



Effect of Biostimulation on Response of Immature Teeth with Necrotic Pulp and Apical Periodontitis to Regenerative Endodontic Therapy in Immature Dogs Teeth

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ABSTRACT

Purpose: This study was carried out to evaluate the effect of biostimulation on the regenerative response of immature teeth with necrotic pulp and apical periodontitis in dogs. **Materials and Methods:** Seventy-two root canals were employed in the present study as follows: Sixty root canals for experimental groups and twelve as positive and negative controls. After the induction of pulp necrosis and apical periodontitis, roots were divided into two groups, group I: Regenerative Endodontic Therapy (RET) with subsequent biostimulation (seven sessions at 808 nm diode laser at output power of 300 mW for 90 seconds) and group II: RET without biostimulation. The groups were followed up for 1, 2 and 3 months (subgroups A, B&C). The maturation of the roots was assessed both radiographically and histologically. All data were statistically analyzed. **Results:** the application of biostimulation in group I demonstrated marked increase in root length, thickness and decrease in apical diameter compared to group II however, it was statistically significant only in subgroup C (12.43% versus 7.66%, 33.09% versus 17.81% and 38.12% versus 23.35 % at third month) respectively ($p < 0.05$). Furthermore, it showed histologically the highest score of vital tissue infiltration and least inflammatory scores which was statistically significant. **Conclusion:** Biostimulation enhanced the response of immature teeth with necrotic pulp and apical periodontitis to regenerative endodontic therapy improving root maturation.

KEYWORDS

*Regenerative endodontic therapy,
biostimulation,
root maturation*

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INTRODUCTION

Pulp necrosis of immature permanent teeth represents a considerable challenge for clinical management as root development ceases and apices remain open. Management of these immature teeth represent a considerable challenge due to the thin dentinal walls which represents low resistance to fracture and lack of apical barrier which limits the mechanical aspects of root canal preparation and obturation⁽¹⁾. Traditionally, these immature apices were managed through long term multiple visits apexification or more recent by single visit MTA apexification⁽²⁾. Regenerative endodontic therapy (RET) is considered a recent treatment modality for the last two decades and has gained wide approval and is performed nowadays routinely⁽³⁾ and has proven the continued root development through the increase in both root canal length and dentinal wall thickness⁽⁴⁾.

Three main components are involved in the process of regeneration, these are stem cells, scaffolds and bioactive/growth factors. Depending on the source of stem cells either exogenous or endogenous, RET can be categorized into cell based or cell free RET⁽⁵⁾. Cell free or in other word cell homing strategy was heavily reviewed through literature and was approved by the European society of endodontics and American association of endodontics while they did not to date approve stem cell transplantation⁽⁶⁾. Thus, all mechanisms and techniques which involves stimulative effect on endogenous stem cells would represents a beneficial adjunctive that adds to the success of the whole procedure.

Recently, laser application in endodontics was well proven. Laser power can be classified either into high power surgical uses or low power biostimulative uses. The later has many synonymous terms like low level laser therapy, low power laser therapy, photobiomodulation, cold laser, soft laser and laser therapy and they all refer to use of laser

for therapeutic purposes at low power output⁽⁷⁾. This includes the use of long wavelength mostly in red and infrared range where it can cause favorable cellular response as increased cellular proliferation and mitochondrial activity and hence adopt its improved inflammatory response⁽⁸⁾.

The synergistic power of biostimulation has been approved in many applications as it reduced post endodontic surgical pain and favored the bone density⁽⁹⁾, enhanced osteointegration around immediate implant placement compared to ozone therapy⁽¹⁰⁾ and enhanced wound healing⁽¹¹⁾. In regenerative endodontics, is worth to be mentioned that the effect of biostimulation was highlighted where large periapical lesion was associated with necrotic immature incisor responded to the disinfection and biostimulation protocol by 980 nm diode laser irradiation with accelerated healing rate⁽¹²⁾. The radiographic and histologic evaluation of regenerative endodontic therapy when biostimulation is accompanied was not sufficiently revealed in the current literature; hence the aim of the present study was to evaluate the effect of biostimulation on the response of immature teeth with necrotic pulp and apical periodontitis to regenerative endodontic therapy.

The null hypothesis of this study was that there was no enhancement in healing response of immature teeth with necrotic pulp and apical periodontitis when using biostimulation.

MATERIALS AND METHODS

Approval for this study was obtained from the research ethics committee in the Faculty of Dental Medicine for Girls Al-Azhar University (REC-EN-21-09). According to sample size calculation, the number of selected teeth incorporated within the study was set to be 72 teeth included in 6 dogs. The sample size was calculated based on the criteria of 80% power of calculation and α level of 0.05 by reviewing data from previous study⁽¹³⁾. Experiments were undergone at Governmental

Veterinary Hospital in Abbasia. A total of three healthy male beagle dogs aged 5-6 months with no sex predilection were included for this study. In each dog, 12 incompletely formed roots of upper and lower premolars were used to sum 72 root canals. Each root was used as a unit of measure for statistical analysis. The double rooted premolars were used in the two experimental groups (30 roots each). While the single rooted premolars were used in the control groups (6 roots each).

