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A Comparison of the Applications Between Different Proportional nHA / PLA in Alveolar Bone Preservation

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Abstract. Objective: To compare the effectiveness of different proportional nHA / PLA application in alveolar bone preservation. Methods: After extraction, apply extraction socket filling based on the alveolar bone defect model due to absorption in Beagle dog. Implant materials are divided into 3 different groups: nHA / PLAI, nHA / PLAII and the control group. Samples of the alveolar bone were collected at Week 4 and 8, respectively for the bone resorption assessment, bone density measurement, and histological examination. Results: After nHA / PLA implantation, the alveolar bone preservation was significantly improved. There was no difference in the alveolar bone preservation between the nHA / PLAI and nHA / PLAII groups. However, the sample which are 8w from group I, have higher bone density and have complete absorption in their dental material nest .Therefore group I is better than group II. Conclusions: The results can provide a reliable basis for the application of alveolar bone preservation in basic research and selection of clinical materials.

Introduction

Alveolar bone is the indispensable place for the tooth survival. Its defects may result in aesthetic impairment and tooth dysfunction, causing a lot of difficulties for the clinical restoration and treatment. In models of alveolar bone defect due to absorption, the alveolar bone is a tooth dependent structure and therefore, after a tooth is extracted, dimensional bone reduction takes place, which may not only affect the required bone mass for the follow-up experiments but also make the morphology of the bone defect area hard to control. The alveolar bone preservation is a practical and feasible way to solve the aforementioned problems, and it aims to minimize external absorption of the ridge and maximize bone formation within the socket [1]. In terms of alveolar bone preservation techniques, graft materials include artificial bone, heterologous bone, and tissue engineering bone, the time of bone substitution is a key issue. There is no study on the material degradation time so far. Systematic studies on how to improve the healing of the extraction socket and shorten the degradation time without leaving residual are still required. Therefore, this study aimed to construct a Beagle dog model to mimic this clinical problem and to observe the effects of filling different proportional nHA / PLA in alveolar bone preservation.

Materials and methods

Animals. Ten 1-year-old beagle dogs weighing approximately 9kg each and possessing a complete set of teeth were included in this study. All animal procedures were performed in accordance with the guidelines of animal center of Chinese PLA general hospital for the care and use of laboratory animals.

Materials. Nano-hydroxyapatite / polylactide (nHA/PLA) with a porosity of approximately 70% was used. The pore size ranged from 100 to 400 microns. According to the molecular size, they were divided into nHA/PLAI (gift from our cooperative institution, Beijing Institute of Graphic Communication) and nHA/PLAII. The nHA/PLAII is the commonly used model of nHA/PLA material.

Animal model. Under general anesthesia, the dogs were anesthetized by an intravenous injection of sodium pentobarbital (0.1ml/kg, 3%; P3761, sigma, USA) and local anesthesia (lidocaine HCL, 2% with epinephrine, 1:80000) was administered by infiltration. An intrasulcular incision was placed in the all mandibular premolar region, followed by elevation of mucoperiosteal flaps. The eight premolars were carefully extracted by dividing crown, and three semicircle defects of 5 mm in radius was created in the bilateral edentulous area. The midpoint of the semicirclar defect was positioned in the middle of the two extraction sockets. These bone defect sites were then randomly divided into three groups on the basis of the material used to fill their sockets: nHA / PLAI, nHA / PLAII, and control groups. These materials were filled into each root of the extraction sockets. The extraction sockets of the control group were left unfilled. The gingiva was sutured using apposition suture. Cefazolin sodium were administered for 3 days after surgery. The animals were given soft diet. All ten animals were euthanized 4w and 8w postoperative. Samples of the mandibular bone were collected and wrapped in saline soaked gauze stored at -20 °.

