Materials Science and Engineering C 45 (2014) 82–88

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Comparison of three calcium phosphate bone graft substitutes from biomechanical, histological, and crystallographic perspectives using a rat posterolateral lumbar fusion model

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ARTICLE INFO

Article history:
Received 1 June 2014
Received in revised form 7 August 2014
Accepted 29 August 2014
Available online 4 September 2014

Keywords:
Spinal fusion
Posterolateral lumbar fusion
Surface treatment
Calcium phosphate cements
Histology
Bioresorption

ABSTRACT

This study evaluated the effectiveness of three calcium phosphate bone graft substitutes with different chemical compositions on spinal fusion using a rat posterolateral lumbar fusion model. Specifically, two recently developed non-dispersive tetracalcium phosphate/dicalcium phosphate anhydrous-based calcium phosphate cements (CPCs), namely a CPC consisting of equimolar amounts of the two compounds (nd-CPC) and a CPC consisting of a two-fold greater amount of dicalcium phosphate anhydrous (DCP-rich CPC), were compared with a commercial calcium phosphate bone graft (c-CPG) consisting of hydroxyapatite (60%) and β-tricalcium phosphate (40%). Single-level posterolateral lumbar fusion was performed at the L4–L5 vertebrae in fifteen adult rats ($n = 5$ for each group). Spinal fusion was evaluated with radiographs, manual palpation, mechanical testing, micro-CT, and histology 8 weeks post-surgery. In particular, the crystallographic phases in the three substitutes were identified before and 8 weeks after their implantation. Manual palpation revealed stable constructs in nearly all of the spine specimens. The stiffness and bending load of fused spines in the two CPC groups were comparable to those in the c-CPG group. The radiographs specifically revealed implant resorption and bone remodeling in the DCP-rich CPC group. Analysis of 3D micro-CT images revealed that the bone volume ratio in the DCP-rich CPC group was significantly greater than those in the nd-CPC and c-CPG groups. Histology showed that the DCP-rich CPC group exhibited the highest degree of bone regeneration and osseointegration. Notably, DCP-rich CPC led to a pronounced phase transformation, generating the greatest amount of poorly crystalline apatite among the three groups, which together with adequate resorption may explain the aforementioned positive findings. We therefore conclude that of the bone graft substitutes considered, DCP-rich CPC has the greatest potential to be used in spinal fusion.

1. Introduction

Spinal fusion surgery is used to treat a variety of disorders associated with segmental instability. Among the various spinal fusion techniques, posterolateral lumbar fusion, which involves placing a bone graft between the transverse processes of the affected vertebrae, is the most commonly used [1]. Although spinal fusion, which occasionally fails due to pseudarthrosis, is a multifactorial process that involves numerous systemic and local factors [2], the bone graft used to form a bridging callus between the transverse processes plays a key role in the success of spinal fusion surgery. Autologous bone grafting remains the gold standard for achieving successful arthrodesis of the spine [3,4]. The use of autologous bone grafting, however, is limited by availability and donor site morbidity [5]. Bone graft substitutes that have been used in clinical practice include allografts, xenografts, demineralized bone matrices, and synthetic biomaterials. Allografts and xenografts also have several drawbacks, including disease transmission, host rejection, and higher infection risk. Synthetic biomaterials, particularly calcium phosphate (CaP) compounds [6], offer numerous benefits, such as unlimited availability, ease of use, and good biocompatibility without concerns of disease transmission.

Of the many synthetic biomaterials available, CaP ceramics, such as tricalcium phosphate (TCP) and hydroxyapatite (HA) in the form of granules or blocks, are widely used in dental and orthopedic

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http://dx.doi.org/10.1016/j.msec.2014.08.065
0928-4931/

