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Effect of local injection of injectable platelet-rich fibrin on eruption rate of teeth (Rabbit animal model)

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Abstract

OBJECTIVE: This study aimed to evaluate the effect of the local injection of injectable platelet-rich fibrin (i-PRF) on the eruption rate of the teeth and evaluation of the effect of i-PRF on the number of odontoblast, cementoblast, osteoblast, osteocyte, and osteoclast cells.

MATERIALS AND METHODS: The samples consisted of 20 male albino rabbits and were divided randomly into the control group (5 rabbits) and i-PRF groups (15 rabbits) sub-divided into week 1, week 2, and week 3 sub-groups. The labial surface of lower right incisors was marked by drilling a hole at the level of the free gingival margin in the mid-line of each tooth with a small round bur, and the eruption rates were measured by measuring the distance from the most apical point of the free gingival margin and the center of the hole with a digital caliper. i-PRF was prepared by centrifuging autologous blood of each rabbit at 1000 rpm for 5 minutes; then the i-PRF layer was collected. The control group received no treatment, whereas i-PRF groups received i-PRF in the following manner: For week 1 groups, a single dose was given at 0 day; for week 2 groups, double doses were given at 0 and 7 days; and for week 3 groups, three doses were given at 0, 7, and 14 days. At the end of experiments for each group, animal scarification was performed, and histological steps were performed.

RESULTS: There was a significant increase in the rate of tooth eruption in i-PRF groups when compared to the control group, and the eruption rate was increased in 107.3%, 144.7%, and 167.5% for week 1, week 2, and week 3, respectively. Concerning the histological finding, the i-PRF groups gave rise to a higher number of odontoblast, cementoblast, osteoblast, osteocyte, and osteoclast cells with a significant difference when compared to the control groups ($P \leq 0.05$).

CONCLUSIONS: Applying i-PRF significantly increased the rate of teeth eruption at all-time intervals. Platelet-rich fibrin injection can be an effective method for acceleration of teeth eruption.

Keywords:

Acceleration of tooth eruption, eruption rate, incisor teeth, injectable platelet-rich fibrin

Introduction

Tooth eruption is a developmental process that happens because of the movement of the tooth from its development area within the dental arches to its final position of occlusion.[1] It is a continuous process that occurs over a broad chronological age range and ends only with the loss of the tooth and is considered as an important milestone during child development.[2] The complex and finely regulated process of eruption strongly affects the normal development of the craniofacial complex.[3] The process of tooth eruption can be divided into three phases: Pre-eruptive phase, eruptive phase, and post-eruptive phase.[4] There are multiple theories that discuss the mechanism of eruption, such as the root formation theory, hydrostatic pressure theory, bone remodeling theory, periodontal ligament traction theory, dental follicle theory,

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neuromuscular theory, and innervation-provoked pressure theory.[5] Also, tooth eruption is a unique biological process which is controlled by cascades of molecules and signaling pathways.[6] These biological molecules control the cellular activity responsible for eruption.[7] Disruption of the eruption process may lead to delayed eruption or complete failure of eruption, which have a significant impact on a child's proper health.[8,9] The potential of the best control along the eruptive process and maintaining the inclusive health of teeth is a major reason to new research in this field. A delay or failure of eruption results in orthodontic and prosthodontic problems with difficult therapeutic interventions and minimum promise of perfect correcting. A variety of agents have been used either locally or systemically, which affect the rate of teeth eruption.[10] There are many methods used to measure the eruption rate of the teeth, such as bur marks and radiographic, optical, and fluorescence markers, but the bur marks method is the easiest, less expensive, and reliable method for calculation of eruption rate.[11-13]

