The Saudi Dental Journal (2023) **35**, 753–759

King Saud University

The Saudi Dental Journal

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A comparative clinico-radiographic analysis of regenerative endodontic procedure on immature necrotic permanent teeth using blood clot and PRF as scaffold: A retrospective study

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Received 29 January 2023; revised 24 May 2023; accepted 28 May 2023
Available online 2 June 2023

KEYWORDS

Platelet-Rich Fibrin;
Induced bleeding;
Blood clot;
Regenerative endodontics;
Sodium Hypochlorite;
Calcium hydroxide

Abstract *Objectives:* To evaluate the clinical and radiographic success rate of blood clot and platelet rich fibrin (PRF) as a scaffold system in regenerative endodontic procedure in immature traumatized necrotic teeth..

Materials and methods: This retrospective study examined the records of 28 subjects with necrotic immature open apex due to trauma treated with blood clot or PRF as a scaffold in regenerative endodontic procedure. The disinfection was carried out with calcium hydroxide as an intra-canal medicament. The clinical outcome was recorded using a binary variable of presence or absence of pain and intra-oral swelling. The radiographic outcome was recorded for periapical healing using Ørstavik's Periapical Index and apical response using Chen and Chen index. Comparison of frequencies of categories of variables with groups was done using the chi-square test. The pairwise comparison of time intervals was done using Wilcoxon Signed Ranks Test.

Results: Intra-group comparison of pain and intra-oral swelling had highly significant ($p = 0.000$) results for both groups over a period of 12 months. Inter-group comparison for clinical outcomes was insignificant. For the radiographic outcome, there was a statistically non-significant difference for periapical healing and apical response for both intra-group and inter-group comparisons.

Conclusion: Both PRF and blood clot have a favourable outcome for a regenerative endodontic procedure with a disinfectant protocol using calcium hydroxide as intracanal medicament.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2023.05.026

Considering the cumbersome procedure involved in procuring PRF scaffold, especially in children, inducing bleeding can be considered a recommended method for a regenerative endodontic procedure.

1. Introduction

Traumatized necrotic immature permanent teeth often exhibit thin and weak dentinal walls, which increases their vulnerability to fracture when undergoing conventional endodontic therapy (Murray, 2018). These traditional approaches aim to create an apical barrier in immature teeth against which the root canal obturation material can be condensed, a procedure known as apexification. Such an apical barrier can be achieved within a few months with the use of calcium hydroxide (multivisit apexification), or immediately with mineral trioxide aggregate (MTA) (single-visit apexification) (Nazzal and Duggal, 2017). While the treatment effectively resolves the infection and alleviates associated signs and symptoms, it does not support ongoing root development. Consequently, the teeth retain their delicate and weakened dentinal walls, thereby increasing the susceptibility to root fractures (Pereira et al., 2021).

Currently, regenerative endodontic procedure (REP) has gained popularity over the traditional approaches as it is a biological procedure that stimulates the pulp–dentine complex. It facilitates root formation with a significant increase in the thickness of the root dentinal wall. This process involves an amalgamation of three essential components within the root canal: a scaffold for newly forming tissue, stem cells, and growth factors (Nazzal and Duggal, 2017; Zbańska et al., 2021).

An ideal scaffold enables the binding and localization of cells, delivers growth factors, and possesses biodegradable properties. Although blood clot as a scaffold offers procedural ease and the presence of fibrin for tissue formation, it lacks adequate growth factors and can yield unpredictable outcomes due to its unstable and uncontrolled nature. (Nazzal and Duggal, 2017; Wigler et al., 2013; Zbańska et al., 2021). Hence, it has been suggested to evaluate the success of REP using a new scaffold system with a more reliable outcome (Nazzal and Duggal, 2017).

Platelet Rich Fibrin (PRF) has emerged as a scaffold in REP due to its rich content of growth factors. It is convenient to prepare, eliminates the need for biochemical manipulation of blood, and is completely derived from the patient's own blood (autologous) (Shivashankar et al., 2017). The naturally occurring fibrin mesh with abundant growth factors relays prolonged activity and thus helps in tissue regeneration. It is a minimally invasive technique with good clinical outcomes and limited risks. (Maniyar et al., 2018).

