

# Clinical Evaluation of Photobiomodulation Therapy on Regeneration of Necrotic Mature Permanent Teeth

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## Article information

Received: 25 December 2022

Revised: 31 January 2023

Accepted: 31 January 2023

## Key words

Non-vital Mature Permanent Teeth.

Photobiomodulation.

Regenerative endodontics.

## Abstract

**Introduction:** Regenerative endodontics was suggested as alternative treatment for necrotic mature permanent teeth to overcome the drawbacks of conventional treatment methods and showed better results when combined with photobiomodulation therapy (PBMT), so the aim of this study was to evaluate the clinical effect of photobiomodulation therapy on regeneration of necrotic mature permanent teeth.

**Materials and Methods:** 24 patients with age 9-13 were selected and randomly divided into two groups; control group (n=12), patients were treated with regenerative endodontics procedures (REPs) using blood clot scaffold, and study group (n=12), patients were treated with the same REPs as control group then treated with PBMT. In each group evaluation of pain, swelling, sinus tract, mobility, and cold sensibility test were performed

**Results:** Data analysis revealed that there was resolution of pain, swelling, and sinus tract in all cases during the follow up periods from the start to the end, no recurrence or pathological signs changes were detected clinically. Also, it revealed normal physiologic mobility of all subjects before and after revascularization procedures. For cold sensibility test, 6(60%) of cases in each group showed positive responses with no significant difference between both groups (p=1). There was a significant difference between values at different intervals in each group (p<0.001).

**Conclusion:** The results of this study showed that photobiomodulation therapy could accelerate the clinical success of regeneration of necrotic mature permanent teeth.

## 1. Introduction

Traumatic dental injuries and carious lesions in mature permanent teeth cause loss of vitality and periapical pathosis. Treatment of such cases was usually performed by conventional endodontic therapy, but it increases the risk of tooth fracture and remove the immune mechanism of teeth. So, regenerative endodontics was suggested as alternative treatment aiming to regenerate new living tissues and repair the damaged tissue. Regenerative endodontics is defined as biologically based procedures designed to replace damaged structure including root structures as well as cells of the pulp -dentine complex<sup>[1]</sup>.

Regenerative endodontics is mainly applied in management of necrotic immature permanent teeth depending on the creation of a bacteria free environment and introduction of stem cells, scaffolds, growth factors, and showed promising results, that encourage the researchers to study its application in mature permanent teeth to avoid the step of shaping of root walls that weakens dentine walls and to eliminate the use of artificial biocompatible filling material which can evoke foreign body reactions that may cause persistence of periradicular lesions<sup>[2]</sup>.

The application of REPs in mature permanent teeth is supported by possibility of releasing growth factors from dentine walls by

EDTA irrigation during disinfection step and regeneration without scaffold is possible<sup>[3]</sup>. Also, histological studies proved that the apical foramen as small as 0.32 mm did not prevent ingrowth of new tissues, in addition, stem cells, scaffold, and growth factors can be provided by provoked bleeding which is induced in the canal space by mechanical stimulation of periapical tissues<sup>[4,5]</sup>.

The use of laser in endodontics and its encouraged results supported the use of PBMT in REPs. PBM is a form of light therapy that utilizes non-ionizing light sources to promote biostimulator effects on different cell types, like stimulating cell growth, increasing cell metabolism, and improves dentoalveolar-derived mesenchymal stem cells viability and proliferation<sup>[6]</sup>.

Despite the increased interest in studying the effect of regenerative endodontics using blood clot as a scaffold in necrotic mature permanent teeth and the positive effect of the PBM on the dental pulp regeneration, few reports had addressed the clinical effect of PBMT on REPs in necrotic mature permanent teeth, therefore the current study was designed to clinically evaluate the effect of PBMT on of regeneration of necrotic mature permanent teeth. The null hypothesis was that

no statistically significant difference would be found between the tested groups.

### **Materials and Methods**

The Ethical Committee of Scientific Research of Faculty of Dentistry, Minia University approved the protocol of this parallel, randomized clinical trial with 1:1 allocation ratio (reference number 264/2019). The parents were asked to sign informed consent form laid down by research ethical committee, Faculty of Dentistry, Minia University.

