Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 3150–3156
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

The effect of hyaluronic acid on brushite cement cohesion

M.H. Alkhraisat [a,*], C. Rueda [a], F.T. Mariño [b], J. Torres [c], L.B. Jerez [d], U. Gbureck [e], E.L. Cabarcos [a]

[a] Departamento de Química-Física II, Facultad de Farmacia, UCM, 28040 Madrid, Spain
[b] Faculty of Dentistry, McGill University, Montreal, Que., Canada
[c] Departamento de Ciencias de la Salud III, Facultad de Ciencias de la Salud, URJC, Alcorcon-Madrid, Spain
[d] Departamento de Estomatología III, Facultad de Odontología, UCM, 28040 Madrid, Spain
[e] Department of Functional Materials in Medicine and Dentistry, University of Würzburg, 97070 Würzburg, Germany

Received 15 December 2008; received in revised form 2 March 2009; accepted 1 April 2009
Available online 7 April 2009

Abstract

The improvement of calcium phosphate cement (CPC) cohesion is essential for its application in highly blood perfused regions. This study reports the effectiveness of hyaluronic acids of different molecular weights in the enhancement of brushite cement cohesion. The cement was prepared using a powder phase composed of a mixture of β-tricalcium phosphate and monocalcium phosphate monohydrate, whereas the liquid phase was formed by 0.5 M citric acid solution modified by the addition of hyaluronic acid of different molecular weights. It was found that medium and high molecular weight hyaluronic acid enhances the cement cohesion and scarcely affects the cement mechanical properties. However, concentrations >0.5% (w/v) were less efficient to prevent the cement disintegration. It is concluded that hyaluronic acid could be applied efficiently to reduce brushite cement disintegration.

Keywords: Brushite; Calcium phosphate cement; Cohesion; Hyaluronic acid; Molecular weight

1. Introduction

Polymethylmethacrylate (PMMA) bone cement is the material of choice in vertebroplasty for the treatment of metastatic and cystic lesions as well as osteoporotic spine fracture [1]. However, the high compressive strength and stiffness of PMMA causes a biomechanical mismatch between treated and untreated vertebral levels allowing vertebral collapse [2]. Its highly exothermic setting reaction carries the risk of localized thermal tissue necrosis [3,4]. Furthermore, allergy to PMMA bone cement or its components has also been reported [5,6]. These drawbacks have encouraged scientists to develop an alternative to non-resorbable PMMA bone cement.

Many studies addressed these problems by examining ex vivo the biomechanical properties of several resorbable cements such as carbonated apatite cement (Norian, Cupertino, CA), bioactive cements (Orthocomp; Orthovita, Malvern, PA), calcium sulphate cements (MIIG X3; Wright Medical Inc., Arlington, TN), and calcium phosphate cements (α-BSM; ETEX Corp., Cambridge, MA). These studies reported that all these cements possess biomechanical profiles comparable with PMMA in simulated cadaveric vertebral compression fractures [3,7–9]. Moreover, using a canine vertebroplasty model, the strength and bone response of a biologically active CPC (BoneSource; Stryker-Leibinger, Freiburg, Germany) was compared with a conventional PMMA cement (PMMA, Simplex P; Stryker, Allendale, NJ) [1]. Vertebral body compressive strength testing revealed no significant differences between the cements. Histological analysis showed the formation of a thin fibrous membrane around PMMA

* Corresponding author. Tel.: +34 91 3941751; fax: +34 91 3942032.
E-mail address: mkhresat@farm.ucm.es (M.H. Alkhraisat).

1742-7061/$ - see front matter © 2009 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2009.04.001

cement, whereas the CPC underwent resorption and remodeling with vascular invasion and bone ingrowths [1].

The use of osteotransductive calcium phosphate cements in vertebroplasty is limited by their low injectability and disintegration. Liquid-phase separation, termed filter-pressing, is a frequent problem for cement injectability [10]. Viscosity-enhancing agents such as hyaluronic acid and chondroitin-4-sulphate are used to improve the injectability of brushite (ChronOs Inject, Synthes-Norian) and apatite (Biopex, Mitsubishi Materials) cements, respectively [10]. The adhesiveness of brushite cements due to the presence of sodium hyaluronate supported intimate contact between the cement and bone surface [11]. Moreover, the addition of hyaluronic acid to an injectable brushite cement had little effect on its osteoconductive properties, except for a slight decrease in initial resorption rate [12].

