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Research Article

RADIOGRAPHIC EVALUATION OF NANOCRYSTALIN HYDROXYAPATITE EFFECTIVENESS ON OSTEOGENESIS AFTER SURGICAL REMOVAL OF MANDIBULAR THIRD MOLAR

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ABSTRACT

Introduction: The aim of this study was to evaluate the effectiveness of nano-hydroxyapatite (nano-HA) in the healing process and restitution of extracted tooth sockets after surgical removal of mandibular third molars.
Materials and methods: In a double-blinded study, participants with bilateral impacted mandibular third molars went through surgical extractions, with one socket receiving nano-HA and the other acting as a control. The surgeon and the participant were both unaware of the study or control side. In order to produce nano-HA, aqueous suspension H_3PO_4 (0.6m) was gradually added to hydroxide calcium aqueous solution. Data was analyzed with t-test and the significance level was set at a p-value less than 0.05, while parallel periapical radiographs were used to observe the changes.
Results: Thirty participants (mean age 27 yr) underwent surgical removal of the mandibular third molars. There was no radiolucency line in any of the cases among experimental group. The mean absorption rate was 1.2722mm in the experimental group and 2.1056mm in control group. The data is significant, yet the decreasing height in the case towards control side was lower, although it's not notable.
Discussion: Based on the results of the present study, nano-HA may act as a safe alternative for autogenous bone graft.

INTRODUCTION

Restorating and/or reconstructing osseous defects have been a challenge in dental procedures. Among various methods of grafting, transplanting the graft from another part of the recipient's own body or known as autogenous bone graft, has been proved to be the best.[1-3] However, there are several serious limitations when it comes to practical usage of this method; providing a sufficient quantity of autogenous bone is a time-consuming process that increases the operative time, blood loss, cost and patient chronic pain considerably. In addition, the process needs second incision and is usually very difficult and sometimes impossible.[1- 9] Also transplanting the graft from a donor of the same species, known as allograft, there is a possibility of disease transmission and variable antigenicity.[10,11] In order to eliminate the mentioned obstacles, the use of alternative graft materials is increasingly becoming of interest.

The ideal graft material would be osteoinductive, osteoconductive, osteogenic, biocompatible, mechanically stable, disease free, relatively inexpensive, quite easy to apply and noncarcinogenic or toxicant.[12,13]

Hydroxyapatite has shown promising results in recent studies, which is the mineral form of calcium apatite that is naturally found in bones, teeth, and coral reefs. Due to the high similarity of HA to mineral portion of the bone, several studies have been conducted to investigate if this substance can fulfill the mentioned criteria. The chemical formula of HA is $Ca_5 (PO_4)_3 (OH)$ also written as $Ca_{10} (PO_4)_6 (OH)_2$ to specify the existence of two molecules in each single cell. As a biomaterial substance, HA has various specific properties and the most

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important is its high biocompatibility. HA is naturally bioactive, which means it can potentially tie with the living tissues without creating a collagen layer and it is assumed that it has the potential to make direct chemical bonds with hard tissues. This mineral substance is also biodegradable and within a certain period of time, during the implantation process, it could be replaced with regenerated bone.[14,15]

As a limitation, it can be noted that HA has low biodegradation properties at micron-sizes. This adverse property reveals itself in orthopedic surgery regarding bone implant and improvement, which is due to the low range of bone reconstruction.[16] Although titanium dental implants that are coated with micron-sizes of HA can eventually unify with the bone, yet the mentioned disadvantage might decrease the rate of success.

Biodegradation property of HA, however, could be the result of grains decreasing to nano-sizes. Bone integration can be achieved three or four times faster at nano-sizes in comparison to micron-sizes. Nano-HA has structural similarities with biological apatite of hard body tissues. Its pore structure and biochemical properties are similar to human cancellous bone. Moreover, its osteoconductivity and osteoinductivity characteristics can direct and stimulate the bone formation. Due to the distinctive features of nano-crystalline HA, this substance can be used in the construction of various items such as nano-composites, bone grafts, and orthopedic implant pieces.[17,18] Considering the similar nature, structure, and dimension of biological HA to mineral portion of a bone, it can contribute to the reduction of healing time and the increase of treatment reliability.

Permanent teeth extraction, especially on third molars, might lead to several complications such as infection and dry socket. Several methods have been introduced to prevent the mentioned problems, especially when the defect is large, the procedure is traumatic and there is going to be an implant or prosthesis placement, thus the use of a graft material might be beneficial. The present study was conducted to assess the healing and reconstructing effect of nano-HA application as a graft material on third molar sockets.

MATERIALS AND METHODS

The present study was conducted at Oral and Maxillofacial Department, Mashhad University of Medical Sciences, Mashhad, Iran. The study protocol was approved by the ethical board of Mashhad University of Medical Sciences and Written informed and detailed consent was obtained from all the patients.

