Original Article

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Dentistry Section

Efficiency of Plasma Rich Fibrin in Accelerating Canine Retraction with Correlation of Timing of Extraction: A Split-mouth Randomised Controlled Trial

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ABSTRACT

Introduction: The chief concern for any patient seeking orthodontic treatment is the duration of the treatment. Various methods have been proposed to accelerate orthodontic tooth movement. In recent years, injectable Platelet-Rich Fibrin (i-PRF) has been shown to enhance cellular activity by increasing bone turnover and promoting healing.

Aim: To study the effectiveness of submucosal injection of i-PRF in accelerating canine retraction and to compare the effectiveness of i-PRF in immediate post-extraction and four weeks post-extraction.

Materials and Methods: The study was designed as a pilot split-mouth randomised controlled trial and was conducted in the Department of Orthodontics and Dentofacial Orthopaedics in CSI College of Dental Sciences and Research, Madurai, Tamil Nadu, India during the period October 2021 to February 2022. A sample size of 12 patients aged 13-21 years who required all first premolar extraction as a part of treatment was enrolled. All four quadrants of each patient were included, and each quadrant was categorised into one of four groups. All four quadrants of each patient were included and allocated to four groups using simple randomisation (lot method). Groups 1 and 2 were from the maxillary arch, while Groups 3 and 4 were from the mandibular arch. Groups 1 and 2 differed by intervention, whereas Groups 3 and 4 received the same intervention but differed in extraction timing to compare immediate and delayed extraction. Individual canine retraction was attempted using a NiTi closed coil spring. The rate of tooth movement was assessed 21 days (T-1) after the intervention; the intervention was then repeated and reassessed at day 42 (T-2). One-way Analysis of Variance (ANOVA) was done for comparison of intragroup variables, followed by a post-hoc Tukey test.

Results: A statistically significant difference in canine retraction (T0-T2) was observed among the study groups ($p<0.001$). The intervention groups (groups 1, 3, and 4) demonstrated significantly greater canine retraction than the control group (group 2). Post-hoc analysis confirmed significantly greater canine retraction in each intervention group compared with the control group. Furthermore, comparison between the healed extraction socket with i-PRF (group 3) and the immediate extraction socket with i-PRF (group 4) at the incisal level during T0–T2 revealed a statistically significant difference ($p=0.011$), with greater canine retraction observed in the immediate extraction group. No statistically significant difference was observed between groups 1 and 4 in canine retraction; however, these groups differed in extraction/intervention timing and arch location, indicating that immediate extraction itself did not influence the amount of canine retraction, whereas i-PRF administration significantly enhanced tooth movement.

Conclusion: It can be concluded that injectable PRF has been shown to increase the rate of orthodontic tooth movement and that i-PRF administered immediately after extraction was associated with greater canine retraction than i-PRF administered in the healed extraction site.

Keywords: Bone turnover, Canine tooth, Healed socket, Orthodontic tooth movement

INTRODUCTION

The chief concern for any patient seeking orthodontic treatment is the duration of the treatment and aesthetic outcome. Balancing both is a great challenge for orthodontists. Uribe F et al., demonstrated, in a survey, that the classic 24-month orthodontic treatment duration seemed too long for patients seeking orthodontic therapy [1]. So, the need for acceleration is intended, which is done by increasing osteoclastic activity that causes an increase in bone resorption, facilitating an acceleration in tooth movement and overcoming the adverse effects of prolonged treatment duration such as increased risk of dental caries, gingival recession and root resorption [2].

Platelet-Rich Plasma (PRP) is an autologous concentration of platelets within a small volume of plasma that is considered a rich source of autologous Growth Factors (GFs). GFs act as natural biologic mediators, thereby regulating the key cellular events in the process of tissue repair and regeneration, as it induces the intracellular signalling pathways that result in the activation of genes, which ultimately change cellular activity along with its phenotype. PRF membrane can release high quantities of GFs, such as Transforming Growth Factor-beta 1, Platelet-Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) [3]. Platelet-based preparations obtained from patients' blood prove to be a safe alternative to other bio-modulating agents. The i-PRF has been developed through a modification in the spin centrifugation forces and has been shown to promote cellular activity by increasing bone turnover and the rate of healing. PRF is effective in producing: a) dose-dependent osteoinductive effects on osteoblasts and viable periodontal ligament cells, along with bone marrow mesenchymal stem cells. It acts as a potential: (b) anti-inflammatory; (c) anti-infective; and (d) analgesic [4]. So, based on this evidence, i-PRF can be considered for studying its efficiency in accelerating tooth movement, as it is proven to be safe, effective for 7-10 days, a good source of GFs and has great potential to promote osteoclasts. This study was planned with i-PRF as it was a comparatively less invasive procedure with better acceleration of orthodontic tooth movement. This study not only aimed to study the effects of i-PRF on orthodontic tooth movement but also evaluated whether it has a synergistic effect when the intervention of i-PRF was done on the day of extraction.

MATERIALS AND METHODS

The study was designed as a split-mouth randomised controlled trial, with the sample selected from patients undergoing fixed orthodontic treatment from the Department of Orthodontics and Dentofacial Orthopaedics in CSI College of Dental Sciences and Research, Madurai, Tamil Nadu, India, from October 2021 to February 2022. The ethical clearance for the study was obtained with the ref no: CSICDSR/IEC/0158/2021 dated 04.09.2021, and patient consent to participate in the study was obtained in writing before their inclusion in the study, with due confidentiality of details obtained for the study.