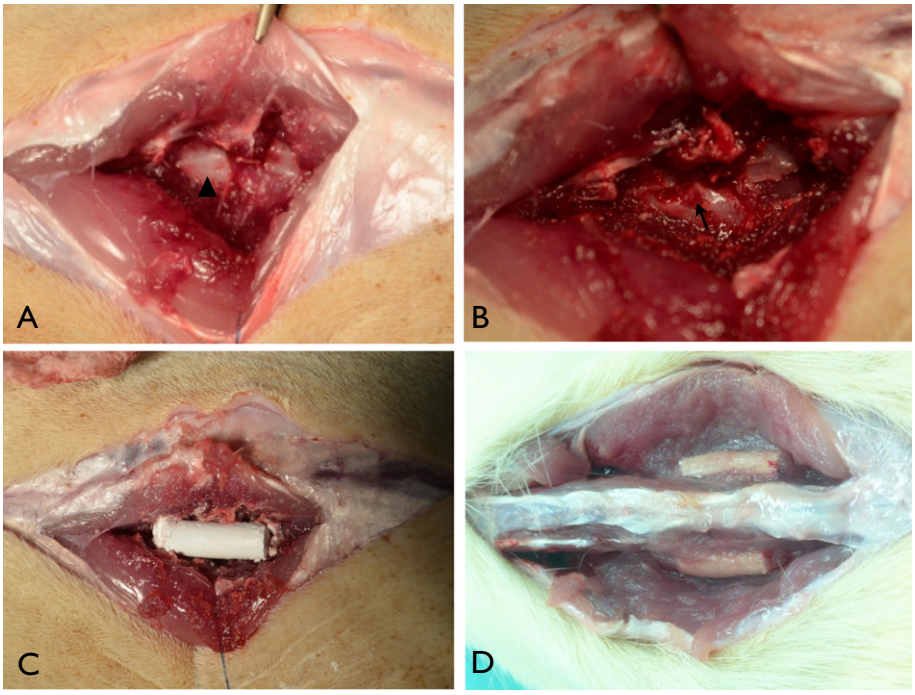

Fig. 1. Photographs showing the posterolateral lumbar fusion procedure. (A) The L4–L5 transverse process was exposed and identified. The arrowhead indicates the L5 transverse process. (B) Decortication with a low-speed burr was performed until the dorsal cortical bone was removed. The arrow indicates the decorticated L5 transverse process. (C) Bone graft (0.25 mL for each side) was placed onto the L4–L5 transverse process; nd-CPC is shown as an example. (D) After 8 weeks, the implanted site was exposed and the lower part of the spine (L1–L6) was harvested.

applications. Nevertheless, the clinical use of these ceramics has been partially replaced by calcium phosphate cements (CPCs) [7–9]. In general, CPC is inexpensive and is easy to handle and deliver. CPC is prepared as a paste which allows for the perfect filling of a bone defect or for injection into a bone defect using a minimally invasive approach. For most clinical applications, however, CPC requires washout resistance; that is, the ability to set in a liquid without disintegration. This issue can be overcome by adding gelling agents, such as hydroxypropyl methylcellulose and chitosan lactate, to the hardening solution to prepare non-dispersive CPCs. On the other hand, it was shown that growing nanocrystals on the surfaces of the reactants of a CPC significantly increases particle interlocking of the reactants and thus imparts washout resistance to the CPC [10].

Another issue regarding the use of CPCs in clinical applications is their resorption rate. It has been suggested that an appropriate resorption rate, which likely varies with the intended application, is the key to achieving optimum clinical results [7]. The *in vivo* bioresorption of a CPC partially depends on the solubility of the constituent phases and the final phase of the cement [11]. Although the most popular CPC, denoted herein as conventional CPC, is prepared by mixing tetracalcium phosphate (TTCP) powders with an equimolar amount of either anhydrous or dihydrous dicalcium phosphate powders (DCPA or DCPD, respectively) in an aqueous solution, CPCs with various compositions have been developed to adjust the resorption rate [12,13].

This study aims to investigate the effectiveness of three CaP bone graft substitutes with different chemical compositions, specifically with various Ca/P ratios, on spinal fusion using a rat posterolateral lumbar fusion model. Two recently developed TTCP/DCPA-based CPCs, namely a CPC consisting of equimolar amounts of the two compounds (nd-CPC) and a CPC consisting two-fold greater amount of DCPA (DCP-rich CPC) with initial Ca/P ratios of 1.67 and 1.50, respectively, were compared with a commercial calcium phosphate bone graft (c-CPG) consisting of HA (60%) and β-TCP (40%) and having an initial Ca/P ratio of 1.62. Both CPCs possess washout resistance due to nanocrystals grown on the surfaces of the reactants of the cements [14,15]. Although the two CPCs exhibited mechanical properties and *in vitro* cell interactions comparable to those of the conventional CPC [16], phase changes during their bioresorption *in vivo* remain unclear. Moreover, little is known regarding the bone fusion capability of the CPCs in mechanically and biologically harsh environments, specifically in posterolateral lumbar fusion surgery.

2. Materials and methods

2.1. Preparation of nd-CPC and DCP-rich CPC

The TTCP powder was prepared *via* the reaction sintering of dicalcium pyrophosphate ($Ca_2P_2O_7$; Alfa Aesar, MA) and calcium carbonate ($CaCO_3$; Shimakyu's Pure Chemicals, Japan) [16]. The median particle size of the TTCP powder was 12.6 μm. Commercial DCPA (Janssen Chemical Co., Japan) was ground using a mortar and pestle to prepare a powder with a median particle size of 2.1 μm.

The two CPCs were made non-dispersive by growing nanocrystals on the surface of one of their reactants. Surface-modified TTCP (mTTCP) [17] and surface-modified DCPA (mDCPA) [18] were prepared according to previously established methods. The nd-CPC essentially consisted of equimolar amounts of mTTCP and DCPA powders, whereas the DCP-rich CPC was a mixture of TTCP and mDCPA powers with a mDCPA-to-TTCP molar ratio of two. Phosphate buffer (1 M, pH = 5.6) was used as a hardening solution with a powder/liquid ratio of 3.0 g/mL. Prior to the operation, the powders were stirred in the hardening solution for 2 min. Then, the paste was loaded into a 1 mL syringe with the needle removed for injection. The working/setting times for the nd-CPC and the DCP-rich CPC are 13.5 ± 3.0 min/25.5 ± 3.5 min and 10.5 ± 1.0 min/27.0 ± 1.0 min, respectively, which are not significantly different ($n = 5$). The compressive strengths of the nd-CPC and the DCP-rich CPC after 24 h of immersion in physiological solution at 37 °C are 57.1 ± 12.5 MPa and 53.2 ± 8.6 MPa, respectively, which are not significantly different ($n = 10$) as well [10,13]. The commercial CaP bone graft (Bicera BC-B01, 5 × 5 × 20 mm bulk dimensions, Yuan Li Biotech, Taiwan), denoted as c-CPG, consists of HA (60%) and β-TCP (40%) with a 70% porosity and a total Ca/P ratio of 1.62. c-CPG samples