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Macro-porous calcium phosphate scaffold with collagen and growth factors for periodontal bone regeneration in dogs

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Abstract

The periodontal bone regeneration has always been considered as a major challenge in maxillofacial clinic. Unfortunately, current treatments cannot achieve optimal therapeutic effect. Self-setting calcium phosphate bone cement (CPC) is regarded as promising material to solve periodontal problem due to its good biocompatibility, mineral similarity to the natural bone's composition. Moreover, it can be arbitrarily shaped, form bio-resorbable hydroxyapatite (HA) *in situ* to fill complex periodontal bone cavities perfectly. So far, there are lots of related *in-vitro* investigations; however, *in-vivo* experiments which could precisely show the real effects of CPC's internal performances are rarely reported. Therefore, in this study, CPC were implanted into bilateral mandible defect of 8 beagles to evaluate its degradation ability and osteogenesis through Micro-CT scan and histological morphology analysis at determined time. Because of the intrinsic drawbacks of raw CPC, reinforced ingredients including collagen and growth factors were selected to synthesis different modified-CPC systems. The results illustrated that the samples' degradation ability could be largely increased by nearly 60% with the help of macro-porous structure and collagen. In addition, by using the same ingredients, the osteogenisis of those samples could be promoted by 12%. What's more, growth factors were proved to be the most important factor to increase the CPC's new bone formation ability (22% more new bone could be obtained after using growth factors after 6 months implantation). The data obtained from histological analysis has presented the similar changing trend. Overall, these results illustrated macro-porous structure; collagen and growth factors could both effectively promote the CPC's degradation ability and osteogenesis *in vivo,* which suggested the macro-porous CPC with collagen and growth factors would be promising as bioactive materials for periodontal bone tissue regeneration.

Keywords: Periodontal bone regeneration; Calcium phosphate bone cement; Biodegradation ability; Osteogenesis

1. Introduction

The periodontal tissue defect, which is often caused by tumor, injury and congenital malformation, is regarded as a major problem in maxillofacial clinical research [1]. As traditional treatment, autograft is considered as the golden standard. However, intrinsic disadvantages such as insufficient grafting tissues, postoperative hemorrhage, inflammation and donor-site disability still restrict its clinical application [2]. Besides, allograft and xenograft are also adopted clinically but neither can achieve desirable effect due to the risk of immunological rejection and disease infection [3,4]. Therefore, scientists begin to focus on the research of alloplasm materials which could be used as another kind of alternative bone tissue. They have made great efforts to synthesis ideal materials that could overcome most of disadvantages mentioned above [5,6].

According to recent reports, calcium phosphate bone cement (CPC) is seemed to be a promising bone substitute due to its excellent biocompatibility and bioactivity. Meanwhile, its mineral similarity to the composition of natural bone could afford sufficient Ca^{2+} and PO_4^{3-} ions during the bone regeneration process [7,8]. In addition, CPC is a mixture by blending reactive calcium phosphate powder and aqueous solution, which means that it can form injectable paste that could easily be shaped to fill the irregular bone defect [9].

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CPC was firstly reported by Brown and Chow in 1983 and soon became the market product since 1990s [10]. Although having a host of advantages, CPC still has its inherent drawbacks, mainly including poor mechanical properties that will be improper for some load-bearing circumstance, undesirable degradation ability which will lead to poor osteogenesis performance [11]. Therefore, in order to overcome CPC's drawbacks and expand its clinical application, nowadays, a great number of scientists pay increasingly close attention to the CPC modification research. Xu's work has clearly illustrated if collagen were added into this material to form a novel composition system, the CPC's elastic modulus and flexural strength could be largely ameliorated indicating its potential application in moderate stress-bearing circumstance. Moreover, this composite could enhance cell's adhesion and proliferation ability which also showed its promising use in tissue repairing field [12].

Meanwhile, some researchers used the pore-forming agent to transform dense CPC to porous structure, which could increase the permeability of environmental medium and finally promote the material's degradation performance [13]. Further research showed that proper porosity and pore size could improve the cells' adhesion, proliferation, differentiation behavior on the surface of CPC. Moreover, it could induct new-born tissues to grow into the inner side of CPC and form stable mechanical combination between tissues and materials. Some other researchers added the related growth factors into CPC system to increase the osteogenic efficiency [14–16].

