The Immunohistochemical Study of Periodontal Tissues Regenerated by Nano Calcium Phosphate Bioceramics

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Abstract. The aim of the experiment was to study the immunohistochemical reaction the periodontal tissues regenerated by nano biphasic calcium phosphate bioceramics (NBCP). Two lateral third incisors were randomly selected and operated as experimental group in two beagle dogs. As control the contralateral third incisors of the same jaw were done nothing. In experimental group, alveolar bone defects were made surgically at the labial aspects of the teeth. After root conditioning, the defects were filled with NBCP. 16 weeks after operation, the dogs were killed. The immunohistochemistry examination was used to observe the newly formed periodontium in the experimental group and the normal periodontium in the control group. The positive expressions of osteocalcin (OCN) in newly formed periodontium were the same to that in normal periodontal tissues.

1. Introduction
Periodontitis is an important concern in dental health. Regeneration of lost periodontium is the focus of periodontal therapy. There are many treatment methods to achieve periodontal tissue regeneration. such as guided tissue regeneration, implantation of scaffold materials, growth factors and combination of these approaches in clinical practice. However, complete periodontal tissue regeneration has not been achieved yet[1-4].

Among these treatment methods, bone grafts are widely used to treat periodontal osseous defects in clinical practice. Calcium phosphate ceramics have been studied extensively[5,6]. In our former studies, the efficacy of NBCP has already been proven on periodontal regeneration[7] The propose of this study was to study the immunohistochemical reaction the periodontal tissues regenerated by NBCP.

2. Material and Method
Two 1.5-year-old male beagle dogs (10.3, 10.7 kg) were used as subjects. The dogs had healthy teeth. The study protocol was approved by the Animal Ethical Committee of Shandong University of TCM (Shandong, China). NBCP was produced by Sichuan University (Figure.1).
The operations were performed by a surgeon masked to the specific experimental conditions. Two third incisors were selected randomly as experimental group. The contralateral tooth of the same jaw was not operated as control. Under general anaesthesia, mesial vertical incisions were cut. Then a full-thickness mucoperiosteal flap was raised to expose labial alveolar bone of the third incisors. A rectangular piece of the bone of 3 mm wide 4mm high, which is deep into the root surface, was removed. At the bottom of the defect, a horizontal reference notch were made with a high-speed handpiece under saline irrigation (fig. 1c). Periodontal ligaments were removed thoroughly. The root surface in defect was conditioned with EDTA for 2 minutes. The surgical sites were cleaned with sterile saline. NBCP granules immersed in the sterile saline were filled in the alveolar bone defects. The incisions were sutured tightly.

The dogs were given Penicillin G (800,000 IU) administered intramuscularly at once after operation. The incision were rinsed by 0.2% chlorhexidine and smeared with iodine once a day until sutures were removed 2 weeks later. The dogs were fed with soft-food in order to minimize mechanical trauma to the incisions during the experiment.

16 weeks later, the dogs were killed. The experimental teeth together with surrounding tissues were harvested. The specimens were fixed in 4% paraformaldehyde, then put 37 °C thermostat, EDTA decalcification, paraffin embedded, sliced. With a microtome, 7μm histological sections were cut in the labio-lingual direction, parallel to the axes of the teeth. The immunohistochemistry examination of OCN was used to observe the changes in newly formed periodontium in the experimental group and the normal periodontium of the contralateral teeth in the control group. The photos were taken by a digital camera of light microscope.

3. Experimental Results
All operations went smoothly. The wounds healed well. During the experiment, no inflammation or exposure of NBCP were observed.

The immunohistochemistry photos of the two groups were observed. Mainly occurring in cementoblasts and osteoblasts, OCN protein was weakly positive in periodontal tissues which appeared light brown on the substrate. The positive expressions of OCN in the newly-formed periodontium were the same to that in the normal periodontal tissues. There was no difference between the two groups.
4. Discussion
Bone grafting is the common modality of therapy to achieve effective periodontal regeneration. Potential infection of allografts and limited availability of autografts produce an increasing demand on synthetic bone grafts. Biphasic calcium phosphate bio ceramics consist of highly stable hydroxyapatite and biodegradable beta-tricalcium phosphate, which are similar to chemical composition of natural bone. With excellent biocompatibility, bioactivity and bone conductivity, they have been studied extensively and widely used in bone tissue engineering[5].

Nanomaterials are different from traditional materials because of their microstructure and huge surface area. Many researches have reported that the surface of nanomaterials have a great influence on cell adhesion and growth[8,9]. There are abundant micropores in the walls of interchannelled macropores. The macropores can provide spaces for bone in-growth. The micropores in the wall of macropores can transport body fluid to facilitate bone tissue regeneration[10].

Combining tissue engineering principles with nanomaterial technology, nano bone graft materials is researched as an interdisciplinary research hotspot. In our previous study, NBCP used in this experiment can lead to periodontal regeneration[7]. With a high porosity of 85%, NBCP has a huge surface area to enhance its degradation and to accelerate interface bonding reaction.

OCN is mainly synthesized and secreted by osteoblast and cementoblast during the late stage of differentiation, deposited mostly in bone extracellular matrix and little into blood circulation. Widely found in bones, dentin and cementum of vertebrates, OCN is one of the most abundant non-collagen proteins in mineralized tissues, which makes the main physiological role of maintaining normal bone mineralization rate and inhibiting abnormal HA crystal formation and is consider as the most characteristic phenotype on osteoblast. Adopting osteoblastic phenotype, periodontal ligament cells can also synthesize and excrete OCN to promote the mineralization process of alveolar bone and cementum. OCN in periodontal membrane is mainly synthesized during the late stage of differentiation and released in some parts [11]. The immunohistochemistry examination were used to observe the changes in newly formed periodontium. No significant differences were found between the two groups.(fig. 2) . Based on these findings, it seems that NBCP, as a promising bioceramic, may provide a new therapy method for the patients who have lost his periodontal tissues.

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