Effect of biphasic calcium phosphate nanocomposite on healing of surgically created alveolar bone defects in beagle dogs

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Abstract. The aim of the present study was to investigate the effect of porous biphasic calcium phosphate nanocomposite (nanoBCP) scaffolds bioceramic. Alveolar bone defects were surgically created bilaterally at the buccal aspects of the upper second premolar in fourteen beagle dogs. After root conditioning with ethylenediaminetetraacetate (EDTA), nanoBCP was randomly filled in the defects and nothing was put into the contralaterals as controls. Dogs were killed at the 12th weeks. Histological observations were processed through a light microscopy. The results revealed that a great amount of functional periodontal fissures formed in the defects in the nanoBCP groups while minimal bone took shape in the controls. In this study, nanoBCP has proved to work well as a biocompatible and osteoconductive scaffold material to promote periodontal regeneration effectively.

1. Introduction
Regenerative periodontal therapy aims at the restitution of periodontal tissues that have been lost following inflammatory periodontal disease. This type of healing should result in regeneration of cementum, a functionally oriented periodontal ligament, and the alveolar bone [1, 2]. At present, the hotspot in the area of periodontal regeneration focuses on the biomaterial scaffold graft on alveolar bone defects to provide adequate space for periodontal regeneration. Both limited availability of autografts and potentiality of infection of allografts produce an increasing demand on synthetic bone grafts. Among various artificial bone materials, calcium phosphate bioceramic is similar to the animal bone in chemicophysics compositions, which is thus noticeable [3]. Due to a good many advantages including its excellent bioactivities, biocompatibility, osteophony and osteoinductivity, NanoBCP is expected to be an ideal tissue engineering scaffold material [4-6].

2. Material and method

2.1 Biomaterial preparation
In our study, the nanoBCP bioceramic was supplied by the Material Lab of West China College of Stomatology Sichuan University (Figure.1a). The proportion of hydroxyapatite(HA) / β-tricalcium phosphate is 4/6 with 40% NanoHA. The 3-dimensional images from µCT (µCT80, Scanco Medical AG, Basserdorf, Swiss) scan and reconstruction revealed the porosity of the material was 85% and the
macropores were interconnected. The diameter of macropore ranged from 116 to 515 μm (Figure. 1b, c). The observation of nanoBCP by SEM revealed that the distribution of macropores was uniform and micropores was distributed widely in the wall of macropores. The size of micropores ranged from 100 nm to 10μm.

![Figure 1.](image)

2.2 Animal preparation
Fourteen healthy male beagle dogs of 1.5 years old with the weight of 9.4± 1.5kg were selected in this experiment. They were offered by the Experimental Animal Center of Sichuan University (Chengdu, China). The experimental protocol was approved by the Animal Ethical Committee for Animal Research of Sichuan University (Chengdu, China).

2.3 Surgical procedure
The dogs were anesthetized with an intravenous injection of 3% pentobarbital sodium (Shanghai, China, 30 mg/kg). Following mesial vertical incisions, a full-thickness mucoperiosteal flap was raised to expose the buccal mesial aspect of maxillary second premolars, where a rectangular piece of the bone, about 4 mm long, 3 mm wide and deep into the root surface was removed with an osteotome. Reference notches indicating the bottom of the defects were made on the respective root surfaces with a high-speed handpiece under saline irrigation (Figure.2a). Periodontal ligaments were scaled thoroughly. Root surfaces in defects had been conditioned with EDTA for two minutes. The surgical sites were rinsed thoroughly with sterile saline. NanoBCP was trimmed into appropriate patches or particles and immersed into sterile saline. Then it was randomly filled in the defects and was impacted lightly to eliminate any gap or void that might appear at the interfaces (Figure.2b). The contralaterals were filled with void as controls. Finally, the flap was repositioned and sutured. Sutures were removed 2 weeks later. During the entire experiment chemical plaque control (0.2% chlorhexidine rinsing twice a week) was performed. The beagle dogs were fed with soft-food diet and water throughout the experiment.
The alveolar bone block about 3mm wide, 4mm high and deep into the root surface was removed from the buccal mesial aspect of the upper second premolars. The horizontal notch was made on the bottom of the root surface in bone defects; (b) nanoBCP was filled in the right bone defect.

