

Assessment of Osteogenic Material with and Without Low Intensity Laser Activation in Acceleration of Healing Intra-bony Defects

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Abstract

The purpose of this study was to evaluate the efficacy of a new material with or without Low intensity laser treatment in accelerating bone formation of an apical radiolucency through a surgical procedure. In this study a total of 30 upper anterior maxillary teeth with periapical lesions ranging from 5-8mm in diameter. Patients were divided randomly into three groups: Group A: Curettage and Filling osseous defect with Nanobone graft only (n=10). Group B: Curettage and treating the Osseous defect with Laser only (n=10). Group C: Curettage and Filling Osseous defect with Nanobone graft and treating the defect with Low intensity laser (n=10). Cone beam was taken to determine the presence of periapical radiolucency and its approximate sizes, all patients were followed up using CBCT at 3, 6 and 12 months interval for evaluation of the bone density and size of the radiolucent area. Results: The results of our present study in case of panoramic view (CBCT), it was found that group A (NBG) and C(L+NBG) showed increase in the grey scale value (bone density), while the third group B(LG) had shown lowest grey scale value, on the other hand, in case of cross-section view (CBCT), it was found that group C(L+NBG) had shown the highest grey scale value regarding the other two groups (NBG, LG). Conclusion: The use of Nano-bone graft and Low intensity laser technique in endodontic surgery is considered an optimum choice, as they both accelerate bone and tissue healing, the use of Low intensity laser decrease the post-operative pain and symptoms, the use of CBCT is considered the only effective method for measuring the bone density or the grey value.

Key Words: Low intensity laser, Curettage without apicectomy, Putty nanobone graft

Introduction

Bacterial infection of the dental pulp may lead to periapical lesions. They are generally diagnosed either during routine dental radiographic examination or following acute pain in a tooth [1].

After failure of root canal treatment, apical surgery is the last available resort to resolve inflammatory processes in the periapical zone. Apical surgery consists of exposing the apex of the involved tooth, curetting the periapical tissues, apicectomy and, ultrasonically preparing the apex [2] followed by retrograde filling. Many materials were used to enhance bony healing such as Bioactive Calcium phosphate ceramics are the largest family of alloplastic materials such as HA and TCP, they had been used in many as an enhancement of bone fill after periapical surgery [3-6]. However, many researchers have suggested the use of bone substitutes to fill the space created after removal of periapical pathological lesions surgically in order to speed up the healing process [7,8].

However, one of the drawbacks of traditional apical surgery is affecting the tooth integrity (Crown-root ratio) and slow intra-bony formation. So, an Alternative to preserve teeth integrity was to perform periapical curettage without apicectomy [9].

Recently nanotechnology was introduced in dentistry; which is the creation and utilization of materials, devices and systems through the control of matter on the nanometer-length scale [10].

Development of Osteo-conductive and Osteo-inductive materials in a gel or putty form can allow easy delivery of the material into the bony defect with a syringe. One of these biomaterials are the Nano Bone, consisting of nano crystalline hydroxyapatite (nHA) embedded in a silica gel matrix, which offers several of the advantages of nano structural biomaterials [11-13].

NanoBone used in this study was a synthetic and absorbable bone auto graft substitute with osteoconductive and osteoinductive properties.

Laser is an acronym for light amplification by stimulated emission of radiation. It is classified according to power into low power lasers (LPL) which is also called low intensity lasers (LIL) which is used in biomodulation, stimulation of healing, decreasing inflammation and pain relief [14].

The beneficial effect of LIL (Optodan) on bony healing was proven by many authors [14-18]. It was observed that the collagen production, osteoblastic activity and increased bone mineral density. Cone beam computed tomography allowed for 3D measurements which solved most of the measurement problem done with 2D image and low radiation dose in comparison with CT [19]. So the main objective of this study was to evaluate the efficacy of a nano bone (SBX putty) material with or without application of low intensity laser (LIL) and in accelerating bone formation of an apical defect through apical curettage without apicectomy after conventional root canal treatment.

