

## Brushite–collagen composites for bone regeneration

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### Abstract

Brushite-based biomaterials are of special interest in bone regeneration due to their biocompatibility and biodegradability; on the other hand, collagen is a well-known osteoconductive biomaterial. In the present study a new brushite–collagen composite biomaterial is reported. This new biomaterial was prepared by combining citric acid/collagen type I solutions with a brushite cement powder. The obtained biomaterial was a cement paste, with improved handling properties. The effect of collagen on the setting reaction of brushite cement was studied, and was found to speed up the cement setting reaction. The cement paste set into a hard ceramic material within  $18.5 \pm 2.1$  min and had compressive strength similar to that of spongy bone ( $48.9 \pm 5.9$  MPa in dry conditions and  $12.7 \pm 1.5$  MPa in humid conditions). The combination of collagen with citric acid revealed an interesting synergistic effect on the compressive strength of the composite material. Moreover, this new biomaterial had excellent cohesion properties (ninefold better than brushite cement), and high cellular adhesion capacity (threefold higher than brushite cement). The composite biomaterial described in this study combines good handling properties, compressive strength, cohesion and cell adhesion capacity, along with the osteoconductive and biodegradable properties inherent in brushite and in collagen-based biomaterials.

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### 1. Introduction

Complications associated with the use of autologous bone in the repair or replacement of tissue lost through injury or disease has driven the search for alternative sources of graft material. Different biomaterials have been used as scaffolds for bone tissue engineering. Bioceramics containing hydroxyapatite (HA) or tricalcium phosphate (TCP), or composites that combine the best properties of both components are among the principal inorganic bone graft substitutes [1].

Collagen is a widely investigated extracellular matrix biomaterial with well reported potential in the field of tissue engineering. Collagen coatings are known to enhance early cell adhesion and proliferation on HA and TCP, increasing the *in vivo* osteointegration, osteoconduction and osteoregenerative capacity of these materials [2,3].

For these reasons, composite materials containing calcium phosphates and collagen have been developed [4] using methods such as particle–gel mixing and powder compression [5–7].

In the mid-1980s Brown and Chow developed HA cements with acceptable mechanical properties for orthopedic and dental applications [5]. Recently, collagen–HA bone cements have been developed in order to combine the osteoconductive properties provided by collagen with the favorable mechanical properties of HA cements [8,9].

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Unfortunately, HA- and TCP-containing materials have a limited resorption rate in vivo due to the low solubility of these minerals at physiological pH.

Among the different biocompatible calcium phosphate phases present in human bone, dicalcium phosphate dihydrate (brushite) has a higher solubility than HA at physiological pH and an ideal in vivo resorption rate that can match the rate of new bone formation. Besides having mechanical properties similar to those of cancellous bone, brushite-based orthopedic cements are known to be biocompatible, osteoconductive and bioresorbable [10,11]. Therefore, brushite-containing materials are especially interesting for bone regeneration procedures [1].

Interestingly, type I collagen and brushite particles (DCPD) are both predominant in fracture callus [12,13]. The main structural framework of a human fracture callus consists of disordered, mineralized collagen fibrils containing calcium phosphate crystals. Brushite particles are found in the noncollagenous organic matter around nonmineralized, ordered collagen fibrils, and it is believed that these particles probably serve as the reservoir of calcium and phosphate ions for subsequent mineralization [14].

Deposition of DCPD mineral compensates for the insufficient mineralization of the collagen fibrils, making bone repair faster [14]. Fracture callus in children in fact contains much more brushite than that of adults, and it is for this reason, among others, that bone repairs itself twice as rapidly in children than in adults [15]. Therefore, a brushite–collagen biomaterial would be of interest as a means to produce a fast bone repair by simulating the bone-healing response.

Surprisingly, very few studies have focused on developing brushite–collagen composites for bone regeneration purposes. Recently, Jayaraman and Subramanian reported the first and only previously reported brushite–collagen composite by precipitating brushite crystals in a collagen gel placed in a crystallization chamber [16]. This biomaterial presented interesting biological properties, but the authors did not define its mechanical properties.

