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Self-setting bioactive calcium–magnesium phosphate cement with high strength and degradability for bone regeneration

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Abstract

Calcium phosphate cement (CPC) has been successfully used in clinics as bone repair biomaterial for many years. However, poor mechanical properties and a low biodegradation rate limit any further applications. Magnesium phosphate cement (MPC) is characterized by fast setting, high initial strength and relatively rapid degradation in vivo. In this study, MPC was combined with CPC to develop novel calcium–magnesium phosphate cement (CMPC). The setting time, compressive strength, phase composition of hardened cement, degradation in vitro, cells responses in vitro by MG-63 cell culture and tissue responses in vivo by implantation of CMPC in bone defect of rabbits were investigated. The results show that CMPC has a shorter setting time and markedly better mechanical properties than either CPC or MPC. Moreover, CMPC showed significantly improved degradability compared to CPC in simulated body fluid. Cell culture results indicate that CMPC is biocompatible and could support cell attachment and proliferation. To investigate the in vivo biocompatibility and osteogenesis, the CMPC samples were implanted into bone defects in rabbits. Histological evaluation showed that the introduction of MPC into CPC enhanced the efficiency of new bone formation. CMPC also exhibited good biocompatibility, biodegradability and osteoconductivity with host bone in vivo. The results obtained suggest that CMPC, having met the basic requirements of bone tissue engineering, might have a significant clinical advantage over CPC, and may have the potential to be applied in orthopedic, reconstructive and maxillofacial surgery.

Keywords: Magnesium phosphate; Calcium phosphate; Calcium–magnesium phosphate cement; Biodegradability; Biocompatibility

1. Introduction

Calcium phosphate biomaterials, such as hydroxyapatite (HA) and tricalcium phosphate (TCP), have been widely used as bone substitute materials in clinic applications because of their excellent biocompatibility and osteoconduction [1–5]. Mostly, they are applied in the form of blocks or granules [6]. The incorporation of blocks may be limited due to the difficulty in filling irregular-shaped bone defects [7,8]. Some cases have presented secondary migration of granules and relatively lower mechanical strength compared to blocks [9–11]. Recently, much attention has been paid to calcium phosphate cements (CPCs).

The first CPC was reported in 1986 by Brown and Chow [12]. It consisted of tetracalcium phosphate (TTCP, $Ca_4(PO_4)_2O$) and dicalcium phosphate anhydrous (DCPA, $CaHPO_4$) [12]. The CPC powder could be mixed with an aqueous medium to form a paste that could conform to the bone cavity even for irregularly shaped cavities. The CPC paste could then set in situ to form HA ($Ca_{10}(PO_4)_6(OH)_2$) as the final product. This is the main compound in the mineral phase of bone and teeth [13–16]. Due to its high compatibility, osteoconductivity and bone replacement capability, CPC is a promising candidate for a wide range of clinical applications [12–16]. However, the currently used CPC has some limitations

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due to its poor initial mechanical properties, low biodegradation rate in vivo and relatively long setting time [17,18]. In clinical applications, a long setting time may cause the cement paste to crumble when it comes into contact with physiological fluids or when bleeding occurs due to the difficulty in achieving complete hemostasis in some cases [19–22]. Thus, it is desirable to develop a rapid-setting cement which could provide relatively high initial mechanical strength shortly after placement in a defect site.

Due to its characteristics of quick setting and development of high early strength, magnesium phosphate cement (MPC) is used as a rapid-repair material for deteriorated bridge decks, pavements, airport runways, etc. [23–25]. The main components of MPC are dead-burnt magnesia (MgO) and acid ammonium phosphates, particularly ammonium dihydrogen phosphate ($NH_4H_2PO_4$) [26–29]. The MPC powders react in the presence of water to form $NH_4MgPO_4{\cdot}6H_2O$ (struvite) as the final product [30]. During the last few decades, much work has been done to study the kinetics and mechanism of the reaction, microstructure, strength, porosity and hydration products [31–33]. However, little has been done to investigate its use as a biomaterial. Since 1999, our laboratory has been studying the biomedical applications of MPC. In 2001, Liu disclosed a magnesium phosphate cement as inorganic bone adhesive and its use in human hard tissue repairs [34]. This kind of MPC could set in 13.8 min and reached a strength of 35 MPa after hydration for 30 min. The results of MPC implanted into cavities in the femoral condyle of rabbits showed that the biocompatibility of MPC with the surrounding tissue was good, without showing any obvious foreign body reaction, inflammatory reaction or tissue necrosis during the 3 months implantation. Wu et al. studied the effect of this kind of MPC on fixing fractures of the rabbit tibia plateau [35,36]. The results showed that MPC degraded gradually and the in-growth of new bone was detected at 3 weeks after operation. At 6 weeks, the fracture line had disappeared. At 9 weeks after operation, the fracture was healed without displacement and the MPC was completely absorbed.

