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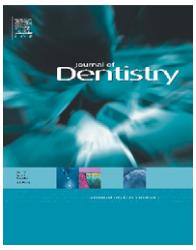

Calcium phosphate bone cement with 10 wt% platelet-rich plasma *in vitro* and *in vivo*

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ABSTRACT

Objectives: The aim of this study was to evaluate the performance of a 10 wt% platelet-rich plasma (PRP) additive composite with calcium phosphate cement (CPC) *in vitro* and *in vivo*.
Methods: The *in vitro* testing of modulus, the apatite conversion rate, morphology, cell and alkaline phosphatase (ALP) activities, and *in vivo* testing of histological examinations between two groups of 10 wt% PRP/CPC and CPC were characterised and compared.
Results: Although the crystallite morphologies showed a retarded effect in the PRP/CPC group *in vitro*, the modulus results showed that the 10 wt% PRP/CPC group had a significant reduction in strength but had no significant changes in the relative conversion ratio of the apatite phase with CPC only. The osteogenic evaluation of ALP expression was significantly increased by the PRP additives group with stem cells (D1) cultured for different periods (2–32 days). Our histological examinations showed that greater remodelling and the phenomenon of isolated/detached CPC particles were significantly observed at 9 weeks after implantation when the 10 wt% PRP/CPC composite was used.
Conclusion: The results demonstrate that CPC may be a potential candidate as a carrier with PRP additives for bone regeneration.

1. Introduction

Calcium phosphate bioceramics are highly biocompatible materials that have been extensively employed in clinical practises for years. These ceramics are increasingly being used in implantable biomaterials and are available in powder, granular, block or paste/slurry form. In recent years, calcium phosphate bone fillers have been employed extensively orthopaedics, for example, with periodontal defect repair, as scaffolds for bone reconstruction, and in orthopaedics.[1–6] Because the main inorganic mineral components of bone and teeth are impure forms of hydroxyapatite (HA), the use of calcium phosphates for restorations leads to better osteoconductive properties compared with other commonly used biomaterials (for example, zirconia, alumina).[1–6]

A suitable biomaterial for tissue engineering should support target cell growth and osteogenic differentiation. Material composition is thought to play an important role in providing specific adhesion characteristics for cells within tissues. Therefore, to exhibit the desirable functional characteristics of bone regeneration, synthetic biomaterials should possess or combine similar properties of the suitable environment for bone cell growth and differentiation. Accordingly, growth

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factors (GFs) are usually introduced into biomaterial composites. GF presentation within a novel biomaterial design can influence the chemotaxis, differentiation, proliferation and synthetic activity of bone cells and thereby physiologically regulate bone reconstruction and remodelling.[7] Numerous GFs, such as the specific protein bone morphogenetic protein 2 (BMP-2) composite, with calcium phosphates appear to significantly enhance bone growth.[8–10] BMP-2 and BMP-7 have actually been approved for bone grafts with specific indications in the U.S. and Europe for clinical applications.[11] However, the high cost of purified growth factors is the main issue that limits their use in general clinical applications.

Platelets may secrete multiple GFs, such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), platelet-derived epidermal growth factor (PDEGF), platelet-derived angiogenesis factor (PDAF), insulin-like growth factor 1 (IGF-1), and platelet factor 4 (PF-4), which have been reported to be an effective way to induce tissue repair and encourage regeneration.[12–16] An excellent clinical property of platelet-rich-plasma (PRP) is that it can be used autogenously and thus presents no risk in disease transmission. Therefore, PRP may provide a convenient and cheaper source for a tissue regeneration enhancer. Furthermore, PRP has the ability to release multiple GFs and is expected to have a higher tissue regeneration rate compared with a single GF.[17] However, the role of PRP as a promoter of bone healing remains controversial. For example, studies have indicated PRP may not be an appropriate adjunct to a demineralized bone matrix in some clinical applications, and this inconsistent response may be due to a rapid turnover of growth factors.[18] Many studies have attempted to combine the advantages of PRP with calcium phosphates, such as when PRP/β-tricalcium phosphate (β-TCP) composite material was applied for sinus floor augmentation, which led to the formation of new bone at a rate about 8–10% higher than β-TCP only.[7,19] However, combining PRP and a porous HA granule led to no significant difference when compared with the ceramic without PRP on bone ingrowth in rabbits, which was contrary to the expected result.[20] PRP improved the proliferation of mesenchymal stem cells (MSCs) on both β-TCP and calcium-deficient hydroxyapatite (CDHA) scaffolds but had a weak influence on osteogenic properties, which are also the recommended properties for PRP additives.[21]