Sample size calculation: The present study was conducted as a pilot study with 12 patients contributing 48 canines, with 12 canines allocated to each of the four groups. The sample size was calculated using G*Power software based on the study by Erdur EA et al., [5]. Assuming a detectable mean difference of 0.50 mm between the study groups with a pooled standard deviation of 0.40 mm, a significance level (α) of 0.05, and a study power of 80%, the required sample size was estimated.

Inclusion criteria: Patients aged between 13-21 years undergoing orthodontic treatment following extraction of all first premolars with an MBT appliance 0.022 slot, in whom levelling and alignment are completed, and who are planned to proceed with individual canine retraction were included.

Exclusion criteria: Syndromic patients; patients with systemic conditions such as osteoporosis or asthma that affect orthodontic tooth movement were excluded. Lactating and pregnant women; patients with anaemia (Hb <10g/dL), patients with blocked-out or impacted canines; patients with pre-existing mobility of the tooth; mutilated malocclusion were not considered.

Study Procedure

Preparation of i-PRF: The precursor of i-PRF is PRP. PRP is prepared by using the double-spin technique suggested by Marx RE; however, i-PRF is prepared through a single centrifugation at low speed (approximately 60 g, 700 rpm for three minutes in the centrifuge), as described by authors such as Miron RJ et al., [6,7].

A venous blood sample of 10 mL was taken from each patient using a 30 mL injection syringe from brachial vein of the left arm in 10 mL i-PRF tubes without any anticoagulant, and it was immediately centrifuged at 700 rpm for three minutes at room temperature using a PRF duo centrifuge [Table/Fig-1]. The supernatant straw-coloured fluid, which is rich in platelets, was collected as the i-PRF from those test tubes into 23-gauge sterile syringes.



[Table/Fig-1]: Double centrifuge machine.

Intervention and control group: For patients included in the study, initially two first premolars, both right and left of the upper arch, were extracted and categorised as group1 and group 2. Allocation to groups was done using simple randomisation (lot method for side determination). One first premolar of the mandibular arch was also extracted in the same week, 3 days post-extraction of groups 1 and 2 and was categorised as group 3. After a minimum of four weeks, the remaining first premolar of the mandibular arch was extracted and categorised as group 4. Groups 1, 3,4 all received the intervention (i-PRF), and group 2 remained as the control, receiving saline. This is a single-blinded study, with the participant being blinded to the intervention side. In the maxillary arch the major comparison is between left and right side where in both the premolars were extracted on same day with one group receiving the intervention of i-PRF and the other remained a control but with respect to mandibular arch the major distinction is between right and left-side who received same intervention of i-PRF but had one healed socket and one immediate extraction site. This comparison was used to analyse if i-PRF has a synergistic effect in accelerating orthodontic tooth movement. The Consolidated Standards of Reporting Trials (CONSORT) flow chart CONSORT flow should state that each patient contributed four quadrants (n=12 patients), with 12 quadrants allocated to each group. Groups 1 and 2 were maxillary and differed by intervention; groups 3 and 4 were mandibular, received the same intervention, and differed in extraction timing. No patients were lost to follow-up or excluded from analysis, explained in [Table/Fig-2].

Intervention protocol: The intervention side was anaesthetised with local anaesthesia lignox 2% A. Then 0.25 mL of i-PRF was injected sub mucousally on both buccal and palatal side along with an intraligamentary injection on the middle, distobuccal, and distopalatal areas of the respected distal surfaces of the canine [Table/Fig-3a,b,4].

