochetes, accounting for up to 50% of the microbial population. Intergroup comparison of microbiological parameters (spirochete count) revealed that all three experimental groups exhibited statistically significant differences compared to the control group at 1 month post-treatment. However, at 3 months post-treatment, only Experimental Group I (i-PRF group) retained statistical significance. Although the specific component of i-PRF responsible for its antimicrobial effects remains unclear,

several mechanisms have been proposed. These include the production of reactive oxygen species, such as hydroxyl radicals, superoxides, and peroxides, as well as the binding and internalization of bacteria, and the release of antimicrobial peptides. Additionally, white blood cells within i-PRF may engulf platelets, participate in antibody-dependent cell-mediated cytotoxicity, and directly interact with microorganisms, potentially leading to pathogen elimination.

Additionally, the release of myeloperoxidase, antigen-specific immune responses, and activation of antioxidant-responsive elements have been proposed by Choukroun J. *et al*. (33). Additionally, Karde *et al*. (34) discovered that i-PRF outperformed conventional platelet concentrates in terms of both platelet count and antibacterial activity.

Reduction of spirochetes at subgingival level has also been carried oxygen releasing gel group. According to Delibrator *et al*. (28) and Koul A. *et al*. (19), the reason could be role of oxygen in enhancing the cellular mechanisms which involved in cicatrization such as angiogenesis. The heme peroxidase family member lactoperoxidase (LPO), which is found in lactoferrin, catalyses the oxidation of thiocyanate to hypothiocyanate in the presence of hydrogen peroxide and has antibacterial qualities. It has also been shown that the LPO thicyanate-hydrogen peroxide combination decreased the development and viability of oral bacteria.

Commercially available high-molecular-weight HA products are highly biocompatible in gingival tissues and enhance tissue regeneration, which supports the use of HA gel as an adjuvant to SRP. (21,35-37) The bacterial profile of the regions receiving subgingival administration of HA as an adjuvant to SRP before and after therapy did not significantly change.

The limited duration of the follow-up period in the present study may have constrained the evaluation of the long-term efficacy of local drug delivery agents (LDDAs) on clinical outcomes. The microbiological analysis of subgingival plaque, specifically the assessment of spirochetal populations, is contingent upon the accuracy of diagnostic methodologies employed, such as dark field microscopy, which is influenced by the sensitivity and specificity of these tools. The absence of advanced molecular techniques, such as polymerase chain reaction (PCR), may have contributed to potential misclassification or underreporting of the microbial burden. Future investigations should focus on integrating clinical, microbiological, and genetic parameters to elucidate the optimal selection of LDDAs tailored to individual patient profiles, thereby advancing personalized therapeutic strategies.

CONCLUSION

The local drug delivery agents evaluated in this study, namely injectable platelet-rich fibrin (i-PRF), oxygen-releasing gel, and hyaluronic acid (HA) gel, demonstrated considerable therapeutic efficacy. The application of oxygen-releasing gel in conjunction with scaling and root planing (SRP) resulted in superior clinical outcomes. Microbiologically, i-PRF emerged as a viable and effective therapeutic modality for the management of periodontal pockets.

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