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Original Research

Evaluation of the Effects of Nano-honey Scaffold on Reconstruction of Calvarial Defects in Rat

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Abstract

Bone defects have caused many problems. Restoration of bone defects with great gaps, has been a huge problem for many patients. Nowadays, different ways are used for bone restorations that cause difficulties; so there is tendency to use organic drugs such as propolis. The aim of this study was investigating the effects of nono-honey on calvarial defect. In this research 20 Wistar male rats were divided into two control and nano-honey groups. After general anesthesia, to access the calvaria, the midline was cut. We turned the skin and periosteum over and dig a fossa in parietal bone with electronic handpiece with 1000 round per minute (depth 5 mm and length 6 mm). After 8 weeks, histopathologic samples were provided and the new vessels, neutrophils, lymphocytes, macrophage, fibroblast, granulation tissue and formation of fresh bone trabeculae were measured. We observed that average number of neutrophils in nano-honey group was lower than control group, expressively ($p = 0.030$). The average of bone trabuculation in nano-honey group was more than other group ($p < 0.001$). Other histological factors was not significantly different ($p > 0.05$). In this study, it was shown nano-honey has positive effects on restoration of hard and soft tissues. Since this research was carried out on rats more study and examinations on human beings are needed. Furthermore, adding osteoconductive and stimulant of regenerating bones in order to evaluate the effects on restoration is recommended.

Keywords: Nano-honey, Reconstruction, Calvarial defect, Rat

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Introduction

Today bone defects have caused many clinical, social and economical problems for different reasons such as secondary physiological loss after missing tooth, trauma, bone pathologies and infection [1]. Bone restoration includes a series of regular biological inducing and directing bone growth with different types of cells and intra or extracellular molecular signals in a schedule which maximize bone regeneration and its function [2]. Restoration of bone defects with great gaps, has been a huge problem for many patients. In tissue engineering the ideal regeneration includes anatomical and missed tissue restoration [3]. That, in bone problems, means regenerate bone with osteoblasts and proper biological connection with bone tissues around lesions. Despite of many studies, there has not been a huge development in treating this lesions [4].

Nowadays different ways are used for bone restorations. Autogenous bone, which is the gold standard and a measure of the comparison and success of new methods is used but according to limitation of this method, other methods have been more common [5].

Scaffolds, growth factors and osteoblasts are sides of tissue engineering and used in regenerating missing bones [6]. Allograft, zenograft and synthetic materials of hydroxyapatite are the most common materials as scaffolds in regenerating bones [7]. Mineral structures and similarity to bones are two factors that recreate the guiding point for bone regenerating of this materials but in critical defect size, they are not enough to regenerate [8].

Drugs have harmful side effects for people, so there is attraction to organic drugs such as propolis [9]. Honey provides resistance to oral pathogens and protects moisture lesions with antibacterial effects [10]. Honey provides resistance to 60 species of bacteria like aerobic and non-aerobic [11], gram positive and gram negative [12,13]. Also, it is a good barrier to prevent infection with high viscosity [14,15]. Its immune modulatory effect improves the restoration procedure [16].

There have been few studies about the effect of nono-honey to improve extent of fresh bone trabeculae and accelerating bone tissue repair. Thus, this article investigates the effects of nono-honey on calvarial defect.

Methods

Surgeries and Treatment Group

In this research 20 Wistar male rats (weigh between 200 to 250g) were divided into two control and nano-honey groups. Before starting the procedure rats were examined by a veterinarian for public health and eligible status for the study. Then, with intraperitoneal injection of ketamine 100 mg/ml and xylasein100 mg/ml were placed under general anesthesia. The rats' hair was shaved and they became ready for surgery. To access the calvaria, the midline was cut. We turned the skin and periosteum over and dig a fossa in parietal bone with electronic handpiece with 1000 round per minute (depth 5 mm and length 6 mm). In control group there was no material and in other group they placed nano-honey scaffold. Then, we returned the per-

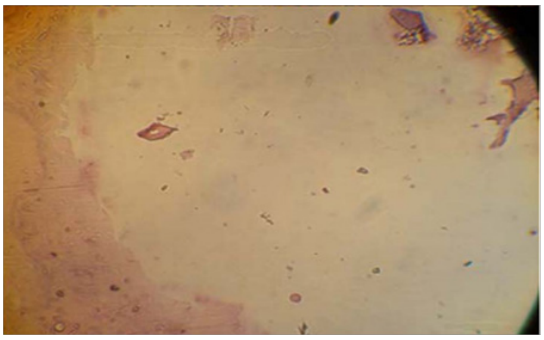
iosteum and suture the skin.

Scaffold Preparation

Nano-honey was produced as follows: to construct the scaffolds, honey was produced by the col-gel method as a porous polymer nano-scale biocomposite. The col-gel method is a chemical approach used to synthesis various nano-structures. In this method, the molecular precursor is dissolved in water or alcohol using heat and stirring. It is then converted to gel by hydrolysis/alkylisation. The gel is then dried, which can be done by burning ethanol for alcoholic beverage. The dried gel is converted into the powder from which is then heated to obtain calcite. The col-gel method is inexpensive and the chemical composition of the products can be properly controlled due to the low reaction temperature. Popularity and industrial application of the col-gel method are than other available methods. Large volumes of high-quality nano-particles (producing particles of the same size) can currently be produced using the col-gel method. The cytotoxicity of the scaffold was investigated by Multi Interface Test Tool (MITT).