#### **Sample size calculation**

Sample size calculation was carried out using power analysis (G\*Power version 3.1.9.7) that was planned to have sufficient power of 80%, adopting an alpha level of (0.05) a beta of (0.2) and calculated based on the results of a previous study<sup>[1]</sup>, the predicted sample size was a total of 24 cases to apply a statistical test of the null hypothesis that there is no difference would be found between the tested groups<sup>[7]</sup>.

#### **Study setting**

24 patients were selected randomly from the outpatient clinic of Pediatric Dentistry and Dental Public Health Department, Minia University. A total of 4 patients (2 patients from each group) could not be reached after the first visit and only 20 patients attended the follow-up examination.

#### **Inclusion criteria**

- Patients with age from 9-13 years and free from any systemic diseases or genetic disorders.
- Tooth with mature root apex (apical opening <1 mm)
- Traumatically or cariously exposed non-vital single rooted teeth with or without apical abscess.

#### **Exclusion criteria**

- Unrestorable teeth
- Patients allergic to medicaments or antibiotics necessary to complete the procedure.
- Tooth with vital pulp or incomplete root formation.
- Uncooperative patient

#### **Randomization and allocation concealment**

Simple randomization was used to allocate patients using the sealed envelope method with 1:1 allocation ratio into 2 groups (n=12) based on the treatment protocol. In control group, teeth were treated with REPs using blood clot scaffold, whereas in study group, teeth were treated with the same REPs as in control group then exposed to PBMT.

#### **Sequence generation**

Sequence generation was done for the patient number (1-24) using computer sequence generation (www.random.org). The sequence generator icon was chosen from the home page, the

sample size was specified, and ordered in two columns. The result was then copied to control group and study group with randomized patients' numbers (12 numbers in each group).

#### **Allocation concealment mechanism**

Each of the 24 papers numbered from 1 to 24 was individually packed in opaque envelopes after folding each paper eight folds. Each patient picked an envelope after their enrolment in the study and before the start of second visit. Numbers in the envelope determined which group was determined for the patient.

#### **Treatment of the selected necrotic mature teeth was conducted according to The American Association of Endodontics**

All patients in both groups were treated with the same REPs, then study group was exposed to PBMT after application of coronal plug material. Patients were anaesthetized using 3% mepivacaine with vasoconstrictor (Mepivacaine HCL 3%, Alexandria Co. pharmaceutical, Alexandria, Egypt). Teeth were isolated with rubber dam. A conventional access cavity was prepared, and the canal was slowly irrigated with 1.5% sodium hypochlorite (NaOCl; Clorox Co, 10th of Ramadan, Egypt) (20 mL/canal, 5 minutes), followed by saline (El Fath for Pharmaceutical & Cosmetics Industries, Alexandria, Egypt; 20 mL/canal, 5 minutes), and gentle irrigation with 20 mL of 17% EDTA (Prevest Den, Jammu, India).

After canal dryness by paper points, double antibiotic paste was prepared by mixing metronidazole (Flagyl 500 mg; Aventis, Cairo, Egypt) and ciprofloxacin (Ciprocin 500 mg; EPICO, Cairo, Egypt) with concentration 1 mg/mL, then it was applied up to CEJ. Access cavity was sealed by dry cotton and a temporary restorative material. The patient was dismissed for 1-month then examined for response to treatment. Complete resolution of signs and symptoms including pain, swelling, and sinus tract was considered success.

After 1-month, patients were injected with 3% mepivacaine without vasoconstrictor (Mepivacaine HCL 3%, Alexandria Co. for pharmaceutical), teeth were isolated by rubber dam, and temporary filling material was removed. Copious, gentle irrigation with 1.5% sodium hypochlorite (NaOCl; Clorox Co, 10th of Ramadan, Egypt) (20 mL/canal, 5 minutes), followed by saline (El Fath for Pharmaceutical & Cosmetics Industries, Alexandria, Egypt; 20 mL/canal, 5 minutes), and gentle irrigation with 20 mL of 17% EDTA (Prevest Den, Jammu, India). After canal dryness by paper points, bleeding was obtained into root canal by rotating a pre-curved K- file 2 mm beyond the apex. A dry cotton pellet was placed into the canal to permit the formation of blood clot. MTA (Rootdent, TehnoDent, Russia) was applied over the formed blood clot in both Groups<sup>[8]</sup>, then the access cavity was sealed with glass ionomer (NOVA Glass-F; IMICRYL, Konya/ Turkey)<sup>[9]</sup>. All patients on both groups were called back after 3, 6, 9, and 12 months for follow-up.

