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# Biocompatibility and osteogenicity of degradable Ca-deficient hydroxyapatite scaffolds from calcium phosphate cement for bone tissue engineering

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**Abstract**

Ca-deficient hydroxyapatite (CDHA) porous scaffolds were successfully fabricated from calcium phosphate cement (CPC) by a particle-leaching method. The morphology, porosity and mechanical strength as well as degradation of the scaffolds were characterized. The results showed that the CDHA scaffolds with a porosity of 81% showed open macropores with pore sizes of 400–500 μm. Thirty-six per cent of these CDHA scaffolds were degraded after 12 weeks in Tris–HCl solution. Mesenchymal stem cells (MSCs) were cultured, expanded and seeded on the scaffolds, and the proliferation and differentiation of MSCs into osteoblastic phenotype were determined using MTT assay, alkaline phosphatase activity and scanning electron microscopy. The results revealed that the CDHA scaffolds were biocompatible and had no negative effects on the MSCs in vitro. The in vivo biocompatibility and osteogenicity of the scaffolds were investigated. Both CDHA scaffolds and MSC/scaffold constructs were implanted in rabbit mandibles and studied histologically. The results showed that CDHA scaffolds exhibited good biocompatibility and osteoconductivity. Moreover, the introduction of MSCs into the scaffolds dramatically enhanced the efficiency of new bone formation, especially at the initial stage after implantation (from 2 to 4 weeks). However, the CDHA scaffolds showed as good biocompatibility and osteogenicity as the hybrid ones at 8 weeks. These results indicate that the CDHA scaffolds fulfill the basic requirements of bone tissue engineering scaffold.


*Keywords:* Ca-deficient hydroxyapatite scaffold; Mesenchymal stem cells; Biocompatibility; Osteogenesis; Bone tissue engineering

## 1. Introduction

Several calcium phosphate cements can self-harden to form hydroxyapatite (HA) and possess excellent osteoconductivity and biocompatibility [1,2]. A calcium phosphate cement (CPC) that initially contains an equimolar mixture of tetracalcium phosphate (TECP) and dicalcium phosphate anhydrous (DCPA) hardens in about 30 min after mixing the powder with water; this cement has unique in vivo properties: slow resorption and replacement by new bone formation with no loss in volume [3]. However, for certain clinical applications a more rapid resorption and replacement by new bone is desirable [4].

It is known that TECP/DCPA cements do not necessarily react in equimolar proportion (Ca/P = 1.67) and that calcium-deficient apatite rather than stoichiometric HA may be formed [5]. HA from this CPC system exists over a range of compositions typically characterized by a Ca/P molar ratio of 1.50–1.67 [6]. Driessens thought that the variation in the molar ratio of calcium to phosphate greatly affected the solubility of the product due to calcium deficiency in the HA crystal [7]. Others suggested that CPC with Ca/P of 1.50 degraded faster than the sample with

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Ca/P of 1.67 when CPC was implanted in vivo, and the degradation rate in vivo could be controlled to a certain extent by changing the Ca/P ratio in the sample [8]. Therefore, the composition of synthetic apatite, such as Ca/P ratio, can be varied to suit specific in vivo applications. This permits tailoring of the properties of CPC implants, such as resorption rate.

Previous studies have shown that calcium phosphate ceramic implants with macropores (>100 μm) allowed ingrowth of bone tissue with functional Haversian systems and facilitated osteointegration [9]. Although the presence of macropores in CPC is not critical to implant resorption and replacement by bone, incorporation of macropores in CPC is likely to promote the process. As a result of the setting and hardening mechanisms operating in CPC, through dissolution and precipitation processes, CPC has an intrinsic microporosity [10]. However, since one of the requisites for bone tissue engineering scaffolds is to have a macroporous structure, it is necessary to be able to create macropores in the material. In general, it is accepted that the pore size should range between 100 and 600 μm [11]. Several attempts have already been made to improve the resorption behavior of CPCs, e.g. by increasing the porosity of the material [12,13]. Porous scaffolds in bone tissue engineering require three-dimensional interconnected porous structure, which could provide sufficient space for cell migration, adhesion, and the ingrowth of new bone tissue [14]. CPC has a composition and structure very close to natural bone mineral and therefore has been considered to be the ideal material to build bone tissue engineering scaffold [15]. In this study, an alternative route is presented to develop macroporous calcium-deficient hydroxyapatite (CDHA) scaffolds with a Ca/P ratio of 1.5 at low temperature from CPC. It is presumed that the decreased Ca/P ratio of CPC precipitates in such cement formulations promotes the resorbability of these materials after implantation, and macropores were built into implants to facilitate cell and vascular ingrowth.

