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Strontium-modified premixed calcium phosphate cements for the therapy of osteoporotic bone defects

A. Lode [a], C. Heiss [b,c], G. Knapp [b,c], J. Thomas [d], B. Nies [e], M. Gelinsky [a], M. Schumacher [a,*]

[a] Centre for Translational Bone, Joint and Soft Tissue Research, Medical Faculty and University Hospital, Technische Universität Dresden, Dresden, Germany
[b] Department of Trauma, Hand and Reconstructive Surgery, University Hospital Giessen-Marburg GmbH, Campus Giessen, Germany
[c] Experimental Trauma Surgery, Justus-Liebig-University of Giessen, Germany
[d] Leibniz Institute for Solid State and Materials Research (IFW), Dresden, Germany
[e] INNOTERE GmbH, Radebeul, Germany

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ABSTRACT

In this study a premixed strontium-containing calcium phosphate bone cement for the application in osteoporotic bone defects has been developed and characterised regarding its material and in vitro properties as well as minimally invasive applicability in balloon kyphoplasty. Strontium was introduced into the cement by substitution of one precursor component, $CaCO_3$, with its strontium analogue, $SrCO_3$. Using a biocompatible oil phase as carrier liquid, a cement paste that only set upon contact with aqueous environment was obtained. Strontium modification resulted in an increased strength of set cements and radiographic contrast; and the cements released biologically relevant doses of Sr^{2+}-ions that were shown to enhance osteoprogenitor cell proliferation and osteogenic differentiation. Finally, applicability of strontium-containing cement pastes in balloon kyphoplasty was demonstrated in a human cadaver spine procedure. The cement developed in this study may therefore be well suited for minimally invasive, osteoporosis-related bone defect treatment.

Statement of Significance

Strontium-releasing calcium phosphate bone cements are promising materials for the clinical regeneration of osteoporosis-related bone defects since they have been shown to stimulate bone formation and at the same time limit osteoclastic bone resorption. Today clinical practice favours minimally invasive surgical techniques, e.g. for vertebral fracture treatment, posing special demands on such cements. We have therefore developed a premixed, strontium-releasing bone cement with enhanced mechanical properties and high radiographic visibility that releases biologically relevant strontium concentrations and thus stimulates cells of the osteogenic lineage. In a pilot experiment we also exemplify its excellent suitability for minimally invasive balloon kyphoplasty procedures.

1. Introduction

Osteoporosis (OP) is a systemic, metabolic disease characterised by a decrease in bone mass and deterioration of bone micro architecture [1]. With around 200 million cases worldwide OP is amongst the most frequent chronic bone disorders and place an enormous socio-economic burden on healthcare systems [2,3]. The pathogenesis of OP includes an imbalance of bone homeostasis, in particular a decrease in new bone formation and concurrent increase in bone resorption [4,5], that results in an increased risk of bone fractures. OP-related bone defects are frequently augmented using bone cements. A typical example for large OP-related bone defects are vertebral fractures [6]. A current treatment standard for such vertebral fractures are minimally invasive balloon kyphoplasty (BKP) procedures that employ polymethylmethacrylate (PMMA) cements to augment the bone [6,7]. In brief, BKP is a technique where vertebral body shape is restored under radiographic control by inflation of a percutaneously inserted balloon and subsequent reinforcement of the vertebra by cement augmentation [6]. Although PMMA is currently the material of choice, in particular for elderly patients, it bears potential cytotoxicity and an exothermic setting reaction of methylmethacrylate that eventually can lead to inflammatory reactions and even osteonecrosis.

* Corresponding author.
E-mail address: matthias.schumacher@tu-dresden.de (M. Schumacher).

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Furthermore, PMMA is non-resorbable and thus prevents bone remodelling. This has encouraged the development of calcium phosphate (CaP) bone cements especially for younger patients, where a full remodelling is desirable. The intrinsic material properties of CaP cements may have superior properties, and accordingly several studies have dealt with their application on BKP [8–10]. Conventional CaP cements are powder mixtures mainly comprised of calcium orthophosphates that, upon mixture with aqueous liquids, form a paste that self-set into a stable calcium phosphate phase within a short time (hence powder-liquid, pl-type cements) [11].

One example for synthetic CaP cements that can actively promote a specific tissue response and thus enhance bone, a concept that has been termed "next generation" biomaterials [12], are strontium-substituted CaP cements (SrCPC) that upon implantation release biologically active Sr^{2+} [13], an ion that has been successfully administered in OP therapy due to its dual effect on bone metabolism: It has been shown to stimulate proliferation and osteogenic differentiation of osteoblasts and to enhance bone formation [14–16] as well as to reduce the resorptive activity of osteoclasts [17,18].