These teeth were assigned to two groups. Group I: disinfection of root canal through double antibiotic paste (DAP) with subsequent biostimulation. Group II: disinfection of root canal through DAP without biostimulation. Positive control: Samples of this group represent teeth with induced periapical infection without any treatment procedures and left open. Negative control: Samples of this group represent normal teeth that were left intact for normal maturation. The experimental and control groups were subdivided into three subgroups (A, B, and C) based on the length of the post-treatment evaluation period, which was 1, 2 and 3 months; respectively.

Induction of periapical lesions⁽¹⁴⁾

Premedication of dogs with I.V infusion of a blend of atropine sulfate (Atropine®CID Co, Egypt) at dose of 0.05 mg/ kg and diazepam (Neuril®Memphis Co Egypt) at a dosage of 1 mg/kg was achieved. Anesthesia was actuated quickly by I.V infusion blend of ketamine HCl (Ketamine® Sigmatec Co, Egypt) of dosage of 10 mg/kg and Xylazine (Xylaject® Adwia Co, Egypt) (1mg/kg). The anesthetic profundity was kept up with thiopental sodium 2.5% (Thiopental sodium® Sandoz, Austria) at dose of 25 mg/kg given intravenously. Exposing the pulp chambers was achieved by a #2 round bur in a high-speed hand piece under non septic conditions.

After pulp chamber exposure, disruption of pulp tissue inside root canals was performed with the

aid of stainless steel endodontic hand file (#30), then the root canals were irrigated with tap water and the access cavities were left open to the oral environment for three weeks and the animals were given carprofen as analgesic (Rimadyl tablets®: Zoites, USA) at a dose of 4.4 mg/kg given as single dose on daily bases for a week. Under the previously mentioned anesthesia regimen, infected teeth were re-entered under aseptic condition achieved with cotton roll isolation and surface disinfection with 0.12% chlorhexidine and tincture of iodine. All the root canals were irrigated using 1.5% NaOCl (10mL) per root canal.

Root canal preparation and disinfection

Mechanical preparation of the root canals was not performed in any of the samples. Samples of the experimental group were disinfected through antibiotic paste. The double antibiotic paste was prepared as following: equal weights of Ciprofloxacin 250 mg and Metronidazole 500 mg are crushed and the powder is mixed with propylene glycol until a homogenous mixture is obtained⁽¹⁵⁾. Under general anesthesia, surface disinfection of teeth was done. After reopening the root canals, they were irrigated with 10 mL of 1.5% NaOCl, for around 5 minutes using the side vented needle 1 mm short of the apex, passively to allow for none or minimal irrigant extrusion into the periapical area. DAP mixture was placed intra the canals by injecting through the canal by a 20" gauge sterile plastic syringe. Finally, the access cavities were closed by the temporary filling and teeth were left for 3 weeks before second intervention.

Treatment modalities⁽¹⁶⁾

Under general anesthesia, reopening of the access was done under complete aseptic condition. Irrigation with 10ml of 17% EDTA in Copious and gentle way and then 10 ml of saline irrigation were performed followed by dryness with paper point. Bleeding into the canal system was induced due

to overinstrumentation which was achieved by rotating a precurved K file passing the root apex by 2 mm with the aim of filling the entire canal with blood to the level of the cement-enamel junction. Then bleeding was allowed to stop to form a blood clot at a level where the MTA (Bio MTA, Cerkamed, Poland) is 3-4 mm thick. On the other hand, after using the MTA coronal plug, the teeth were filled with glass ionomer (GC Fuji, GC America, Alsip, IL) as a permanent restoration.

Laser biostimulation⁽¹⁷⁾

Before sessions of biostimulation, the dogs were anesthetized with the injection of ketamine hydrochloride (5 mg/kg) and xylaject (1 mg/kg). Samples of the experimental group I were irradiated by low power diode laser, with a wavelength of 808 nm and output power of 300 mW. The energy fluency 27 J/cm² was directed at the buccal side, in continuous wave mode for 90 seconds. The tip of the hand piece of the laser device was placed with no contact. Low level laser therapy (LLLT) was started shortly after regenerative procedure and was continued by every other day for total of seven sessions.

Methods of evaluation

Radiographic evaluation:⁽¹⁴⁾

Digital radiograph with RVG using parallel technique via film holder device were taken before and after induction of the periapical lesion and compared with the follow up radiographs (1, 2 and 3 months). ImageJ analysis software (ImageJ v1.44, US National Institutes of Health, Bethesda, MD, USA) was used for converting photos into 32-bit TIFF files, while TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland) was used for standardization of images. The following parameters were evaluated: increase in root length, increase in root thickness and decrease in apical diameter.

Increase in root length: Calculations of the

difference in root length as well as percentage of change were done of measurements of pre and post operative follow up radiographic images. Root length was measured as straight line from the cemento-enamel junction (CEJ) to the radiographic apex of the tooth.

Increase in root thickness: Increase in root thickness was measured at fixed point 5 mm apical to CEJ and calculations of difference in root thickness and percentage of change were done of measurements of pre and post operative follow up radiographic images.

Decrease in the apical foramen diameter: Calculations of difference in diameter of apical foramen and percentage of change were done of measurements of pre and post operative follow up radiographic images. Blind evaluation of periapical radiographs were carried out by two examiners for the previous parameters.

