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Properties of injectable ready-to-use calcium phosphate cement based on water-immiscible liquid

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ABSTRACT

Calcium phosphate cements (CPCs) are highly valuable materials for filling bone defects and bone augmentation by minimal invasive application via percutaneous injection. In the present study some key features were significantly improved by developing a novel injectable ready-to-use calcium phosphate cement based on water-immiscible carrier liquids. A combination of two surfactants was identified to facilitate the targeted discontinuous exchange of the liquid for water after contact with aqueous solutions, enabling the setting reaction to take place at distinct ratios of cement components to water. This prolonged the shelf life of the pre-mixed paste and enhanced reproducibility during application and setting reactions. The developed paste technology is applicable for different CPC formulations. Evaluations were performed for the formulation of an α-TCP-based CPC as a representative example for the preparation of injectable pastes with a powder-to-carrier liquid ratio of up to 85:15. We demonstrate that the resulting material retains the desirable properties of conventional CPC counterparts for fast setting, mechanical strength and biocompatibility, shows improved cohesion and will most probably show a similar degree of resorbability due to identical mineral structure of the set products.

1. Introduction

When acquired or congenital bony defects cannot heal during the natural regeneration process due to being of critical size, the application of bone substitution materials becomes necessary. These materials replace missing bone in the host tissue and stimulate the healing process by mechanical and structural support. This supporting effect can be attended by natural as well as artificial bone substitute materials and in a variety of ways. Due to the limited availability of autologous and allogeneic bone, combined with the related side-effect upon harvesting and poorly standardized quality, intensive research is being carried out into synthetic alternatives.

Calcium phosphate cements (CPCs) have gained clinical acceptance as valuable bone substitution biomaterials for over 20 years [1]. The term characterizes chemical formulations in the CaO–H_3PO_4–H_2O system. In general a mixture of several calcium phosphate salts in powder form and an aqueous solution is prepared to obtain a liquid or pasty matter, which transforms during the so-called setting reactions. The final product of this precipitation is a calcium phosphate solid body of either brushite or apatite depending on the composition of starting materials [2]. Owing to the similarities with the natural bone mineral phase, the hydroxyapatite(HAp)-forming compositions of CPCs show good biocompatibility, osteoconductivity and the possibility of getting resorbed in vivo [3–5].

Indications for clinical application of CPCs include irregular defects in bone, e.g. due to fracture, degenerative bone resorption, tumor, cyst removal or support of osteosynthetic device fixation [6]. For minimal invasive applications, i.e. spinal applications, vertebroplasty, bone void filling in closed fracture sites and the reinforcement of osteoporotic bone the CPC system has to fulfill distinct handling requirements, especially injectability [1,7]. Therefore, the consistency and cohesive properties are of special interest. Conventional powder/liquid-CPCs (pl-CPCs) exhibit intrinsic problems in handling since the paste must be prepared before implantation and cement setting starts immediately after mixing the powder and the aqueous component (Fig. 1a). Material properties change continuously during the working phase. This leaves only a limited window of preparation and application time to the surgeon, which in addition may be variable due to changes in environmental conditions. Consequently, quality features such as homogeneity and mechanical strength of the final implant may vary in a wide range, causing unexpected clinically relevant failures [8,9].

In order to overcome these major drawbacks, so-called ready-to-use cements were developed. The concept has been shown to work with single calcium phosphate phase powders or mixtures

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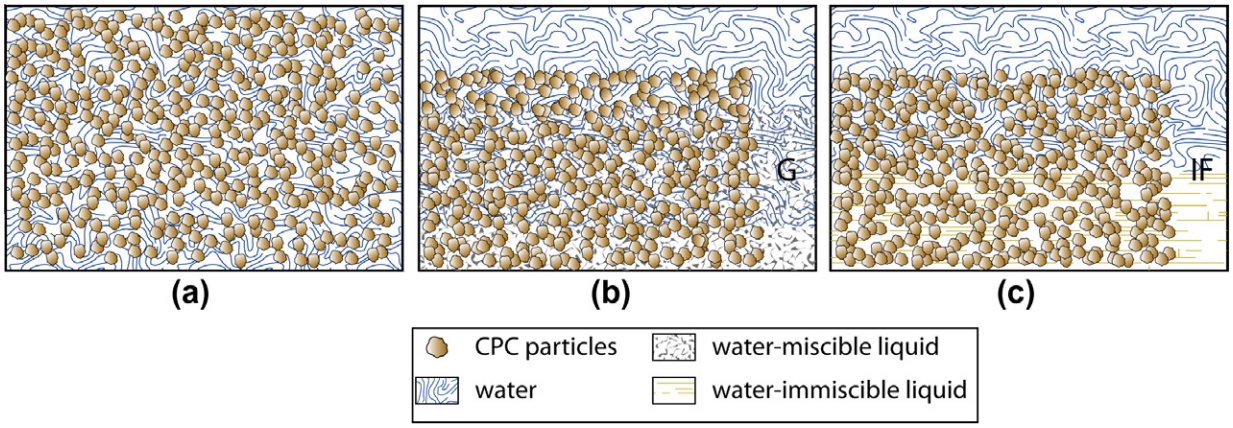