Along with the use of an appropriate scaffold system, an efficient and effective disinfection protocol that controls the infectious process is currently considered an inevitable requirement to achieve pulp regeneration as the persistent infection hinders stem cell attachment (Diogenes et al., 2013). American Association of Endodontists (AAE) in 2021 recommends the use of calcium hydroxide or a low concentration of triple antibiotic paste as an intra-canal medicament. The advantage of calcium hydroxide over triple antibiotic paste (TAP) is that it is non-toxic to stem cells and promotes the proliferation of stem cells of the apical papilla (Rai and Rangappa, 2021).

Though several studies have been reported in the literature that compared the treatment outcomes of the blood clot and PRF as a scaffold system in REP, the results of these studies are ambiguous. Most of these studies have shown better healing with PRF but with an insignificant difference with blood clot and thus it does not substantiate the widespread use of PRF. Hence, this retrospective study aims to evaluate the clinical and radiographic success rate of the regenerative endodontic procedure using blood clot and PRF as a scaffold system using calcium hydroxide as an intra-canal medicament.

2. Materials and methods

This study protocol was approved by the institutional review board and ethical committee with the reference number ECR/1652/Inst/KA/2022/23/03–005. The regenerative endodontic procedure was performed by postgraduate students under the supervision of the professor of the Department of Pedodontics and Preventive Dentistry from January 2014 to June 2022.

2.1. Inclusion criteria

- Immature traumatized anterior permanent teeth.
- Cases where the regenerative endodontic procedure was performed in children with age 7–15 years.
- PRF or blood clot as a scaffold.
- Cases where AAE protocol 2016 or 2018 with calcium hydroxide as intra-canal medicament has been followed.
- Minimum 6 months of follow-up.

2.2. Exclusion criteria

- Immature necrotic permanent teeth due to carious exposure.
- Cases where any antibiotic intra-canal medicament was used.
- Cases with incomplete clinical dental records, poorly processed periapical radiographs, or distorted images.
- Cases that have been included in any other research.

2.3. Treatment protocol

The AAE protocol of 2016 and 2018 was followed. (American Association of Endodontists, 2016, American Association of Endodontists, 2018.)

2.4. Sample size determination

The sample size was determined using the expected proportion of an outcome in each group's values. The z variate of alpha error is a constant with a value of 1.96 and the z variate of beta is a constant with a value of 0.84. The value of the proportions of the variable was taken from the existing literature (Pereira et al., 2021). A minimum of 14 subjects per group completing the study gave good external validity.

2.5. Data collection

The data was collected from the patient's records which included gender, age, signs and symptoms (spontaneous pain, sinus tract, abscess, and tooth mobility), and the radiographs.

2.6. Evaluation of clinical outcome

The teeth were examined for pain and intra-oral soft tissue swelling and were recorded using present or absent (binary variable) at baseline, 3 months, 6 months, and subsequent follow-up.

2.7. Evaluation of radiographic outcome

Intraoral digital periapical radiographs were evaluated for the periapical radiolucency with Ørstavik's Periapical Index (Ørstavik et al., 1986) (Table 1) and the apical response with Chen and Chen index (Chen et al., 2012) (Table 2) at 3 months, 6 months, and subsequent follow-up.

2.8. Reliability and measurement error

Both intra-examiner and inter-examiner reliabilities were tested using intra-class correlation coefficients (ICC) using a two-way mixed and absolute agreement model.

2.9. Statistical analysis

Data obtained was compiled on MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States) and was subjected to statistical analysis using a statistical package for social sciences (SPSS v 26.0, IBM). The chi-square test was employed to compare the frequencies of variable categories across different groups. Pair-wise comparison of time intervals was done using Wilcoxon Signed Ranks Test. For all the statistical tests, $p < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving power to the study as 80%.

Table 1 Ørstavik's Periapical Index for periapical radiolucency.

Score	Criteria
1	Normal periapical structures
2	Small changes in bone structure
3	Changes in bone structure with some mineral loss
4	Periodontitis with well-defined radiolucent areas
5	Severe periodontitis with exacerbating features

Table 2 Chen and Chen Index for apical response.

