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A Comparison of Osseous Defects Healing After Surgical Enucleation of Periapical Lesions in The Presence of Hydroxyapatite and Nanohydroxyapatite (A Clinical Study)

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KEYWORDS

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Nano- hydroxyapatite

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ABSTRACT

Aim: to evaluate the bone healing in failed endodontically treated teeth after surgical enucleation of periapical lesions and packing of hydroxyapatite, and nanohydroxyapatite powder periapically. **Subjects and methods:** the study was established on sixteen patients having periapical radiolucency in single rooted teeth. The selected teeth were divided into two groups: Group A and Group B; of 8 teeth each. All the teeth were retreated in two visits. In the first visit the old filling was removed then irrigation with sodium hypochlorite was done. Di-antibiotic paste (ciprofloxacin and metronidazole) was used to fill the canals for 10 days. In the second visit the canals were obturated with Pro Taper gutta-percha points and root canal sealer followed by surgical intervention in the same day. Apicoectomy and periapical curettage were established. In both the groups, after preparation of root end cavity, it was filled with MTA followed by placement of regeneration material in the curetted periapical defect (hydroxyapatite (group A) and nanohydroxyapatite (group B)). In both groups, patients recall visits were scheduled at 1, 3, and 6 months time intervals for clinical and radiological evaluation. **Results:** after one month; there was a statistically significant difference between the median percentage changes in lesions size in the two groups. After three and six months; there was no statistically significant difference between the two groups. **Conclusion:** It was concluded that nanohydroxyapatite produced faster bone regeneration in the first three months than hydroxyapatite. However, hydroxyapatites produce bone regeneration after six months.

INTRODUCTION

Bacterial infection of the dental canal may lead to periapical lesions[1]. Most periapical lesions (>90%) could be classified as dental granulomas, radicular cysts or abscesses[2,3]. Endodontic therapy success depends on complete periapical repair and regeneration.

Most of the time periapical lesions may achieve successful healing after non-surgical root canal treatment[4]. However, some cases with persisting symptoms or recurrent infection requires periradicular

surgery in order to eliminate the source of irritation, and promote healing by removing pathological lesion[5]. Bone regeneration is a very slow process.

Many materials were used to improve bone healing. One of the largest family of alloplastic materials is bioactive calcium phosphate ceramics, i.e., hydroxyapatite (HA) and tricalcium phosphate (TCP). They had been used many times for accelerating bone growth after periapical surgery[6-9]. Many researchers suggested that bone substitutes application after surgical enucleation of periapical lesion speed up the healing process[10,11].

Recently nanotechnology was introduced in dentistry for which was found to possess unique properties related to its small size and large surface area[12].

SUBJECTS AND METHODS

The present *in vivo* study was established on sixteen patients having periapical lesion related to single rooted teeth taken from the outpatient clinic of endodontic department of faculty of dental medicine Minia University. Ethical clearance was taken from the Ethical Committee of the Institute with approval number: 238.

Periapical radiographs and CBCT were taken for all patients to measure the approximate size of the intra-bony defect (cross-sectionally, sagittally and coronally views). The measurements were taken from 3 different dimensions, views or angulations and the mean of the 3 measurements gave us the approximate size of the defect.

All patients were suffering from a necrotic tooth or previously treated single canalled teeth with periapical lesions size range equal to or more than 5mm in diameter.

Patients of total of 16 affected single rooted teeth were randomly splitted into 2 groups (8 teeth each).

Procedure:

First visit:

Periapical radiographs were taken for all the patients. All patients were anaesthetized by buccal infiltration technique using local anesthesia; articaine in 4% solution with epinephrine in concentration of 1: 100000. Access cavity was prepared. Rubber dam was placed. Working length was measured by periapical radiographs and an apex locator (Woodpecker). Cleaning and shaping were done using Protaper rotary files mounted on E- Connect endomotor.

In stable cases sodium hypochlorite (NaOCl) irrigation was used, while saline irrigation was used in swelling cases, followed by NaOCl irrigation in the following visits.

After using paper points to dry the canals a Di-antibiotic paste (ciprofloxacin and metronidazole) was introduced into the canals using lentulo spiral. A piece of cotton was applied in the pulp chamber and resin-modified glass ionomer was used to seal the cavity for 10 days.

