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Regenerative Ability of Immature Teeth with Necrotic Pulp in Single and Multiple Visits

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ABSTRACT

Background: Restoration of pulp-dentine complex cells, root structure, and dentin by the use of stem cells, scaffolds, and growth factors is the goal of regeneration, a set of biological processes. Dentists often face difficulties while treating immature teeth with necrotic pulp. **Objective:** This study aimed to examine the regeneration potential of immature teeth over the course of one visit versus numerous visits.

Materials and Methods: Forty participants were recruited from the Clinic of the Faculty of Dentistry at Ain Shams University and Future University in Cairo, Egypt, all of them had immature, non-vital single rooted teeth exhibiting evidence of periapical disease (2-4 mm diameter) and no symptoms.

Results: Neither Group I nor Group II significantly differed from one another in root length gain or loss over time. Also, the size of the tip.

Conclusion: In light of the data presented here, it appears that single visit regenerative procedure is successful as multivisit approach in immature pulp regeneration. Apical closure was slightly faster in single visit approach than multivisit.

Keywords: Immature teeth, Necrotic pulp, Single visits, Multiple visits.

INTRODUCTION

It is difficult for a dentist to treat immature teeth that have necrotic pulp. First, disinfection of these canals is very difficult since the use of aggressive instrumentation will lead to further weakening of the already thin root and will eventually lead to root fracture [(1)]. Second, obturating these canals is very difficult because the wide apex provides no apical stop for root filling material before invading the periodontal tissues [(2)].Historically apexification was introduced, its aim is the formation of a calcific barrier at the root terminus most often made of cementum like tissues, however due to the week root formed, and several cases of fracture were reported after apexification [(3)]. Then, MTA apexification came along, providing a two-visit apexification alternative so the vulnerable tooth may be fixed right away, but without improving the root length and thickness [(4)].

Recently, regenerative endodontics was suggested. Regeneration refers to a series of physiologically driven processes aimed at replacing impaired structures, such as dentin, root structure, and cells within the pulp-dentine complex. This is achieved through the utilization of stem cells, scaffolds, and growth factors [(5)]. Regenerative procedure depends mainly on disinfection rather than instrumentation. The most commonly used intra-canal medicaments are calcium hydroxide and triple antibiotic paste that consist of metronidazole, ciprofloxacin and minocycline. The minocycline was reported to make discoloration, so it was removed from the paste, and the paste was called double antibiotic paste [(6)].

Calcium hydroxide was considered a goal standard material for several decades due to its high alkalinity giving it excellent antibacterial properties also causing initial necrosis followed by repair and hard tissue formation [(7)].

On the other hand, two-visit REPs have a number of limitations, including the requirement for more visits, the implantation of temporary restorative materials, patient compliance, and an extended duration of therapy. Discoloration of the tooth, lower fracture resistance of the tooth, unfavorable influence on the survival of apical papilla stem cells, and their retrieval from the root canal walls are some other concerns that are associated with the use of intracanal medicament in REP. One possible solution to these problems is the use of REPs that only require one visit from the patient, which would result in considerable time and financial savings for both the patient and the dentist. As a result, it is important to determine whether an approach consisting of a single visit for REPs is both successful and safe [(8)].

The study's goal was to compare the effectiveness of treating immature teeth over the course of a few visits with that of treating them over the course of a few months.

MATERIALS AND METHODS

Materials:

Figure 1: photograph showing injectable calcium hydroxide paste.

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Figure 2: photograph showing mineral trioxide aggregate.

Methods:

Participants in the study were from the Clinic of the Faculty of Dentistry at Ain Shams University and Future University in Cairo, Egypt. All of the participants had immature, non-vital single rooted teeth and presented with evidence of periapical disease (2-4 mm diameter). Every patient and their legal guardians were asked in-depth questions about their medical and dental histories, and the research team only included patients who were in good overall health.

Inclusion criteria: Male or female, age from 8 – 18 years. Non vital permanent central anterior teeth with exposed pulp and having immature apex.

Exclusion criteria: Patients with systemic antibiotics within last 2 months and patients with hypersensitivity to antibiotic paste component. Patients with systemic diseases that impair healing. Traumatized tooth that had been avulsed and replanted**.** Tooth that is primary or mature with closed apex or non-exposed pulp or fracture through root or non-restorable crown or highly mobile and tooth with resorbed root or lesion, which was very big and did not resolve.

Classification of cases: A total of 40 patients are split evenly between two groups of 20 each:

- **Group 1:** 20 non-vital permanent central anterior teeth with exposed pulp and having immature apex treated in single visit.
- **Group 2:** 20 non-vital permanent central anterior teeth with exposed pulp and having immature apex treated in multiple visits.