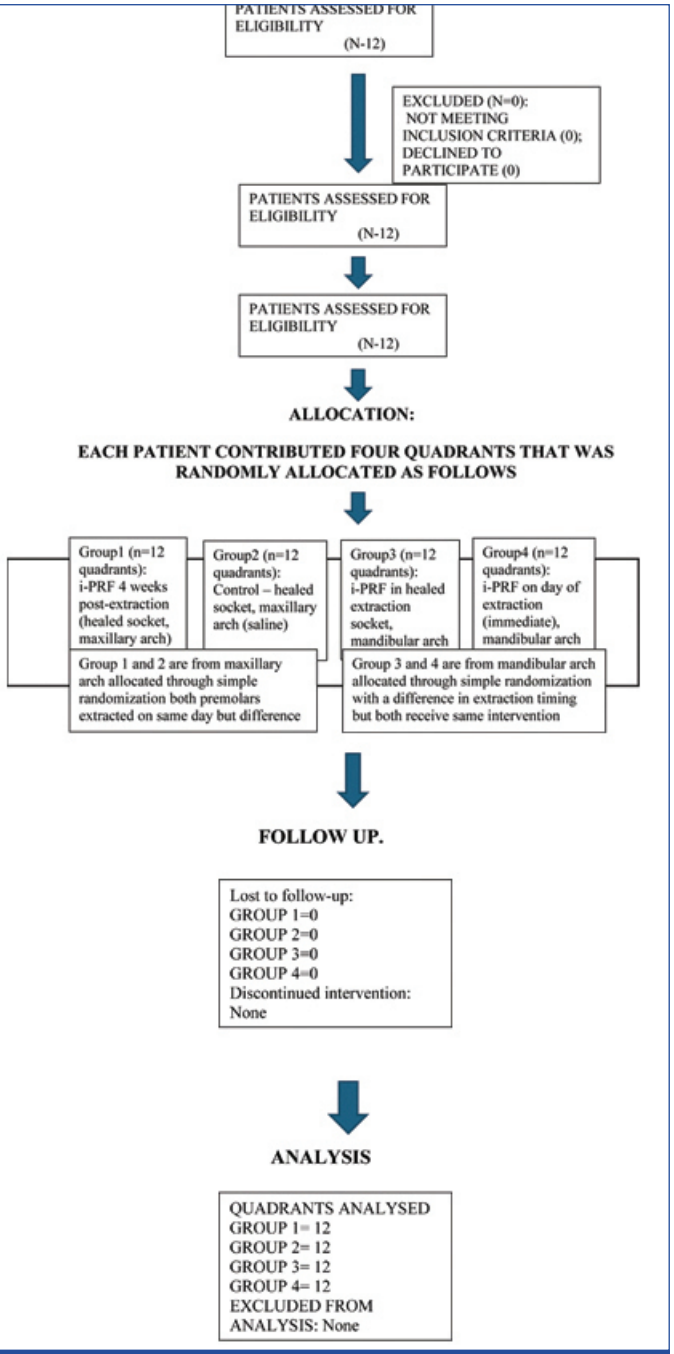
The control side was anaesthetised and injected with isotonic saline following the same protocol as in the intervention site for blinding purposes. This is a single-blinded trial in which the participants were blinded to the intervention side. Patients in group 1, group 3 and group 4 were given injectable PRF intervention, and group 2 remained as the control, wherein 1.5 mL of saline was given. Then all four canines were retracted individually using a NITI closed coil spring with an average force of 150 g. Intervention was given on day 0 and day 2. Groups 1, 3 and 4 received intervention of i-PRF.

Records: Alginate impressions were made at the start of the study, day 0 (T-0), day 21(T-1) and day 42 (T-2). Lateral Cephalogram (Ceph) and OPG were taken on day 0 and day 42 (T-0 and T-2). For differentiation between the right and left canine and molars in both OPG and lateral ceph, stainless steel jigs were customised in two specific designs [Table/Fig-5a,b].

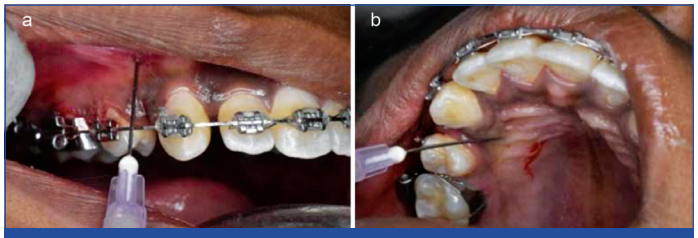
Measurements made: From the OPG, the change in the mesiodistal angulation of the canine and molar was determined with respect to the intercondylar axis as the reference to determine the tipping of the tooth [Table/Fig-6].

From the lateral Ceph, we measured the distance between the canines, molars and the nasion perpendicular, and the difference in value between pre and post showed the rate of canine retraction and anchorage loss, respectively [Table/Fig-7a,b].

In the model, two lines were drawn: one perpendicular to the incisal edge of the lateral incisor passing through its midline, dividing it into two halves and the other through the canine tip, bisecting the crown into two halves. The distance between these two lines was measured at each time interval, and the differences in the values between the time intervals were obtained from which the rate of canine retraction can be identified (B) [Table/Fig-8].



[Table/Fig-2]: CONSORT flow diagram.



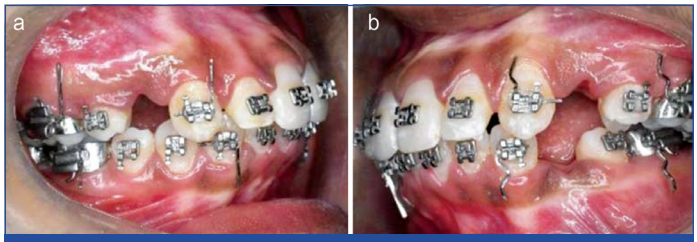
[Table/Fig-3]: a) Injection of i-PRF on buccal side of canine; b) Injection of i-PRF on palatal distal side of canine.

Angulation changes between the line drawn through the mesial and distal edges of the canine and the line drawn perpendicular through the midpalatal raphe were measured, and any change in angulation gave a picture of canine rotation (A). Anchorage loss was measured as the change in distance between the horizontal line drawn through the third palatal rugae and a horizontal line through the mesiobuccal cusp tip of the molar extending to the midpalatine raphe, and any difference in value between the time intervals indicates anchorage loss (C) [Table/Fig-8]:

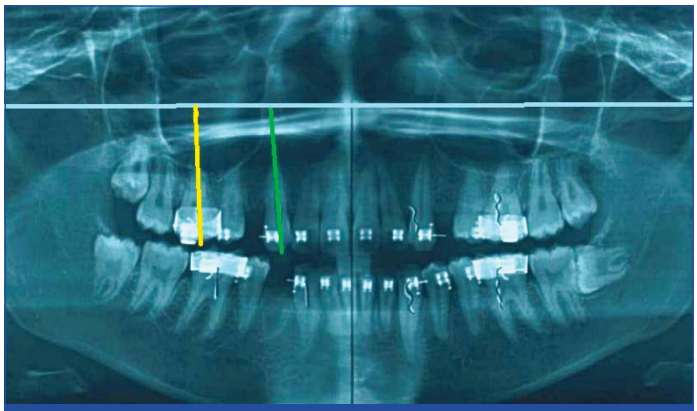
Parameters measured from models at three time frames: T-0 (day 0), T-1 (day 21) and T-2 (day 42) [Table/Fig-8].