Score	Criteria
Type 1	Increased thickening of the canal walls and continued root maturation.
Type 2	No significant continuation of root development with root apex becoming blunt and closed.
Type 3	Continued root development with the apical foramen remaining open.
Type 4	Severe calcification (obliteration) of the canal space.
Type 5	A hard tissue barrier formed in the canal between the coronal MTA plug and the root apex.

3. Results

From 2014 to 2022, 107 cases reported to the department with a history of trauma with an immature apex, on vitality testing 44 cases were necrotic, and 28 cases were included in the study (Fig. 1).

The inter-examiner reliability was excellent (ICC = 0.97) and intra-examiner reliability was highly reliable (ICC = 0.95 and 0.97).

The intergroup comparison for the age (Table 3) and gender of the participants was statistically insignificant.

The intra-group comparison of pain and intra-oral swelling had a statistically highly significant difference between the time intervals ($p = 0.000$) with a higher frequency of pain and intra-oral swelling present at baseline and absent at 12 months for both PRF and blood clot group (Table 4). The inter-group comparison of pain and intra-oral swelling was statistically non-significant for the frequency of pain ($p = 0.705$ at 12 months) and intra-oral swelling ($p = 0.705$ at 12 months) between the groups at all the time intervals.

The intra-group comparison was statistically insignificant for periapical healing with a p-value of 0.496 for the PRF group and 0.159 the for blood clot group over a period of 12 months. Even for the apical response for PRF and blood clot group, the data was statistically insignificant ($p = 0.066$ and $p = 0.265$). The pairwise comparison of time intervals was statistically significant in the blood clot group between the time intervals of 3 months and 6 months ($p = 0.034$), whereas it was non-significant for the PRF group.

The inter-group comparison for periapical healing ($p = 0.084$ at 12 months) and apical response ($p = 0.722$ at 12 months) was statistically insignificant between the groups at all time intervals.

3.1. Sensitivity analysis for different follow-up periods

A sensitivity analysis was carried out to assess the effect on the obtained results due to attrition of sample size at 12 month follow-up period. It revealed that the association between all the clinical and radiographic parameters with time was robust at 6 months of follow-up and is unlikely to be influenced by the inclusion or exclusion of cases with longer follow-up durations.