Methods of evaluations: Patients are contacted at predetermined time intervals of 3, 6, 9, and 12 months to ensure their health and wellbeing. The subsequent steps comprised the following:

Clinical assessment: History taking and a physical examination (including percussion and periapical palpation of soft tissue) can reveal important information about the patient's condition, such as the presence or absence of pain, edema, or sinus tracts during a clinical assessment.

Radiographic assessment : Root length, root thickness, and apical diameter were all found to have increased and decreased respectively in accordance with the standard radiographic assessment.

Sample size calculations: G*Power 3.1 (Heinrich Heine University, Dusseldorf, Germany) was used for the power calculations. Each group, according to the sample size calculation, needed at least 15 instances.
so a total sample size of 40 patients was calculated,to compensate for drop outs.

Ethical consideration: In accordance with Helsinki, all required steps were taken following clearance by a University-Level Ethics Committee in line with guidelines from Ain Shams University School of Dentistry. The study protocol was explained in details to all patients. Patients or guardians signed informed consent of proposed treatment or outcomes and possible complications might occur. The data obtained from patients as well as the results of the follow up were kept confidential.

Statistical analysis

It was determined whether the numerical data were normally distributed by inspecting the distribution of the data, computing the mean and median values, and employing the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results of these procedures were provided as mean and standard deviation. The data were found to have a non-parametric distribution, hence the Mann Whitney U test was utilized in the analysis. The level of significance was determined at $p \leq 0.05$. IBM9 SPSS 10 Statistics version 26 for Windows was used to complete the statistical analysis, and the results are depicted in figures 1 and 2 correspondingly.

RESULTS

Changes in root length between 0 - 3, 3-6, 6-9 and 9-12 months were not statistically different amongest Groups I and II (p=0.077 and p=0.332, respectively) (Table 1).

Table (1): Root length changes in millimeters and percentages over time for both groups, with means and standard deviations (SDs)

Difference points	change of root length (mean±SD)		P-value
	Group (I)	Group (II)	
Baseline- 3 months	(0.4±0.16mm) (4.67±1.79%)	(0.39±0.26mm) (4.27±3.65%)	**0.994ns**
3- 6 months	(0.58±0.15mm) (6.28±1.31%)	(0.48±0.16mm) (4.82±1.32%)	**0.691ns**
6-9 months	(0.4±0.16mm) (4.16±1.54%)	(0.3±0.24mm) (3.03±2.79%)	**0.445ns**
9-12 months	(0.32±0.18mm) (3.2±1.75%)	(0.33±0.17mm) (3.2±1.59%)	**0.984ns**
P value	0.077ns	0.332ns	

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

In terms of percentage root-length change, there was no discernible difference between Groups I and II (p=0.082) (Table 2).

Table (2): Total percentage change of root length for both groups

Percentage change of apical diameter (mean±SD)		P-value
Group (I)	Group (II)	
1.7±0.48mm 17.12±4.05%	1.52±0.60mm 14.18±6.88%	0.082ns

The groups (0-3 m), (3-6 m), (6-9 m), and (9-12 m) did not differ significantly in root thickness change (p=0.908 in group I and (p=0.541 in group II) (Table 3).

Table (3): Change in root thickness in mm & percentage for both groups at different time intervals.

Difference points	change of root thickness (mean ± SD)		P-value
	Group (I)	Group (II)	
Baseline- 3 months	(0.30±0.16mm) (19.70±7.69%)	(0.24±0.17mm) (11.80±7.31%)	**0.06ns**
3- 6 months	(0.24±0.19mm) (14.81±11.93%)	(0.22±0.09mm) (10.35±6.55%)	**0.365ns**
6-9 months	(0.26±0.15mm) (13.47±9.13%)	(0.16±0.09mm) (5.92±2.70%)	**0.136ns**
9-12 months	(0.26±0.13mm) (12.24±7.78%)	(0.15±0.10mm) (5.34±2.62%)	**0.072ns**
P value	0.908 ns	0.541ns	

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Changes in root dentin thickness showed no significant difference between groups I and II (p=0.072) (Table 4).

Table (4): Total percentage change of root dentin thickness for both groups

Percentage change of dentin thickness (mean±SD)		P-value
Group (I)	Group (II)	
1.06±0.26mm 47.77±12.30%	0.77±0.08mm 29.54±7.73%	0.072ns

Change in apical diameter was not significantly different between the 0-3, 6-9- and 9-12-months groups (p=0.129 in group I and p=0.202 in group II) (Table 5).