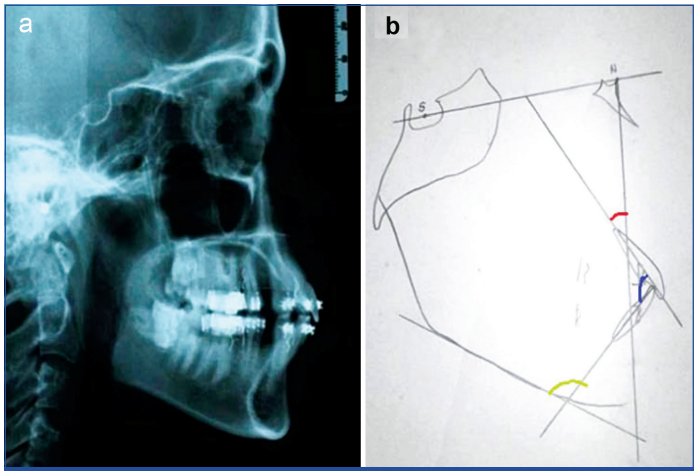
[Table/Fig-4]: Injection of i-PRF in an immediate extraction socket.



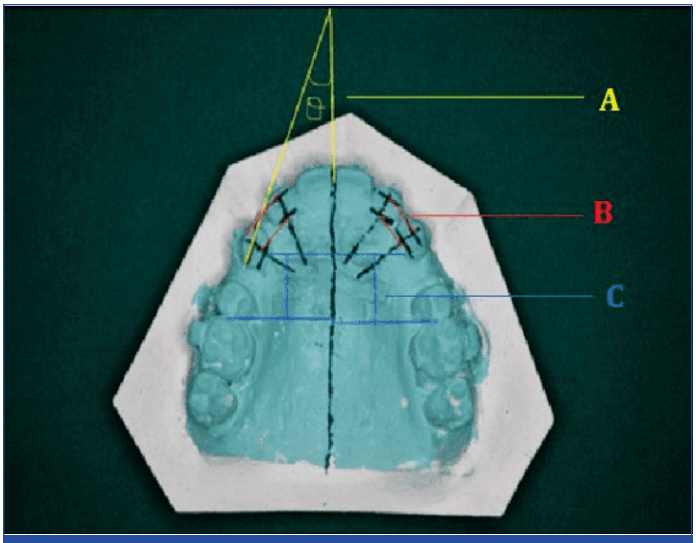
[Table/Fig-5]: a) Stainless steel jigs in canine and molars for differentiation between right and left-side made of same wire dimension but different configuration- right side; b) Stainless steel jigs in canine and molars for differentiation between right and left side made of same wire dimension but different configuration- left-side.



[Table/Fig-6]: OPG with the intercondylar axis that is used as a reference, along with its measurement lines. The light blue horizontal line is the intercondylar axis, with the green lines showing the long axis of the canine and the yellow line depicting the long axis of the molar and the angle between these lines gives the inclination of canine and molar.



[Table/Fig-7]: a) Lateral Ceph with stainless steel jigs for side differentiation; b) Cephalometric tracing used for linear and angular measurement, with red angle indicating the angulation of upper incisor to S-N plane, green angle depicting angulation of lower incisor to tangent of mandibular body and blue depicting interincisal angle. Linear measurement is the distance between the perpendiculars to the corresponding jigs placed on the molar and canine, which will give the estimation of canine retraction and anchor loss.



[Table/Fig-8]: Models with the reference lines marked that are used to measure the rate of canine retraction (B), canine rotation (A) and anchorage loss (C).

1. Anchor loss of molars;
2. Canine rotation;
3. Canine retraction at incisal, middle and cervical region.

Parameters measured from OPG and Lateral Ceph at two time frames: T-0 (day 0) AND T-2 (day 42) [Table/Fig-6,7].

1. Nasion perpendicular to canine and molar (linear measurement);
2. Angular changes in molar and canine in relation to intercondylar axis;
3. Inclination changes in the incisor.

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for Social Sciences (SPSS) version 16.0 (IBM Corp., Armonk, NY, USA). Intergroup comparisons were performed using One-way ANOVA followed by Tukey post-hoc analysis. A p-value<0.05 was considered statistically significant.

RESULTS

A total of 12 patients (48 canines) were included in the study. The intervention groups demonstrated greater canine retraction than the control group throughout the study period.

Canine rotation: No statistically significant intergroup differences were observed in canine rotation during the T0-T1 (p=0.251) and T1-T2 (p=0.321) intervals [Table/Fig-9].