In order to utilize CaP cements for BKP or other minimally invasive surgical techniques, they need to fulfil several requirements apart from general considerations regarding their biocompatibility. As summarized by Lewis et al., a high radiographic visibility, ease of preparation and application as well as mechanical properties that enable immediate reinforcement of the vertebral body are amongst the key requirements [19]. In this context, the limitations of pl-type cements are the need of mixing the precursor powder with the aqueous carrier liquid immediately prior implantation and the narrow time window for application. In contrast, a cement for minimally invasive application is ideally provided in a syringe and can be supplied via a small diameter nozzle directly into the defect without time constraints. This requires precise control of the cements paste characteristics, in particular its rheological properties determining extrudability, and setting, which has to be delayed to prevent premature hardening within the syringe, but should start directly after extrusion. A number of concepts have been developed to optimise CaP cements for minimally invasive application. For example, the use of setting retardants and polymeric additives [20] as well as non-aqueous carrier liquids [21–23] has been evaluated. As described by Heinemann et al. [24], oil-based carrier liquids allows the preparation of extrudable, ready-to-use cement pastes based on well-established CaP cement precursors. In a previous study we showed that such pasty CaP cements (p-type cements, pCPC) are suitable for extrusion-based 3D plotting and hence applicable via small diameter nozzles, cytocompatible and, although cell proliferation undergoes a prolonged lag-phase in direct contact with the cement, cells of the osteogenic lineage can differentiate into osteoblast-like cells on the material [25]. We further characterised the setting behaviour of pCPC and found that a setting regime of combined initial setting in humidity and subsequent ageing in direct contact to aqueous liquid is preferential to avoid crack-formation that derives from material swelling during penetration of water into the cement [26]. Since BKP is performed under radiographic control, a potential cement for this application should exhibit a suitable radiopacity. Due to the chemical resemblance of CaP cements to the mineral part of natural bone, they often cannot be distinguished from bone by X-ray imaging. Therefore, several radiopacifiers, e.g. zirconia [23] or strontium compounds [27–29] have been added to CaP cements to enhance their radiopacity.

Based on a previously developed, pl-type SrCPC, we now have developed a premixed, strontium-substituted CaP bone cement paste to be used for the therapy of osteoporotic bone defects as well as bone reconstruction procedures where an additional stimulus for new bone formation is desirable. Herein we describe this development and aim to characterise the new cement with respect to its material and in vitro characteristics in comparison with a Sr-free control group. Finally, we demonstrate the general applicability of the newly developed cement in balloon kyphoplasty in a pilot experiment on human cadaver spine.

2. Materials and methods

2.1. Cement and sample preparation

The precursor powder used to prepare premixed CaP cements in this study is based on the cement originally described by Driessens et al. and sets via the hydrolysis of α-tricalcium phosphate (α-TCP) to Ca-deficient hydroxyapatite (HA) [30]. As described previously, we prepared a strontium-substituted cement precursor by replacing $CaCO_3$ with $SrCO_3$ [31], which resulted in a strontium content of 2.21 at.% in the cement powder. The exact composition is detailed in Table 1. Then, precursor powders were mixed with 2.5 wt-% finely ground K_2HPO_4 and subsequently dispersed in a carrier liquid consisting of Miglyol 812 with 14.7 wt-% Cremophor ELP (BASF) and 4.9 wt-% Amphisol A (Brenntag AG) to obtain a cement paste with a final solid content of 86 wt-% as described by Heinemann et al. [24]. The resulting paste-like cements are denoted as pCPC for the strontium-free and pS100 for the strontium-containing cement.

Samples were prepared using silicone moulds (cuboids of $12 \times 6 \times 6$ mm^3 for porosity and mechanical characterisation, disc-shaped of 10 mm diameter and 5 mm height for radiographic characterisation and with 6 mm diameter and 1 mm thickness for cell culture experiments) and allowed to set in water-saturated atmosphere for 3 days at 37 °C (samples denoted as "initial"). This approach was chosen in order to avoid uncontrolled ion release as well as crack formation in the samples [26,31]. For analysis of phase composition, porosity and mechanical characteristics samples were either directly characterised or further aged in 10 ml per sample modified simulated body fluid (mSBF) prepared according to the protocol described by Oyane et al. [32] in sealed containers for 7, 14 and 21 days.

2.2. Cement characterisation

2.2.1. Paste characterisation

Cement pastes were characterized regarding their extrudability using a custom-made experimental setup (see Supplementary Fig. S1a). Therefore, 1 ml cement paste was filled into 1 ml syringes

Table 1
Composition of pCPC and pS100 cement precursors (α-tricalcium phosphate: α-TCP; calcium hydrogen phosphate: DCPA; hydroxyapatite: HA, calcium carbonate: $CaCO_3$; strontium carbonate: $SrCO_3$).

	Composition (wt.%)					Sr-content
	α-TCP	DCPA	HA	$CaCO_3$	$SrCO_3$	at.%
pCPC	60.0	26.0	4.0	10.0	–	–
pS100	57.3	24.8	3.8	–	14.1	2.21

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