The effect of adding hyaluronic acid to calcium phosphate on periapical tissue healing following periradicular surgery in dogs

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Abstract

Objective: This study aimed to evaluate the effect of adding exogenous hyaluronic acid (HA) to Beta-tricalcium phosphate (CP) on osseous tissue healing of induced apical lesions following periradicular surgery in a dog model.

Methods: In the first and second sessions, periapical lesions were induced by exposing pulp cavities of selected teeth for 7 days then sealing them with glass ionomer for 60 days. Root canals were then cleaned, shaped and obturated. Surgical treatment included buccal osteotomy to expose the root apex and root-end resection and filling. Osteotomy cavities were randomly allocated to two study groups 12 samples each according to the graft materials; CP or combination of HA and CP. The graft materials were prepared and applied in each animal in alternate quadrant in a randomized manner. Animals were sacrificed after 60 days and bone treated cavities were prepared for histological study and histomorphometric analysis for the area percentage of new bone tissue and trabecular bone thickness.

Results: All samples displayed signs of regeneration as newly formed bone tissue and fibrovascular connective tissue within the treated cavity sites with complete resorption of the implemented materials. The newly formed bone consisted mainly of osteoid bone trabeculae with some more mature dense bone present at the periphery of cavity site. There was no significant difference in the percentage of newly formed bone tissue (P > 0.05) and bone trabecula thickness (P > 0.05) between the two study groups.

Conclusions: Addition of exogenous HA to CP after periradicular surgery did not improve the histological outcome of osseous tissue healing in a dog model.

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Keywords: Hyaluronic acid; Hyaluronan; Calcium phosphate; Bone grafts; Alloplasts; Periapical lesion induction

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1. Introduction

Periapical surgery with root-end resection is indicated for endodontically treated teeth with periapical pathology where an orthograde revision cannot be done or not able to resolve the periapical disease [1]. In such conditions, surgical approach provides better access to clean the root surface and apical lesion, to eliminate the source of inflammation and reactive periradicular tissue and to prepare and seal the apical portion of the root canal system [2]. This will allow the regeneration of hard and soft tissues, including the formation of a new attachment apparatus [3]. To promote bone regeneration following periapical surgery, bone graft materials are used especially when the surgical site is compromised [4].

Bone grafts fall into four general categories: autografts, allografts, xenografts, and alloplasts [2,5]. The role of these materials in regenerative procedures is based on that; they possess the osteogenic potential (contain bone-forming cells), they are osteoinductive (contain bone inducing substances), or they are osteoconductive (serve as a scaffold for bone formation) [6]. Autograft and allograft remain the most effective grafting materials because they have more potentials for bone regeneration [2,4]. However, alternatives such as alloplast have been used because of donor site morbidity, limited supply with increased cost of autographs and risk of disease transmission and response rejection by allografts [2,4,7]. An alloplast is a synthetic or inert foreign body material that has osteoconductive capacity [2]. Its distinct advantage over autograft and allograft is that it can be produced in unlimited quantities without risk of disease transmission [7,8]. Currently used alloplasts include; coralline hydroxyapatite, collagen-based matrices, calcium sulfate, and tri-calcium phosphate [2,7].

Hyaluronic acid (HA) is also known as hyaluronan or hyaluronate is a high molecular weight polysaccharide (glycosaminoglycan) and a major component of extracellular matrix almost in all living tissues [9,10]. It plays a critical part in the function of extracellular matrix and tissue matrix, including tissue hydrodynamics and cell migration, proliferation and differentiation [9,11]. HA has an important anti-inflammatory role through inhibition of tissue destruction and facilitation tissue healing [9,12]. Consequently, application of exogenous HA in the treatment of inflammatory processes is shown in different medical fields such as orthopedics, dermatology and ophthalmology [9,10].

In the dental field, HA demonstrated beneficial effects in the treatment of gingivitis and periodontitis as well as periodontal surgery [13–15]. It demonstrated bacteriostatic effect on bacterial strains commonly found in gingival lesion and periodontal wound [16]. In addition, it has been used as a carrier for demineralized bone allograft without reducing its effectiveness for sinus lift augmentation [17]. Furthermore, it has been proposed as a scaffold for regeneration therapy of dental pulp because of its appropriate structure, biocompatibility and biodegradation [18].