The innovative advancement in the field of platelet-rich fibrin (PRF), such as injectable PRF (i-PRF), has paved the way for the usefulness in the uses of platelet concentrate.[14] This liquid form of PRF is prepared by using blood without any additives or anti-coagulant and at low-speed centrifugation (700 rpm for 3 minutes) in plastic tubes.[15] The resultant liquid PRF is a concentrate of platelets, leukocytes, and their growth factors in a liquid fibrinogen-based matrix. It maintains its liquid form for about 15 to 30 minutes at room temperature, and the fibrin clot forms thereafter.[16] It has advancement through injecting patients autologous PRF in affected areas of soft tissue, mucous membrane, or skin.[17] The main components of i-PRF have been noted as being key components assisting in tissue regeneration, and these include platelets, leukocytes, fibrin matrix, stem cells, and growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), insulin growth factor-1 (IGF-1), epidermal growth factor (EGF), tumor necrosis factor-α (TNF-α), platelet-derived growth factor (PDGF), interleukin-4 (IL-4), interleukin-1 (IL-1), and interleukin-6 (IL-6).[18,19] The powerful benefits of i-PRF have been greatly investigated in dental fields (e.g., in grafting of bone and regeneration, in oral medicine, in implantology, in periodontal therapy, in orthodontic fields, and in the field of facial rejuvenation.[20]

The aims of this study are to evaluate the effect of the local injection of i-PRF on the eruption rate of the teeth and the effect of i-PRF on the number of odontoblast, cementoblast, osteoblast, osteocyte, and osteoclast cells.

Materials and Methods

Animal experimental groups

The sample size was calculated according to the method described by Charan and Kantharia, 2013.[21] The sample consisted of 20 apparently healthy mature male albino rabbits with an average weight of 1700 gm ± 2 gm and ranging between 1500 and 1900 gm. The animals were obtained from a local farm with the help of a veterinarian. After that, the rabbits were housed in cages prepared for this experiment with good ventilation and temperature and a stable protocol of feeding.[22] The animals were kept for 2 weeks for hybridization to a new position before experiments. Then, the sample was randomly divided into two groups: a control group (5 rabbits) which received no treatment and an i-PRF group sub-divided into three sub-groups: i-PRF week 1 sub-group (5 rabbits), i-PRF week 2 sub-group (5 rabbits), and i-PRF week 3 sub-group (5 rabbits).

Anesthesia

Each animal was anesthetized with intramuscular injection of a mixture of ketamine and xylazine. Each dose was calculated according to the weight of the rabbit as follows: Ketamine 35 mg/Kg in combination with xylazine 10 mg/kg to produce anesthesia for half an hour.

Measurement of eruption rate

At zero day of the experiment for all groups, the lower right central incisor was marked by drilling a small hole on the mid-point of the cervical third near the free gingival margin using a low-speed handpiece (Coxo, China) with a small round bur in a perpendicular direction to the labial surface of the tooth [Figure 1a]. This hole moved incisively because of continuous eruption, so the eruption rate was calculated by measuring the distance between the deepest border of the free gingival margin and the middle point of the hole using a digital

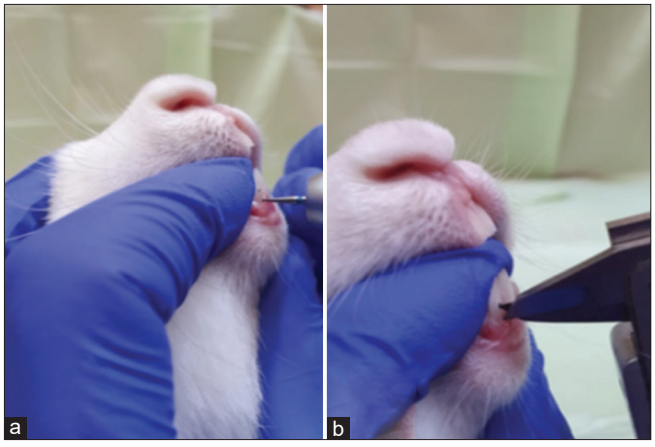

Figure 1: (a) Marking the lower rigrt incisor. (b) Measurement of the eruption rate using a digital caliper

caliper (China) in part of millimeters [Figure 1b].[23,24] Each distance was measured three times, and the mean value was used for data computations. The measurement of eruption rate was repeated two times per week,[12,25] and this means that