The cement cohesion, defined as the ability to stay in one piece during setting [10], is a determinant factor for CPC applications in highly blood perfused regions, such as vertebroplasty. Small volumes of intravascular CPC resulted in right ventricle occlusion and massive pulmonary embolism [13]. The authors hypothesized that cement microparticles might also act as a scaffold for platelet aggregation, and that the coagulation cascade might be enhanced by free calcium ions [13]. Furthermore, CPC particles <10 μm decreased the viability and proliferation of osteoblasts, reducing the production of extracellular matrix [14].

Bohner et al. developed a method, based on measuring the change in the cement paste weight caused by the sedimentation of small particles [15], to quantify calcium phosphate cement cohesion. A previous study [16] showed that this property is inversely related to the water solubility of calcium carboxylate for citric, glycolic and tartaric acids. Thus, the highest solid weight loss corresponded to cements set with citric acid. Furthermore, the cohesion was better for cements that had a shorter final setting time (FST). In addition, viscosity-enhancing agents improved the cement paste cohesion, but their effect was less pronounced in the set cement.

In many applications, the cement paste is injected into an aqueous environment and has to harden in these conditions. Thus, a high-viscosity paste is expected to perform better than a low-viscosity paste [17]. The use of high molecular weight hyaluronic acid was effective in improving the cohesion of brushite cement set with citric acid, as a result of increasing the viscosity of cement paste. However, the biological response of hyaluronic acid depends on its molecular weight. High molecular weight hyaluronic acid (>400 kDa) is antiangiogenic [18], anti-inflammatory [19] and inhibits phagocytosis by monocytes [20]. However, low molecular weight hyaluronic acid can promote angiogenesis and proinflammatory responses [21].

The purpose of this study is to address the effect of the concentration and molecular weight of hyaluronic acid (the viscosity-enhancing agent) on the cohesion of brushite cement. As the highest weight loss was recorded for cements set with citric acid (in comparison with the other carboxylic acids), these cements were used as a model to elucidate the effect of hyaluronic acid on cement cohesion. For that, the viscosity of the citric acid solution used as liquid phase was modified by adding different concentrations of hyaluronic acids of low, medium and high molecular weights. The resultant cement paste was then aged for 24 h at 37 °C and, subsequently, the cement physico-chemical properties and solid weight loss were characterized.

2. Materials and methods

Calcium carbonate ($CaCO_3$), dicalcium phosphate dihydrate (DCPD; $CaHPO_4{\cdot}2H_2O$), monocalcium phosphate monohydrate (MCPM; $Ca(H_2PO_4)_2{\cdot}H_2O$), sodium pyrophosphate ($Na_2H_2P_2O_7$), hyaluronic acids of 300, 750 and 1649 kDa (all from Sigma–Aldrich), and citric acid (from Panreac) were purchased and used without further purification.

The brushite-cement powder phase was composed of β-tricalcium phosphate (β-TCP) and MCPM in a molar ratio 1.32. β-TCP was produced by solid-state reaction of stoichiometric amounts of $CaCO_3$ and $CaHPO_4{\cdot}2H_2O$ at 900 °C for 14 h (Eq. (1)):

$$CaCO_3 + 2CaHPO_4 \cdot 2H_2O \rightarrow \beta\text{-}Ca_3(PO_4)_2 + 5H_2O + CO_2 \quad (1)$$

Pyrophosphate ions 0.54% (w/w) were used as a retardant of the setting reaction. The cement liquid phase was prepared by adding hyaluronic acids of different molecular weights (300, 750 and 1640 kDa) at concentrations of 0, 0.05, 0.1, 0.25 and 0.5% g ml^{-1} to a 0.5 M citric acid solution. In addition, for the 1640 kDa molecular weight, concentrations of 0.4, 0.5, 0.6, 0.8 and 1.6% were also used.

The cement setting reaction (Eq. (2)) was started by mixing the cement powder and liquid phase on a glass slab for 30 s at a powder to liquid (P/L) ratio of 2.5.

$$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O \quad (2)$$

After mixing, the cement paste was filled into cylindrical silicone molds with an aspect ratio of 2:1. The cements were tested for their FST according to the international standard ISO1566 for dental zinc phosphate cement at room temperature and humidity [22]. The cement is considered to be set when the Vicat needle 1 mm in diameter and loaded with 400 g fails to mark a visible circular indentation on the cement surface. The cement's mechanical properties were characterized by the diametrical tensile strength measured on a Pharma Test PTB 311 equipment after sample ageing in water for 24 h. Viscosity at zero shear rate was measured at 25 °C for citric acid solutions with different hyaluronic acids on a Rheostress 1 rotational rheometer (HAAKE, Karlsruhe, Germany) using a distance of 1 mm between the two plates.

ID	Title	Pages
1126	The effect of hyaluronic acid on brushite cement cohesion	7