Considering the advantages and disadvantages of both CPC and MPC, it was assumed that a combination of MPC with CPC might result in a novel calcium–magnesium phosphate cement (CMPC) with improved properties. It is expected that the CMPC will have a shorter setting time and better mechanical properties than either CPC or MPC alone. Moreover, the CMPC might have significantly improved degradability in comparison with CPC due to the relatively fast degradation of MPC. Besides the setting time, mechanical strength and degradability, the biocompatibility of CMPC is an essential aspect that should be taken into consideration when suggesting its use as a biomaterial. The aim of the present study was to prepare CMPC and investigate its setting time, mechanical properties, in vitro degradability and in vitro biocompatibility by cell culture, as well as in vivo biodegradation. The hard tissue response to the CMPC was also investigated.

2. Materials and methods

2.1. Preparation and characterization of the cement samples

Generally, both CPC and MPC consist of powder and liquid phases. The CPC powder prepared in our laboratory was composed of TTCP and DCPA in an equivalent molar ratio, using preparation methods obtainable from the relevant literature [37]. Briefly, TTCP was synthesized by a solid-to-solid reaction between calcium phosphate and calcium carbonate at a temperature of 1500 °C for 8 h. Dicalcium phosphate dehydrate (DCPD, $CaHPO_4{\cdot}2H_2O$) was prepared from ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) and calcium nitrate ($Ca(NO_3)_2$) in an acidic environment. DCPA was obtained by removing the crystallization water in DCPD at 120 °C.

The MPC powder fabricated in our laboratory was composed of MgO and $(NH_4)H_2PO_4$ in a molar ratio of 3.8:1 [34]. The MgO was prepared by heating basic magnesium carbonate pentahydrate ($(MgCO_3)_4{\cdot}Mg(OH)_2{\cdot}5H_2O$) in a furnace at 1500 °C for 6 h. The resultant powder was first cooled to room temperature and then grounded in a planetary ball mill for 5 min, followed by sieving through 200 and 300 meshes, respectively. The grains in the range of 200 and 300 meshes were kept for further experiment. All the chemicals used were purchased from Sinopham Chemical Reagent Co. Ltd.

The self-setting CMPC comprised a powder and a liquid phase. The CMPC powders ($CMPC_{3/1}$, $CMPC_{1/1}$ and $CMPC_{1/3}$) were prepared by mixing CPC and MPC powders at different weight ratios. For example, the CMPC powder consisting of 50 wt.% CPC and 50 wt.% MPC was termed $CMPC_{1/1}$. Pure MPC and CPC powders were used as controls. The compositions of different cement powders are shown in Table 1. Deionized water was employed as the cement liquid for CPC, MPC and CMPC samples. Based on the workability of cement pastes, the powder to liquid (P/L) ratio of 8 g ml^{-1} was selected for MPC, $CMPC_{3/1}$, $CMPC_{1/1}$ and $CMPC_{1/3}$ samples. The optimum P/L ratio for CPC sample was 3 g ml^{-1}.

The cement powder was mixed with water for 1 min at the given P/L ratio using a spatula to form a paste. The paste was then loaded into a stainless-steel mold and periodically packed by means of a stainless-steel rod to about 2 kg. The sample was transferred to a glass tube, which was then sealed with plastic films and stored at 37 °C in a 100% humidity box for set periods of time. After setting for 24 h, the composition of the hardened cement body

Table 1
Composition of different cements

Cement	CPC (wt.%)	MPC (wt.%)
CPC	100	0
$CMPC_{3/1}$	75	25
$CMPC_{1/1}$	50	50
$CMPC_{1/3}$	25	75
MPC	0	100

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