- For i-PRF week 1 sub-group, the eruption rates were measured on day 3 and 7 and the animal scarification was made on day 7.
- For i-PRF week 2 sub-group, the eruption rates were measured on days 3, 7, 10, and 14 and the animal scarification was made on day 14.
- For the control group and i-PRF week 3 sub-group, the eruption rates were measured on days 3, 7, 10, 14, 17, and 21 and the animal scarification was made on day 21.

A new mark was drilled at every measurement. All these procedures were performed under anesthesia. The amount of eruption rate in millimeters per day (mm/d) for each week was calculated.

Blood collection and i-PRF preparation

5 ml of blood from each rabbit was collected by the cardiac puncture method and put in plastic tubes of 10 ml without an anti-coagulant (i-PRF; Vacutainer; BD Biosciences, Allschwil, Switzerland) and then centrifuged at 1000 rpm for 5 minutes at room temperature. At the end of centrifuging, the i-PRF layer, which is the upper orange fluid, was aspirated and injected immediately in administration sites [Figure 2].[26,27]

Administration protocol of i-PRF

1 ml of i-PRF was delivered using a disposable one-unit insulin syringe 26-gauge; 0.5 ml was injected in the labial vestibule approximately to a root apex of the lower right central incisor, and the other half of the dose was injected in the lingual vestibule in the same manner. A single dose was given at 0 day for the week2 sub-group, double doses were given at 0 and 7 days for the week 3 sub-group, and three doses were given at 0, 7, and 14 days.

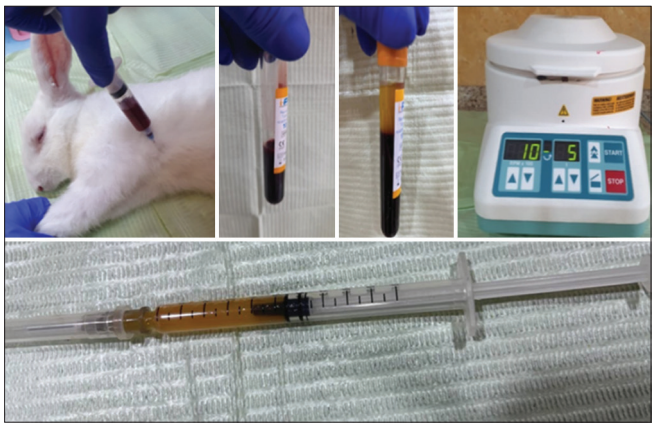

Figure 2: Preparation steps of i-PRF

Histological procedures

At the end of the experiment, the animals were sacrificed, and the lower jaws were dissected out and cleaned by running water. The specimen then immediately was placed in a labeled glass vial containing 10% neutral-buffered formalin solution for 48 hours. Then, the specimens were decalcified and dehydrated and then embedded in paraffin with a conventional technique. A5 μm thick longitudinal serial sections were cut in the labiolingual direction of mandibular right lower incisors with supporting structures and stained with hematoxylin and eosin for examination.[28]

Examination of the slides was performed using a light microscope with a color USB digital image camera (Omax 9.0 M Pixels, China), and histomorphometric analysis was performed by a software program (Omax Toup View program). A5 slides for each specimen were examined by a specialist in histology for counting the numbers of the odontoblast, cementoblast, osteoblast, osteoclast, and osteocyte cells.

Ethics committee

All described experiments were approved by the research ethics committee of University of Mosul/College of Dentistry, REC reference number: UoM.Dent/A.L.33/21 in 29/03/2021.

Statistical analyses

The results obtained for the eruption rate and histological finding were analyzed using IBM SPSS statistics, version 25 (IBM Corporation, USA). The statistical analyses of the data for the eruption rate and histological finding among different groups were performed by ANOVA and Duncan multiple range test. Values of $P \leq 0.05$ were considered statistically significant.

