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EFFECT OF EMDOGAIN AND DIODE LASER IRRADIATION ON PERIODONTAL HEALING OF REPLANTED TEETH AFTER EXTENDED EXTRA - ORAL DRY TIME (AN EXPERIMENTAL STUDY)

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KEYWORDS

Alveolar bone density, Avulsion, Dental trauma, Diode laser, Emdogain.

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ABSTRACT

Dental injuries usually affect one or two of the anterior teeth, and especially the maxillary central incisors. The aim of the experimental study was to radiographically evaluate the effect of both Emdogain with and without high power Diode laser irradiation Emdogain with and without high power Diode laser irradiation on replanted teeth of rats teeth of rats after extended extra oral dry time. **Material and methods:** The study was conducted on extracted maxillary incisors of 42 adult male Albino rats weighing 160–250 g. The rats were assigned to three groups 14 rats in each, then replanted after 75 minutes as follow ; **Group I** (Control group) no treatment for the root surface was performed on the extracted upper right central incisors and alveolar wound before their replantation. **Group II** (EMD) Emdogain was applied on root surface and alveolar wound. **Group III** Diode laser then Emdogain were applied on root surface and alveolar wound.Replantation was done then splinting of teeth. Half of each group was euthanized after 4 weeks, while the other half after 8 weeks. The jaws were dissected out for radiographic examination using standardized periapical technique. **Results:** The radiographic analysis showed that the alveolar crest bone density in group III was the highest and less in the group II, while the least density was in the group I**. Conclusion**: Application of EMD to the root surface has limited inductive effects on bone while using EMD with Diode laser leads to better PDL healing and formation of bone in comparison to using EMD only or without treatment of root surface.

INTRODUCTION

Trauma to the face or teeth can be caused by auto accidents, falls, or injury from a variety of sports. The incidence of injuries to primary teeth increases from 1 year of age, and most traumas involve children younger than 4 years of age [(1)]. n the permanent dentition, the most accident-prone time is between 8 and 10 years of age.Boys appear to sustain injuries to permanent teeth twice as often as girls Dental injuries usually affect one or two of the anterior teeth, and especially the maxillary central incisors [(2)]. Classification of traumatic dental injuries comprises trauma to the hard dental tissues, the pulp, the periodontal tissues, the supporting bone and to gingiva and oral mucosa. It reflects the application of international classification of diseases to dentistry and stomatology by World Health Organization (WHO).It comprises seven types of tooth fractures, six types of luxations, eight types of damage to supporting bone and three to oral mucosa or gingiva.Avulsion is

one of the injuries to the periodontal tissues [3,4] and is defined as the complete displacement of a tooth from its socket in alveolar bone owing to trauma. The ideal treatment for permanent teeth consists of replantation which radiographically improve the prognosis. Deciduous teeth should not be replanted due to the risk of damaging the permanent tooth germ[5].

The frequency of the reported incidence of tooth avulsion ranges from 1-16% of all traumatic injuries to permanent dentition.The prognosis of the treatment as well as the survival of an avulsed tooth in the mouth depends on intrinsic and extrinsic factors, such as the duration of the tooth's extra-alveolar period and replantation time, its storage medium, PDL status and duration of splinting [6].

In case of delayed replantation higher risk of treatment failure is antipated therefore, a variety of adjunctive treatments have been proposed to increase possibilities of the survival of replanted teeth[16].

Substances that have been used for treatment of the root surface of replanted teeth in an attempt to increase their retention rate, are:[7]

Formol acid solutions (such as citric acid, hydrochloric acid, acidulated fluoride and neutral fluoride), Alkaline substances (such as calcium hydroxide and sodium hypochlorite), Antibiotics (such as tetracycline and rifacin),Antibiotic/corticosteroid combination, Corticosteroid, Alendronate,Vitamin C, Carbonic anhydrase inhibitor (acetazolamide),Basic fibroblast growth factor, Enamel matrix derivatives protein (Emdogain) and Diode laser.

EMDs contain amelogenin as major component as well as other enamel matrix component such as enamelins, tuftelin,amelin and ameloblastin. It stimulates the regeneration of periodontal tissue including the a cellular cementum, PDLs and alveolar bone.On its application on the root surface it inhances formation of cementum either cellular or a cellular with large numbers of inserting collagen fibers. It also inhibits epithelial cell growth which interfere in the regeneration process.[8]The effect of EMD on periodontal healing and root resorption after tooth replantation has been investigated in many preclinical and clinical studies. **EMD** is convenient and effective to use in difficult to reach areas such as interproximal areas and defects located under bridgework, In addition to the possibility for the use of EMD in periodontal defects is expanding[9].