Histopathological evaluation:⁽¹⁸⁾

Dogs were euthanized at the end of each evaluation period using an overdose of the anesthetic solution thiopental sodium. After resection of jaws, they were fixed in 10% buffered formalin solution. The fixed tissues were decalcified in mixture of formic acid and sodium citrate solution for 120 days, dehydrated, and embedded in paraffin blocks which were then sectioned (5 μ m) and stained using hematoxylin and eosin stain and thus examined under Olympus light microscope (BX60, Olympus Corporation, Japan).

Samples were evaluated for statistical analysis for the following parameters by two examiners blinded to the experimental groups for the histological evaluation and according to the following scoring systems:

1. Presence or absence of vital tissue in the canal space: Absent (0), tissue in growth in the apical third of the canal space (1), tissue in growth in

the middle third of the canal space (2), tissue in growth in the cervical third of the canal space(3)⁽¹⁹⁾.

2. Presence or absence of new hard tissue: Absent (0), partial formation (1), Complete formation of new hard tissue (2)⁽²⁰⁾.
3. Apical closure: No apical closure (0), evidence of apical closure (1)⁽¹⁹⁾.
4. Inflammation(Intensity of inflammatory cell infiltrate):Absent or very few cells (0), mild (1), moderate (2) and severe (3)⁽²¹⁾.

Statistical analysis

All data were coded, tabulated and analyzed using Statistical package for Social Science (SPSS 15.0 for windows; SPSS Inc, Chicago, IL, 2001). The normality of distribution parameters was evaluated by One-Sample Kolmogorov-smirnov. The statistical significance of the difference between two experimental group means was assessed by the Independent-Samples T Test while One-Way ANOVA test was used to assess the statistical significance of the difference between more than two group means. One-Way ANOVA Post Hoc Tests was used once differences among the means was determined, which included both post hoc tests and pairwise multiple comparisons. For non-parametric data, data comparison was performed by using Mann-Whitney test, significance difference was set at an alpha level of 0.05.

RESULTS

First: Radiographic evaluation

All the experimental groups showed various degrees of root maturation manifested in the increase

of both root length and dentine thickness and apical closure (Fig. 1 A-H). The increase in root length and thickness and decrease in apical diameter in all groups and subgroups are manifested in table (1).

Regarding the experimental groups, group I (RET with subsequent biostimulation) showed the highest increase in root length and thickness and decrease in apical diameter, whereas group II (RET without subsequent biostimulation) showed less root maturation where there was a significant difference between groups I and II in the subgroup C ($P \leq 0.05$).

The positive control group showed no increase either in root length, thickness or apical closure among all subgroups, while on the other hand, the highest increase in root length and thickness with signs of apical closure was demonstrated by the negative control group which was significant among all subgroups ($P \leq 0.05$).

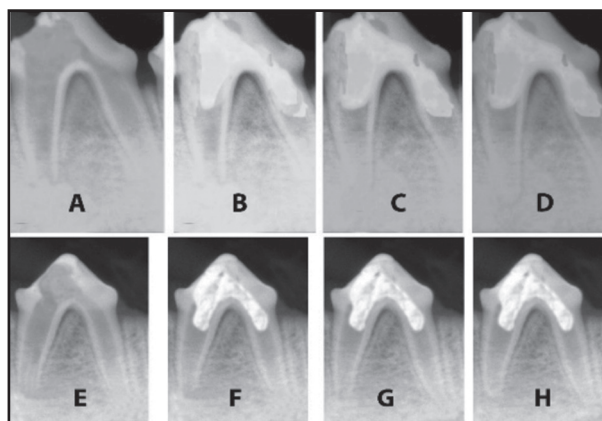


Figure (1) Representative radiographs of group I. (A) preoperative (B) one month,(C) two months and (c) three months showing improvement in root length, root thickness, apical diameter after three months while (E,F,G&H) represent preoperative, one month, two months and three months radiographs of group II

Table (1): Mean Value and (SD) of increase in length, thickness & decrease in apical diameter and percentage of change for Group I, II, Negative control & Positive control