One of the most important aspects of bone tissue engineering is the introduction of bioactive cells into the three-dimensionally porous scaffold [16]. Mesenchymal stem cells (MSCs) are present in many human tissues and can be directly derived from marrow. MSCs serve as a readily available source of undifferentiated cells that are capable of giving rise to diverse tissues, including bone, cartilage, muscle and other tissues of mesenchymal origin [17]. Moreover, MSCs do not appear to be rejected by the immune system, allowing for large-scale production and appropriate characterization, and the subsequent ready availability of allogeneic tissue repair enhancing cellular therapeutics [18]. As MSCs present more advantages than other cells, they have already been widely used in bone tissue engineering [19]. The superiority of MSCs has encouraged us to introduce them into CDHA scaffolds for tissue-engineering applications. Therefore, in the present study, we investigate the biocompatibility of CDHA scaffolds using MSCs cultured by in vitro tests, and study the in vivo biocompatibility and osteogenicity of these MSC-hybridized CDHA scaffolds in an animal model.

## 2. Materials and methods

### 2.1. Fabrication of CPC powders

CDHA scaffolds fabricated from CPC were based on TECP and DCPA, and all the calcium phosphates were prepared in our laboratory. The details of the preparation method were described by Liu et al. [20]. The CPC powders were prepared by mixing TECP and DCPA in a molar ratio of 1:2, and the final product of hydration was CDHA with a Ca/P ratio of 1.50. The CPC with a Ca/P ratio of 1.67 was prepared by mixing equimolar amounts of TECP and DCPA, and the final product of hydration was the stoichiometric HA (used as control).

### 2.2. Fabrication of CDHA scaffolds

The CDHA scaffolds with a Ca/P ratio of 1.5 were prepared by a particulate-leaching method. Briefly, the CPC powder was mixed with distilled water using a spatula at a powder/liquid mass ratio of 3:1 to form a paste. Sodium chloride (NaCl) particles sieved with diameters of 400–500 μm as porogen were added into the CPC paste. The mixture of CPC paste/NaCl was placed in stainless steel molds of different sizes, and the mixture was molded under a pressure of 2 MPa. After storage in beakers in a constant temperature oven at 37 °C and 100% relative humidity (RH) for 2 days, the samples were then immersed in deionized water to leach out the porogen. Finally, they were vacuum-dried to obtain sponge-like scaffolds. HA scaffolds were prepared using the same method as the control. After preparation, the surface morphology of the porous scaffolds was examined by scanning electron microscopy (SEM, JSM-6360LV, JEOL, Japan).

### 2.3. Porosity and mechanical strength of scaffolds

The Archimedes method was used to measure the porosity of the CDHA scaffolds in distilled water. After the scaffolds had been vacuum-dried at 50 °C for 48 h, their dry weight was recorded as $m_1$. The samples were then immersed in water under vacuum to force the liquid into the pores of the scaffolds until no bubbles emerged from the scaffolds. Then, the samples were reweighed in water to produce the measurement $m_3$. After that, the scaffolds were carefully removed from the beaker and surface saturated water droplets were dabbed off. They were quickly reweighed in air to produce the measurement $m_2$. The porosity of the open pores in the scaffold can then be calculated via the following formula:

$$\text{Porosity} = (m_2 - m_1)/(m_2 - m_3) \times 100\%,$$

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