Table (5): Change in apical diameter in mm and percentage change for both groups at different time intervals

Difference points	change of apical diameter (mean± SD)		P-value
	Group (I)	Group (II)	
Baseline- 3 months	(0.26±0.15mm) (22.24±11.22%)	(0.08±0.02mm) (8.76±4.89%)	**0.045***
3- 6 months	(0.12±0.09mm) (9.41±3.85%)	(0.12±0.07mm) (11.25±10.47%)	**0.54ns**
6-9 months	(0.12±0.10mm) (8.22±5.27%)	(0.09±0.05mm) (6.43±2.72%)	**0.27ns**
9-12 months	(0.10±0.04mm) (6.67±2.55%)	(0.05±0.04mm) (3.87±1.82%)	**0.142ns**
P value	0.129ns	0.202ns	

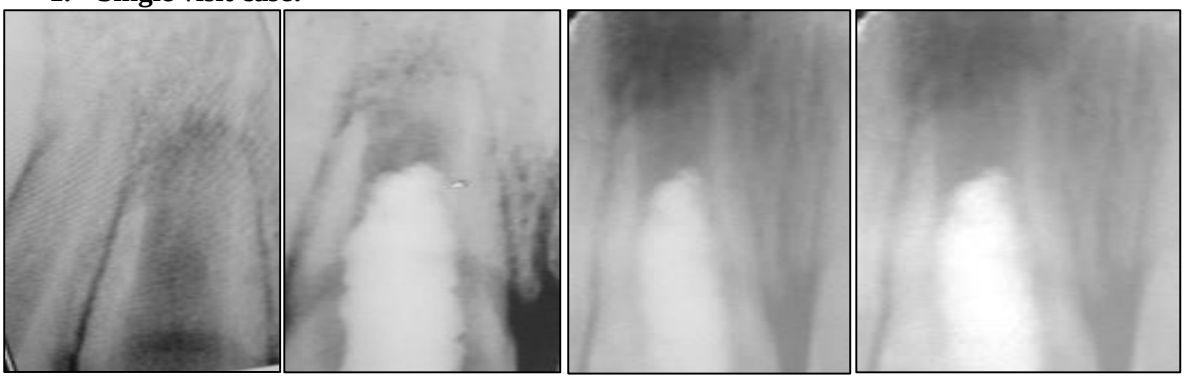
When comparing the percentage change in apical diameter between groups I and II, there was no statistically noteworthy disparity (p=0.103) (Table 6).

Table (6): Total percentage change of apical diameter for both groups

Percentage change of apical diameter (mean±SD)		**P-value**
Group (I)	**Group (II)**	
0.6±0.22mm 39.77±10.30%	0.35±0.61mm 26.54±13.15%	0.103ns

REPRESENTATIVE CASES

1. **Single visit case:**

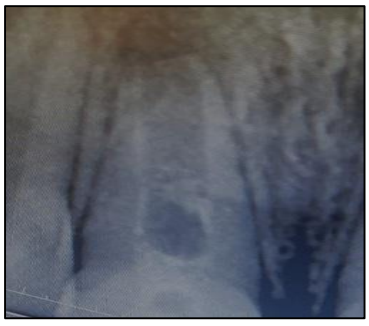


A (0-3M) B (3-6M) C(6-9M) D(9-12)

Figure (1): Periapical radiograph showing a representative case of group I (single visit) at 3,6,9 and 12 months respectively.

2. **Multiple visit case:**

A (0-3M) B (3-6M) C (6-9M)



D (9-12M)

Figure (2): The provided image is a periapical radiograph that depicts a representative case belonging to group II (multiple visit) at 3,6,9 and 12 months respectively.

DISCUSSION

Endodontic treatments now include revitalization methods as an alternative to apexification for immature teeth with pulp affection. Many difficulties arise for the dentist when dealing with pulp affection in juvenile teeth brought on by caries, dental trauma, or developmental abnormalities. Conventional root canal therapy is not optimal for teeth with short roots, thin dentine walls prone to fracture, and large root canals and apices [(9)].

This stem cell induction from the apical papilla constitutes the biological basis of a method called "revascularization," which seeks to (re)generate pulp-like tissue inside the root canal. Pain and swelling subside, and periapical lesions recover, according to research by **Hargreaves *et al.*** [(10).] Root length and thickness can both be increased through rejuvenation, a process known as "maturogenesis" .

In infected immature teeth, the potential for revascularization has been thought to be lost because mechanical instrumentation and irrigation with sodium hypochlorite has been evidenced to be ineffective in root canal disinfection. However, the use of intracanal medications is crucial in preventing bacterial reinfection [(11)].