Sample measurements. Extraction socket measurements included (i) The vertical height from the edge of the buccal alveolar bone to the mandibular edge, (2) the vertical height from the edge of the lingual alveolar bone to the mandibular edge, and (iii) the horizontal width between the buccal and lingual bone plate. Measurements were taken after tooth extraction (time 0), at Week 4 and Week 8. Volume of bone resorption = measurement value after extraction – measurement value at Week 4 or Week8. Measurements were taken for three times and the average value was calculated.

Radiographic analysis. The specimens were scanned by micro-computed tomography (GE healthcare RS-9Micro-CT, GE healthcare, Canada), with a voltage of 85 KV, a current of 15 mA. Measurements were performed for three areas of each grafted site: the center point, 0.7mm medial and distal to the point. The region of interest (ROI) was set as an area of 2 × 4mm in the alveolar crest perpendicular to the long axis of extraction socket. The ROI image within a selected complete image was extracted using image analysis software (Micro View2.1, GE healthcare).

Histological analysis. Preparation of undecalcified bone grinding slices: bone tissues were fixed in 10% neutral formalin for 48h and cut into 0.5mm thick bone slices using Leica1600 sawing slicer. The samples were fixed in 10% neutral formalin again for 48h, then dehydrated, infiltrated, embedded, and mounted. Then the samples were grinded and polished and stained with methylene blue staining. The extraction socket bone healing and graft material degradation were observed.

Statistics. Data analysis was performed using SPSS 19.0 software. The endpoint differences between the groups were analyzed using Student's t-test ($p < 0.05$). Data are expressed as mean ± standard deviation.

Results

Alveolar bone absorption. After extraction, the alveolar bone width and height were reduced due to absorption. At Week 4 and Week 8, the alveolar bone width variation in experimental groups (I and II) was significantly less than that in the control group. There was no significant difference between groups I and II (Table 1). The alveolar bone height variation data showed that buccal bone absorption was more than that on the lingual side. Compared with the control group, bone absorption in groups I and II was significantly reduced by 0.698mm and 0.711mm at Week 8, respectively. At Week 4, there was no significant difference between the

Table 1. The alveolar bone width variation in the extraction socket ($\bar{x}$ ±s, mm)

Groups	Week 4	Week 8
Control	0.845±0.175	1.516±0.338
Group I	0.475±0.151	0.818±0.234
Group II	0.487±0.204	0.805±0.299
*t*c1	3.576	3.799
*p*c1	0.007*	0.005*
*t*12	0.106	0.074
*p*12	0.919	0.943
*t*c2	2.977	3.524
*p*c2	0.018*	0.008*

Note:***P** < 0.05 indicates a statistically significant difference.

experimental groups and the control group. However, the results showed that the treatment had certain effects reducing the bone height variation. At Week 4, there was no significant difference in the bone absorption improvement between groups I and II (Table 2).

Table 2. The alveolar bone height variation in the extraction socket ($\bar{x}$ ±s, mm)

	Buccal side		Lingual side	
Groups	Week4	Week8	Week4	Week8
Control	1.117±0.285	1.880±0.413	0.913±0.198	1.506±0.282
Group I	0.758±0.222	1.202±0.233	0.598±0.270	0.974±0.175
Group II	0.799±0.305	1.189±0.181	0.612±0.251	0.929±0.233
tc1	2.221	3.197	2.105	3.586
pc1	0.057	0.013*	0.068	0.007*
t12	0.246	0.097	0.082	0.339
p12	0.812	0.925	0.936	0.743
tc2	1.7	3.428	2.109	3.523
pc2	0.128	0.009*	0.068	0.008*

Note:*P ＜0.05 indicates a statistically significant difference

Table 3. Bone mineral density measurement of the extraction socket ($\bar{x}$ ±s, mg / cc)

Groups	Week 4	Week 8
Control	21.601±11.677	21.601±11.677
Group I	83.188±18.445	398.539±63.632
Group II	79.495±16.269	342.927±52.191
tc1	6.308	9.166
pc1	0.000*	0.000*
t12	0.336	1.511
p12	0.746	0.169
tc2	6.465	8.683
pc2	0.000*	0.000*

Note:*P < 0.05 indicates a statistically significant difference

Bone mineral density measurement. At Week 4 and Week 8, the bone mineral density measurement of the extraction socket showed that the bone mineral density in the experimental groups was significantly higher than that in the control group. The bone mineral density in group I was higher than that in group II, but the difference was not statistically significant (Table 3).