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Material and Methods

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Minya University.

Patients with total of 30 affected upper anterior teeth (Not treated before) (Age ranging from 20-30 years free from medical conditions) with long standing peri-apical lesions ranging from 5 to 8 mm in size. They were divided into three groups randomly with total of 10 teeth for each group.

Material used in this study was optodan (LIL) and ARTOSS (GmbH) putty (Nano bone graft).

Material Composition

Optodan, Labtajm LTDKiev, Ukraine

- Gallium Aluminum Arsenide.
- Power: 13 mw (0.013w).
- Mode: CW and Pulsed.
- Wave length: 980 nm.

NanoBone Putty, Artoss GmbH, Rostock, Germany

- Nanocrystalline Hydroxyapatite (ncHA)
- Similar size, chemistry, and morphology as HA in human bone
- No bonding between nanocrystals-unsintered Autologous proteins adsorb rapidly to surface

AmorphousSilica Gel Matrix (ASG)

- Highly nanoporous with large internal surface area
- Holds HA nanocrystals in place
- Extremely hydrophilic
- Releases SiO₂ triggering angiogenesis, the basis for bone formation
- Is rapidly replaced by autologous organic matrix

Inclusion Criteria for patients according to which patient were included in the study

- Patients in this study were free from any systemic chronic debilitating diseases that may contraindicate the use of local anesthesia, or has negative effect on healing such as (uncontrolled diabetes, immune diseases, uncontrolled cardiovascular diseases and blood coagulation problems.)
- All of them cooperated in this study and come regularly for follow-up visits up to the end of the study period.
- Patient present in this study with a radiolucent area related to the upper anterior teeth with ranging from 5-8 mm.
- Teeth didn't suffer from any abnormalities or procedure errors.
- All patients that participated in this study were informed in detail about the procedure and the materials used and were asked to sign a written consent form.
- All reasonable steps to protect the security of personal information and privacy of the patient protected health information will be taken.

- All data were kept confidential and the Faculty of Research Ethics Committee reviewed the proposal.

Exclusion criteria

- Shouldn't be taking chemotherapy or radiotherapy to the head and neck region in the 12-month period prior to the treatment.
- Shouldn't be taking any drugs which may affect the healing process e.g. systemic steroids or anticoagulant therapy.
- Patients suffering from periodontal problems and bad oral hygiene have been excluded.

Then all patients were undergone for CBCT to determine the approximate size of the intra bony defect (The estimated size were measured from Cross-Sectional, Sagittal and Coronal views) and it is approximate, because the measurements were taken from three different dimensions, views or angulation and the mean of the three measurements give us the defect's size. Also to collect data about the tooth to be treated (root angulation, morphology, relation to the adjacent teeth or other structures) (*Figure 1*).

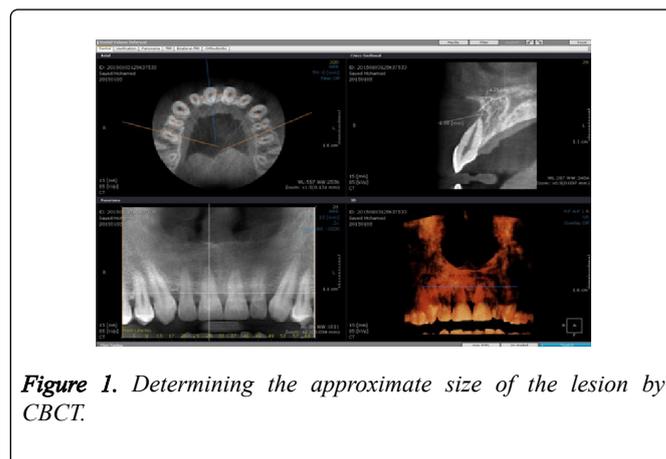


Figure 1. Determining the approximate size of the lesion by CBCT.