#### **Photobiomodulation therapy**

The regenerative procedures in study group were followed by photobiomodulation therapy on the apical root areas of the buccal and lingual surfaces at 48 hours intervals for 2 weeks.

Photobiomodulation was done by diode semiconductor laser with 810 nm wavelength, Gallium- Aluminum –Arsenide laser (eleixxon\_Claros\_Pico, Germany) and output power of 300 mW. The laser beam was delivered with a biostimulation tip of 0.6 cm diameter in contact with the tissues, and the teeth were irradiated with a continuous emission of the laser,  $E = 2.7 \text{ J}$  and total dose of each application was  $4 \text{ J/cm}^2$  [10].

### Statistical analysis

Categorical data were presented as frequency and percentage values and were analyzed using Fisher's exact test for intergroup comparisons and Cochran q test. The significance level was set at  $p < 0.05$ . Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows [11].

### Results

Statistical analysis of clinical signs and symptoms in tested groups revealed:

1- Clinical examination for pain, swelling, and sinus tract of the subjects before REPs revealed the presence of pain, abscess, sinus tract in 3 (30%) of cases in group I (control group) and 4 (40%) of cases in group II (study group). After disinfection procedures, there was resolution of the clinical signs and symptoms in all cases and during the follow up periods from the start to the end, no recurrence or pathological signs changes were detected clinically. Also, clinical evaluation of all subjects before and after REPs revealed normal physiologic mobility. According to these results, clinical success was considered as 100%.

2- There was no response for cold sensibility test during the first 6 months for both groups, while 3(30%) of cases in group II (study group) showed positive response at 9 months ( $p = 0.210$ ). At 12 months follow up, 6(60%) of cases in both groups showed positive response ( $p = 1$ ). There was a significant difference between values at different intervals within the same group ( $p < 0.001$ ). Table (1)

**Table (1):** Frequency and percentage values for cold sensibility test at different follow up periods

Interval	Cold sensibility test		Control group	Study group	p-value
Baseline	Negative	N %	10 <sup>A</sup> 100.0%	10 <sup>A</sup> 100.0%	NA
	Positive	n %	0 0.0%	0 0.0%	
3 months	Negative	N %	10 <sup>A</sup> 100.0%	10 <sup>A</sup> 100.0%	NA
	Positive	n %	0 0.0%	0 0.0%	
6 months	Negative	n %	10 <sup>A</sup> 100.0%	10 <sup>A</sup> 100.0%	NA
	Positive	n %	0 0.0%	0 0.0%	
9 months	Negative	n %	10 <sup>A</sup> 100.0%	7 <sup>AB</sup> 70.0%	0.210ns
	Positive	n %	0 0.0%	3 30.0%	
12 months	Negative	n %	4 <sup>B</sup> 40.0%	4 <sup>B</sup> 40.0%	1ns
	Positive	n %	6 60.0%	6 60.0%	
p-value			<0.001*	<0.001*	

Values with different superscript letters within the same vertical column are significantly different, NA: Not Applicable, \*, significant ( $p \leq 0.05$ ) ns; non-significant ( $p > 0.05$ )

### Discussion

Root Canal Treatment has been used for many years as a traditional protocol for the treatment of necrotic mature permanent teeth; however, it involves several drawbacks, so,

REPs have been suggested as an alternative treatment to overcome these drawbacks. Most of studies of regenerative endodontics have been focused on the treatment of immature necrotic permanent teeth as stem cells of the apical papilla that possess regenerative potential are found near their root apices coupled with their wide-open apices, which allow more stem cells to be recruited to the root canals providing a basis for the success of this treatment modality [12,13].

In contrast to a growing body of published regenerative endodontic cases in immature necrotic teeth, limited reports have attempted REPs for necrotic mature permanent teeth [14,15]. Therefore, the present study was conducted to clinically evaluate the effect of PBMT on regeneration of necrotic mature permanent teeth.

According to eligibility criteria, the average age of the patients was maintained between 9 and 13 years old to ensure the completion of root development and closure of the apex, also, the older ages have certain physiological changes occurs result in thickening of the apical cementum and deviation of the apical foramina, which could affect the migration of mesenchymal stem cells into the root canal [16,17].