Histological observation. Histological examination of extraction socket showed that the volume of new bone in the control group at week 8 was enhanced more significantly than week 4, but still less than group I and group II . At Week 4, new bone developed along the nHA / PLA material in the extraction socket in groups I and II, presence of graft materials was observed. At Week 8, new bone formed, but the osteocytes were not mature. No graft material was observed in group I, and in group II, the residual graft material and newly formed bone had interlacing arrangement (Figure 1).

Discussion

Alveolar ridge defect is one of the most common mandibular defects. Effective preservation and restoration of the alveolar bone morphology and function may lay a solid foundation for later restorative treatment and basic research. In recent years, preventing the absorption of the remaining alveolar bone has become a research focus [2].

Human bone tissue consists of inorganic and organic components, in which the inorganic component is mainly hydroxyapatite [3]. It has been reported in the literature that [4] graft materials are the key factor determining the success of surgery. HA hydroxyapatite is a kind of artificial bone with good osteoinductivity and biocompatibility. Moreover, it is not limited by the resource and is non-immunogenic. It is easy to mold according to clinical needs [5]. It can create favorable conditions

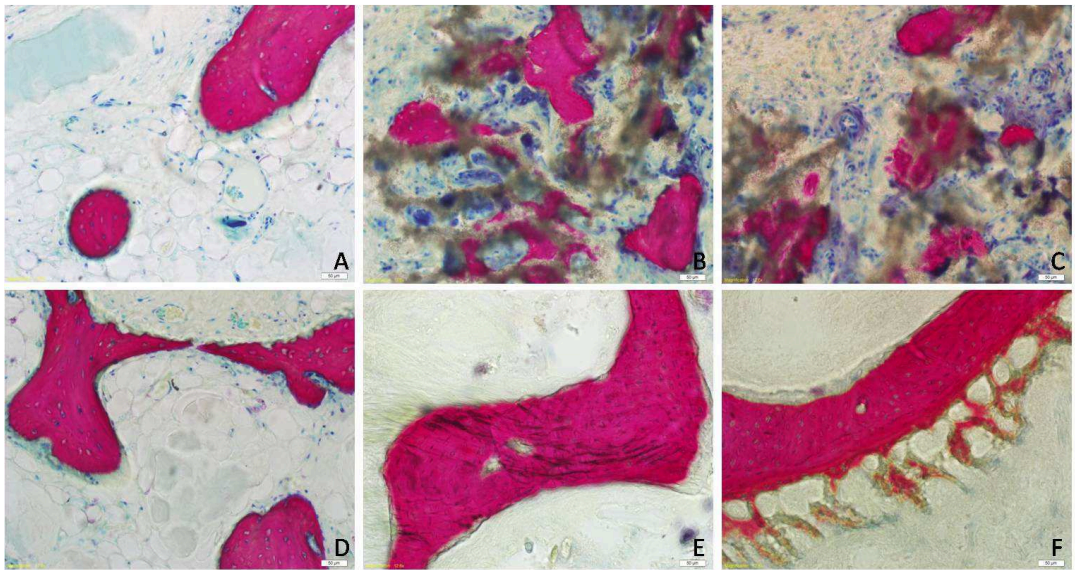

Fig.1 Histological examination of extraction socket (methylene blue staining, ×20). A: Control group, the new bone started developing at week 4. B&C: Group nHA/PLA I and nHA/PLA II, the new bone developed along the implantation material at week 4. D: Control group, the volume of new bone enhanced significantly at week 8. E: Group nHA/PLA I, the new bone developed well and no nHA / PLA material residue at week 8. F: Group nHA/PLA II, the residual material and new bone interlaced arrangement at week 8.