Emdogain requires little or no preparation time, no mixing of materials is required and no specialized products or equipment are necessary.It allows for rapid placement and little time in manipulation of material into a periodontal defect, resulting in limited exposure of open tissues.It doesnot affect tissue integrity beyond that of normal flap surgery and no follow up surgery is necessary [10].

LASER (Light Amplification by Stimulated Emission of Radiation).

Many different wavelengths are utilized in medicine, the first such wavelength, 1064 nm, was approved by the FDA specifically for dentistry was in 1989. Perhaps the most important principle of lasers for a practitioner to understand the laser-tissue interaction [11].

Photobiostimulation

Its application can be summarized as the control/eradication of disease, the control/relief of pain, and the restoration of form/function. The inter-relationship of any stimulus with injury, cellular response and pain can be the product of the nature and potency of the stimulus and the ability of the tissue to respond Research on peri-apical lesions has shown that there is a correlation between the release of cellular and biochemical mediators and the nature of the injury, with acute and traumatic injuries re-

sulting in greater reactive processes, compared to chronic pathogenesis [12].

Using Diode laser irradiation as root surface treatment prior to delayed replantation has shown[13] reduction in the occurrence of external root resorption and to inhibit ankylosis, where laser promote fusion and melting of dental structure, thus make it homogeneous and favoring the adhesion of connectives tissue fibers and cells and the new cementum formation, this render the root surface more resistant to microbial and clastic cell action.It is important to understand that the laser is not just a hot instrument. Heat is not produced until the tissue absorbs a particular wavelength. The absorbed laser energy causes the tissue temperature to rise slowly, causing a series of tissue changes. First, simple warming occurs from normal body temperature of 37 degrees centigrade up to 60 degrees centigrade. The next warming stage (60-90 degrees) brings about coagulation and protein denaturation. Finally at 100 degrees centigrade, vaporization begins as the intra and extracellular water of each cell begins to boil away [23]. Each of these stages has specific biologic phenomena: Coagulation prevents hemorrhage, protein denaturation prevents bacterial cell division as well as sealing of nerve endings. Vaporization facilitates incision into tissue, ablation of structures and sterilization of the surface. Laser energy that is not absorbed will be frequently reflected, transmitted and scattered. The occurrence of these phenomena depends on the tissue composition as well as the melanin, blood, mineral, and water content of the irradiated tissue [14]. Low-level laser (e.g diode laser) most significant uses are photobiostimulation, composite resin curing, caries detection,photo-activated disinfection (PAD) and laser scanning (restorative dentistry, orthodontics) [15].

Radiographic investigation

Radiographs are required to assess the healing and/or the development of post-trauma complications. **The ideal (2-D) radiographic examination** include **two views** of the injured tooth from different angles, ideally at right angles to one another, but more usually with the x-ray tube head in two different positions in the vertical plane. For example in the anterior region a **periapical** (paralleling technique) film and an standard **occlusal** film. Also soft tissue radiograph of the lip to locate avulsed tooth. Recently (3-D) radiograph assessed (cone beam CT) is considered a replacement multiple (2-D) examination. [16] The radiographic findings in post-trauma complications in replantation includes root resorption ,infection and ankylosis.Reproducible views could be done to provide a base-line assessment and to allow subsequent follow-up evaluation. Views of the chest and/or abdomen if a tooth or foreign body is thought to have been inhaled or swallowed, including: Soft tissue lateral and antro- posterior (AP) of the larynx and pharynx ,postro- anterior(PA) of the chest, antro- posterior (AP) of the abdomen.[17]

MATERIALS AND METHODS

The present study was carried out in the the animal house of Faculty of Dentistry, Suez Canal University after the approval of the Research Ethical Committee (REC) Faculty of Dentistry Suez Canal University (27 / 2017).

The study was done on 42 adult male Albino rats weighing 160–200 g and divided into three groups (two experimental groups and one control group). Rats were caged in wire top cages where seven rats were housed per cage in the animal house of Faculty of Dentistry, Suez Canal University. They were supplied natural diet and tap water throughout the experiment, they were maintained under good ventilation.