Previous studies demonstrated the ability of exogenous hyaluronic acid in enhancing bone healing both experimentally [11,19–22] and clinically [14,15,23]. In animal studies, HA recorded significant bone mineralization acceleration compared to untreated bone cavities (11). The adjunctive use of HA to grafting process when combined with autografts and allografts recorded favorable results. Previous studies recorded the capacity of HA in supporting the significant bone formation when combined with bone marrow stromal cell and basic fibroblast growth factors [21], recombinant human bone morphogenic protein –2 [20] and spongiosal bone graft [22]. Clinically, application of HA in combination with autologous bone demonstrated good capabilities in accelerating bone formation when used in extractive socket and periodontal bony defect [14,15,23].

Experimental evaluation of the efficiency of different materials and techniques requires conditions similar to clinical environments for its relevance interpretation. Therefore, inducing one of the situations requiring the use of grafting materials when tested in the animal model such as periapical lesion is required. Periapical lesion induction was done previously to evaluate periradicular tissue response to bone grafts in apical surgery of dogs’ teeth [24].

Based on these data, and considering that, no previous study exists on the use of HA combined with alloplast as adjunctive to grafting process, the purpose of this study was to histologically examine the hypothesis that adding exogenous hyaluronic acid (HA) to Beta-tricalcium phosphate (CP) could enhance osseous tissue healing of induced apical lesions following peria-radicular surgery in a dog model.

2. Materials and methods

2.1. Experimental animal and materials

Animal right commity of Faculty of Veterinary Medicine (Cairo University) approved the
experimental protocol of this study, which is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experimentation. The study was conducted on four dogs of one year old including the following teeth; second, third and fourth mandibular premolars and second, third maxillary premolars.

The materials used in the present study were an ester of hyaluronic acid (HA) with benzyl alcohol with a concentration 20 mg/2 ml in a gel form and pure-phase beta-tricalcium phosphate (CP). CP should be mixed with fresh blood from the defect or with venous blood at about a 1/3 blood to 2/3 CP ratio [25]. In this study, HA was mixed with CP in the same ratio instead of blood.

2.2. Operative procedures

All experimental procedures were performed under general anesthesia that was achieved by intramuscular injection of 0.5 ml (20 mg) 2% Xylazine base and intravenous injection of ketamine at 15 mg/kg. At surgical time, Local infiltration of 2% lidocain with nor-epinephrine (1:100,000) was administered for hemostasis and reduction of operative pain. Periradicular lesions were induced by opening the pulp chambers of experimental teeth, removing pulp tissue and leaving the access cavities exposed to oral flora for 7 days. Access cavities were then closed with cotton and filled with glass ionomer for 60 days. Following the induction procedure, all infected root canals were cleaned and shaped in a crown down approach using Gates Glidden drills and K files in conjunction with frequent irrigation with 5.25% sodium hypochlorite. Root canals were dried and sealed with calcium hydroxide paste. After 7 days, calcium hydroxide was removed and root canal filling with gutta percha and sealer was performed using cold lateral technique. Upon completion of the root canal treatment, a full thickness mucoperiosteal flap was reflected exposing the buccal cortical bone covering the root surfaces. A # 2 rounded low speed bur with copious saline irrigation was used to create an osteotomy cavity (approximately 15 × 15 mm) and exposing the apical third of the root apex with total 24 osteotomy cavities. Fig. 1 showed steps of the surgical procedures. The most apical 2 mm of each root was resected with minimal bevel, periradicular area was thoroughly curetted, and root-end cavity was prepared and retrofilled with MTA Angelus. Osteotomy cavities were randomly allocated to two study groups 12 samples each according to the graft materials; CP or combination of HA and CP. The graft materials were applied in each animal in alternate quadrant in a randomized manner. The osteotomy cavity was then covered with a piece of a resorbable collagen membrane that was cut to overlap the osseous margin by 3 mm. The flap was then coronally repositioned, sutured and compressed with the moist gauze. Ibuprofen was given twice daily for 2 days. Animals were placed on a soft diet for two days post surgically followed by a regular diet for 60 days.