Fig. 1. Schematic illustration of the characteristic cement setting conditions for pl-CPC (a), ready-to-use paste-CPC based on water-miscible liquid (b) and ready-to-use paste-CPC based on water-immiscible liquid (c). The insets represent the gradient forming between the liquids (G in b) or the sharp interface (IF in c) between liquids.

of several components on both brushite and apatite cements [10]. On the one hand, ready-to-use cements can be obtained by stabilizing the different calcium phosphate reactants as separated liquid or pasty components, with at least one of them containing an aqueous liquid, which is needed to initiate the cement setting reactions after mixing the components. The preparation process is fast and reproducible since two liquid phases can be mixed more homogeneously than powder with liquid as performed for conventional CPC. Furthermore, this strategy allows usage of dual chamber syringes equipped with a mixing device, meaning reduced paste processing/handling time, less risk of contamination, enhanced reproducibility and immediate injection of the mixture in the host tissue defect. Another innovative approach for the preparation of ready-to-use cements is the use of non-aqueous yet water-miscible liquids, e.g. glycerine [11] or polyethylene glycol (PEG) [12] to prepare the CPC paste (Fig. 1b). Miscibility with water and biocompatibility of the components are considered to be the most important requirements for these systems [9]. As a result, the paste preparation is done under defined conditions, pre-mixed paste can be stored until use avoiding the powder and liquid mixing during surgery while retaining the adjusted viscosity and injection time for the surgeon is prolonged since the cement setting reaction only starts when delivered to the aqueous environment of the defect site [11–13]. This process involves a continuous increase of the water content in the CPC paste, which implies unpredictably changing chemical conditions for the cement setting reaction (characterized by the gradient G in Fig. 1b). Since the quality (e.g. strength, homogeneity) of the final biomaterial is very sensitive to the setting reactions, better control of the water balance is desirable for CPC-based bone substitution materials. Moreover, Ginebra et al. stressed the lack of experience concerning unpredictable moisturization during storage and the influence of exceeding expiry dates [9].

The aim of the present study was to develop a flexible system which allows the surgical injection of ready-to-use CPC cement, independent of the CPC formulation. The α-TCP based CPC formulation of Biocement D [14] was used as principal cement component. The strategy is based on the use of water-immiscible carrier liquids for the preparation of paste-CPC (Fig. 1c). As a result the cement setting reaction proceeds only after definitive substitution of the carrier liquid in paste-CPC by water, both liquids separated by a sharp interface (IF in Fig. 1c). This facilitates a defined CPC/water reaction as either the carrier liquid or water is in contact with the cement powder. The present study summarizes our efforts to prepare a ready-to-use and fully injectable CPC with long shelf life based on a conventional and clinically established α-TCP based composition.

2. Materials and methods

2.1. Cement formulation, powder preparation, paste preparation, and application

The composition of the powder used for the preparation of pl-CPC as well as for paste-CPC was similar to the formulation of Biocement D originally developed by Driessens and co-workers [14] and contained 60 wt.% α-TCP (α-$Ca_3(PO_4)_2$), 26 wt.% DCPA ($CaHPO_4$), 10 wt.% calcium carbonate ($CaCO_3$), and 4 wt.% precipitated HA (pHA). The dry powder was mixed in an agate ball mill (Pulverisette 5, Fritsch, Germany) with 30 g agate balls (Fritsch) at 200 rpm for 45 min. Particle size distribution of the resulting CPC powder was determined using a laser particle sizer system (Analysette 22, Fritsch). Manual mixing of the CPC powder and 4% Na_2HPO_4-solution resulted in pl-CPC with liquid to powder ratio L/P = 0.4 ml g^{-1}. Paste-CPC contained the described CPC powder with addition of 2.5% finely ground K_2HPO_4 in an oil-based suspension (synthetic short chain triglyceride Miglyol 812 with 8-12 C saturated fatty acids) at different powder/oil phase ratios. The oil phase contained two surface-active agents, 14.7% (w/w) castor oil ethoxylate 35 (Cremophor ELP, BASF, Germany) and 4.9% (w/w) hexadecyl-phosphate (Cetyl-phosphate, Amphisol A, Brenntag AG, Germany). CPC powder and oil phase were mixed in a stainless steel mixer (Stephan Mischer, Stephan Machinery GmbH, Germany) until homogeneity. A fraction of 400 g of the mixed paste was then transferred into a 500 ml zirconia beaker and further mixed and homogenized with 8 zirconia balls of 100 g each in a planetary ball mill (Pulverisette 5) for 3 h at 300 rpm. The obtained paste-CPC was transferred into either 3 ml syringes (Medmix, Switzerland) or 2, 5 or 10 ml syringes (Braun-Inject, B. Braun, Germany). Syringes were stored in sealed plastic containers with added desiccated silica pads. Paste-CPC was either tested directly or after sterilization by gamma-irradiation at 25 kGy (Synergy Health Radeberg, Germany).

2.2. Paste-CPC stability

Modifications of paste-CPC were obtained by varying the powder/oil phase ratio and by manual admixing of additional CPC-powder in an agate mortar. The method of stability testing was based on accelerated sedimentation. Therefore 2 g of paste-CPC were transferred to 15 ml tubes followed by centrifugation at 700 g for 20 min. The paste-CPC was considered stable when no oil drops separated from the paste after upside down mounting of the centrifuged tube.