An access cavity through the lingual surface of the anterior teeth, rubber dam were applied, working length will be determined by an apex locator (MortialII) and cleaning and shaping was done in a crown down technique using Next rotary files (Dentsply) mounted on X- Smart endomotor. Root canals were irrigated with Sodium hypochlorite in stable cases, while in swelling cases, first with saline and then followed with Sodium hypochlorite. Then root canals were dried and filled with Di-antibiotic paste (Metranidazole and Ciprofloxacin) using lentulo spiral and access were sealed with Resin-modified Glass inomer (Fuji2) For 10 days, then Patients were examined clinically after 10 days to ensure that there was no swelling, exudate or foul odour with paper points or pain with percussion .if there were no symptoms, the patients were recommended to rinse with 0.2% chlorhexidine mouth wash one day before the day of obturation and surgery and if there is symptoms, canals were irrigated, dried, filled with di-antibiotic paste for also 10 days and access sealed with Fuji2 (usually this protocol continuous depending on the virulence factor of the bacteria, patients immunity, giving a chance for the inter canal medication to show its effect and most cases took from 4 to 6 weeks for the symptoms to subside. The next step, the canals were obturated by Pro Taper

Next GuttaPercha Points and Adseal Resin-based root canal sealer followed by the surgery in the same day.

Group A: 10 upper anterior teeth were selected and the full muco periosteal flap (Modified Rectangular Flap) [20] were considered, followed by apical curettage and then the osteoconductive and osteo inductive material was applied (Nano bone putty, ARTOSS (GmbH), patients were instructed to apply cold compresses 15 min every 1 hour from 3 hours, a course of antibiotics, anti-inflammatory and analgesic were considered for 5 days.

Group B: 10 upper anterior teeth were selected and the full mucoperiosteal flap were considered, followed by apical curettage and then the low intensity laser were applied for four sessions, two sessions was applied at the same day, the first session was applied immediately after suturing, the second session after 45minutes, the third session and the fourth session was at day four. The duration for each session was 5 minutes; patients were instructed to apply cold compresses 15 min every 1 hour from 3 hours, a course of antibiotics, anti-inflammatory and analgesic were considered for 5 days.

Group C: 10 upper anterior teeth were selected and full mucoperiosteal flap were considered, followed by apical curettage and then the osteoconductive and osteoinductive material (Nano bone putty, ARTOSS GmbH) were applied with the low intensity laser treatment for four sessions, two sessions was applied at the same day, the first session was applied immediately after surgery, the second session after 45 minutes, the third session and the fourth session was at day four. The duration for each session was 5 minute; patients were instructed to apply cold compresses 15 min every 1 hour from 3 hours, a course of antibiotics, anti-inflammatory and analgesic were considered for 5 days.

One week after surgery, CBCT was taken as a baseline and followed up for 3, 6 and 12 months respectively for evaluation of the bone density (Grey value).

CBCT Measurement

Regarding the bone density, it was calculated using the Hounsfield Unit through the ROI (Region Of Interest) within

Table 1. Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between rate of increase in grey scale values per month in the three groups.

View	LG		NBG		L + NBG		P-value
	Mean	SD	Mean	SD	Mean	SD	
Panoramic	31.6B	4.3	46.6A	11.3	48.0 A	15.1	0.031*
Cross-Sectional	29.7B	4.6	36.4B	10.5	47.4A	6.2	0.019*

*: Significant at $P \leq 0.05$, *: Significant at $P \leq 0.05$, Different superscripts in the same row are statistically significantly different.

Clinical evaluation

Some cases were edematous (24h) after surgery and the intensity of pain varies from none to mild, except in case 4 in group A there was moderate edema with mild pain, case 2 in

the software(On-demand software, OnDemand3D Technology Inc, Irvine, California.), where the mean pixel gray scale values of serial ROIs can be analyzed to determine whether changes in radio densities have occurred or not [20-24].