Accordingly, studies using PRP *in vitro* and *in vivo* have failed to demonstrate an efficacy for bone healing "unless PRP is combined with other biomaterials to control the growth factor releasing". Calcium phosphate bone cement (CPC) is an ideal composite matrix that can be handled in a slurry form. Cement preparations have the major advantages that they can be moulded into complex shapes and that they can serve as injectable bone grafts.[22–24] The application of injectable materials could shorten operation times and minimize damaging effects to tissues, which would allow the patient to achieve a more rapid recovery.[17] Because the blood volumes of small animal models are too small to allow the production of a sufficient amount of autologous PRP, animal studies with the rabbit that combine CPC pastes, and PRP are rarely found *in vivo*. The 0–15 wt% amount of PRP additives has been tested in our pilot study (0–15 wt% PRP in CPC) *in vitro*. Our preliminary results showed that 10 wt% PRP in a CPC sample was the proper amount to not lead the pastes into dispersion when the sample was demoulding at 30 min after mixing and was immediately immersed in simulate body fluid (SBF) at 37 °C. The aim of the present study was to further investigate the effectiveness of a physiochemical property *in vitro* and the regenerative procedure *in vivo* based on combined CPC bone paste and PRP. The hypothesis was that they use of CPC with a 10 wt% PRP formation would enhance bone regeneration *in vivo*.

2. Methods and materials

2.1. Material preparation

The tetracalcium phosphate ($Ca_4P_2O_9$, abbreviated as TTCP) powder with a controlled mean particle size of 10.1 ± 0.7 μm was fabricated following the method from the reaction of dicalcium pyrophosphate ($Ca_2P_2O_7$; Sigma Chemical Co., St. Louis, MO) and calcium carbonate ($CaCO_3$) (Katayama Chem. Co., Tokyo, Japan).[25] Developed CPC with a nanocrystallite treatment on the surfaces of powders had demonstrated excellent mechanical properties, and the same procedures were employed.[6] Briefly, the process for preparing the CPC for this study required soaking TTCP powder through a 3 M diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) solution for 5 min, which was then filtered and dried immediately. The powder was mechanically ground to the mean particle size distribution of CPC (~3 μm), vacuum-packed and γ-ray-sterilized (20 kGy) (China Biotech Co., Taiwan). Functionally inactivated purified PRP powder (~5 μm) was prepared according to the method of Su et al., and platelet concentrates were subjected to a solvent/detergent treatment, oil extraction, hydrophobic interaction chromatography, and sterile filtration.[26]

2.2. In vitro physiochemical measurements

Both CPC powders, the CPC only and 10 wt% PRP/CPC samples, which contained 0.300 g of TTCP-based CPC with 0.030 g of PRP, were mixed with 0.12 mL of hardening solution (1 M $(NH_4)_2HPO_4$) at a pH of 8.1 to form a slurry. The ratio of PRP additives composited to CPC bone cement was 10 wt% and was designated the 10 wt% PRP/CPC group. To test their physiochemical properties, all pastes were well mixed for 1 min, moulded (6 mm × 3 mm (diameter × depth)) at 0.7 MPa pressure, and demoulded at 30 min after mixing to let the sample has an initial strength. Then, the sample was immersed in simulate body fluid (SBF) of Hanks' solution[27] at 37 °C for different periods of time (0.5, 1, 2, 4, 8, 16, and 24 h, 2, 4, 8, 16, and 32 days). For mechanical measurements through immersion, the immersion ratio was set at 1 g of sample to 10 mL of SBF for different immersion periods. The diametral tensile tests and recordings of stress versus strain of the wet specimens were measured immediately after the different immersion times were reached using a desktop mechanical tester (LLOYD instruments, Tokyo, Japan) at a crosshead speed of 2.0 mm/min. The modulus of elasticity was defined as follows:

$$\text{Modulus of elasticity } E = \frac{\sigma(t)}{\varepsilon_{\text{offset}}}$$

where $\sigma(t)$ is the stress (force: N/area: m^2; unit: Pa) and ε is the strain (deformation relative to the original sample length) at a