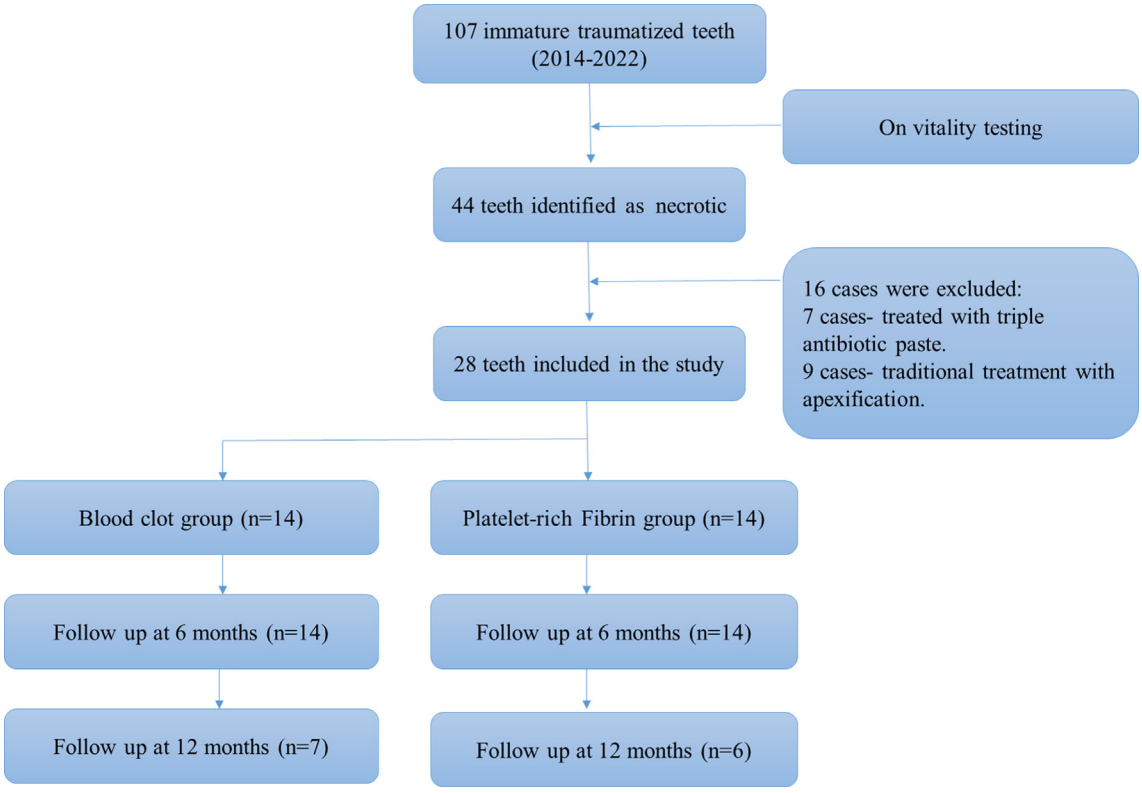

Fig. 1 Flowchart depicting process of recruiting included subjects.

Table 3 Inter-group comparison of age.

	Group	N	Mean	Std. Deviation	Std. Error Mean	t value	p-value of *t*-test
Age	PRF	14	10.57	1.989	0.532	0.188	0.852#
	Blood clot	14	10.43	2.027	0.542		

4. Discussion

The objective of this retrospective study was to evaluate the clinical and radiographic success rate of two scaffold systems for the regenerative endodontic procedure in immature necrotic teeth with trauma as an etiological factor. The present study had strict inclusion criteria for eliminating the heterogeneity in the outcome as the success of the regenerative endodontic procedure depends not only on the scaffold system but also on a number of factors like disinfection protocol, the width of apical foramen, and age of the patient (Bansal et al., 2015).

As stated earlier, age and sex are important factors that can influence the outcomes of regenerative endodontics. In the present study, there was no significant difference in age and sex between the Platelet Rich Fibrin (PRF) and blood clot groups. This suggests that clinical and radiographic outcomes were not influenced by age or sex as independent variables in the present study.

One of the important clinical outcomes of regenerative endodontics is pain resolution. The results of our study showed that the use of PRF and blood clot as a scaffold in regenerative endodontics resulted in significantly lower pain scores (absence) compared to the baseline at all the time periods (3 months, 6 months, and 12 months). These findings suggest that both PRF and blood clot as a scaffold may have a more sustained analgesic effect. Intra-oral swelling is another clinical parameter that is commonly assessed in regenerative endodontic cases. Both PRF and blood clot groups showed a significant reduction in swelling compared to baseline at all the time periods. However, the PRF group showed a more consistent and significant reduction in intra-oral swelling compared to the blood clot group. This may be attributed to the anti-inflammatory properties of PRF, which have been shown to reduce inflammation and promote tissue healing. (Maniyar et al, 2018) The results of the present study are in accordance with studies conducted by Narang et al. where they found complete resolution of pain and swelling for the cases treated with PRF and blood clot with immature necrotic permanent teeth. (Narang et al., 2015). Ulusoy et al. observed that there was complete resolution of pain and swelling by the 6 months of follow-up with blood clot and PRF. (Ulusoy et al., 2019).

Radiographic assessment is crucial in evaluating the success of regenerative endodontics. In our study, we compared periapical healing and apical response in PRF and blood clot

Table 4 Intra-group comparison of pain and intra-oral swelling for Platelet Rich Fibrin (PRF) and blood clot versus time.