Cephalometric variables: N-canine measurements demonstrated a statistically significant difference in canine retraction among the four groups (p<0.001). No significant intergroup difference was observed for N-molar measurements (p=0.827), indicating satisfactory anchorage control [Table/Fig-10].

Tukey's post-hoc analysis showed significantly greater canine retraction in the intervention groups than in the control group. Significant pairwise differences were observed between group 1 and group 2 (p<0.001), group 1 and group 3 (p<0.001), and group 3 and group 4 (p=0.027). No significant difference was found between group 1 and group 4 (p=0.649) [Table/Fig-11].

OPG variables: No statistically significant intergroup difference was observed in canine angulation (p=0.354). Molar angulation showed a statistically significant intergroup difference (p<0.001) [Table/Fig-12].

Tukey's post-hoc analysis demonstrated that the significant difference in molar angulation was observed between Group 1 and 3 (p=0.017), Group 2 and Groups 4 (0.042) and Groups 3 and 4 (<0.001) whereas the remaining pairwise comparisons were not statistically significant [Table/Fig-13].

Rate of canine retraction: The rate of canine retraction differed significantly among the four groups during the T0–T1, T1–T2, and T0–T2 intervals (p<0.001). The greatest canine retraction was observed in group 4, followed by group 1 [Table/Fig-14].

Intergroups comparison		N	Mean±SD	Std. Error	95% Confidence interval for mean		Min	Max	p-value
					Lower bound	Upper bound			
Canine rotation (T1-T0)	Group 1	12	-1.17±0.937	0.271	-1.76	-0.57	-3	0	0.251
	Group 2	12	-1.50±0.905	0.261	-2.07	-0.93	-3	0	
	Group 3	12	-1.00±1.128	0.326	-1.72	-0.28	-3	0	
	Group 4	12	-0.75±0.622	0.179	-1.14	-0.36	-2	0	
	Total	48	-1.10±0.928	0.134	-1.37	-0.83	-3	0	
Canine rotation (T2-T1)	Group 1	12	-0.33±0.492	0.142	-.65	-0.02	-1	0	0.321
	Group 2	12	-0.67±0.778	0.225	-1.16	-0.17	-2	0	
	Group 3	12	-0.92±1.084	0.313	-1.61	-0.23	-3	0	
	Group 4	12	-0.75±0.622	0.179	-1.14	-0.36	-2	0	
	Total	48	-0.67±0.781	0.113	-0.89	-0.44	-3	0	

[Table/Fig-9]: Intergroup comparison for canine rotation performed using One-way ANOVA test.

Intergroup comparison		N	Mean±SD	Std. Error	95% Confidence interval for mean		Min	Max	p-value
					Lower bound	Upper bound			
N-canine (T1-T0)	Group 1	12	-3.083±1.0836	0.3128	-3.772	-2.395	-6.0	-2.0	<0.001
	Group 2	12	-1.208±1.1172	0.3225	-1.918	-0.499	-4.0	1.0	
	Group 3	12	-1.333±0.7785	0.2247	-1.828	-0.839	-3.0	0.0	
	Group 4	12	-2.583±1.1645	0.3362	-3.323	-1.843	-6.0	-2.0	
	Total	48	-2.052±1.2975	0.1873	-2.429	-1.675	-6.0	1.0	
N-molar (T1-T0)	Group 1	12	0.50±0.674	0.195	0.07	0.93	0	2	0.827
	Group 2	12	0.7500±0.86603	0.25000	0.1998	1.3002	0.00	2.00	
	Group 3	12	0.75±0.866	0.250	0.20	1.30	0	2	
	Group 4	12	0.92±1.676	0.484	-0.15	1.98	0	6	

[Table/Fig-10]: Intergroup comparison for cephalometric variables (N-canine, N-molar) performed using One-way ANOVA test.

Multiple comparisons							
Dependent variables	Group (I)	Group (J)	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
N-canine (T1-T0)	Group 1	Group 2	-1.8750	0.4274	<0.001	-3.016	-0.734
		Group 3	-1.7500	0.4274	<.001	-2.891	-0.609
		Group 4	-0.5000	0.4274	0.649	-1.641	0.641
	Group 2	Group 3	0.1250	0.4274	0.991	-1.016	1.266
		Group 4	1.3750	0.4274	0.013	0.234	2.516
	Group 3	Group 4	1.2500	0.4274	0.027	0.109	2.391
N-molar (T1-T0)	Group 1	Group 2	-0.25000	0.44559	0.943	-1.4397	0.9397
		Group 3	-0.25000	0.44559	0.943	-1.4397	0.9397
		Group 4	-0.41667	0.44559	0.786	-1.6064	0.7731
	Group 2	Group 3	.00000	0.44559	1.000	-1.1897	1.1897
		Group 4	-0.16667	0.44559	0.982	-1.3564	1.0231
	Group 3	Group 4	-0.16667	0.44559	0.982	-1.3564	1.0231

[Table/Fig-11]: Intergroup comparison for cephalometric variables (N-canine, N-molar) performed using post-hoc Tukey test (after One-way ANOVA test).