Second Visit:

After 10 days clinical examination for patients was done. If tooth was asymptomatic, the patients were instructed to use mouth rinsing (0.2%chlorhexidine) one day before obturation and surgery day. If symptoms (swelling, exudate or bad odor) still persist, repeat the steps of the first visit (NaOCl irrigation + dry + di-antibiotic paste + seal) for another 10 days. Usually, this protocol continued for a period of time depending on bacterial virulence and patient's immunity to allow the intracanal medication to produce its effect. Symptoms relieved after 4 to 6 weeks in the majority of cases. Finally, canal obturation with Pro Taper gutta-percha points and root canal sealer was done then starting surgical procedure in the same day.

Under strict aseptic conditions surgical procedures were done. Profound buccal and palatal/lingual infiltration with local anesthesia was injected using articaine 4% solution with 1: 100000 epinephrine. After 15 minutes a full mucoperiosteal

flap (modified rectangular flap) was raised. Apical curettage and root end resection with fissure bur mounted to 45° surgical handpiece with saline irrigation were performed. Finally root end cavity was prepared with ultrasonic tips and filled with MTA.

Group A: the osteoconductive and osteoinductive hydroxyapatite powder were applied in the bony cavity. Group B: the osteoconductive and osteoinductive nanohydroxyapatite powder were applied in the bony cavity.

Wound was sutured, patients were informed to apply cold packs 15min every 1 hour for 3 hours (at first day of surgery). A course of antibiotics (Augmentin 1gm every 12 hours and Flagyl 500 mg every 8 hours for 5 days), together with anti-inflammatory and analgesic (Bi profenid 150 mg every 8 hours for 5 days) were given orally. Patients were instructed to rinse their mouth three times daily (one day after surgery) with warm saline and 0.1% chlorohexidine gluconate for 10 days. All sutures were removed after 10 days.

In both groups, patients recall visits were scheduled after 1, 3, and 6 months for clinical and radiological examination. On each visit, clinical examination regarding postoperative discomfort, pain, sensitivity to percussion, and presence/absence of swelling were established. Digital radiographs were obtained throughout the study for both groups (A and B) with a paralleling technique [13]. Using size 2 charged couple device (CCD) intraoral digital sensor with XCP, RINN crop film holding system. The sensor was positioned in the mouth parallel to the long axis of the tooth being imaged. The x-ray tube was aimed at right angle to both the tooth and the sensor. After 6 months CBCT was taken to evaluate bone density and confirm bone healing. Linear radiographic measurements (in mm) were made on digital periapical radiographs to assess the surface area of the bony defect and compared with preoperative radiograph[14-16].

Table (1) *Group A case radiographs*

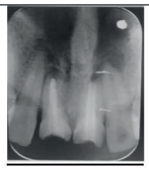
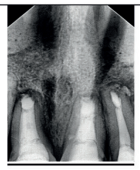
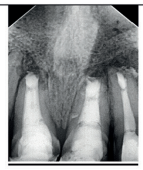
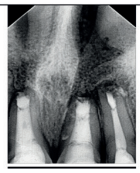
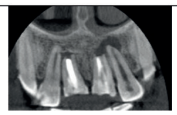
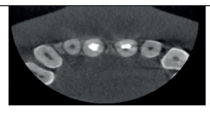
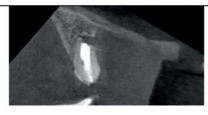
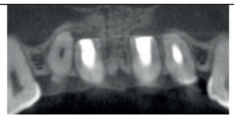
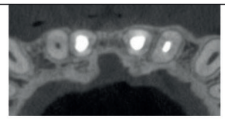
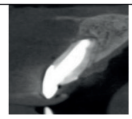
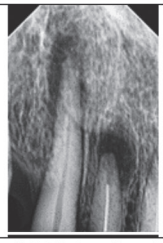
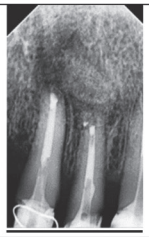
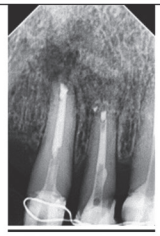
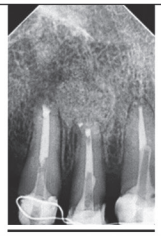
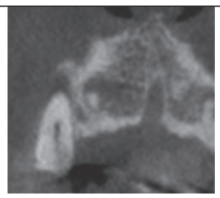
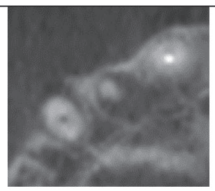
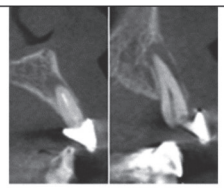
Pre-operative	After 1 month	After 3 months	After 6 months
			