Since the European Society of Endodontology recommends against using mechanical instrumentation of the root canal walls during the initial visit, disinfection of the canals between visits should be accomplished solely via means of intracanal medications and irrigation fluids . Not only should mechanical instrumentation be avoided lest it further damage the already fragile root canal walls, but it should also be avoided so as to preserve the health of the stem cells in the apical tissues . **Lin *et al.*** [(12)] described a clinical example of ineffective regenerative endodontic therapy due to the existence of bacterial biofilms that remained firmly attached to the apical canal walls despite the absence of mechanical debridement. This was despite the fact that the apical canal walls had not been subjected to any form of debridement. This was caused by the absence of mechanical debridement [(12)].

With a side-vented needle placed 2 mm above essential tissue and 20 ml of 1.5-3% sodium hypochlorite irrigated over the course of 5 minutes, because of its efficiency in cleaning juvenile root canals, NaOCL solution was employed for extensive passive irrigation during revascularization therapy and without the requirement for synergistic irrigation, was found to drastically reduce bacterial infection within the canal. NaOCl was the sole irrigant used in 65% of regenerative endodontic clinical articles. The dosage of sodium hypochlorite shows the compromise that must be struck between thorough disinfection and the protection of delicate tissues [(13)]. The cytotoxic effects of sodium hypochlorite on vital tissue were reduced by irrigating the area with 5 ml of sterile physiological saline and paper-point dryness. Allowing the blood to fill the canal to about 2 mm below the gingival edge, we induced bleeding by mechanically irritating the periapical tissue and rotating an apically prebent file (size 40 Hedström). Regenerative endodontic therapy includes an induced bleeding step, which promotes the concentration of progenitor and stem cells into the area of the root canal and helps to facilitate the regeneration of tissue. Stem cells can enter the canal area with the help of a sturdy blood clot, which not only acts as a scaffold but also carries growth and differentiation factors for the cells it contains.

"The American Association of Endodontics clinical considerations for a regenerative procedure" states that the major objective of regenerative endodontic procedures is the resolution of apical periodontitis and the disappearance of any clinical signs or symptoms. Root expansion and/or canal wall thickening should be considered as secondary outcomes. Since the beginning of treatment till now, all of the cases in our study have remained symptom-free and fully functional. Roots in the cases matured significantly throughout the course of a year of observation [(14)].

In our study we compared between single and multiple visits regeneration. All of the teeth in this investigation had necrotic pulps before they were disinfected. Therefore, The ingrowth of progenitor cells from the periapical area is the only explanation that makes sense for the repair and improvement of periapical radiolucencies during the process of ongoing thickening of root walls and apical maturation that is evaluated radiographically. There are three stages in root development: the thickening of the root wall, the extension of the root, and the narrowing of the canal at the root's apex. Root lengthening and apex development are two of the many roles played by the apical papilla and the Hertwig epithelial root sheath.

In the majority of our patients, the apical lesion improved or disappeared entirely after 9 months. Twelve months following surgery, root extension and apical closure had occurred, along with root canal wall thickening. This is in agreement with what has been stated in other publications [(15)]. In contrast, Strindberg's seminal research showed that full recovery from periapical radiolucent lesions in people can take as long as 4 years.

Our findings showed that throughout the course of the entire follow-up period, all groups experienced statistically significant increases in root length and root thickness while experiencing statistically significant decreases in apical diameter. In regards to regenerative endodontic cases, our findings were consistent with those of **Bose *et al.*** [(16)], **Cehreli *et al.*** [(17)], **Tawfik *et al.*** [(18)], and **Estefan *et al.*** [(19)]. Quantitative examination of regenerative endodontic results was demonstrated in these discussions of 155 researches, reporting significant gains in length and thickness compared to retrospective investigations. The findings of this study are congruent with the findings of a number of writers

who have claimed an increase in length and thickness without providing quantitative data.

While, our findings contradict those of **Lin *et al.*** [12] who documented a failed revascularization instance in which a tooth that had previously been asymptomatic for 16 months became painful and swollen in a specific area after receiving treatment. Because the biofilm and bacteria in the dentinal tubules were not mechanically removed during root canal disinfection, revascularization failed in this case.

Our study is in agreement with **Gamze *et al.*** [20] who described that PRP and Biodentine were used successfully in a single visit to treat necrotic pulp in three immature mandibular molar teeth, considering the role of irrigation of 2.5% sodium hypochlorite, sterile saline and 17% EDTA solutions in canal disinfection, without the use of intracanal medications, which may have negative consequences on stem cells differentiation and the possibility of infection in between visits and taking in consideration the range size of the periapical lesions (2-4 mm) and the asymptomatic condition of the patients, all of these reasons contribute to the success of single visit regeneration [20].

CONCLUSIONS

The findings of this investigation suggest that single visit regenerative procedure is successful as multivisit approach in immature pulp regeneration. Apical closure was slightly faster in single visit approach than in multivisit.

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