Results

The amount of eruption rate

Descriptive statistics (mean ± SD), ANOVA, and Duncan's multiple range test of eruption rate (millimeter per day) for control groups and i-PRF groups are presented in Table 1 and Figure 3. i-PRF accelerated

Table 1: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of eruption rate (mm/d) for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	0.41	0.01	0.0000	D
	2	0.38	0.03		D
	3	0.40	0.01		D
i-PRF	1	0.85	0.03		C
	2	0.93	0.04		B
	3	1.07	0.02		A

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

the rate of tooth eruption and had the highest mean of eruption rate with a significant difference when compared to the control group. i-PRF week 1 showed a 107.3% increase in eruption rate, i-PRF week 2 showed a 144.7% increase in eruption rate, and i-PRF week 3 showed a 167.5% increase in eruption rate compared to the control group.

The results of the present study showed that the eruption rate increased when the dose of i-PRF was repeated so that the i-PRF week 3 group gave rise to the highest eruption rate with a significant difference with control groups, followed by the week 2 group and finally the week 1 group, whereas the eruption rate for the control group was already stable.

Histological finding:

Regarding the histological findings, the statistical analysis of odontoblast and cementoblast cell number showed that the i-PRF groups had a significantly higher number of odontoblast and cementoblast cells than the control groups at the three time intervals, whereas control groups gave rise to the lowest number of these cells [Tables 2 and 3].

For osteoblast, osteoclast, and osteocyte cell numbers, there was a significant difference in the numbers of osteoblast, osteoclast, and osteocyte cells in the i-PRF groups at a three time intervals when compared to the control group [Tables 4-6] [Figures 4 and 5].

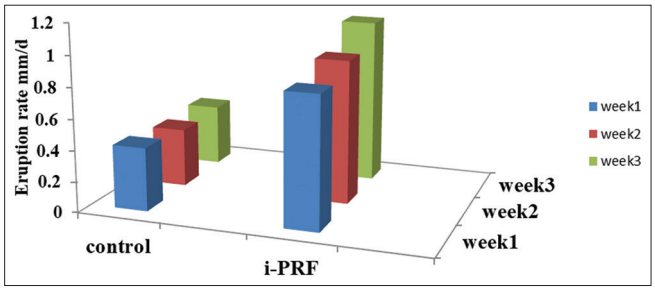

Figure 3: Histogram demonstrated eruption rate (mm/d) of the control group and i-PRF groups in week 1, week 2, and week 3

Discussion

Tooth eruption is a unique biological process which occurs over a broad chronological age range and can be influenced by a number of factors. Variation in the normal eruption of teeth is a common finding, but significant deviations from established norms should alert the clinician to use some procedures to maintain the child's health and normal development of dentofacial structures.[29,30]

Rodents have a property of continuously erupting incisors, and there is no effect of the consistency of the diet and the age of rodents on the eruption rate.[31,32] Rodent incisors have an odontogenic region, so the dental follicle and enamel organ are maintained in this region throughout life. Therefore, the rodents' incisors make an excellent model to study the eruption process mechanisms in its pre-eruption and post-eruption stages.[12,23] Thus, the experimental research of tooth eruption has been performed on it. Rabbits possess continuously erupting teeth displaying the total life cycle of teeth development from inception to maturity.[13,33]

The use of platelet concentration that gives a wide range of growth factors and cytokinesis has increased