Study Design

This research was a split mouth, double-blind clinical trial.

Production Method of HA

In order to prepare the hydroxyapatite, aqueous suspension H3PO4 (0.6m) is gradually added to hydroxide calcium aqueous solution in stoichiometric proportion of calcium to phosphor. After powder synthesis was performed in 42°C, it was mixed in a certain condition and for adequate period of time (Fig 1).

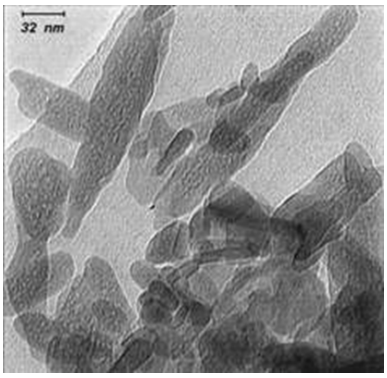

Fig1 TEM image of nano FHA

By adding NaOH, PH solution reached approximately to 10. It became dried after remaining at 80° C for 24 hours. High power grinder was used for grinding the produced powder.

Study Sample

The study was carried out on patients in need of surgical extraction of bilateral impacted third molars with similar erupted state and comparable condition, within the period of October 2011 to October 2012. A total of 30 patients aged between 20 to 30 year (mean age 27 y) both male and female, with no systemic complication were selected. The exclusion criteria included any history of alveolar osteitis (AO), pericoronitis around mandibular wisdom tooth or any lesion on panoramic radiograph, lactating or pregnant mothers, oral contraceptive users, smokers, recreational drug users and those who had any complications during the process of extraction.

Surgical Procedures

All extractions were performed by the same surgeon in one sitting according to the standard extraction procedure.

Surgical Protocol

Removal of the impacted teeth were performed under local anesthesia by using 0.2% lidocaine and Epinephrine [1:100000]. After sulcular incision was made from the midbuccal surface of second molar to the distal surface of the third molar, it was extended to the anterior surface of ramus at a 45 angle. To prevent further periodontal complications, gingival wedge was omitted from the distal surface of second molar. Ostectomy was carried out by using a round bur (with abundant irrigation and sterile normal saline) following elevation of muccoperiosteal flap and exposure of the underlying bone. The extraction was performed by cutting the tooth into pieces for minimizing the trauma. Then, the residual dental follicle was also excised and the cavity was meticulously rinsed with sterile normal saline again (Fig 2).

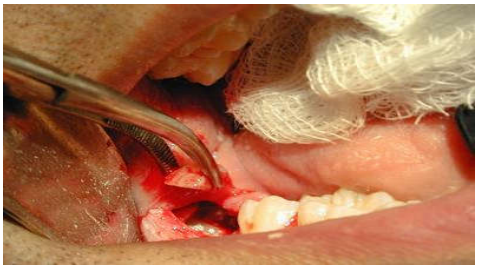

Fig2 Insertion of nanoHA into the socket

Tooth sockets of right or left sides of mandible were randomly selected as case or control groups. Subsequently, in the case group (n=30), sockets were filled with nano-HA granules and covered by a collagen membrane (Duo-Teck, Italy), followed by flap repositioning and wound closure, using 3-0 silk suture. On the other hand, in the control group (n=30), sockets were just covered with the membrane and were sutured with 3-0 silk, similar to the case sockets.

All patients were prescribed amoxicillin (500 mg, Tid, n=20) and acetaminophen (325mg, Tid, for 3 days).

Fig3 Radiography Prior To Extraction

Written postoperative instructions were given to the patients while they were asked to return postoperatively after a week for stitch removal and after 90 days for doing a radiographic assessment.

Radiographic evaluation

Prior to the surgery, patients were guided to obtain periapical or panoramic images. Furthermore, the images were taken just after the surgery and at the end of third month from both control and experimental sides.

Postoperative examinations

Clinical outcome including pain, infection and inflammation were examined while stitch removal, one week after the surgery.

Statistical analysis

T-test was used for statistical evaluation. The level of significance was set at a p-value less than 0.05 while parallel periapical radiographs were used to observe the changes.

RESULTS

The study sample consisted of 30 patients, 12 males and 18 females, with mean age of 27 years. For follow up sessions, after 90 days, 3 patients did not double back.

Clinical Results

At the stitch removal session, after a week, 3 membrane exposures were observed (2 in experimental and 1 in controlled group). There were no local inflammation nor infection in the cases. We observed that the severity of pain in both test and control group was equal.

Radiographic Results

Three factors were evaluated for each patient

1) Evaluation of the presence or absence of the radiolucent lines
2) Assessing the bone height changes
3) Comparison of the opacity grade between the pre-operation radiographs and postop third month radiographs in the case and control groups

By comparing all the images, it showed that radiopacity has been formed and there was no radiolucent line between nano-HA and the tooth socket and its walls in any cases. All the radiographies were prepared by the parallel technique. First, an imagery line that goes across the end of distal and mesial root of 7th tooth was considered as a fixed line (with no alteration), then the bone crest of extracted third molar was measured as a line, in which the modification has been occurred.