Extraction of a Right maxillary incisors of all rat were performed as following:

A. Rats were anesthetized under intramuscular general anesthesia in pectoral muscle, using ketamine and xylazine at 0.7:0.5 ml ratio and in dose of 0.1 ml per100g of weight.

B. Extraction of the maxillary right central incisor was done by laxation of the tooth by using amalgam carver as an elevator to break down the periodontal ligament. Followed by extraction using orthodontic adam pliér.

C. After extraction, the tooth was left on an external napkin for 60 minutes.

D. The dental papilla of each extracted tooth was removed using a scalped blade number 15.

E. Then, pulp exacerbation was done by removal of the pulp using endodontic files after passing of the pre determined time period (60 minutes),the pulp was removed via the apical foramen with a precurved 25-mm length number #20 K file

F. The root canals were irrigated with 5 ml of saline solution then dried with absorbent paper points and filled with calcium hydroxide paste with idoform Metapex.

Then, rats were classified into three groups 14 rats in each group :

- **Group I** (Control group) no treatment for the root surface and alveolar wound were performed on the extracted upper right central incisors before their replantation.
- **Group II** (EMD) Emdogain were applied to root surface and alveolar wound of the extracted upper right central incisors before their replantation.
- **Group III** Diode laser then emdogain were applied to root surface where diode laser (810nm,contionous mode,1.0w,30s) and topical application of Emdogain gel were performed on the extracted upper right central incisors before their replantation.

G. Replantation of the maxillary central incisor was done for all groups after 75 minutes of the beginning of the experiment and splinting of the replanted incisor to the neighboring maxillary central incisor was performed as the following:

1. Acid etch (meta) was applied to the tooth crown then washed away after 15 sec.
2. The bonding agent (3m) was applied and light cured for 20 sec.
3. The flowable composite and light cured for 40sec.
4. Splintting was left for four weeks.

H. Intramuscular dose of antibiotic (amoxicillin 1gm) in dose of 0.005ml per 100g of body weight were administrated for 4 days twice daily.

Half of the rats each group were euthanized after 4 weeks of the beginning of the experiment, while the other half was after 8 weeks of the beginning of the experiment.

The rats were sacrificed by cervical dislocation. The maxillary jaw of the upper incisors regions of the sacrificed animals were dissected out for standardized digital radiographic examination.

- A Size 2 intraoral Kodak Films were taken for the maxilla of each group using de- gotzen x-ray machine and they were placed on horizontal table and the maxillary occlusal plane of the rat was positioned parallel to and resting on the film.
- The central x-ray beam was placed with vertical angulation 60° to the film.
- The x-rays were digitized using a Canon MP280 and analyzed using Digora software 2.7.

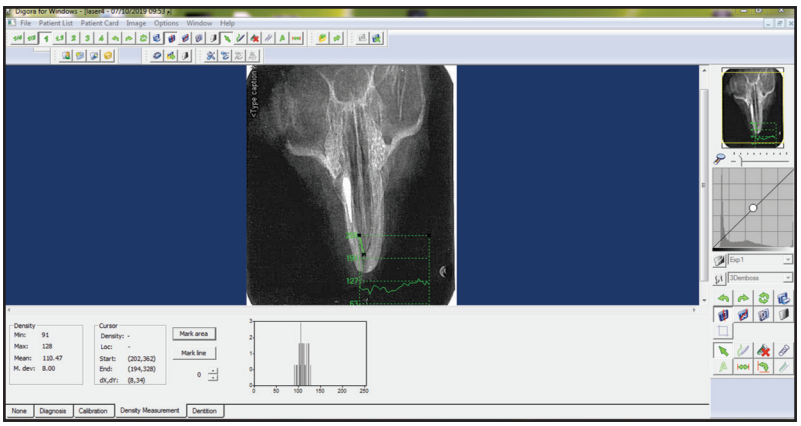

Fig. (1) A photograph showing digora software and how the measurements were done

For the alveolar density measurement: a vertical line 1cm was drawn from the crest of the alveolar process and extending apically mesial to the replanted tooth.

The average radiographic bone density was assessed after 4 weeks and 8 weeks where a line was calculated as a numerical gray scale value by quantifying the image on 256 grey scales. Zero scale was given to the totally black regions (totally radiolucent), while 225 was given to the totally white areas (totally radiopaque).