Group	Parameter		Time				Chi-Square value	p value of Chi-Square test
			B	3 M	6 M	12 M		
PRF	Pain	Nil	0	0	0	8		
		Absent	3	14	14	6	62.444	0.000**
		Present	11	0	0	0		
		Total	14	14	14	14		
	Intraoral swelling	Nil	0	0	0	8		
		Absent	9	14	14	6	38.794	0.000**
		Present	5	0	0	0		
		Total	14	14	14	14		
Blood Clot	Pain	Nil	0	0	0	7		
		Absent	1	14	14	7	65.438	0.000**
		Present	13	0	0	0		
		Total	14	14	14	14		
	Intraoral swelling	Nil	0	0	0	7		
		Absent	10	14	14	7	27.581	0.000**
		Present	4	0	0	0		
		Total	14	14	14	14		

groups at different time intervals. When evaluating periapical healing, our results showed that there were no significant differences between PRF and blood clot groups at any time point. Both groups showed comparable periapical healing outcomes with no significant differences in mean scores. This suggests that both PRF and the blood clot can be effective scaffolds for promoting periapical healing in REP. After 12 months, the PRF group exhibited changes in bone structure (Score 2) in all cases, while the blood clot group showed a varied distribution of scores: 14% had normal periapical structure (Score 1), 43% had changes in bone structure (Score 2), and 43% had changes in bone structure with mineral loss (Score 3). Similarly, apical response, which reflects the healing of the apical tissues, showed no significant differences between PRF and blood clot groups at any time point. Both groups showed similar apical response and hence can promote tissue healing in REP. For the apical response at the end of 12 months in the PRF group, 34% of cases had increased thickening of canal wall and root maturation (Type 1 response), 16% had closed blunt apex (Type 2 response), and 50% had continued root development with apex remaining open (Type 3 response). In the blood clot group, 44% of cases had increased thickening of canal wall and root maturation (Type 1 response), 14% had closed blunt apex (Type 2 response), 28% had continued root development with apex remaining open (Type 3 response), and 14% had hard tissue barrier formed in the canal between the coronal MTA plug and the root apex (Type 5 response). This suggests that 50% of cases treated with PRF had continued root development as opposed to 28% of cases in the blood clot group. (Fig. 2) These findings were in accordance with the study conducted by ElSheshtawy et al. and Ulusoy et al., where periapical healing and root lengthening were better with PRF than blood clot, however, the results were not significant (ElSheshtawy et al., 2020; Ulusoy et al., 2019). The results of the present study were also in accordance with a recent systematic review and *meta*-analysis by Tang et al., where 16 studies were included and it suggested that there was no statistically significant difference in periapical healing between PRF and blood clot but the healing time was faster for the PRF group (Tang et al., 2022). Although there were no significant differences in periapical healing and apical response between different time intervals within each group, the p-values were close to the significance level in some cases. This suggests that there might be a trend towards better periapical healing and apical response in the PRF group.

In addition to appraising clinical and radiographic efficacy, a crucial aspect of regenerative endodontic treatments is regaining pulp vitality. In our study, all tested teeth displayed a negative response for pulp vitality, which aligns with the findings reported by ElSheshtawy et al. (2020) who also observed a lack of response in 100% of their cases. However, these outcomes contradict the results of Ulusoy et al. (2019), where 86% of the cases exhibited a positive response to pulp vitality testing. This discrepancy may be attributed to variations in the duration of follow-up periods, as our study and ElSheshtawy et al. (2020) had a 12-month follow-up period, whereas Ulusoy et al. (2019) followed up for 27 months. Additionally, incorrect placement of mineral trioxide aggregate (MTA) could contribute to generating false negative responses.

This study was subject to several limitations. Firstly, the strict inclusion criteria resulted in a limited number of cases being included, leading to a smaller sample size. The inability to conduct histological evaluations of the samples and the availability of only a limited number of cases for the 12-month follow-up also posed constraints.

5. Conclusion

Within the limitations of the present study, it can be concluded that:

- Both PRF and blood clot have a favorable clinical and radiographic outcomes for regenerative endodontic procedures with a disinfectant protocol of calcium hydroxide as an intracanal medicament.