Subgroups Groups		Subgroup A (1 month)	Subgroup B (2 months)	Subgroup C (3 months)	P value
Root length	GI	0.82 ± 0.13 ^{Aa} 4.42%	1.35 ± 0.3 ^{Ba} 7.21%	2.34 ± 0.43 ^{Ca} 12.43%	0.004*
	GII	0.58 ± 0.26 ^{Aa} 2.99%	1.05 ± 0.37 ^{Ba} 5.85	1.4 ± 0.36 ^{Cb} 7.66	0.02*
	Negative Control	1.13 ± 0.38 ^{Ab} 8.22%	1.87 ± 0.4 ^{Bb} 12.59%	3.13 ± 0.74 ^{Cc} 22.76%	0.01*
	Positive Control	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ad} 0%	-----
	P value	<0.001*	<0.001*	<0.001*	
Dentin Thickness	GI	0.21 ± 0.04 ^{Aa} 7.94%	0.43 ± 0.04 ^{Ba} 16.22%	0.88 ± 0.16 ^{Ca} 33.09%	<0.001*
	GII	0.22 ± 0.10 ^{Aa} 7.67%	0.38 ± 0.16 ^{Ba} 13.43%	0.50 ± 0.15 ^{Cb} 17.81%	0.02*
	Negative Control	0.36 ± 0.07 ^{Ab} 12.04%	0.80 ± 0.21 ^{Bb} 26.69%	1.49 ± 0.55 ^{Cc} 50.35%	0.01*
	Positive Control	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ad} 0%	-----
	P value	<0.001*	<0.001*	<0.001*	
Decrease in Apical diameter	GI	0.41 ± 0.13 ^{Aa} 11.42%	0.80 ± 0.1 ^{Ba} 22.08%	1.39 ± 0.13 ^{Ca} 38.12%	<0.001*
	GII	0.32 ± 0.7 ^{Aa} 6.30%	0.50 ± 0.09 ^{Ba} 14.54%	0.81 ± 0.14 ^{Cb} 23.35%	<0.001*
	Negative Control	0.68 ± 0.26 ^{Ab} 22.14%	1.19 ± 0.36 ^{Bb} 38.64%	1.64 ± 0.22 ^{Cc} 52.35%	0.01*
	Positive Control	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ac} 0%	0 ± 0 ^{Ad} 0%	-----
	P value	<0.001*	<0.001*	<0.001*	

*Significant difference as $P < 0.05$

Means with the same superscript letters were insignificantly different (uppercase in row / lowercase in column)

Means with different superscript letters were significantly different (uppercase in row / lowercase in column)

Second: Histopathological findings

Group I: (RET with subsequent biostimulation)

In subgroup A, vital tissue was formed at the apical third, while no evidence of formation of hard tissue, open apex and moderate inflammation near the root apex were present (Fig. 2A).

In subgroup B, the middle third showed vital tissue infiltration. Partial hard tissue, signs of apical closure as well as moderate inflammation towards root apex were noticed (Fig. 2B).

In subgroup C, whole pulp space exhibited vital tissue infiltration and differentiated odontoblast-like cells. Apparent increase in vascularity, complete hard tissue formation, signs of apical closure in addition to mild inflammatory cells were noticed towards the root apex (Fig. 2C).

Group II: (RET without biostimulation).

In subgroup A, absence of both vital tissue infiltration and hard tissue formation, open apex and severe inflammatory infiltration towards root apex were noticed (Fig. 2D).

In subgroup B, vital tissue formation near apical third, no hard tissue formation, open apex and moderate inflammation towards the root apex were seen (Fig. 2E).

In subgroup C, exhibited vital tissue infiltration at apical two-thirds, partially formed hard tissue, signs of apical closure and moderate inflammation (Fig. 2F).

Group I showed newly formed hard tissue and signs of apical closure which was more obvious than group II. The canals of these teeth were filled with well-formed vascularized pulp like tissues, showing numerous fibroblasts, newly formed capillaries, blood vessels and extracellular matrix.

At three months, in group I, the histological results revealed complete closure of the apices by calcified tissues, root canal walls thickening through formation of considerable thickness of dentin like mineralized tissue between the wall of the canal (Fig. 2C)

The statistical analysis of the histological results is demonstrated in table (2).

Table (2): Mean Value and (SD) of scores of vital tissues, hard tissue, apical diameter & inflammatory cells count

Groups	Subgroups	Subgroup A (1 month)		Subgroup B (2 months)		Subgroup C (3 months)		P value
		M	SD	M	SD	M	SD	
Vital tissue	GI	1.000 ^A	0.816	1.500 ^B	1.08	2.600 ^C	0.516	0.002*
	GII	0.400 ^A	0.516	1.300 ^B	0.675	1.900 ^C	0.568	0.001*
	P value	0.12		0.68		0.02*		
Hard tissue	GI	0.400 ^A	0.516	1.100 ^B	0.738	1.800 ^C	0.422	0.0001*
	GII	0.200 ^A	0.422	0.700 ^B	0.483	1.200 ^C	0.632	0.003*
	P value	0.48		0.24		0.05*		
Apical diameter	GI	0.200 ^A	0.422	0.600 ^B	0.516	0.900 ^C	0.316	0.008*
	GII	0.100 ^A	0.316	0.400 ^B	0.516	0.700 ^C	0.483	0.028*
	P value	0.73		0.48		0.48		
Inflammatory cells count	G I	2.100 ^A	0.568	1.500 ^B	0.527	0.800 ^C	0.632	0.001*
	G II	2.700 ^A	0.483	2.200 ^B	0.632	1.800 ^C	0.632	0.003*
	P value	0.04*		0.03*		0.007*		

*Significant difference as $P < 0.05$

Means with the same superscript letters were insignificantly different while means with different superscript letters were significantly different