Data collection and Statistical Analysis

After 8 weeks [17] rats died because of phenobarbital injection and calvaria is removed for histopathological examination. Examples are fixed in 10% formalin. Then demineralized in EDTA, dehydrated by alcoholic suspensions and they placed in paraffin. They cut out coronal plate 5-7 mm and colored by H&E for investigation by optical microscope. We analyzed the results with SPSS v. 23. Data were analyzed using one way ANOVA. We measured the new vessles, neutrophils, lymphocytes, macrophage, fibroblast, granulation tissue and formation of fresh bone trabeculae (Figure 1).



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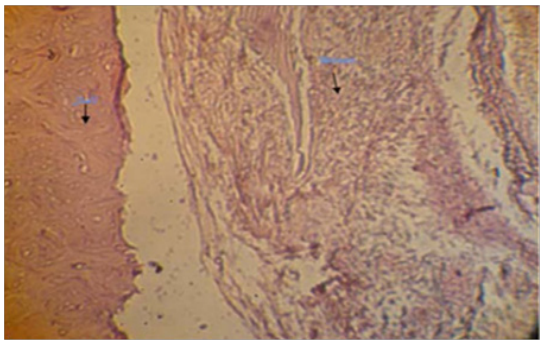

(b

Figure 1. a) Light microscopy image show the amount of bone formation in the calvarial defect. Control group. b) Light microscopy image show the amount of bone formation in the calvarial defect. Nano-honey scaffold group.

Results

We observed that average number of neutrophils in nano-honey group was lower than control group, expressively ($p = 0.030$). The average of bone trabuculation in nano-honey group was more than other group ($p < 0.001$). Other histological factors were not much different ($p > 0.05$) (Table 1).

Table 1. The results of measuring of histopathological variables.

Histopathological variables	Nano-honey group (n = 10) Standard deviation ± Average	Control group (n = 10) Standard deviation ± Average	P-value
Number of newly formed vessels	0.76±2.71	0.98±3.57	0.091
Neutrophil	1.11±3.71	0.82±5.00	0.030
Lymphocyte	1.53±5.00	1.57±5.14	0.866
Macrophage	4.39±27.29	5.40±31.14	0.168
Fibroblasts	5.57±64.00	5.62±58.71	0.102
Extent of granulation tissue	0.07±0.32	0.06±0.40	0.062
Formation of bone trabecula	0.10±2.89	0.21±2.42	<0.001

Discussion

In this article the influence of nano-honey scaffold was studied. Better therapeutic effect of nano-honey scaffold on bone healing was seen. Also, lower inflammation was indicated in nano-honey group. There are many articles about other scaffolds on restoration of bone lesions but none of them studied nano-honey scaffold. This study is important because maintaining bone defects, alveolar bones and jaws on restorations for prosthetics is important.

Honey is one of the materials which is investigated because of its multiple healing properties. Animal and in vitro studies express many healing properties of propolis [18]. In addition of antibacterial effects, honey stimulates the granulation of tissues, epithelium, angiogenesis and moisturizing to prevents dryness [19]. It has antioxidant effect by having phenolic materials. This prevents oxygen reactive species that neutrophils and macrophage produce that destruct the tissues [20].

In different surgery methods screws, metal sutures, and metal plates are needed for regenerating broken bones and torn ligaments.Sometimes long term effects of this method are harmful and second surgery is needed for removing the particle [21]. There were side effects like pain [22], concentration of textured metals [23,24], rubbing [25], hypersensitivity to titanium [26,27], interference with radiotherapy and photography [23,28,29], growth constraints in children [30,31], infection and sudden pressure in the area when removing the particle [32] (in order to reduce the harmful effects).

Today, tissue engineering tries to reduce the pain with replacement materials to restore the tissue [33]. In order in this study, nono-honey has positive effects on restoration of hard and soft tissue. Studies have shown the effects of propolis on restoration of surgery and hard tissue lesions. Margo and Cravalho [16] washed the patients' mouth with propolis alcoholic extract to investigate the restoration of alveolar lesions after extraction. After 7, 14, 30 and 45 days patients returned for histopathologic evaluations. The mouthwash minimized the time of restoration with anti-inflammation and anti-pain effects. Also, it stimulates the volume of restoration of lesions after extraction of tooth.

Investigation of exfoliative cytology suggests the lesions epithelized and had extension in bone trabucles.

In Hemalatha et al [34] study the positive effect of honey in restoration of hard and soft tissue in dental socket is reported according to the Turnbull, Landry and Howley index. Furthermore, Okeiny et al [35] showed that honey decreases the time of the bone restoration.

Because this research has been conducted on rats, more studies and examinations on human beings are needed. Furthermore, adding osteoconductive and stimulators of regenerating bones in order to evaluate the effects on restoration is recommended.

Conflict of Interest

None.

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