2.3. Specimens preparation for histological study and histomorphometric analysis

Sixty days post-surgically, animals were sacrificed by administrating an overdose of sodium pentobarbital. Immediately after sacrifice, animals' jaws were carefully dissected, and the experimental teeth with their surrounding bone tissue were block-sectioned using electric surgical saw. Blocks were immediately fixed in 10% formalin, then decalcified in ethylene dioxide tetra acidic acid (EDTA) for 12 weeks. After complete decalcification, blocks were routinely processed and embedded in paraffin. Six μm sections were cut in a buccolingual direction, mounted on glass slides, deparaffinized, hydrated and stained. Hematoxylin and eosin stain (HE) was used for histological evaluation. Histomorphometric analysis was carried out on the healing sites. In each HE stained slide, three digital images were captured at (×100) magnification. The area percentage of newly formed bone tissue within the osteotomy cavity (calculated as percentage of newly formed bon/surface area of the cavity) and trabecular bone thickness in the healing sites were recorded and expressed as mean and standard deviation (SD). Student t-test was used for comparing quantitative variables between the two study groups at \( P \leq 0.05 \).

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1 Curavisc, Germany.
2 Curasan, Inc, U.S.
4 Rompun, Bayer, Wuppertal, Germany.
5 Vitremer; 3M/EP AG.
6 Brasseler, Savannah, GA, USA.
7 Mani, Japan.
8 Clorox, Alexandria detergent of chemicals company, Alexandria, Egypt.
9 Pulpdent, Pulpdent Corp., Watertown, MA.
10 Endo Fill, Dentsply dental products.
11 Londarin, PR, Brazil.
12 Zimmer Dental, Freiburg, Germany.
3. Results

Histological examination of all treated cavities sites demonstrated newly formed bone tissue with complete resorption of implemented materials (Figs. 2 and 3). The newly formed bone consisted mainly of osteoid bone trabeculae with some more mature lamellar bone present at the periphery of cavity (Figs. 2 and 3).

Cavities treated with CP showed more regularly arranged osteoid bone trabeculae separated by marrow spaces (Fig. 2). Bone trabeculae with embedded osteocyt cells and lined with osteoblast cells were seen (Fig. 2A and B). Dens bone formation with the appearance of cortical bone was observed at the periphery of cavity site (Fig. 2C). Bone trabeculae were seen coalescing together in certain areas and separated by marrow spaces in other sites (Fig. 2C).

HA and CP treated cavities revealed nearly similar histological picture to CP treated cavity; osteoid bone trabeculae dispersed within the bony defect and separated by marrow spaces (Fig. 3). However, marrow spaces separating the bone trabeculae were wider than CP treated group and with more vascular fibrous connective tissue (Fig. 3A and B). The newly formed bone trabeculae contain osteocytes and lined with cementoblast cells. Coalescence of thick bone trabeculae containing variable size marrow spaces was recorded.
At the defect periphery, dense bone with embedded osteocytes was seen (Fig. 3C).

The Mean and SD of the area percentage for new bone tissue and trabecular bone thickness were recorded in Table 1. HA and CP treated cavities demonstrated lower percentage of new bone tissue and lower mean of bone trabeculae thickness within the formed tissue components compared to CP treated cavities. However, Student t-test didn’t show any significance difference between the two study groups (P > 0.05).

4. Discussion

The mechanisms by which HA promote bone formation were attributed to some actions. HA accelerated bone regeneration by means of chemotaxis, proliferation and successive differentiation of mesenchymal cells [11]. It significantly increased alkaline phosphatase and hence stimulate cell mineralization [20]. HA allowed the early deposition of osteoid tissue by providing a scaffold on which osteoprogenitor cell attached and so stimulated osteoblastic differentiation [21]. This action is supported by Aslan et al. [22] who have noted that HA stimulated bone healing through accelerating the three phases of healing; inflammation, proliferation and migration of mesenchymal cells with the production of bone matrix. Additionally, hyaluronic acid holds the advantage of complete degradation that allows its complete replacement with newly formed bone tissue [19]. Furthermore, the anti-inflammatory and antibacterial ability of exogenous HA may reduce the postsurgical inflammation and bacterial contamination when added to the surgical site and finally improve the surgical outcomes [12–14].