2.4 Specimen obtainment
Fourteen dogs were euthanized with an overdose of 3% pentobarbital sodium at the 12th week postoperatively. The experimental teeth, together with their surrounding hard and soft tissues, were harvested and trimmed to the appropriate size. The specimens had been fixed in 70% ethanol for 3 days, dehydrated with increasing concentrations of ethanol, and then embedded in methylmethacrylate without decalcification. Buccal-lingual sections were cut parallel to long axis of the teeth with a microtome (Leica SM 2500E, Germany) set at 5 μm. The sections were stained with hematoxylin-eosin (H&E) staining and observed under light microscope. The images were captured by a digital camera connected with the light microscope.

3. Experimental results

3.1 Clinical observations
All surgeries went smoothly and the clinical incision healing was uneventful in all the animals. No complications, such as allergic reactions to material, abscesses and exposure of nanoBCP, occurred throughout the experimental period.

3.2 Histological observations
NanoBCP group: alveolar bone defects had about 60-86% bone-filling. The new bone possessed a normal structure. There was no significant difference between the new alveolar bone and the host one near the notch, which showed nanoBCP has excellent biocompatibility. The cementum mixed with inserted collagen fibers formed on the root surface of the defect. The width of new PDL was normal and uniform. One end of the newly formed periodontal ligament (PDL) fiber was inserted into the newborn cementum, the other end was embedded in the new adjacent alveolar bone. Thus, a new functional periodontal supporting tissue formed (Figure. 3 a,b).

In the control group: alveolar bone defects had about 10-26% bone-filling, which just covered the notch partly or fully at most. The limited new cementum deposited someplace on the local root surface of the notch, which had no collagen fibers embedded in (Figure. 3 c,d). The newly formed fiber bundles were thin and little, which were parallel to the root surface. It means the healing was still a periodontal repair via a long junctional epithelium instead of a new attachment.

4. Discussion
There are mainly two kinds of materials used as the tissue engineering scaffold at present: organic polymer material and inorganic material. The polymer materials, such as collagen, fibrin, polylactic acid, chitosan and alginate, have been widely applied as scaffolds [7, 8]. Although they meet the requirements of bone tissue engineering in many ways, they still have many defects, including poor mechanical properties, acid products of degradation in vivo and aseptic inflammation, to affect growth and reproduction of cells and tissues. Among inorganic materials bioceramic has a positive effect as the tissue engineering scaffold material. However, its high brittleness and slow degradation in vivo affect both bone ingrowth and followed reconstruction. As a result, its application has been restricted in some fields.
Figure 3. Photomicrograph of healing. (Yellow arrow pointed to the notch, NB new bone, DE dentin, PDL periodontal ligament) (a) nanoBCP group: healing resulted in abundant periodontal regeneration and there was no apparent difference between the new bone and the normal one (×100); (b) magnified view of (a), (×200); (c) Control group: the healing was characterized by formation of a long junctional epithelium along the root surface all the way down to the notch. There were limited new alveolar bones formed in the defect (×100); (d) Magnified view of (c) (×200).

In this study, nanoBCP is a kind of new scaffold which has a porous and 3D structure. The porosity of the scaffold was 85% and the pore size was 116-515 μm; the proportion of hydroxyapatite / β-tricalcium phosphate was 4/6 with 40% NanoHA. Because of interconnected macropores and numerous micropores on macropore walls, the large surface area of the material is beneficial to acceleration of interface reaction. The good interconnected pore structure provides larger space for proliferation and attachment of osteoblastic cells and favors both permeation of nutrition composition and formation of vascularization[9]. Due to a good many advantages including its excellent bioactivities, biocompatibility, osteophony and osteoinductivity, nanoBCP is expected to be an ideal tissue engineering scaffold material[10,11]. With the small-size effect and the surface effect, nano-ceramic material shows different characteristics from traditional ceramic. Increase of its specific surface area enhances its guidance ability of histiocytes and its biodegradation. Research has shown...
that microstructure and sub-microstructure on the surface of tissue engineering scaffold have a great influence on cell adhesion and growth.

In this study, histological analysis reveals abundant and homogeneous new alveolar bones formed in the defects of nanoBCP group while little in the controls. The findings of the experiment provide evidence that treatment of acute intrabony defects with nanoBCP can enhance periodontal regeneration. It shows that nanoBCP as a promising bone grafting might provide a favorable choice for periodontal therapy.

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