The measurements were calculated in two stages regarding each patient (in both experimental and control sides). The first calculation was carried out in post-surgery radiography, and the second was done 90 days after the operation; then the results were compared. (Measurements were calculated to tenth of a millimeter, using a caliper and a negatoscope). Results are presented in the table 1 and table 2. The tables show significant height changes in both sides. Although resorption in case towards the control side is lower, yet it's not notable.

Table 1 The mean rate of resorption in case group

	N	Mean	Std. Deviation	Std. Error Mean
Height 1	30	1.2722	0.14874	0 .03506

Table 2 The mean rate of resorption in control group

	N	Mean	Std. Deviation	Std. Error Mean
Height 2	30	2.1056	0.28589	0.06738

DISCUSSION

The aim of the present study was to investigate the effects of nano-hydroxyapatite application on the healing process and restitution of extracted dental sockets after surgical removal of mandibular third molars. The null hypnosis was that the reconstruction of the socket walls, with and without applying nano-HA, would be equal. However, the results were in contrast with the hypnosis; there was no sign of radiolucency line in post-operative radiographs among the experimental group. Radiopacity has been formed and no local inflammation nor infection was observed. The comparison of pre and post-operative radiographs, observed by a professional radiologist, demonstrated a statistically significant difference in the amount of radiopacity between case and control group. Furthermore, post-operative radiographs revealed satisfying restitution of crest height in both groups, although the comparison between them was not statistically significant.

Autograft is considered as the gold standard when it comes to graft materials due to its osteogenicity, osteoconductivity and/or steoinductivity. Various graft materials have been introduced in order to omit the disadvantages of bone grafts such as morbidity, blood loss, operation time, cost and second incision in autogenous bone graft harvest and donor-site complications, disease transmission and variable antigenicity in allografts.[17] Recent papers have reported clinical success in using HA as a graft material. Bucholz *et al.* has reported that coralline hydroxyapatite has equal efficacy to autogenous cancellous bone when used for subchondral support in internal fixation of tibial plateau fractures.[15]

Agrillo *et al.*[16] first investigated the osseous efficacy of a composite implant that was coated with coralline HA in 2002. HA is osteoconductive, biocompatible, bioactive, biodegrade able, and has similar biochemical properties on human cancellous bone. Holmes *et al.*[19] biomechanically compered HA to autogenous iliac crest cancellous bone in a canine model for six months and reported that HA has increased the strength and decreased stiffness over size-matched cancellous. Similar to cancellous bone, CHA is isotropic, which results in greater mechanical strength. However, osteoinductivity can only be achieved in nano-sizes of HA.[17] Other forms of this mineral are not suitable for implant or prosthesis placement since they are unstable and cannot be resorbed; yet animal and histological studies have proved that biodegradation property could be achieved by decreasing the grains to nano-sizes.[14,17]

The present study is the first to address the efficacy of nano-HA in reconstructing the socket walls and preventing the development of bone defects in extracted dental sockets. Although our main purpose was to investigate the efficacy of nano-HA material in any bone defects, yet impacted mandibular third molar surgery was chosen due to its prevalence and also the importance of preserving the socket for future plans regarding implant or prosthesis placement.

Various rates of bone resorption after extraction, has been reported in literature. The rate of resorption in the present study was assessed with parallel intraoral radiograph and the results were 1.27mm in the case group and 2.10mm in the control group. This higher rate of bone resorption could be due to the differences in study designs. For instance, several methods such as CT scan, intraoral radiography and dental impressions have already been used as a post-operative modality to determine radiopacity and the rate of bone resorption. It is impractical to advise a CT scan due to the risk of radiation as well as the excessive costs[20] and extra-oral impressions are not accurate in the reproduction of detail. Furthermore, previous studies have tried to extract the teeth with minimum pressure while removing the impacted teeth are much more traumatic. In addition, the thickness of cortical and cancellous bone is different in various regions of alveolar bone.

Garcia *et al.*[21] investigated the efficacy of HA granules APAFILL-G as a filler to prevent the resorption of alveolar bone after tooth extraction in 33 participants, which were having implant surgery. Radiographs that were taken a year after the operations revealed a continuous radiopacity between the bone and the implant, while there was no evidence of resorption in any of the cases.

CONCLUSION

Our data support the results of other investigators, who have demonstrated that supplemental metaphyseal grafting can effectively decrease the duration of healing process and prevent bone defects. HA graft has also been reported to be successful in the reconstruction of metaphyseal defects in long bone fractures.[22] It has been used in cages, impacted into the hollow center[15] and in cervical spine fusion.

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