The critical step for ensuring the success of the regenerative endodontic treatment is the disinfection of the root canal as the infection inhibits regeneration, repair, and viability of the stem cells. So, in the current study, 1.5% of NaOCl to adequately eradicate the bacteria and allows attachment of stem cells to root dentine. Then, irrigation with saline was performed to reduce any prolonged toxicity of NaOCl that could diminish the regeneration responses and decrease the risk of possible precipitates of irrigants. Irrigation using EDTA 17% can decalcify the surface of the root canal dentine to reveal its collagen fibers to release the growth factors that enhance the cellular differentiation [18,19].

The root canal medicated with the double antibiotic paste has proved similar effectiveness as triple antibiotic paste in eliminating bacteria from the infected dentine of root canals, without tooth discoloration [20]. Also, MTA is a hydrophilic biocompatible material that allow survival of stem cells and regeneration of new tissues and it was placed directly into the root canal over the blood clot to obtain bacterium-tight coronal plug which is essential for revascularization procedures as it prevent bacterial invasion into the pulp space [21].

In the study group, PBMT using 810 diode laser was selected as it is portable, inexpensive device and energy density of 2 to 4 J/cm<sup>2</sup> was suggested by previous studies when used directly on teeth or indirectly above the apex [22,23]. Also, 4 J/cm<sup>2</sup> energy density for 48 hours intervals was suggested to prevent the accumulated dose of energy that result in the bio-inhibition range [10]. Also, the infrared wavelength (700-1000 nm) is preferred to treat deeper tissues as it penetrates 2 mm before losing 37% of its intensity, so it can reach dental pulp cells [24]. Moreover, the optical probe was applied in close contact to the tissues to minimize light reflection so increase the number of light photons that penetrate the tissues to reach the target tissue [25].

The clinical evaluation of patients in this study showed that PBMT accelerate the response of the treated teeth to the regenerative procedures in view of resolution of signs and symptoms, and cold sensibility test that was earlier in study group than control group.

The results of the current study after 12 months follow up showed that 6(60%) of cases showed positive response to cold sensibility testing with no significant difference between both

groups. These results are compatible with Paryani and Kim 2013<sup>[26]</sup>, and Nageh et al. 2018<sup>[12]</sup>, who reported that 60% of their cases regained tooth sensibility at the end of 12 months, indicating the presence of vital tissues. However, it was inconsistent with the case series by Saoud et al. 2015, 2016,<sup>[5, 27]</sup> who reported negative responses to sensibility tests after 26 months and it may be due to the use of NaOCl irrigation in concentration 2.5% and metapaste as a disinfectant dressing which are different from the disinfection protocol in the current study. Moreover, Nagas et al. 2018<sup>[28]</sup>, reported negative responses to sensibility tests after 60 months of follow-up that may owing to the different concentration of NaOCl 5.25%, that was proved to be destructive to stem cells.

It was observed that the response to cold sensibility test in the control group were delayed compared to study group at 9 months of evaluation. This advance positive response in study group to sensibility test may be explained by the stimulatory effect of PBM on stem cells from the apical papilla that may mediate cold responses.<sup>[29]</sup>

Moreover, the response to sensibility test in control and study group were also delayed from normal teeth which may be explained by the presence of the coronal plug material that makes it difficult for the vital pulplike tissue response to be felt immediately. Furthermore, the regenerated vital pulplike tissue may have an immature innervation system; hence, the response initially is delayed<sup>[5]</sup>.

There is a gap of knowledge regarding the exact mechanism of regaining tooth sensibility after REPs. However, nerve regeneration could be attributed to the blood clot being a rich source of endogenous growth factors (transforming growth factor beta platelet-derived growth factor, vascular endothelial growth factor, and basic fibroblast growth factor) that may aid in neurogenesis<sup>[30]</sup>.

In addition, the use of 17% EDTA not only allows the release of the dentin-derived growth factors that could promote cell proliferation, migration, and odontoblastic differentiation but also it may promote neuronal growth and axonal regeneration<sup>[19]</sup>.

## Conclusions

Within the limitation of the current study and according to their results, the following can be concluded:

1. Regenerative endodontics can be considered as a successful alternative treatment for necrotic mature permanent teeth.
2. Photobiomodulation therapy was found to be an effective way to improve the REPs.

Further research and clinical trials are required to evaluate REPs in necrotic mature permanent teeth and the effect of photobiomodulation on regeneration process.

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