

# In vivo testing of nanoparticle-treated TTCP/DCPA-based ceramic surfaces

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## Abstract

This study reports the development of a non-dispersive calcium phosphate cement (nd-CPC) paste containing tetracalcium phosphate and anhydrous dicalcium phosphate that can be used as a filling material in dental and orthopedic applications. The nd-CPC bone cement is compared with two commercial materials, OsteoSet<sup>®</sup> and Collagraft<sup>®</sup> bone grafts. Gross examination of retrieved implants/bone composite samples indicated that none of the implants in this study evoked an inflammatory response. The OsteoSet<sup>®</sup> (calcium sulfate) implant was resorbed too quickly to allow for osteo-remodeling, and it led to the formation of fibrous connective tissue in the fracture site, which remained even 24 weeks after implantation. Histological examination revealed that nd-CPC and Collagraft<sup>®</sup> (hydroxyapatite/tricalcium phosphate/collagen) had greater remodeling and osteoconductive activity than OsteoSet<sup>®</sup> at both 12 and 24 weeks after implantation. Greater remodeling activities were found with nd-CPC cement than with the other materials at 12 weeks after implantation, and the Fourier transform infrared absorption band of carbonate or cellulose derivatives grew from 6 weeks to 24 weeks after implantation in nd-CPC cement. These findings show that nd-CPC compares favorably to commercial bone remodeling materials, and the fact that it is in a paste formulation makes it an ideal material to fill regeneration defects.

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## 1. Introduction

An ideal reconstructive material for bone defects must fill in well, harden in situ, cause no side effects such as inflammation, promote no connective tissue growth, be degradable and be replaced by osseous tissue. These requirements must be met for biomaterials to be osteoinductive. After implantation in bone defects, materials must be rapidly integrated into new bone by the cellular activity of osteoclasts and osteoblasts, which carry out local bone remodeling.

Calcium phosphate cements (CPCs) can be handled in paste form, providing the advantage of the ability to mold the material during an operation [1–5]. However, many CPC pastes tend to disintegrate upon early contact with blood or other aqueous fluids, which inhibits the clinical use of these materials in bone repair, reconstruction and augmentation. To address this problem, some methods have been developed to allow cement setting in a wet medium after reaction at the implantation site. These methods involve adding cohesion promoters and organic compounds to the CPCs [6,7].

There are many approaches for mixing additives into calcium phosphate powders to shorten the setting time of CPC. Such additives have been shown to effectively reduce the setting time to 1–15 min [8–11]. However, these modifications often reduce the compressive strength of the cement

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[12,13]. In addition, in many cases, the CPC remains dispersed when in contact with blood or an aqueous agent. Several attempts have been made to solve the dispersion problem by adding organic materials such as collagens. The hydroxyl group of CPC can bond with the polymers, and the resulting modified CPC can harden in situ in an aqueous environment. The effect of these additives on the biocompatibility of CPC, however, remains to be determined [6,14–16].

Our previous studies provided detailed microstructural information about the setting/hardening behavior at the early stages of the dissolution and precipitation processes of the TTCP/DCPA-based CPC [17]. The results suggest that, upon mixing of TTCP and DCPA with phosphate hardening solution, the TTCP rapidly dissolves and the local concentration between particles rises. Supersaturated calcium and phosphate ion concentrations were observed to result in precipitation, predominantly on the surface of DCPA particles. Few apatite crystals were observed on the surface of the TTCP powder. The nanosized nucleation was difficult to identify due to its low quantity, and the phase was not stable at the initial reaction time. After 20 min of reaction, the fine (typically <100 nm) apatite crystals were clearly observed by transmission electron microscopy (TEM).

After these TTCP and DCPA particles were bridged by the nanoparticles, the cement was set with an initial compressive strength. This means that, in the later stage of the reaction, DCPA particles were linked together by the extensive growth of nanocrystals/whiskers with a Ca/P ratio very close to that of hydroxyapatite (HA). In addition, the larger TTCP particles are locked in place by the bridging apatite crystals/whiskers, and the CPC is set and will not dissolve when immersed in solution.