• **Right central lesion:** 5.2mm • **Left central and lateral lesion:** 10.2 mm	• **Right central lesion:** 2.5 mm • **Left central and lateral lesion:** 6.87 mm	• **Right central lesion:** 1.2 mm • **Left central and lateral lesion:** 4.68 mm	• **Right central lesion:** almost zero • **Left central and lateral lesion:** 0.1 mm
CBCT			
Before			
Coronal	**Axial**	**Sagittal**	
			
After			
Coronal	**Axial**	**Sagital**	
			

Table (2) *Group B case radiographs*

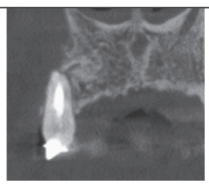
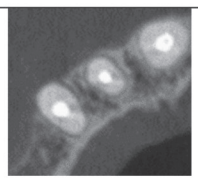
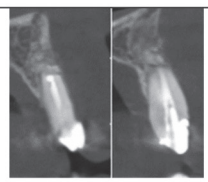
Pre-operative	After 1 month	After 3 months	After 6 months
			
Upper right lateral and canine lesion: 15.25mm	**Upper right lateral and canine:** 5.7mm area of low density bone	**Upper right lateral and canine:** 2.3mm area of low density bone	**Upper right lateral and canine:** no lesion incresed bone density

CBCT

Before

Coronal	Axial	Sagittal
		

After

Coronal	Axial	Sagital
		

Exploring numerical data for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed non-normal (non-parametric) distribution. Data were presented as mean, standard deviation (SD), median and range 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 when Kruskal-Wallis or Friedman's test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Lesions size (mm)

a. Comparison between the groups

Pre-operatively, after one, three as well as six months; no statistically significant difference between median lesions size in the two groups was found (*P*-value = 0.554, Effect size = 0.079), (*P*-value = 0.122, Effect size = 0.053), (*P*-value = 0.205, Effect size = 0.007) and (*P*-value = 0.096, Effect size = 0.073), respectively (Table 3).

Table (3) *Descriptive statistics and results of Kruskal-Wallis test for comparison between lesions size (mm) in the three groups*

Time	Hydroxyapatite		Nano-Hydroxyapatite		*P*-value	*Effect size (Eta Squared)*
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)		
Pre-operative	7.9 (2.8)	6.7 (5.2-12.5)	10.2 (4.8)	8.3 (5-18.7)	0.554	0.079
1 month	5.2 (1.9)	5.3 (2.3-7.5)	3.5 (3.7)	4.1 (0-10.3)	0.122	0.053
3 months	2.3 (2)	2.4 (0-5.2)	1.3 (1.9)	0 (0-5.6)	0.205	0.007
6 months	0.3 (0.6)	0 (0-1.8)	0.2 (0.5)	0 (0-1.5)	0.096	0.073

*: Significant at P ≤ 0.05

b. Changes within each group

In hydroxyapatite as well as Nano-Hydroxyapatite groups; there was a statistically significant change in lesions size by time (*P*-value <0.001, Effect size = 0.978) and (*P*-value <0.001, Effect size = 0.878), respectively. Pair-wise comparisons revealed that there was a statistically significant decrease in lesion size after one month, from one to three as well as three to six months (Table 4).

b. Percentage reduction in lesions size

Percentage reduction in lesions size was calculated as follows:

$$\frac{\text{Pre-operative size}}{\text{Pre-operative size} - \text{Post-operative size}} \times 100$$

After one month; there was a statistically significant difference between median percentage changes in lesions size in the two groups (*P*-value = 0.003, Effect size = 0.382). Pair-wise comparisons between groups revealed that there was nano-hydroxyapatite showed statistically significantly higher median percentage reduction in lesions size than hydroxyapatite group.

After three as well as six months; there was no statistically significant difference between median percentage decreases in lesion sizes in the two groups (*P*-value = 0.077, Effect size = 0.092) and (*P*-value = 0.096, Effect size = 0.073), respectively.