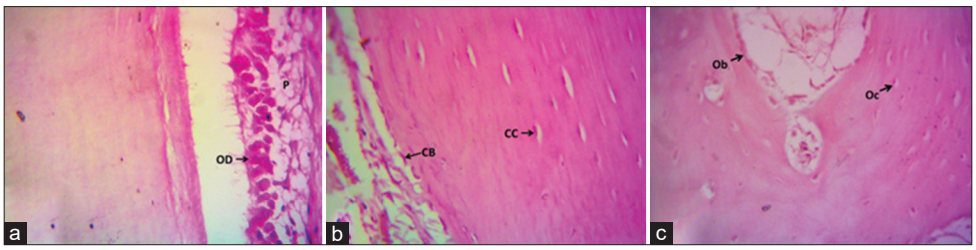

Figure 4: Photomicrograph (H and E stain, 400X) of tooth of the control group at week 3 shows (a) Odontoblast (OD), the pulp (p). (b) Cementoblast (CB) and cementocyte (CC). (c) Osteocytes (Oc) and osteoblasts (Ob)

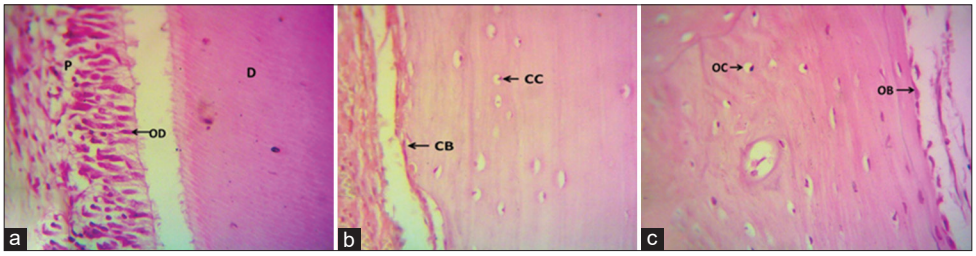

Figure 5: Photomicrograph (H and E stain, 400X) of tooth of the i-PRF group at week 3 shows (a) Odontoblast (OD, pulp (P), dentin (d). (b) Cementoblast (CB) and cementocyte (CC). (c) Osteoblasts (OB) and osteocytes (OC)

Table 2: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of odontoblast numbers for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	8.72	0.83	0.0000	D
	2	8.72	0.83		D
	3	8.72	0.83		D
i-PRF	1	66.00	5.52		B
	2	74.00	7.65		A
	3	43.20	6.50		C

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

Table 3: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of cementoblast number for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	4.20	0.84	0.0000	C
	2	4.20	0.84		C
	3	4.20	0.84		C
i-PRF	1	7.00	1.00		B
	2	7.00	1.41		B
	3	10.00	2.12		A

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

Table 4: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of number of osteoblasts for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	6.64	0.33	0.0000	D
	2	6.64	0.33		D
	3	6.64	0.33		D
i-PRF	1	20.00	2.55		A
	2	15.00	0.96		B
	3	11.72	0.83		C

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

Table 5: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of number of osteoclasts for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	0.40	0.14	0.0000	C
	2	0.40	0.14		C
	3	0.40	0.14		C
i-PRF	1	1.00	0.00		B
	2	0.80	0.45		B
	3	1.80	0.45		A

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

to accelerate wound healing and tissue repair and is also used in the field of regeneration in dentistry and medicine.[34] i-PRF is an autologous, injectable, low-cost, easily prepared, repeatable, and minimally invasive procedure and had a slow release of growth factor.[35] For these reasons, we preferred to use i-PRF in this study.

Teeth eruption is a biological process which is regulated by different molecules and cytokinesis which act on dental follicles and tooth-supporting structures. Thus, the analysis of our results demonstrated that i-PRF increased and accelerated the rate of tooth eruption, and this is because of the presence of a variety of growth factors and cytokines with a high concentration in i-PRF, which stimulated cell migration, proliferation, and differentiation. This result is supported by Miron *et al*., 2017,[26] who stated that i-PRF had the ability to secrete higher quantities of growth factors and produce cell migration and expression of andcollagen1, PDGF, and TGF-β. Also, Wang *et al*., 2018[36] reported that i-PRF increased cell migration, proliferation, and differentiation into osteoblasts and accelerated bone turnover through enhancing cellular activity.