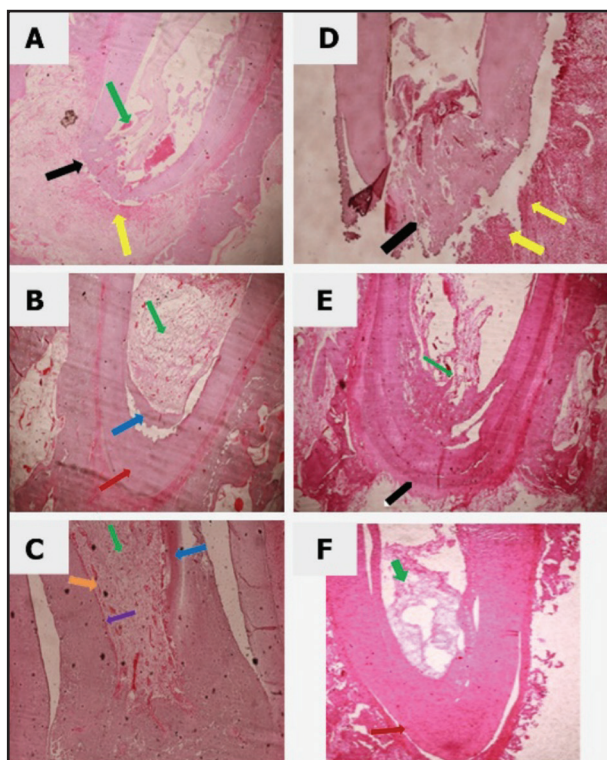


Figure (2) Photomicrographs of **group I**. (A) *Apical third* (after 1 month) showing vital tissue (green arrow), absence of hard tissue, open apex (black arrow) and moderate inflammation (yellow arrow). (B) *Apical half* (after 2 months) showing vital tissue, partially formed hard tissue (blue arrow), signs of apical closure (red arrow) and moderate inflammation. (C) *Apical two-thirds* (after 3 months) showing vital tissue infiltration in entire pulp space (green arrow), odontoblast-like cells (violet arrow), increased vascularity (orange arrow), completely formed hard tissue (blue arrow), signs of apical closure and mild inflammation (H&E, $\times 20$). **Group II**. (D) *Apical third* (after 1 month) showing absence of both vital tissue and hard tissue, open apex (black arrow) and severe inflammation (yellow arrow). (E) *Apical half* (after 2 months) showing vital tissue infiltration (green arrow), absence of hard tissue, open apex (black arrow) and moderate inflammation. (F) *Apical two-thirds* (after 3 months) showing vital tissue infiltration (green arrow), partial hard tissue formation, signs of apical closure (red arrow), and moderate inflammation (H&E, $\times 20$).

DISCUSSION

Regenerative endodontic therapy is now a well established treatment modality as was identified as “biologically based procedures designed to

replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex”⁽²²⁾. This emphasizes the procedure of recruitment of stem cells in the presence of suitable scaffold under the influence of specific bioactive molecules or growth factors.

The stimulation of endogenous stem cells from apical papilla or other near sources to migrate and populate root canal space in the strategy of cell homing was widely accepted as a base for modern concept of dental pulp regeneration⁽²³⁾. Although cell free approach is considered more as a repair rather than true regeneration process, it is still accepted due to successful clinical outcomes as well as proven continued root development and apical foramen closure in contrary to cell based RET which is still faced by technical and ethical issues⁽²⁴⁾.

Root canal disinfection is a major prerequisite for the success of the whole procedure and the negative effect of the presence of residual infection was highlighted with significantly less mineralized tissue and lack of radiographic growth⁽²⁵⁾. Commonly, predictable root canal disinfection was carried out by placement of tri antibiotic paste⁽²⁶⁾, however its shortcomings encouraged its replacement with double antibiotic paste through exclusion of minocycline. These shortcomings include dentin staining causing crown discoloration⁽²⁷⁾, interferes with the release of dentin growth factors⁽²⁸⁾ as well as the vascularization inhibition due to minocycline presence⁽²⁹⁾.

It is crucial to choose the proper concentration of irrigant that minimally interfere with stem cell migration, adhesion and root canal space population. Sodium hypochlorite irrigation of concentration of 1.5% represented a low toxic effect on stem cell and the maximum expression of dental pulp stem cells was associated with that low concentration as compared with least survival of stem cells when irrigated with 6% and 3%⁽³⁰⁾.

Blood clot has been chosen as a scaffold for the present study. Evidence revealed no particular

advantage of other scaffolds as platelets rich plasma or fibrin over blood clot^(31,32). Moreover it revealed superiority over both sodium hyaluronate:chitosan or pectin:chitosan scaffold⁽³³⁾.

Dogs have been chosen as an animal model for the present study due to similarity in teeth composition and growth pattern with humans. Moreover, they have a rapid healing rate, large number of teeth that could be employed in the study as well as the availability of suitable cavity size that would facilitate the conduction of the study⁽¹⁸⁾.

Low level laser therapy (LLLT) has a proven stimulatory effect on regenerative processes^(34–36) without causing any significant temperature increase. It enhances cell proliferation, migration, differentiation as well as enhanced cellular metabolism and protein expression⁽³⁷⁾. The enhanced mitochondrial activity was demonstrated through delayed luminance by electron transport system with production of reactive oxygen species (ROS) and ATP as well as the physiological changes of the mitochondria after LLLT⁽³⁸⁾.