Measurements was performed twice by the principle investigator with two weeks intervals between each reading and the average of both readings were used for further statistical analysis.

RESULTS

I-Radiographic Analysis:

After 4 weeks the radiographic analysis showed that the alveolar crest bone denisty in the Diode laser and Emdogain group is the highest and less in the Emdogain group, while the least in the control group**.**

However, no statically signification difference of alveolar bone density was found between group III in comparison to group II and group I, where p value is 0.163. As the mean values are considered significant when the P value < or = 0.05.

Similarly, the radiographic analysis showed in the 8 weeks scarification that the alveolar crest bone density in the Diode laser and Emdogain group is the highest and less in the Emdogain group, while the least were in the control group**.** Although alveolar bone density showed increased in group III in comparison to groups II and group I, no statistically significant results were found where p value was found to be 0.329.

Table (1) *shows Mean±SD of alveolar crest bone density and the P value of the three groups after 4 weeks.*

4 weeks	Control	EMD	Diode laser + Emdogain
Range	59.41–95.26	52.52–118.69	63.66–130.75
Mean ± SD	77.86±12.86	85.12 ± 24.22	100.39±25.11
p. value		**0.163**	

** Significant if P ≤ 0.05*

** Highly significant if p ≤ 0.01*

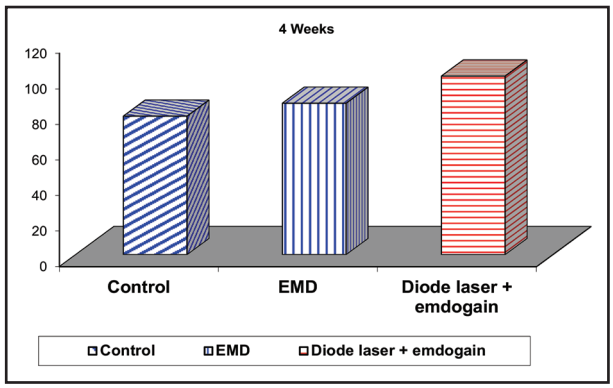

Fig. (2) Shows Bar chart showing the alveolar crest bone density in the three groups after 4 weeks

Table (2) *shows Mean ±SD of alveolar crest bone density and the P value of the three groups after 8 weeks.*

8 weeks	Control	EMD	Diode laser + emdogain
Range	81.38 – 93.2	57.79 – 139.2	62.95 – 141.66
Mean ± SD	88.09 ± 4.28	91.77 ± 27.88	106.42 ± 29.52
p. value		**0.329**	

** Significant if P ≤ 0.05*

** Highly significant if p ≤ 0.01*

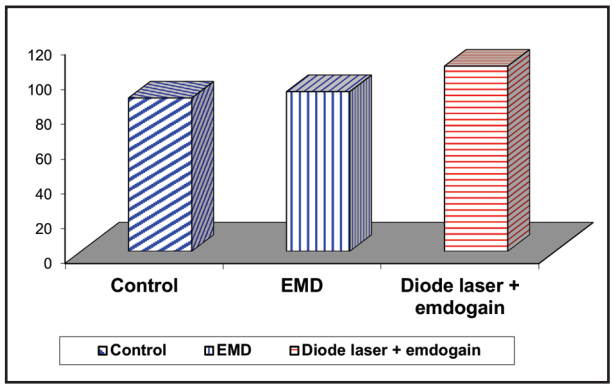

Fig. (3) Shows Bar chart showing the alveolar crest bone density in the three groups after 8 weeks.

DISCUSSION

Avulsion of teeth is a complex traumatic injury characterized by complete displacement of the tooth out of the alveolus, which exposes it to the external environment and causes disruption of its periodontal ligament attachment [18]. The permanent anterior teeth are the most commonly avulsed teeth in the oral cavity, they are not only important for aesthetics but are also essential for speech, mastication, health of the supporting tissues and psychological and mental health of children.

Hence, immediate replantation of avulsed permanent incisors contributes to an improved self-image and enhanced self-esteem in children [19].

Delayed treatment of the avulsed tooth gives very poor prognosis due to dehydration of the tooth and lose of vitality of the periodontal ligament. Therefore the aim of the present study was a way to improve prognosis of delayed replantation through evaluating the effect of both Emdogain with and without diode laser irradiation on periodontal healing of replanted teeth after extended extra oral dry time through radiographic examination.