Intergroup comparison		N	Mean±SD	Std. Error	95% Confidence interval for mean		Min	Max	p-value
					Lower bound	Upper bound			
Canine angulation (T1-T0)	Group 1	12	.25±1.485	0.429	-0.69	1.19	-2	4	0.354
	Group 2	12	2.17±1.586	0.458	1.16	3.17	0	5	
	Group 3	12	1.58±3.528	1.018	-0.66	3.82	-4	9	
	Group 4	12	1.17±3.298	0.952	-0.93	3.26	-2	8	
	Total	48	1.29±2.657	0.384	0.52	2.06	-4	9	
Molar angulation (T1-T0)	Group 1	12	-0.08±0.515	0.149	-0.41	0.24	-1	1	<0.001
	Group 2	12	0.75±1.215	0.351	-0.02	1.52	0	4	
	Group 3	12	1.33±1.303	0.376	0.51	2.16	0	3	
	Group 4	12	-0.50±1.243	0.359	-1.29	0.29	-3	1	
	Total	48	0.38±1.299	0.187	0.00	0.75	-3	4	

[Table/Fig-12]: Intergroup comparison for OPG variables (canine angulation, molar angulation) performed using One-way ANOVA test.

Multiple comparisons							
Dependent variables	Group (I)	Group (J)	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Molar angulation (T1-T0)	Group 1	Group 2	-0.833	0.456	0.274	-2.05	0.38
		Group 3	-1.417	0.456	0.017	-2.63	-0.20
		Group 4	0.417	0.456	0.797	-0.80	1.63
	Group 2	Group 3	-0.583	0.456	0.580	-1.80	0.63
		Group 4	1.250	0.456	0.042	0.03	2.47
	Group 3	Group 4	1.833	0.456	<0.001	0.62	3.05

[Table/Fig-13]: Intergroup comparison for OPG variables (molar angulation) performed using post-hoc Tukey test (after One-way ANOVA test).

Tukey's post-hoc analysis confirmed significantly greater canine retraction in the intervention groups compared with the control group. Among the intervention groups, Group 1 showed significantly greater retraction than group 3 at the incisal and cervical levels during the T1-T0 interval (p=0.026 and p=0.018, respectively). Group 4 also demonstrated significantly greater retraction than group 3 at the incisal level during the T1–T0 (p=0.011) and T0–T2 (p=0.003) intervals. No significant difference was observed between groups 1 and 4 [Table/Fig 15].

DISCUSSION

The present study was primarily aimed to studying the effect of injectable PRF on orthodontic tooth movement. As suggested by the previous literature evidence, the current study showed there was an accelerating effect on orthodontic tooth movement when

Intergroup comparisons		N	Mean±SD	Std. error	95% Confidence Interval for Mean		Min	Max	p-value
					Lower bound	Upper bound			
Incisal (T1-T0)	Group 1	12	1.50±0.61949	0.1788	1.1065	1.8935	0.90	2.80	<0.001
	Group 2	12	0.683±0.4108	0.1186	0.4223	0.9444	0.2	1.8	
	Group 3	12	0.883±0.5024	0.1450	0.5641	1.2026	0.40	2.30	
	Group 4	12	1.567±0.4997	0.1443	1.2492	1.8842	1	2.70	
	Total	48	1.15833±0.6294	0.0908	0.9756	1.34108	0.2	2.80	
Cervical (T1-T0)	Group 1	12	0.992±0.3502	0.1011	0.769	1.214	0.5	1.6	<0.001
	Group 2	12	0.400±0.3133	0.0905	0.201	0.599	0.1	1.1	
	Group 3	12	0.600±0.2954	0.0853	0.412	0.788	0.2	1.0	
	Group 4	12	0.992±0.2843	0.0821	0.811	1.172	0.5	1.4	
	Total	48	0.746±0.3973	0.0573	0.630	0.861	0.1	1.6	

Incisal (T2-T1)	Group 1	12	1.0875±0.34534	0.09969	0.8681	1.3069	0.50	1.70	0.009
	Group 2	12	0.6833±0.47258	0.13642	0.3831	0.9836	0.20	1.90	
	Group 3	12	0.7583±0.32879	0.09491	0.5494	0.9672	0.30	1.40	
	Group 4	12	1.0917±0.25030	0.07226	0.9326	1.2507	0.70	1.50	
	Total	48	0.9052±0.39444	0.05693	0.7907	1.0197	0.20	1.90	
Cervical (T2-T1)	Group 1	12	.6117±0.25548	0.07375	0.4493	0.7740	0.24	1.00	0.013
	Group 2	12	.3417±0.29064	0.08390	0.1570	0.5263	0.00	1.10	
	Group 3	12	.5167±0.29181	0.08424	0.3313	0.7021	0.20	1.30	
	Group 4	12	.7000±0.20889	0.06030	0.5673	0.8327	0.30	0.90	
	Total	48	.5425±0.28846	0.04164	0.4587	0.6263	0.00	1.30	
Incisal (T2-T0)	Group 1	12	2.5875±0.61761	0.17829	2.1951	2.9799	2.00	4.20	<0.001
	Group 2	12	1.3667±0.78199	0.22574	0.8698	1.8635	0.80	3.70	
	Group 3	12	1.6417±0.59001	0.17032	1.2668	2.0165	1.10	3.10	
	Group 4	11	2.7000±0.70143	0.21149	2.2288	3.1712	1.70	4.20	
	Total	48	2.0606±0.87706	0.12793	1.8031	2.3182	0.80	4.20	
Cervical (T2-T0)	Group 1	12	1.6033±0.45342	0.13089	1.3152	1.8914	0.90	2.50	<0.001
	Group 2	12	.7417±0.47570	0.13732	0.4394	1.0439	0.40	2.20	
	Group 3	12	1.1167±0.41524	0.11987	0.8528	1.3805	0.70	2.30	
	Group 4	12	1.6917±0.42095	0.12152	1.4242	1.9591	1.10	2.30	
	Total	48	1.2883±0.57758	0.08337	1.1206	1.4560	0.40	2.50	

[Table/Fig-14]: Intergroup comparison for rate of canine retraction performed using one-way ANOVA test.