The synergetic effect of biostimulation on regenerative endodontic therapy that was demonstrated in the present study was in agreement with the results of many *in vitro* studies^(34,36,39–42). It enhanced stem cell proliferative capacity of stem cells from human exfoliated deciduous teeth at power as low as 10 mW in two irradiation setting⁽⁴¹⁾. It also significantly increased the differentiation capacity of fibroblast which is a cornerstone cell type in tissue repair/regeneration at power of 0.2-0.5 Watt while not affecting the cell cycle⁽⁴³⁾.

Despite that the laser energy used to study its effect on stem cell proliferation and differentiation in conjugation with pulp regeneration was ranged in the low energy fluencies levels of 1-7 J/cm² in which 5 J/cm² demonstrated the highest stimulative effect in short term evaluation^(44,45) the total energy used for the present study was 27 J/cm². This decision was owing to the fact of light energy attenuation by buccal bone and overlaying mucosa in contrary

to previous preclinical studies. Moreover, it was revealed that irradiation through one mm thickness of dentine disk could reduce energy fluency from 42 J/cm² into as low as 0.3 J/cm² and in accordance with the evidence of maximum mitochondrial activity at irradiation with wavelength of 810 nm and energy fluency 38 J/cm²⁽³⁸⁾.

Noteworthy, the synergistic effect of biostimulation is best exhibited under nutritional deficiency. This was reported as cell proliferation of stem cells upon exposure to biostimulation under nutritional deficiency condition was comparable to positive control⁽⁴⁵⁾. The same effect was reported where the cell growth rate of stimulated stem cells of exfoliated deciduous cells under nutritional deficiency were similar to that of optimum nutrition⁽⁴¹⁾. This could be attributed to the production of low levels of ROS in mitochondria while counteract its inhibitory effect if its threshold was exceeded^(46,47).

The present results were contradicted by Santamaria who reported no additional benefits when connective tissue grafts accompanied by biostimulation. This is best explained by that the biostimulation represents maximum synergistic effect on short term rather than on long term follow up as the outcomes was measured at two years follow up, nevertheless the application of biostimulation demonstrated positive results on 6 months follow up⁽⁴⁸⁾.

The effect of LLLT on pulp regeneration was heavily searched in literature; nevertheless, they mostly have been *in vitro* preclinical studies. The histologic evaluation of regenerated tissue was only revealed in three *in vivo* studies using rats as the animal model^(49–51). To the best available knowledge, the present study is the only one to reveal the effect of LLLT on RET using radiographic and histological both evaluation on the animal model of beagles. They are closer to the human beings; in addition, the full protocol of root canal disinfection as described by AAE was adopted in contrary to revascularization process which was carried on immediately after removal of pulp tissue of molar

roots in rats. So, despite some limitations that have been encountered in the current study as the need for bigger sample size, the present results have paved the way for further investigations in order to confirm the synergistic effect of biostimulation on regenerative endodontics.

CONCLUSION

Biostimulation enhance the response of immature teeth with necrotic pulp and apical periodontitis to regenerative endodontic therapy improving root maturation in immature Dogs Teeth

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DECLARATION

No fund was received for this study.

No conflicts of interest

RECOMMENDATION

Additional investigations are required to demonstrate the effect of biostimulation on regenerative endodontic therapy on randomized controlled clinical trial to better reveal its effect on humans.

REFERENCES

- Shobhana R, Prakash V, Venkatesh A, Vivekanandhan P, Balaji S. A Review On Management Of Open Apex In Permanent Teeth. *Int J Aquat Sci.* 2021;12:2055–61.
- Yoshpe M, Kaufman AY, Lin S, Ashkenazi M. Regenerative endodontics: a promising tool to promote periapical healing and root maturation of necrotic immature permanent molars with apical periodontitis using platelet-rich fibrin (PRF). *Eur Arch Paediatr Dent.* 2021;22:527–34.
- Fouad AF. Contemporary Microbial and Antimicrobial Considerations in Regenerative Endodontic Therapy. *J Endod.* 2020;46:105–14.
- Wikström A, Brundin M, Lopes MF, El Sayed M, Tsilingaridis G. What is the best long-term treatment modality for immature permanent teeth with pulp necrosis and apical periodontitis? *Eur Arch Paediatr Dent.* 2021;22:311–40.
- Ahmed GM, Abouauf EA, Abubakr N, Fouad AM, Dörfer CE, Fawzy El-Sayed KM. Cell-Based Transplantation versus Cell Homing Approaches for Pulp-Dentin Complex Regeneration. *Stem Cells Int.* 2021;2021:1–23.
- Lin LM, Huang T-J, Sigurdsson A, Kahler & B. Clinical cell-based versus cell-free regenerative endodontics: clarification of concept and term. *Int Endod J.* 2021; 54:887–901.
- Musstaf RA, Jenkins DFL, Jha AN. Assessing the impact of low level laser therapy (LLLT) on biological systems: a review. *Int J Radiat Biol.* 2019;95(2):120–43.
- Walski T, Dąbrowska K, Drohomirecka A, Jędruchiewicz N, Trochanowska-Pauk N, Witkiewicz W, et al. The effect of red-to-near-infrared (R/NIR) irradiation on inflammatory processes. *Int J Radiat Biol.* 2019;95:1326–36.
- Metin R, Tatli U, Evlice B. Effects of low-level laser therapy on soft and hard tissue healing after endodontic surgery. *Lasers Med Sci.* 2018;33:1699–706.
- Karaca IR, Ergun G, Ozturk DN. Is Low-level laser therapy and gaseous ozone application effective on osseointegration of immediately loaded implants? *Niger J Clin Pract.* 2018;21:703–10.
- Amadio EM, Marcos RL, Serra AJ, dos Santos SA, Caires JR, Fernandes GHC, et al. Effect of photobiomodulation therapy on the proliferation phase and wound healing in rats fed with an experimental hypoproteic diet. *Lasers Med Sci.* 2021;36:1427–35.
- Yadav S, Nawal RR, Talwar S, Verma M. Low-level laser therapy for management of large periapical lesions associated with open apex cases. *Indian J Dent Res.* 2020;31:334–6.
- Pagliarin CML, Londero C de LD, Felipe MCS, Felipe WT, Danesi CC, Barletta FB. Tissue characterization following revascularization of immature dog teeth using different disinfection pastes. *Braz Oral Res.* 2016;30:1–10.
- El Halaby HM, Abu-Seida AM, Fawzy MI, Farid MH, Bastawy HA. Evaluation of the regenerative potential of dentin conditioning and naturally derived scaffold for necrotic immature permanent teeth in a dog model. *Int J Exp Pathol.* 2020;101:264–76.