Statistical analysis

Numerical data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. All data showed non-normal (non-parametric) distribution.

Data were represented by mean and standard deviation (SD) values. Kruskal-Wallis test was used to compare between the three groups. Friedman's test was used to study the changes by time within each group. Dunn's test was used for pair-wise comparisons.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

In the present study, the total of 30 Patients was enrolled in this evaluation returned for scheduled follow up at the periods of evaluation. According to WAD [25] classification for criteria for follow up, the results were:

Successful outcome

No signs or symptoms present on follow up observations with complete resolution of the apical lesion with bone formation in all cases.

Uncertain outcome

No signs or symptoms, but the apical lesions didn't resolve completely, no cases in this study had shown this.

Unsuccessful outcome

Tenderness on percussion and the size of the lesion increase, no cases in this study had shown this.

group C with moderate pain and edema, but, during the follow up period in this study all cases in all groups didn't show any signs or symptoms of infection.

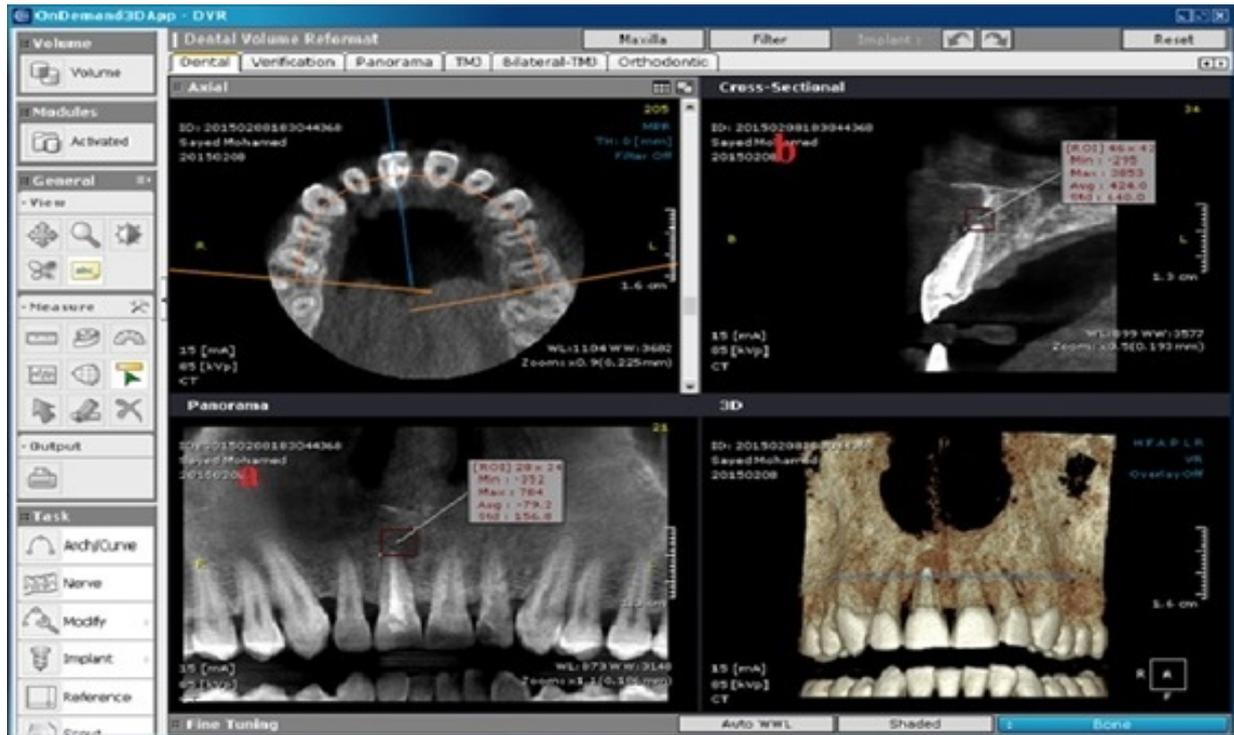


Figure 2. (A) Panorama and (B) Cross-Sectional, In Panoramic view, shows the Baseline of periapical radiolucent area related to upper right Central Incisors (Group C LIL + Nanobone). The ROI were fixed at 28 x 24 and the Average of the grey value was -79.2. While in the Cross-Sectional View the ROI were fixed at 46x42 and the Average of the grey value were 424.1.

Table 2. Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between grey scale values in the three groups (Panoramic view).

Time	LG		NBG		L + NBG		P-value
	Mean	SD	Mean	SD	Mean	SD	
Base line	291.5	130.8	245.7	110	201.2	141	0.399
3 months	382.8	94.1	493.9	117.6	359.4	134.6	
6 months	524.9	166.5	672.1	126.5	545.1	117.1	
12 months	670.2	135.5	804.9	118.3	777.2	162.3	

*: Significant at P ≤ 0.05

Table 3. Mean, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between grey scale values in the three groups (Cross-Sectional view).

Time	LG		NBG		L + NBG		P-value
	Mean	SD	Mean	SD	Mean	SD	
Base line	420.2	134.3	470.5	142	326.6	56.2	0.189
3 months	529.5	123.6	644.4	172	428.5	83.7	
6 months	635	122.8	804.6	194.3	667	128	
12 months	776.3	98.3	907.7	192.6	895.8	42.9	

*: Significant at P ≤ 0.05

CBCT evaluation