Multiple comparisons							
Dependent variables	Group (I)	Group (J)	Mean difference (I-J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
Incisal (T1-T0)	Group 1	Group 2	0.8166	0.2096	0.002	0.2569	1.3763
		Group 3	0.6166	0.20961	0.026	0.05698	1.1763
		Group 4	-0.0666	0.2096	0.989	-0.6263	0.4930
	Group 2	Group 3	-0.20	0.2096	0.776	-0.7596	0.3596
		Group 4	-0.8833	0.2096	0.001	-1.4430	-0.3236
	Group 3	Group 4	-0.6833	0.2096	0.011	-1.2430	-0.1236
Cervical (T1-T0)	Group 1	Group 2	0.5917	0.1273	0.001	0.252	0.932
		Group 3	0.3917	0.1273	0.018	0.052	0.732
		Group 4	0.0000	0.1273	1.000	-0.340	0.340
	Group 2	Group 3	-0.2000	0.1273	0.405	-0.540	0.140
		Group 4	-0.5917	0.1273	0.001	-0.932	-0.252
	Group 3	Group 4	-0.3917	0.1273	0.018	-0.732	-0.052
Incisal (T2-T1)	Group 1	Group 2	0.40417	0.14625	0.040	0.0137	0.7947
		Group 3	0.32917	0.14625	0.126	-0.0613	0.7197
		Group 4	-0.00417	0.14625	1.000	-0.3947	0.3863
	Group 2	Group 3	-0.07500	0.14625	0.956	-0.4655	0.3155
		Group 4	-0.40833	0.14625	0.037	-0.7988	-0.0178
	Group 3	Group 4	-0.33333	0.14625	0.119	-0.7238	0.0572
Cervical (T2-T1)	Group 1	Group 2	0.27000	0.10773	0.073	-0.0176	0.5576
		Group 3	0.09500	0.10773	0.814	-0.1926	0.3826
		Group 4	-0.08833	0.10773	0.845	-0.3760	0.1993
	Group 2	Group 3	-0.17500	0.10773	0.376	-0.4626	0.1126
		Group 4	-0.35833	0.10773	0.009	-0.6460	-0.0707
	Group 3	Group 4	-0.18333	0.10773	0.335	-0.4710	0.1043
Incisal (T2-T0)	Group 1	Group 2	1.22083	0.27613	0.001	0.4829	1.9588
		Group 3	0.94583	0.27613	0.007	0.2079	1.6838
		Group 4	-0.11250	0.28233	0.978	-0.8670	0.6420
	Group 2	Group 3	-0.27500	0.27613	0.753	-1.0129	0.4629
		Group 4	-1.33333	0.28233	0.001	-2.0878	-0.5788
	Group 3	Group 4	-1.05833	0.28233	0.003	-1.8128	-0.3038
Cervical (T2-T0)	Group 1	Group 2	0.86167	0.18045	0.001	0.3799	1.3435
		Group 3	0.48667	0.18045	0.047	0.0049	0.9685

		Group 4	-0.08833	0.18045	0.961	-0.5701	0.3935
	Group 2	Group 3	-0.37500	0.18045	0.176	-0.8568	0.1068
		Group 4	-0.95000	0.18045	0.001	-1.4318	-0.4682
	Group 3	Group 4	-0.57500	0.18045	0.014	-1.0568	-0.0932

[Table/Fig-15]: Intergroup comparison for rate of canine retraction performed using post-hoc Tukey test (after One-way ANOVA test).

an intervention with i-PRF was given. The unique point that differs from previous studies is that our study also found that there was a synergistic effect of immediate extraction and i-PRF on accelerating tooth movement. So, the study demonstrated that performing extraction and intervention with i-PRF on the same day might accelerate tooth movement more effectively.

Abbas NH et al., considered the rate of tooth movement with an optimal orthodontic force to be around 1.0 - 1.5 mm in 4 - 5 weeks as a baseline to compare in his study on the effect of corticotomy in accelerating orthodontic tooth movement [8]. Tooth movement depends on magnitude along with duration of orthodontic force, bone quality, age, number, along with shape of roots and type of tooth movement attempted. To prove that an accelerating method is effective, the movement of the tooth must be greater than 1.25 mm in four weeks, which is justified in our study by using a time period of six weeks, and all the groups which were given an intervention of i-PRF showed an average tooth movement greater than 1.99 mm-3.4 mm in six weeks

Che Y et al., studied the quantities of 3 GFs(TGFβ, PDGF-AB and VEGF) released by PRF membrane on seven experimental time lines (20 minutes, one hour, four hours, 24 hours, 72 hours, 120 hours and 168 hours) which showed that the PRF membrane remain in solid form and continues releasing the GFs consistently upto seven to 10 days so several authors advocated repetitive injection of i-PRF to produce sustained release of GFs for 10 days followed by a free period of 10 days to overcome hyalinisation which is justified in this study with two intervention once in three weeks in the total six week time period [9].