15. Abdelrahman MYT, El-Ashry S, El-Batoty K, El-Hady S. Efficiency of Triple Antibiotic Mixture and Propolis as Intracanal Medication in Revascularization process in immature apex: A clinical study. *Glob J Med Clin Case Reports*. 2019;6:019–25.
16. Jang JH, Moon JH, Kim SG, Kim SY. Pulp regeneration with hemostatic matrices as a scaffold in an immature tooth minipig model. *Sci Rep*. 2020;10:1–11.
17. Fekrazad R, Seraj B, Ghadimi S, Dehghan MM. The effect of low-level laser therapy (810 nm) on root development of immature permanent teeth in dogs. *Lasers Med Sci*. 2015;30:1251–7.
18. Abbas KF, Tawfik H, Hashem AAR, Ahmed HMA, Abu-Seida AM, Refai HM. Histopathological evaluation of different regenerative protocols using Chitosan-based formulations for management of immature non-vital teeth with apical periodontitis: In vivo study. *Aust Endod J*. 2020;46:405–14.
19. Tawfik H, Hashem AA, Nagy MM. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. *Int Endod J*. 2013;46:1–13.
20. da Silva LAB, Nelson-Filho P, da Silva RAB, Flores DSH, Heilborn C, Johnson JD, et al. Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing in dogs' teeth with apical periodontitis. *Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology*. 2010;109:779–87.
21. Alcantara BAR, Carli ML de, Beijo LA, Pereira AAC, Hanemann JAC. Correlation between inflammatory infiltrate and epithelial lining in 214 cases of periapical cysts. *Braz Oral Res*. 2013;27:490–5.
22. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative Endodontics: A Review of Current Status and a Call for Action. *J Endod*. 2007;33:377–90.
23. Louvrier A, Terranova L, Meyer C, Meyer F, Euvrard E, Kroemer M, et al. Which experimental models and explorations to use in regenerative endodontics? A comprehensive review on standard practices. *Mol Biol Rep*. 2021;48:3799–812.
24. Lee HN, Liang C, Liao L, Tian WD. Advances in Research on Stem Cell-Based Pulp Regeneration. *Tissue Eng Regen Med*. 2021;18:931–40.
25. Verma P, Nosrat A, Kim JR, Price JB, Wang P, Bair E, et al. Effect of Residual Bacteria on the Outcome of Pulp Regeneration in Vivo. *J Dent Res*. 2017;96:100–6.
26. Montero-Miralles P, Martín-González J, Alonso-Ezpeleta O, Jiménez-Sánchez MC, Velasco-Ortega E, Segura-Egea JJ. Effectiveness and clinical implications of the use of topical antibiotics in regenerative endodontic procedures: a review. *Int Endod J*. 2018;51:981–8.
27. Porter MLA, Münchow EA, Albuquerque MTP, Spolnik KJ, Hara AT, Bottino MC. Effects of Novel 3-dimensional Antibiotic-containing Electrospun Scaffolds on Dentin Discoloration. *J Endod*. 2016;42:106–12.
28. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, et al. Influence of Root Canal Disinfectants on Growth Factor Release from Dentin. *J Endod*. 2015;41:363–8.
29. Dubey N, Xu J, Zhang Z, Nör JE, Bottino MC. Comparative Evaluation of the Cytotoxic and Angiogenic Effects of Minocycline and Clindamycin: An In Vitro Study. *J Endod*. 2019;45:882–9.
30. Martin DE, De Almeida JFA, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. *J Endod*. 2014;40:51–5.
31. Lolato A, Bucchi C, Taschieri S, Kabbaney A El, Fabbro M Del. Platelet concentrates for revitalization of immature necrotic teeth: a systematic review of the clinical studies. *Platelets*. 2016;27:383–92.
32. Riaz A, Furqan •, Shah A, Shah FA. Regenerating the Pulp–Dentine Complex Using Autologous Platelet Concentrates: A Critical Appraisal of the Current Histological Evidence. *Tissue Eng Regen Med*. 2021;18:37–48.
33. Palma PJ, Ramos JC, Martins JB, Diogenes A, Figueiredo MH, Ferreira P, et al. Histologic Evaluation of Regenerative Endodontic Procedures with the Use of Chitosan Scaffolds in Immature Dog Teeth with Apical Periodontitis. *J Endod*. 2017;43:1279–87.
34. Malthiery E, Chouaib B, Hernandez-Lopez AM, Martin M, Gergely C, Torres JH, et al. Effects of green light photobiomodulation on Dental Pulp Stem Cells: enhanced proliferation and improved wound healing by cytoskeleton reorganization and cell softening. *Lasers Med Sci*. 2021;36:437–45.
35. Paula A, Turrioni S, Montoro LA, Gonçalves F, Fátima L De, Almeida D De, et al. Dose-responses of Stem Cells from human exfoliated teeth to Infrared LED Irradiation. *Braz Dent J*. 2015;26:409–15.
36. Ozverel C, Islam A and YH. The biostimulative effectiveness of photobiomodulation therapy application on thawed dental pulp stem cells. *JIOHS*. 2021;14:1–8.