Recent guidelines from the International Association of Dental Traumatology [20] refer to 60 minutes as the extraoral dry time threshold and outline different treatment approaches for avulsion cases with an extraoral dry time of less than 60 min. The healing potential of the PDL tends to decrease exponentially as the dry time increases. Some literature even suggests that if replantation does not occur almost immediately, the healing of PDL is likely to be compromised and low healing rates should be expected, even after only 20 min of extraoral dry time [21].

Enamel matrix derivative (Emdogain) has been successfully used in periodontal regenerative procedures as reported by several investigations[22]. Both animal and human studies have shown that

Emdogain may lead to the formation of periodontium. The use of Emdogain in periodontal regeneration has been suggested because of its biological origin and properties.

It was shown that high-power lasers have antimicrobial activity when applied in the root canal system [23]. In addition,it promotes adhesion of connective tissues fibers and new cementum tissue adhesion. Root surface treatment with high-power diode laser irradiation prior to delayed replantation has shown potential to reduce the occurrence of external root resorption and to inhibit ankylosis[24].

The present study was conducted on central incisors of rats due the ease of manipulation and assessment the radiographic analysis showed the alveolar density was performed by measuring a vertical line 1cm from the crest of the alveolar process and extended apically was drawn from mesial to the replanted tooth. Results of our study showed that the alveolar crest bone density of groups III (Emdogain And Diode laser) is the highest when compared with those of the emdogain group and control group. These results were in agreement with Sculean et al [25] who performed surgical periodontal treatment of deep intrabony defects with EMD to promote periodontal regeneration in a vivo study. The application of EMD in the context of non-surgical periodontal therapy has failed to result in periodontal regeneration alone.they conducted that the combination of EMD and some types of bone grafts/ bone substitutes may result in certain improvements in the soft and hard tissue parameters compared to treatment with EMD alone. Treatment of recession-type defects with coronally repositioned flaps and EMD was found to promote formation of cementum, periodontal ligament and bone, and may significantly increase the width of the keratinized tissue and which was in agreement to our results where the bone density increased when EMD was used in comparison to the control.

This was also in agreement with Esposito *et al*.[26] who studied the use of Enamel matrix derivative (Emdogain) for periodontal tissue regeneration in intrabony defects. In their study they tested whether EMD was effective, and in comparison to guided tissue regeneration, and various bone grafting procedures for the treatment of intrabony defects. Emdogain showed similar clinical results to guided tissue regeneration, but is simpler to use and determines less complications No serious adverse reactions to Emdogain were reported in their trials.

On the other hand, Mahmood *et al*.[27] who reported that 940 nm of diode laser stimulate bone regeneration in dental socket after human tooth extraction using 11.9 W/cm2 power density which was in accordance with our results where the Emdogain and diode laser irradiation applied showed highest bone density results.

In addition this was also in accordance with Biloklytska *et al*.[28] who studied the combined usage of Diode Laser and Emdogain during the surgical phase in treatment of generalized periodontitis and they found significant inflammatory reduction in the periodontal tissues on pre-surgical stage since the initial periodontal tissue condition may influence the healing in postsurgical period. The presented preliminary clinical results showed that additional diode laser usage during pre-surgical and surgical phases may be beneficial due to its bactericidal simultaneous effects in the preservation of marginal periodontal tissues, promotion of progressive regeneration process and long-term of generalized periodontitis. In addition the reduction of main periodontal indices values can be accelerated by diode laser irradiation usage.

Baburao *et al*.[29]showed that root surfaces treated with scaling and root planning followed by diode laser application showed slight to mild superficial improvements as compared to scaling and root planning alone.

In addition to Erica *et al*.[30] who performed a study to evaluate the effects of irradiation with a high-power diode laser on root surface of rats teeth in delayed replantation by radiographic and histomorphometric analyses and they found that the occurrence of external root resorption was reduced compared to no treatment or sodium fluoride treatment follow up period to 60 days.

CONCLUSION

From the results of the study the following can be concluded :- Application of EMD to the root surface has limited inductive effects upon the remaining PDL cells. Using of Emdogain in combination with Diode laser on the root surface and wound socket in extra-oral dry time leads to better periodontal healing and increasing in bone density in comparison to using of Emdogain only.

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