In this paper we exploit the low pH solubility of collagen and the low pH setting of brushite cement to form a self-setting brushite–collagen cement which has not been reported previously. This study investigates the new brushite–collagen composite that would combine the mechanical and biological properties of collagen and brushite cement. Enhancement of setting time, mechanical properties, cohesion properties and cellular adhesion are revealed.

## 2. Materials and methods

### 2.1. Collagen preparation

Bovine collagen was prepared in a similar manner to the method described elsewhere [17], except that acetic acid was replaced by citric acid in the collagen solution. Briefly, type I collagen was extracted from bovine tendons and processed in acetic acid solution to obtain sterile soluble colla-

gen. At first, crude collagen was dissolved in acetic acid, then frozen at  $-20\text{ }^{\circ}\text{C}$  and lyophilized to obtain a sponge, which could be stored at  $-80\text{ }^{\circ}\text{C}$  [17]. Lyophilized collagen was then dispersed in 0.1 M citric acid to obtain a sterile solution of collagen at 3 wt.%. This mildly acidic solution stabilized and dissolved the collagen [17] and, unlike acetic acid, which is normally used, did not have a detrimental effect upon the setting and handling properties of the brushite cement [18].

### 2.2. Brushite cement preparation and characterization

Brushite cement powder was prepared from an equimolar formulation of calcium phosphate monohydrate (MCPM; ABCR GmbH & Co. KG, Karlsruhe, Germany) and  $\beta$ -tricalcium phosphate, prepared as described previously [19].

The cement liquid phase was prepared from type I tendinous collagen (0–3%) to which citric acid was added to make 100–800 mM solutions. Citric acid solutions without collagen were used to make negative control cements.

The different cement liquid phases were mixed with the cement solid phase in a powder to liquid ratio of  $3.5\text{ g ml}^{-1}$ , forming a workable paste that was left to set at room temperature in cylindrical molds of two different sizes:  $6.0\text{ mm } \varnothing \times 12.0\text{ mm}$  and  $6.0\text{ mm } \varnothing \times 3.0\text{ mm}$ . The initial and final setting times of the cements were measured with Gilmour needles. After final setting, the samples were stored at  $37\text{ }^{\circ}\text{C}$  for 24 h before further testing.

The effect of water on brushite–collagen cement was tested by immersing both the control and brushite–collagen (3 wt.% in the liquid phase) samples, set in 0.8 M citric acid, in distilled water at  $37\text{ }^{\circ}\text{C}$  for 24 h, after the initial 24 h storage in dry conditions.

The effect of cross-linking on brushite–collagen composites was also studied by immersing collagen-containing cement samples (3% collagen; 0.8 M citric acid solution), in a 2% glutaraldehyde solution for 24 h at  $37\text{ }^{\circ}\text{C}$  after their initial 24 h storage in dry conditions.

Compressive strength mechanical testing of the  $6\text{ mm } \varnothing \times 12\text{ mm}$  cylindrical samples was measured with a universal testing machine (Instron<sup>®</sup> 5569, Instron Corp., Canton, MA) with a crosshead speed of  $0.1\text{ mm min}^{-1}$ .

A vertical-goniometer X-ray diffractometer (Philips model PW1710, Bedrijven b. v. S&I, The Netherlands), equipped with a Cu  $K_{\alpha}$  radiation source, was used for the powder diffraction pattern collection. Data were collected from  $20^{\circ}$  to  $40^{\circ}$  with a step size of  $0.02^{\circ}$  and a normalized count time of 1 s per step. The phase composition was examined by means of the International Centre for Diffraction Data (ICDD) reference patterns for  $\beta$ -TCP (PDF Ref. 09-0169), DCPA (PDF Ref. 09-0080), HA (PDF Ref. 09-0432) and DCPD (PDF Ref. 09-0077). The morphology of the samples was investigated using field-emission scanning electron microscopy (SEM; Model JSM-840) at a potential of 5 kV and a working distance of 12.0 mm.

ID	Title	Pages
1563	Brushite-collagen composites for bone regeneration	7

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