Erdur EA et al., showed a significant difference in canine retraction between the study group and control group that is in compliance with the current study results [5]. He also attempted to prove that there was an increase in stimulation of inflammatory cytokines, which supported his result (p=0.001). The quantification of cytokines varied in both groups between time intervals T1 and T2, in which IL-1b, MMP8, and RANKL values were statistically significant, while the OPG values were decreased, which was statistically significant.

Shroff B stated that the rate of tooth movement is also affected by the type of movement as teeth move faster by tipping than by translation and in the current study it was observed that when there is greater acceleration in tooth movement it results in tipping and not in bodily movement that is evident from the difference in values obtained from the cervical and incisal aspect of tooth among the group that received intervention but control group also showed tipping only so this may also be attributed to the use of NiTi closed coil spring [10]. Among all the groups, the immediate extraction with intervention showed the maximum amount of canine retraction and was found to be a tipping movement, as clearly seen in the difference between the rate of retraction in the incisal and cervical among all the groups, immediate extraction with intervention showed the maximum amount of canine retraction and was found to be predominantly a tipping movement, as clearly evident from the difference between the rates of retraction at the incisal and cervical aspects. This finding is consistent with the observations of Shroff B, who reported that teeth move faster by tipping than by translation, suggesting that the greater rate of canine retraction observed in the immediate extraction with intervention group may be partly attributable to the predominance of tipping movement [10].

El Timamy A et al., showed that injectable PRP proved to have a faster rate of canine retraction in the first interval by 15% and in the second interval by 5% [11]. Even though the increase was statistically significant in the first month and non-significant in the second month, he concluded a positive correlation between PRP injection and accelerated orthodontic movement. As an unexpected consequence in the third interval following cessation of PRP injections, canine retraction rate on the intervention side was slower compared to the control side by 40%. He stated this could be a negative feedback mechanism with respect to the release of GF, similar to that of a hormonal negative feedback mechanism. We observed in certain cases that there was a decrease in tooth movement just after the second intervention, even when it accelerated after the first intervention, which could be attributed to the negative feedback, which also correlated with Nakornnoi T et al., who observed the same phenomenon while studying around 20 New Zealand rabbits in a split-mouth pattern [12].

Hassler R et al., studied the rate of movement of maxillary canines into healed and recent extraction sites of the first premolar, which was evaluated in 22 patients within the age group 10-27 years [13]. On one side of the dental arch, the first premolar was first extracted. After 86 days, the first premolar of the contralateral side was extracted, and the individual canine retraction was started using the Gjessing canine retraction spring. A higher rate of tooth movement into a recent compared with a healed extraction site was found. These findings support the observations of the present study.

This may be based on the assumption that the centre of resistance of the tooth is usually located 10 mm below the bracket gingival margin. Considering the character of bone to be denser around the apex of the tooth compared to the marginal area, the centre of resistance of the tooth might be located more apically in the recently extracted socket, as stated by Hassler R et al., [13]. For a bodily movement to occur in this area, we require a higher moment-to-force ratio than the NiTi spring actually documented to exert. This insufficient moment-to-force ratio might result in excessive tipping of the tooth more than was intended [14-16]. This would have happened in our study; that is why we found excessive tipping in the recent extraction site compared with the healed extraction site, which is evident from the angulation change observed in the OPG, even though not statistically significant

Among all available methods about non-surgical methods for accelerating tooth movement, injectable PRF is considered a recent invention and has its own benefits that are superior to other techniques in accelerating tooth movement. Scala A et al., in their study, showed that a PRF clot contains numerous cytokines, namely IL-6, IL-1β, IL-1, TNF-α, and healing cytokines, such as IL-4 and VEGF [17]. These components are the reasons why PRF has anti-inflammatory properties, as well as it acts by accelerating angiogenesis [18,19] and increasing the production of fibroblasts along with osteoblasts, as a consequence of which there is enhancement in healing. The i-PRF is a second-generation component of the PRF clot that has all the properties of PRF [20]. Recent meta-analyses and studies have suggested i-PRF is a safe and viable alternative that provides effective acceleration of orthodontic tooth movement [21-23].

Limitation(s)

The present study was limited by its small sample size, short observation period, and split-mouth design. Potential crossover effects of circulating GFs could not be eliminated. Long-term effects on root resorption and periodontal health were not evaluated.

CONCLUSION(S)

Within the limitations of this pilot randomised controlled trial, submucosal administration of i-PRF significantly increased the rate of canine retraction compared with the control group. Furthermore, administration of i-PRF immediately following extraction demonstrated a greater acceleration of tooth movement than administration in healed extraction sites. Although the observed tooth movement was predominantly tipping rather than bodily movement, the findings suggest that i-PRF may serve as a promising minimally invasive adjunct for accelerating orthodontic treatment. Further multicenter studies with larger sample sizes and longer follow-up periods are required to validate these findings.

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