The result showed that the eruption rate was increased over time and week 3 groups gave rise to a higher rate, followed by groups week 2 and week 1. This result is because of the property of i-PRF of slowly releasing growth factors over 14 days and is also because of repeated doses of i-PRF. This result is approved by Thanasrisuebwong *et al*., 2019,[37] who stated that i-PRF showed more efficient biological characteristics and released growth factors in 7 to 14 days. The dose-dependent effect of PRF was investigated by Dohan Ehrenfest *et al*., 2010,[38] who found that doubling PRF results in significantly stimulating the proliferation and differentiation of cells in cell culture during at least 2 weeks. Also, Subramanyam *et al*., 2021[39] reported that administration of three doses has superior outcomes compared to double or single doses and improves the effectiveness of the treatment.

Histologically, the i-PRF groups showed increased numbers of odontoblast and cementoblast cells when compared to the control group, and these results are attributed to the ability of i-PRF to enhance the proliferation and differentiation of odontoblasts and cementoblasts. This result agrees with Kim *et al*., 2017 and Chai *et al*., 2019,[40,41] who investigated the effect of PRF on differentiation of odontoblasts in cell culture of human dental pulp cells and suggested that PRF stimulated reparative dentin formation through odontoblastic differentiation. In addition, Ji *et al*., 2014 and Duan *et al*., 2018[42,43] showed that a thin layer of cellular cementum formed on the root surface and cementoblast cells found on the surface of new cementum in the PRF group. Furthermore, this result is supported by Zhou *et al*., 2017,[44] who stated that the application of PRF in necrotic tooth leads to formation of cementum-like tissue containing cementoblasts at the surface and cementocytes distributed within the bulk of cementum.

One of the tooth eruption theories is bone remodeling theory, which depends on bone formation and resorption

Table 6: Descriptive statistics (mean±SD), ANOVA, and Duncan's multiple range test of number of osteocytes for control groups and i-PRF groups

Groups	Weeks	Mean	SD	*P*	Duncan
Control	1	18.20	1.92	0.0000	D
	2	18.20	1.92		D
	3	18.20	1.92		D
i-PRF	1	27.00	2.74		C
	2	31.60	5.35		B
	3	37.60	2.97		A

Significant difference at $P \leq 0.05$ level. Different letters vertically mean significant difference

around erupting tooth. Our results showed an increase in the number of osteoblast, osteoclast, and osteocyte cells in the i-PRF group when compared to the control group. This explains the effect of i-PRF in increasing the rate of tooth eruption. This result agrees with the result of Kyyak *et al*., 2020 and Shah *et al*., 2021,[45,46] who found that i-PRF enhances human osteoblast activity by increasing cell migration, proliferation, and differentiation. Lee *et al*., 2020[47] showed that i-PRF enhanced new vital bone formation characterized by numerous osteocytes trapped in the osteoid and numerous osteoblasts lined up the osteoid. Furthermore, the i-PRF week 3 sub-group showed a decreasing number of osteoblasts and an increasing number of osteocytes and osteoclast cells, and this result is supported by Mu *et al*., 2020,[48] who stated that there was an increase in the bone remodeling process in i-PRF groups because of a higher osteogenic and osteoclastic activity.

Finally, to the best of our knowledge, this is the first study conducted to evaluate the effect of i-PRF on the eruption rate of teeth. Thus, this study will provide a guideline for future use of i-PRF to accelerate tooth eruption.

Clinical implications

There are many local factors, systemic diseases, and genetic disorders that result in delayed teeth eruption, so this study could give us an idea about the use of i-PRF to accelerate tooth eruption in such cases and maintain the child dental and medical heaths.

Conclusion

This study confirmed that submucosal local injection of i-PRF might increase the eruption rate of the teeth and there has been an increase in the number of odontoblast and cementoblast cells and enhancement of the bone remodeling process through an increase in the number of osteoblast, osteocyte, and osteoclast cells.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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