In general, CPC's modification is well researched and now widely applied in cortical bone, cancellous bone and medullar bone reparation [17–19]. However, with respect to the CPC's research on human's periodontal region, so far, the definitive conclusion still cannot be obtained because there are few reports to focus on this interesting research field.

Until now, the regeneration of periodontal region defect is still a pressing problem both in dental and material fields [20,21]. Many researchers have obtained encouraging *in-vitro* results. However, most of these results have been proved less effective *in-vivo* after further evaluation. The main reasons are listed as follows: first, periodontal region consists of various structures including fibers, bone, membranes, muscles and so on. Second, the micro-environment of this area is also more difficult to understand than other body parts. Finally, this area is proved to be more susceptible to make further complicated research [22–24]. Therefore, more careful and detailed *in-vivo* investigations which could precisely show the real properties such as degradation and osteogenesis in this very special region are essential and important. In this study, dense CPC was selected as control group and three different kinds of CPC composites were prepared: (1) CPCC: porous CPC with collagen; (2) CPCCB: CPCC with growth factor bone morphogenetic protein-2 (BMP-2); (3) CPCCBV: CPCCB with another growth factor vascular endothelial growth factor (VEGF); these samples were implanted into bilateral mandible defect patterns of 8 beagles for 3 or 6 months. After implantation, the samples were taken out and a series of experiments including micro-CT scan and histological morphology analysis were carried out respectively. The objective of this study is to systematically evaluate different CPC samples' *in-vivo* performances mainly including degradation and osteogenesis abilities and then find out the related influence factors. We hypothesized that the macro-porous structure, collagen and growth factors were more effective in promoting both degradation and osteogenic performance in periodontal region than using CPC independently; hoping the collagen modified macro-porous CPC loaded with growth factors could become a promising bioactive material for periodontal bone tissue regeneration.

2. Materials and methods

2.1. Materials

Dense CPC and CPCC (average pore size: 250 μm, macroporosity: 45%)blocks were both obtained from the Department of Endodontics, Prosthodontics and Operative Dentistry University of Maryland. The preparation method is briefly described as follows: CPC powder was synthesized by simply mixing the same weight of tetracalcium (TTCP) and dicalcium phosphate anhydrous (DCPA). Dense CPC was fabricated by adding the CPC powder into distilled water with a mass ratio of 3.0/1 to form a CPC paste. Then, this paste was transferred into molds (rectangular parallelepiped, length × width × height was 8 mm × 5 mm × 2 mm). After 24 h self-setting process at room temperature, these pastes transformed to Dense CPC block. The preparation process of CPCC was listed as follows first, collagen powder (Sigma, St. Louis, MO) with a bundle length of 20–100 μm and diameter of 0.3–1 μm were extracted from the cattle Achilles tendon using a simple method described as the previous report [12]. Then, collagen was added into the CPC paste to form CPC-collagen composite, the mass ratio of collagen was 10%. During this process, 20 wt% mannitol (Kelong Chemical Industry, Chengdu, China) as the pore-forming agent was placed into this system. After transferring to the same molds and waiting for 24 h self-setting, the macro-porous CPCC block was finally obtained. The growth factors bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) were purchased from PeproTech. Inc (USA). Eight beagles (male, 1–1.5 years old, and 10 kg weight) were purchased from Dashuo Animal Experimental Center (Chengdu, China). All other chemicals and solvents were of reagent grade or better.

2.2. Preparation of CPCCB and CPCCBV

BMP-2 and VEGF were firstly centrifuged at the speed of 1000 r/min for 8 min, respectively. After that, BMP-2 was diluted to the concentration of 0.5 mg/ml by adding aqueous solution which contained certain amount of bovine serum albumin (BSA, SIGMA Inc. USA); VEGF was diluted to the same concentration by adding deionized water. Two diluted solution were both kept at 4 °C.