Ceramics International 42 (2016) 15559–15567

Contents lists available at ScienceDirect

Ceramics International

journal homepage: www.elsevier.com/locate/ceramint

The importance of chitosan and nano-TiHA in cement-type composites on the basis of calcium sulfate

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ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form
30 June 2016
Accepted 1 July 2016
Available online 5 July 2016

Keywords:
Chitosan
Titanium doped hydroxyapatite
Calcium sulfate
Bone cements
Nano-hydroxyapatite

ABSTRACT

The objective of this study was to combine titanium doped nano-hydroxyapatite with chitosan and calcium sulfate to obtain cement-type materials with improved cohesion and handling properties. It was proven that the presence of nano-TiHA influenced setting, hardening, microstructure and bioactivity of the cement samples. In the developed composites chitosan played a role of the cohesion promotor and created homogenous organic layers on the materials surfaces. Application of chitosan in acetic acid solution favorably delayed setting process of calcium sulfate dihydrate. The polymer combined with nano-TiHA and calcium sulfate improved handling and workability of the cements without diminishing their mechanical properties. It was suggested that the resorption rate of materials based on calcium sulfate can be optimized by addition of both titanium doped nano-hydroxyapatite and chitosan.

1. Introduction

In the last decade, biomaterials for hard tissue reconstruction have been significantly improved and much advancement has been achieved. Synthetic bioactive bioceramics, including porous and dense calcium phosphate based materials (CaPs), are widely used as bone implants due to their great potential to support regeneration of bone tissue. Especially sintered hydroxyapatite (HA) is commonly applied in bone repair and augmentation for example as scaffolds or coatings on metal implants. Nowadays, substitution of various ions such as: Mg^{2+}, CO_3^{2-}, Co^{2+}, Mn^{2+}, Ag^{+} in crystal structures of calcium phosphates is the subject of intense investigations [1–5]. Recently some attention has been given to titanium as a modifier of HA structure [6–8]. Titanium doped hydroxyapatite (TiHA), revealing good biocompatibility and bioactivity, has been found to be a promising future implant material. TiHA may be synthesized using wet precipitation method [9–11]. Researches regarding titanium doped hydroxyapatite thin films using magnetron co-sputtering were also conducted [12]. Improved biocompatibility of TiHA coatings has been confirmed. *In vitro* observations showed good adhesion and proliferation of human osteoblast cells on the surface of TiHA what makes it promising material for future medical applications [11,12]. Major drawbacks of CaPs bioceramics include brittleness and low mechanical strength, which restricts their use to low load-bearing places in the skeletal system. Furthermore to fit a sintered, preformed ceramic scaffold in bone void, the surgeon has to machine the graft or carve the surgical site [13,14]. In this respect an interesting alternative for classical sintered bioceramics are chemically bonded implant materials, such as calcium phosphate bone cements (CPCs).

Bone cements composed of powder and liquid phase which when mixed together create a moldable paste which may be freely shaped and adjust to the bone defect. After introduction into the defect it sets in situ filling tightly the void and serve as an early support for bone cells. A number of studies regarding chemically bonded materials have already been conducted and especially inorganic-organic composites have attracted significant attention. Recently many combinations of such components have been developed to obtain materials with improved physicochemical and biological properties [15–17]. Nanocomponents (including nano-hydroxyapatite) for chemically bonded materials were also investigated and are believed to be the next step in the field of bone cements [18]. However, still some substantial understandings in terms of setting chemistry and physicochemical properties of bone cements need to be gained. One of the main disadvantages of calcium phosphate bone cements (CPCs) is slow degradation limiting complete regeneration of bone. Ideally, implant material should resorb, degrade and be progressively replaced by the newly forming tissue, whereas the common biodegradable ceramics show too slow or too fast partial degradation which cannot accord with the clinical requirements [19,20]. To increase the degradation rate they can be combined with other components. Calcium sulfate

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http://dx.doi.org/10.1016/j.ceramint.2016.07.003
0272-8842/

is a well-tolerated, biodegradable, osteoconductive bone graft substitute and is believed to promote process of angiogenesis, which plays a key role in regeneration of tissues [21]. Cements only on the basis of calcium sulfate tend to resorb and degrade too fast. Lack of direct contact between biomaterial and bone tissue since the first stage of implantation is one of the major problems in regenerative medicine. Stubbs et al. [22] showed that calcium sulfate applied as paste or pellets supports new bone formation but complete filling of bone defect was not observed due to too fast resorption process. To optimize the degradation rate of calcium sulfate it can be combined with less soluble, inorganic compounds such as CaPs bioceramics [23–28]. Furthermore, in order to improve characteristic of bone cements, mainly handling and mechanical properties, addition of polymers such as sodium alginate [29], sodium hialuronate [30], cellulose and its derivatives [31], glucan [32] or even starch [33] have been proposed. Recently attention has been focused on chitosan and its derivatives. Chitosan, the deacetylated chitin derivative, is known for its biocompatibility as well as the antibacterial and antifungal activity [34–36]. The incorporation of chitosan is a promising way to obtain cytocompatible composites. It has been demonstrated that it improves surgical handling properties, injectability, the degradation rate and mechanical strength of the resulting biomaterials [10,37–41]. Chitosan can be introduced as a solid component, for example in the form of granules or powder, however using chitosan solution as a liquid phase favors its homogeneous distribution in the final material.