Calcium phosphate has been selected for this study as a one of the most commonly used alloplasts and because of its biocompatibility, handling characteristics, porosity, chemical and physical resemblance to bone minerals and potentially unlimited supply at a low price [26,27]. Additionally, previous investigation found that, addition different concentrations of HA solution to CP had a little effect on its porous structure and enhanced its bioactivity demonstrated by the apatite crystal formation on its surface [28].

The dog model has been used previously to evaluate the efficiency of bone graft materials [20,29]. Yet, variations in root canal morphology, bone anatomy and healing process could present difficulties that may bear upon the final results if not properly explored prior to any experiment [29,30]. Other difficulties that encountered during this study and recorded previously were shallow mucobuccal fold, extensive muscle attachment and thick cortical bone of posterior area [29]. These difficulties represented obstacles to the mucoperiosteal flap reflection process and accessibility to root apex as well as root end preparation. Furthermore, excessive bleeding during surgical procedures affected root end visibility that added another difficulty during per-radicular surgery.

Several animal studies have investigated the capacity of HA to augment bone healing based on creating a small bony defect in sterile environments.
In different animal models through creating a small bony cavity and in conjunction with different bone substitutes. The capacity of bone healing and rat of remodeling among different experimental animal species are variable [38]. Rodents animals used in previous investigations such as rat [11,21] and rabbit [22] have faster bone healing capacity [39] that may cause more rapid bone growth and remodeling. Furthermore, different criteria for histological evaluations of bone repair have been utilized. In the present study, the percentage of new bone formation [21] and trabeculare bone thickness was used. In the other study, scoring system was used for histological evaluation [22]. Sensitivities of these different criteria may be varied and might affect the histological outcome.

Another explanation for our finding could be the variations in the formulation, dose and configuration of used HA. It was suggested that HA has a molecular weight-specific and dose-specific mode of action that may enhance the osteogenic and osteoinductive properties of bone graft materials [19]. It significantly increased alkaline phosphatase activity, and hence stimulate cell mineralization in a dose-dependent manner [40]. HA configuration is another factor that can affect its action through allowing more area and volume for more cell attachment, thereby more newly formed tissue [19]. A variety of commercially available preparations of HA derivatives and cross-linked HA materials have been developed in forms such as films, microspheres, liposomes, fibers and hydrogels [9]. In the present study, high molecular weight HA in a gel form was used; in the other studies different configurations have been used such as Autocross linked HA sponge [20] and a biodegradable polymer configured as a non woven mesh [21]. High molecular weight HA in a gel form was used because it recorded promising result of bone fracture healing when combined with fibroblast growth factor-2 [41]. In addition, the gel form allowed easily mixing of HA with CP and facilitated handling the mix into bony cavity.

Regarding the dose, earlier authors presented no details regarding the used dose of HA and specifically the ratio of HA to graft materials [20–22]. So, the dose of HA applied to CP in the present study might be not the optimum level required to achieve significant results especially in a big cavity size. This might explain the presence of more fibrous tissue and large marrow spaces in the CP and HA treated cavities.

Based on the previous, there is a demand for more knowledge concerning the effective formulation, configuration and dose of HA when used in conjunction with graft materials to augment the bone healing process.
5. Conclusions

Under the conditions of this study, adding exogenous HA to β-tricalcium phosphate after periradicular surgery did not improve the histological outcome of osseous tissue healing of induced apical lesion in a dog model. Further studies may be needed to determine the effective formulation including; dose, molecular weight, concentration and configuration of HA-derived materials to produce significant osseous tissue healing effect, taking into consideration the type of graft materials and the size of the surgical wound.

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References