for new bone remodeling and accelerate osteogenesis, promoting the healing of extraction socket [6]. nHA scaffolds have cancellous bone-like structure [7] and it can promote blood clot formation. The blood clot wraps nHA inside, which may speed up the extraction socket repair [8]. Holzapfel [9] and Vagefi [10] have confirmed that filling nHA in extraction sockets can effectively prevent the absorption of the alveolar ridge. Histochemical results showed obvious new bone formation and nHA helps occupy the space for the new bone.

Currently, the materials used for the alveolar bone preservation are bioabsorbable materials including Bio-oss bone dust, β-TCP, and PRP. The degradation time is an important parameter of the bioabsorbable materials. Premature degradation may lead to inadequate time of osteogenesis and poor effects. Excessively long degradation may affect the process of bone substitution, slowing down osteogenesis. For example, nHA first occupies the space for osteogenesis, and it promotes deposition of collagen and minerals, and adhesion of osteoblasts through releasing calcium and phosphate particles [11] to induce osteogenesis. With the degradation, the bone substitution completes and osteogenesis is promoted. Residual graft material is a concern to both clinical implant and restoration and related basic studies after osteogenesis. Residual graft material may affect quality of newly formed bone and the subsequent procedures. No report on the degradation time of graft material in the alveolar bone preservation has been noticed. In previous studies on absorbable bone repair materials, residual material was often observed [12-14]. Frankenburg et al. [15] found that the absorption rate of HA in metaphyseal defect repair was 76 %, 86 %, and 98 % at 6,12, and 52 weeks in dogs, respectively, which is different from what was reported by Frankenburg. The possible reason is species difference and different implant site.

Absorbable artificial bone materials that can adjust the degradation speed are scant. nHA / PLA is a widely applied one. This study compared the effects of different proportional nHA / PLA in the alveolar bone preservation. Compared with the control group, the height and width of the alveolar bone were maintained much better in the experimental groups. The degradation time of nHA / PLAII, i.e., the standard model was about 3.5 months. At Week4, undegraded nHA / PLA material was observed in the tissue slices in experimental groups. At Week8, residual material was only observed in group II, indicating that nHA / PLAI material was replaced by bone tissues and absorption was completed. The bone mineral density results of micro-CT showed no difference between group I and group II at Week 8, but the bone mineral density values were obviously different (Table 3), indicating

that the degradation speed of nHA / PLAI is more conducive to new bone formation and the bone substitution process is faster. nHA / PLAII is a commonly used model nowdays. Its osteogenesis promoting capability has been confirmed in multifaceted studies on bone repair [16, 17]. It has become a more mature osteoinductive graft material. This study compared parameters between group I and II and proved that there was no significant difference in the alveolar bone preservation effects between the two models of materials. In terms of bone substitution speed, the material in group I had better performance. In addition, its degradation within 2 months may shorten the model construction time during experiment and it has no material residue, which may facilitate the subsequent experiments.

Our results showed that although nHA / PLA could inhibit the absorption of the alveolar bone to a certain extent, the bone absorption differences on the buccal and lingual sides were still 1.202 ± 0.233mm and 0.974 ± 0.175mm after 2 months, respectively. To achieve better preservation of the alveolar bone, there is a need to mix nHA / PLA with other bone graft materials or use other new methods [18].

In this study, effects of different proportional nHA / PLA on the alveolar bone preservation were compared. The results showed that nHA / PLAI achieved equivalent osteogenic effect to nHA / PLAII in animal models, and the bone mineral density was higher within the extraction socket. At two months, the material was fully degraded and no material residue was detected. Compared with the traditional material, nHA / PLAI can not only promote bone regeneration and maintain bone remodeling, but also shorten the experiment time greatly. Our results can provide a reliable basis for the application of alveolar bone preservation in basic research and selection of clinical materials.

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