Table (4) *Descriptive statistics and results of Friedman's test for comparison between lesions size (mm) at different follow up times within each group*

Time	Hydroxyapatite		Nano-Hydroxyapatite	
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)
Pre-operative	7.9 (2.8) ^{A}	6.7 (5.2-12.5)	10.2 (4.8) ^{A}	8.3 (5-18.7)
1 month	5.2 (1.9) ^{B}	5.3 (2.3-7.5)	3.5 (3.7) ^{B}	4.1 (0-10.3)
3 months	2.3 (2) ^{C}	2.4 (0-5.2)	1.3 (1.9) ^{C}	0 (0-5.6)
6 months	0.3 (0.6) ^{D}	0 (0-1.8)	0.2 (0.5) ^{D}	0 (0-1.5)
P-value	<0.001*		<0.001*	
Effect size (w)	0.978		0.878	

*: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant change by time

Table 5 *Descriptive statistics and results of Kruskal-Wallis test for comparison between percentage reduction in lesions size (%) in the three groups*

Time	Hydroxyapatite		Nano-Hydroxyapatite		*P*-value	*Effect size (Eta Squared)*
	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)		
1 month	34 (14.6) [B]	35.1 (13.8-57.4)	73.7 (25.5) [A]	62.6 (44.9-100)	0.003*	0.382
3 months	73.8 (21)	69.5 (45.4-100)	90.6 (11.8)	100 (70.1-100)	0.077	0.092
6 months	96.2 (6.9)	100 (79-100)	98.6 (4.4)	100 (87-100)	0.096	0.073

*: Significant at $P \leq 0.05$, Different superscripts in the same row indicate statistically significant difference between groups

DISCUSSION

The optimal goal of periapical surgery is complete regeneration and reconstruction of periapical tissues, including complete repair of osseous defects. Inadequate bone healing is due to ingrowth of connective tissue into the bone space, preventing osteogenesis. To inhibit this soft-tissue ingrowth, bone grafts can be used to occupy the bony space in case of large bony defects[17]. Ideal wound healing would achieve maximum regeneration and minimal repair so that the biological function of the injured tissue would not be jeopardized.

Healing of the damaged tissue either by regeneration or by repair relies on the availability of cell types needed and the presence or absence of signals necessary to stimulate these cells. The tissue-engineering approach to bone regeneration combines three elements which include stem cells or progenitor cells, conductive scaffolds or extracellular matrix, and signaling molecules. Different bone grafts with different properties have been studied and tried in various cases[18].

In this study we compared the healing efficacy of bone when used HA and nano-HA as bone graft after removal of periapical lesion. HA is one of the most promising widely used alloplastic grafts in both the research and clinical fields. It has a similar composition and structure to natural bone mineral and is known to perform direct chemical bond to bone when implanted[19]. The properties of HA ceramics can be enhanced by modifying important parameters of powder precursors such as particle size, particle distribution, and agglomeration[20].

The smaller the particle size, the larger the surface area in volume. Because the cells are too big for the small pores, blood plasma containing all the important proteins is retained in the interstices. The surface of the pores and also of the nanopores is modified in such a way that it "hangs on" to the proteins. Properties of nano-bone graft materials include osteoinductive, fully synthetic, highly porous, nanostructured, absorbs natural proteins into the nanopores, should be degraded by osteoclasts and should have good processability[21-23].

Nano-hydroxyapatite (nHA) possesses unique properties related to its small size and large surface area[24]. Biomaterial researches have focused on the use of nanotechnology in order to enhance bioreactivity. Thus, bone grafts of synthetic nHA crystals are widely utilized in the repair of bony defects[25].

In hydroxyapatite as well as nano-hydroxyapatite groups; there was a statistically significant change in lesion size by time. Pair-wise comparisons revealed that there was a statistically significant decrease in lesions size after one month, from one to three as well as three to six months.

Favorable osteogenic capacity and enhancement of bone regeneration of nHA were explained as the nano-sized particles and their structural similarity to natural bone allow HA nanocrytals to bond firmly to bone. This stimulates the proliferation and metabolism of osteoblasts permitting better osseointegration and strong osteoconductive and osteoinductive ability[26]. It was suggested that when nHA starts to dissolve, it releases calcium ions in the surrounding environment which encourages cell proliferation and osteogenic differentiation. Researchers added that nHA can augment mineral deposition promoting rapid neo-osteogenesis[27]. It was speculated that if HA was placed underneath healthy periosteum and properly vascularized bone, it will release phosphate ions into the medium inducing bone healing[28]. Investigators proposed that nHA induces alveolar osteoblasts to secrete specific bone morphogenic proteins (BMPs) and other growth factors which stimulate and regulate bone regeneration process[28].

In the present study many other common factors in all groups aided in success of surgical treatment. Apical seal can be obtained by the use of root-end filling materials[29]. MTA as retrograde filling material is the most favorable over other materials. MTA in comparison to other root-end filling materials represent the highest healing rates (91.4%) [30]. MTA also represents less leakage than other root-end filling materials[31].