The aim of this study was to apply titanium doped nano-hydroxyapatite and chitosan to improve physicochemical and biological properties of chemically bonded bioceramics on the basis of calcium sulfate. The role of individual components in developed materials was estimated.

2. Materials

Titanium doped nano-hydroxyapatite (TiHA) and calcium sulfate hemihydrate (CSH, Acros Organics) were used to produce the initial solid phases of cements. Nano-hydroxyapatite doped with 2.0 wt% of titanium was synthesized by the wet chemical method following the previously described procedure [10]. Briefly CaO (POCH, Poland), 85% solution of H_3PO_4 (POCH, Poland) and 15% solution of $TiCl_3$ (MERCK, Germany) were used as the substrates. During the precipitation process, pH was kept constant at 11 by addition of ammonia solution. Obtained precipitate was washed with distilled water, filtered and dried at 90 °C. Hydroxyapatite powder was grounded in a ball mill and sieved below 0.063 mm. Three types of TiHA powders: untreated (TiHA-1), calcined at 800 °C (TiHA-2) and at 1250 °C (TiHA-3) were used to prepare initial powder batches.

Chitosan is insoluble in water, but soluble in dilute aqueous acidic solutions, in which glucosamine units ($-NH_2$) can be convert into the soluble protonated form ($-NH_3^+$). The solubility of chitosan depends on its biological origin, molecular weight and degree of acetylation [34–36]. Medium molecular weight chitosan (~100,000 kDa, degree of deacetylation: 75–85%, Brookfield viscosity 200–800 cps in 1% solution in 1% acetic acid at 25 °C) was purchased from Sigma Aldrich. 1.0 wt% chitosan solution was produced by dissolving chitosan powder in 0.3 wt% acetic acid solution. The initial compositions of bone cements are summarized in Table 1.

Table 1
Initial composition of the cements.

Cement	Solid phase (P)	Temperature of TiHA calcination	Liquid phase (L)	L/P [g/g]
CS-A	CSH	–	Distilled water	0.52
CS-B	CSH	–	1.0 wt% chitosan solution in 0.3 wt% acetic acid	0.50
THCS-1	TiHA-1: CSH (2:3)	Non-calcined	1.0 wt% chitosan solution in 0.3 wt% acetic acid	0.60
THCS-2	TiHA-2: CSH (2:3)	800 °C		0.60
THCS-3	TiHA-3: CSH (2:3)	1250 °C		0.48

3. Methods

3.1. Phase composition

The crystalline phases of the initial TiHA powders as well as the set and hardened cement bodies were analyzed by powder X-ray diffraction with CuK_α radiation (D2 Phaser diffractometer, Bruker) within the 2θ range from 10–60° at a scanning speed of 1°/min. The phases were identified by comparing the experimental X-ray diffractograms to the Joint Committee on Powder Diffraction Standards: HA (JCPDS 01-070-0798), α-TCP (JCPDS 00-009-0348), perovskite – $CaTiO_3$ (JCPDS 01-075-0437), bassanit (JCPDS 00-006-0047) and calcium sulfate dihydrate (JCPDS 00-006-0047). Phase quantifications were calculated using the Rietveld method. Hydroxyapatite crystals were observed using transmission electron microscopy (TEM; JEOL JEM 1220).

3.2. Setting times

To produce cement pastes the powder batches were mixed with the liquid phases by a plastic spatula. Appropriate liquid to powder ratios were chosen to obtain optimal consistency and mouldability of the materials. The setting times of cement pastes were determined via Gilmore needles, according to the ASTM C266-08 standard [42]. All the experiments were performed at the room temperature (23 ± 2 °C). Each measurement was repeated 6 times and an average value was calculated.

3.3. Open porosity

The open porosity and pore-size distribution were studied by mercury porosimeter (AutoPore IV 9500). Open porosity and pore size distributions were generated from the pressure versus intrusion data using the Washburn equation [43].

3.4. Compressive strength

The compressive strength was measured using universal materials testing machine Instron 3345 at the crosshead displacement rate of 1.0 mm/min. Cylindrical samples of the cements were prepared of 6 mm in diameter and a height of 12 mm. Differences in compressive strength were analyzed with one-way ANOVA (the mark (*) means statistical significant difference between the results).

3.5. Morphology

The microstructures of fractured, hardened cement bodies and samples after immersion in simulated body fluid were observed