37. Staffoli S, Romeo U, Amorim RNS, Migliau G, Palaia G, Resende L, et al. Review The effects of low level laser irradiation on proliferation of human dental pulp : a narrative review. *Clin Ter.* 2017;168:320–6.
38. Bae Kim H, Youn Baik K, Choung P-HH, Hoon Chung J, Kim HB, Baik KY, et al. Pulse frequency dependency of photobiomodulation on the bioenergetic functions of human dental pulp stem cells. *Sci Rep.* 2017;7:1–12.
39. Bozkurt SB, Hakki EE, Kayis SA, Dundar N, Hakki SS. Biostimulation with diode laser positively regulates cementoblast functions, in vitro. *Lasers Med Sci.* 2017; 32:911–9.
40. Garrido PR, Pedroni ACF, Cury DP, Moreira MS, Rosin F, Sarra G, et al. Effects of photobiomodulation therapy on the extracellular matrix of human dental pulp cell sheets. *J Photochem Photobiol B Biol.* 2019;194:149–57.
41. Moura-Netto C, Ferreira LS, Maranduba CM, Mello-Moura ACV, Marques MM. Low-intensity laser phototherapy enhances the proliferation of dental pulp stem cells under nutritional deficiency. *Braz Oral Res.* 2016;30:1–6.
42. Fernandes A P JM, Marques N.C.T, Machado M., Santos C. OT et al. Effects of low-level laser therapy on stem cells from human exfoliated deciduous teeth. *J Appl Oral Sci.* 2016;24:332–7.
43. Illescas-Montes R, Melguizo-Rodríguez L, Manzano-Moreno FJ, García-Martínez O, Ruiz C, Ramos-Torrecillas J. Cultured human fibroblast biostimulation using a 940 nm diode laser. *Materials (Basel).* 2017;10:1–10.
44. Abdelgawad L., Salah N. AM. Efficacy of Photobiomodulation and Vitamin D on Odontogenic Activity of Human Dental Pulp Stem. *J Laser Med Sci.* 2021; 12:1–8.
45. Ferreira LS, Diniz IMAA, Maranduba CMSS, Miyagi SPHH, Rodrigues MFSDSD, Moura-Netto C, et al. Short-term evaluation of photobiomodulation therapy on the proliferation and undifferentiated status of dental pulp stem cells. *Lasers Med Sci.* 2019;34:659–66.
46. Scialò F, Fernández-Ayala DJ, Sanz A. Role of mitochondrial reverse electron transport in ROS signaling: Potential roles in health and disease. *Front Physiol.* 2017;8:1–7.
47. Hamblin MR. Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. *Photochem Photobiol.* 2018;94:199–212.
48. Santamaria MP, Fernandes-Dias SB, Araújo CF, Lucas da Silva Neves F, Mathias IF, Rebelato Bechara Andere NM, et al. 2-Year Assessment of Tissue Biostimulation With Low-Level Laser on the Outcomes of Connective Tissue Graft in the Treatment of Single Gingival Recession: A Randomized Clinical Trial. *J Periodontol.* 2017;88:320–8.
49. Moreira MS, Sarra G, Carvalho GL, Gonçalves F, Caballero-flores HV, Clara A, et al. Physical and Biological Properties of a Chitosan Hydrogel Scaffold Associated to Photobiomodulation Therapy for Dental Pulp Regeneration : An In Vitro and In Vivo Study. *Biomed Res Int.* 2021;16:1–10.
50. Moreira MS, Diniz IM, Rodrigues MFSD, de Carvalho RA, de Almeida Carrer FC, Neves II, et al. In vivo experimental model of orthotopic dental pulp regeneration under the influence of photobiomodulation therapy. *J Photochem Photobiol B Bio.* 2017 ;166:180–6.
51. Zaccara IM, Jardine AP, Mestieri LB, Quintana RM, Jesus L, Moreira MS, Grecca FS, Martins MD KP. Influence of photobiomodulation therapy on root development of rat molars with open apex and pulp necrosis. *Braz Oral Res.* 2019;33:e084 1-13.