The crown-down technique was used for cleaning and shaping because it permits straight access to the apical region, eliminates coronal interferences, removes the bulk of tissue and microorganisms before apical shaping, allows deeper penetration of irrigants, and allows better control over working length[32].

NaOCl was used as an irrigant because of its broad-spectrum antimicrobial activity as well as its capacity to dissolve necrotic tissue remnants[33].

Using the double antibiotic paste as intra canal medication between visits has bactericidal and bacteriostatic effect providing a clean field for healing and regeneration. Local application of antibiotics has been investigated as intracanal medicaments[34]. The triple antibiotic paste is a combination of metronidazole, ciprofloxacin, and minocycline. It was efficient in reducing viable bacteria in regenerative protocols[35]. Both triple and double antibiotic pastes have antibacterial effect on human dentine so minocycline is not used to overcome discoloration problems[36].

CONCLUSION

It was concluded that nanohydroxyapatite produced faster bone regeneration in the first three months than hydroxyapatite. However, hydroxyapatite produced bone regeneration after six months.

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مقارنه تقييم الشفاء من العيوب العظمية بعد الاستئصال الجراحي للآفات المحيطية في وجود هيدروكسيباتيت ، نانو هيدروكسي أباتيت (دراسة اكلينيكيه)

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الملخص :

الهدف: الهدف من هذه الدراسة هو تقييم التئام العظام في الأسنان المعالجة اللبية بعد الاستئصال الجراحي للآفات المحيطية و إضافة هيدروكسي أباتيت ، نانوهيدروكسي أباتيت.

المواد والأساليب: أجريت هذة الدراسة على سته عشر مريض مصرى تم إختيارهم من قسم علاج الجزور بكلية طب الفم و الأسنان جامعة المنيا على أن يتم تقسيمهم إلى ثلاثة مجاميع متساوية كل مجموعه تحتوي علي ثمانيه مريض وفقا لنوع الماده المعالجه الي: مجموعه أ :مجموعه الهيدروكسي اباتيت و مجموعه ب: مجموعه النانو هيدروكسي اباتيت . تم عمل اعادة علاج جزور للحالات كتالي:

الجلسه الأولي: أزيلت الحشوة القديمة ثم تم الري بهيبوكلوريت الصوديوم. تم تجفيف جميع القنوات وتعبئتها بمعجون ثنائي المضادات الحيوية (ميترونيدازول وسيبروفلوكساسين). الجلسه الثانيه: تم سد القنوات باستخدام PRO TAPER GUTTA-PERCHA وسداد قناة الجذر متبوعًا بالتدخل الجراحي في نفس اليوم. تم إنشاء كشط ذروي جنبا إلى جنب مع استئصال القمة. تم تحضير تجاويف نهاية الجذر في جميع الأسنان. جروب (أ): تم ملء تجاويف نهاية الجذر بـ MTA متبوعًا بوضع هيدروكسيباتيت في الخلل حول الذروي. جروب (ب): تم ملء تجاويف نهاية الجذر بـ MTA متبوعًا بوضع النانو هيدروكسيباتيت في الخلل حول الذروي. في جميع المجموعات ، تم جدولة زيارات استدعاء المرضى في فترات زمنية تتراوح من شهر و ثلاثه و سته أشهر للتقييم السريري والإشعاعي.

النتائج: بعد شهر واحد؛ كان هناك فرق ذو دلالة إحصائية بين متوسط النسبة المئوية للتغيرات في حجم الآفات في المجموعتين. أظهر كلاهما انخفاضًا أعلى معنويًا في النسبة المئوية المتوسطة في حجم الآفات مقارنة بالمجموعة أ (مجموعة هيدروكسيباتيت). بعد ثلاثة إلى ستة أشهر ؛ لم يكن هناك فرق ذو دلالة إحصائية بين متوسط النسبة المئوية للنقصان في حجم الآفات في المجموعتين.

الخلاصة: تم استنتاج أن النانوهيدروكسيباتيت ينتج تجددًا أسرع للعظام في الأشهر الثلاثة الأولى من هيدروكسيباتيت. ومع ذلك، ينتج هيدروكسيباتيت تجديد العظام بعد ستة أشهر.

الكلمات المفتاحية: فجوات عظمية ، الاستئصال الجراحي, آفات المحيطية ، هيدروكسيباتيت ، نانو هيدروكسي أبتايت