(In cross-section View) Treatment of bony defects with L +NBG (groupB) showed the statistically significantly highest

mean rate of increase in mean Grey scale Significant at P≤0.05. As shown in (Table 1 and Figure 2b).

In Panoramic view there was no statistically significant difference between rates of increase in NBG (group A) and L +NBG (group B); both showed the statistically significantly highest mean rate. LG group showed the statistically significantly lowest mean rate of increase in mean Grey scale (Table 1 and Figure 2a).

In regard to the increase of grey value, there was no statistically significant difference between mean Grey scale

values in the three groups through all study periods. In either Panoramic or Cross-Sectional views (Tables 2,3).

Evaluation of the three groups regarding time shown, that, in either panoramic or cross-sectional views with in each group, there was a statistically significant increase in mean grey scale values from base line to 3 months, 3 months to 6 months as well as from 6 months to 12 months (Tables 4,5).

Table 4. Mean, standard deviation (SD) values and results of Friedman's test for comparison between grey scale values at different follow up periods within each group (Panoramic view).

Time	LG		NBG		L + NBG	
	Mean	SD	Mean	SD	Mean	SD
Base line	291.5 D	130.8	245.7 D	110	201.2 D	141
3 months	382.8 C	94.1	493.9 C	117.6	359.4 C	134.6
6 months	524.9 B	166.5	672.1 B	126.5	545.1B	117.1
12 months	670.2 A	135.5	804.9A	118.3	777.2 A	162.3
P-value	<0.001*		<0.001*		<0.001*	

*: Significant at P ≤ 0.05, Different superscripts in the same column are statistically significantly different.

Table 5. Mean, standard deviation (SD) values and results of Friedman's test for comparison between grey scale values at different follow up periods within each group (Cross-Sectional view).

Time	LG		NBG		L + NBG	
	Mean	SD	Mean	SD	Mean	SD
Base line	420.2 D	134.3	470.5 D	142	326.6 D	56.2
3 months	529.5 C	123.6	644.4 C	172	428.5 C	83.7
6 months	635.0 B	122.8	804.6 B	194.3	667.0 B	128
12 months	776.3 A	98.3	907.7 A	192.6	895.8A	42.9
P-value	<0.001*		<0.001*		<0.001*	

*: Significant at P ≤ 0.05, Different superscripts in the same column are statistically significantly different

Discussion

One of the drawbacks of traditional apical surgery is affecting the tooth integrity (Crown-root ratio) and slow intra-bony formation. Hence, an Alternative approach to preserve teeth integrity was to perform periapical curettage without apicectomy [25].