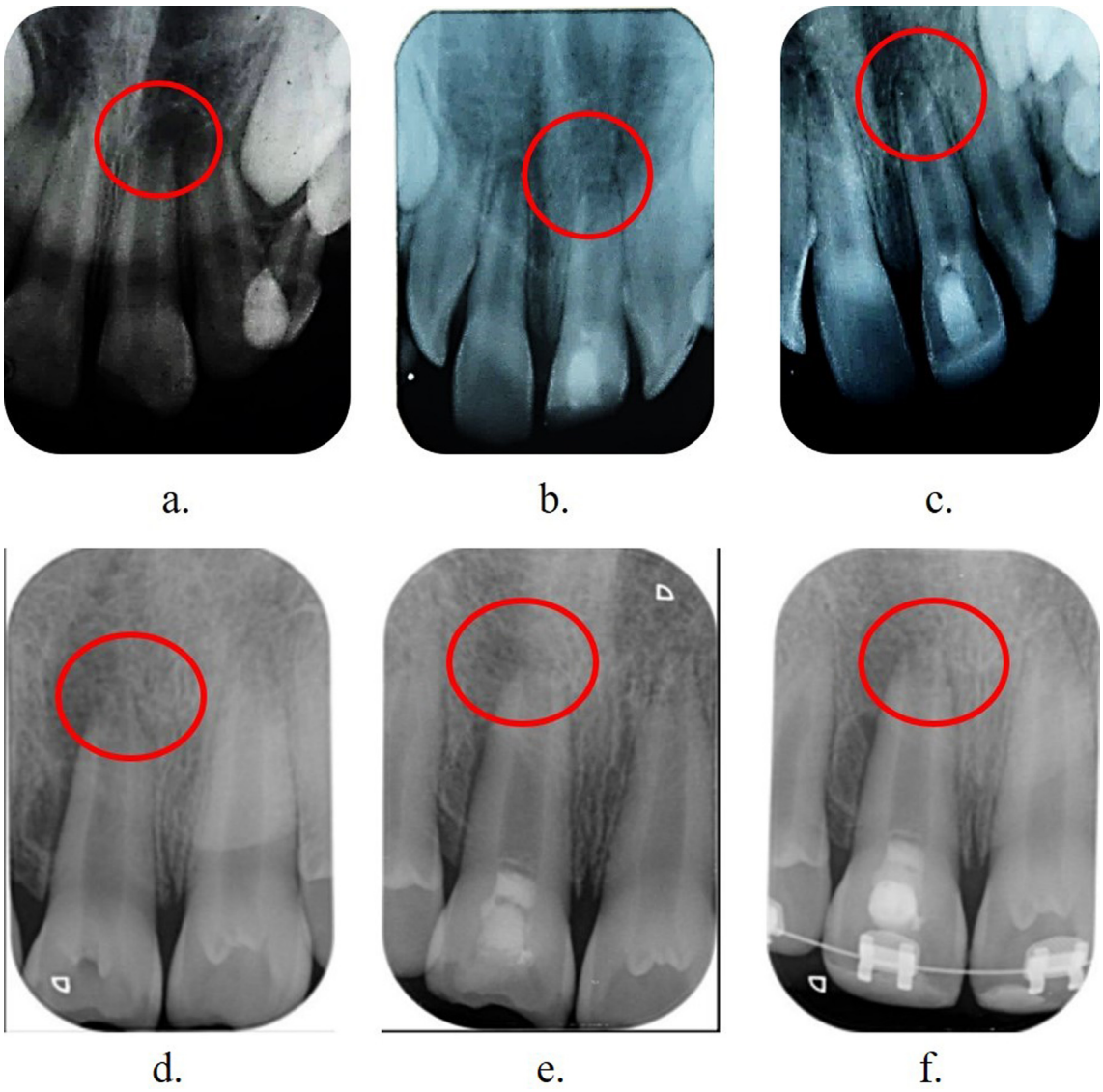

Fig. 2 An intra-oral periapical digital radiograph for periapical healing and apical response in PRF group. a. = at baseline, b. = at 6 months, c. = at 12 months. An intra-oral periapical digital radiograph for periapical healing and apical response in blood clot group. d. = at baseline, e. = at 6 months, f. = at 12 months.

- Considering the cumbersome procedure involved in procuring PRF scaffold, especially in children, inducing bleeding can be considered a recommended method in regenerative endodontic procedures.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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