Next, the mechanisms described above were employed to develop a fast-setting material with a high initial strength, a suitably fast working/setting time, and both rapid and complete apatite phase transformation of nanoparticle-treated non-dispersive (nd)-CPC of TTCP/DCPA-based ceramic. This nd-CPC has no organic additives, as described in our previous studies [18]. Since this nd-CPC cement has not yet been tested in vivo, the aim of this study was to analyze the effects of nd-CPC in vivo and compare its bioresorption rates with those of commercial products.

## 2. Materials and methods

### 2.1. Preparation of nd-CPC

The TTCP powder used for the preparation of CPCs for this study was fabricated in-house following the method reported in Brown and Epstein [19]. The DCPA powder for the preparation of the CPCs is a commercial product (Jassen Chemical Co., Japan). The procedure for preparing nd-CPC has been described in our previous study and patent [17,18]. Briefly, 5 g of c-CPC was stirred in 1.6 ml of 0.25 mM phosphoric acid for 1 min. The resulting mixture

was placed into an oven at 50 °C for 15 min and then mechanically ground for 20 min into fine particles. The resulting nd-CPC powder was dried and ground up to make new reaction surfaces. The hardening solution used to prepare CPCs was 25 mM orthophosphoric acid ( $\text{H}_3\text{PO}_4$ ) with a pH of 1.96.

### 2.2. Implant materials

Prior to animal studies, the nd-CPC powders and hardening solution were sterilized by incubation for 30 min at 160 °C or by autoclaving for 20 min at 120 °C, respectively. The L/P ratio of nd-CPC for implantation was 0.23 ml/0.8 g. This study compared the performance of nd-CPC with two commercially available products: the collagen–calcium phosphate ceramic graft material called Colla-graft® bone graft (Zimmer, Warsaw, IN, USA), which contains 65% HA ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), 35% tricalcium phosphate (TCP,  $\text{Ca}_3(\text{PO}_4)_2$ ) and type I collagen; and OsteoSet® bonegraft substitute (Wright Med Tech Inc, USA), which contains a cylinder of highly pure  $\text{CaSO}_4$ , in which case the sample size used in this study was 4.8 mm  $\varnothing$  with 2.5 mm length. Samples of Colla-graft® were cut into small pieces before implantation to allow small cavities to be filled.

### 2.3. Animal studies and implantation procedures

The animal studies were performed at Kaohsiung Medical University Animal Center, Kaohsiung, Taiwan. Fourteen healthy, male adult (weighing 2.8–3.5 kg) New Zealand white rabbits were used as experimental animals. The rabbits were housed individually in stainless-steel cages and had free access to food and water. An acclimation period of at least 7 days was allowed between receipt of the animals and the start of the study.

Injection sites were shaved and cleansed with 70% ethanol and Betadine TM (povidone iodine 10%). All animals were operated on under general anesthesia. Pentobarbital sodium (0.1 ml/100 g, Tokyo Kasei Kogyo, Tokyo, Japan) was used as general anesthesia, while xylocaine (Fujisawa Pharmaceutical CO., Tokyo, Japan) was used as local anesthesia. To implant cement paste in the medial condyle of the femur, a longitudinal incision was made on the anterior surface of the femur. The inner side of the knee joint of the rabbit was cut to expose the femur. After exposure of the femur, the periosteum was reflected and a 2 mm pilot hole was drilled. The hole was gradually widened with drills of increasing size until a final diameter of 5 mm was reached. A special 5 mm diameter drill burr was used, and a ring was inserted at a depth of 5 mm to ensure appropriate length of the drill hole (Fig. 1a).

The nd-CPC paste was prepared for implantation by mixing with the hardening solution for 1 min and then loading the mixture into a 3 ml syringe for injection into the prepared bone cavity. The injection was conducted carefully in a retrograde manner from the bottom to the

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