In three studies performed by Gotz et al. [26] Harms et al. [27] and Arun et al. [28], they reported a high osteoconductivity of nanocrystalline HA. They reported that the presence of silicate ions appears to promote the process of bone formation and remodeling at the bonen HA interface, as well as induction of angiogenesis, as adequate blood supply is a prerequisite for cellular activity. In contrast to what was observed with other HA-based bone substitute materials, the rapid osseointegration of nanocrystalline HA seemed to prevent its complete degradation. The Nanoboneparticles were completely and firmly embedded within newly formed bone without a detectable fibrous interface and with no indication of an adverse host reaction to the material. These osteoinductive activities are limited to the implantation area

where natural bone incorporating all skeletal bone properties is generated and controlled osteoinduction by active agglomeration of growth factors (BMPs).

Low Level Laser (LLL) is a treatment that utilizes specific wavelengths of light to interact with tissue and is thought to help accelerate the healing process. Two studies performed by, El-Hayes A. et al. [29] Batista et al. [30], they concluded that the LLLT could induce bone formation in the bone defect at a faster rate than PRF, also, they concluded that Laser therapy presented a positive local bio stimulating effect in the early stage of bone healing.

CBCT has been used in many clinical and nonclinical investigations, In the periapical region, the mineral density can be measured in Hounsfield units (HUs) by using CBCT, three studies performed by Papanchev et al. [23], Cassetta et al. [31] and Sadullah Kaya et al. [32], concluded that accuracy of CBCT make it useful substitute for CT in measuring bone density, They concluded that the results of this study support the use of CBCT to measure bone density before and after endodontic treatment.

The result of this work shown that in case of panoramic view (CBCT), it was found that group A (NBG) and C (L+NBG) both showed the statistically significantly highest mean rate, on the other hand, group B (LILG) showed the statistically significantly lowest mean rate of increase in mean Grey scale while in case of cross-section view (CBCT), showed the statistically significantly highest mean rate of increase in mean Grey scale Significant at $P \leq 0.05$. Hence, group C (L+NBG) had shown the highest grey scale value regarding the other two groups A & B (NBG & LILG). Although that, after 12 months, all groups shown full bone formation of the defect as it became indistinguishable from the surrounding bone, this may attributed to the action of nano particles of the nanobone, effect of LIL or the combination of both the LIL and Nanobone graft, this comes in accordance with the result of Ghanaati et al. [33], Gholami G et al. [34], Eldibany RM et al. [35]. They reported that Nanobone accelerated bone healing and improved the quality and quantity of regenerated bone [35]. Also the effect of LIL comes in accordance with results of Cerpa et al. [36] and Acar et al. [37] that reported that the laser improved the acceleration the bone regeneration process.

Regarding or in respect to time, in either Panoramic or Cross-Sectional (CBCT) views within each group, there was a statistically significant increase in mean Grey scale values from base line to 3 months, 3 months to 6 months as well as from 6 months to 12 months, which showed increase of bone formation. This comes in agreement with results of Vinod et al. [38] and Tobon which in the follow-up period of 3, 6 to 12 months radiographically the bone graft became indistinguishable from the surrounding bone, which indicate complete bone regeneration, which stated that bone grafts in periapical surgery demonstrated favorable hard tissue healing [39].

Conclusions

The use of Nano-bone graft and Low intensity laser technique in endodontic surgery is considered an optimum choice, as they both accelerate bone and tissue healing, the use of Low intensity laser decrease the post-operative pain and symptoms, the use of CBCT is considered the only effective method for measuring the bone density or the grey value.

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