Acta Biomaterialia 7 (2011) 3459–3468

Contents lists available at ScienceDirect

Acta Biomaterialia

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

In vitro degradation rate of apatitic calcium phosphate cement with incorporated PLGA microspheres

R.P. Félix Lanao, S.C.G. Leeuwenburgh, J.G.C. Wolke, J.A. Jansen *

Department of Biomaterials, Radboud University Nijmegen Medical Center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 1 March 2011
Received in revised form 24 May 2011
Accepted 31 May 2011
Available online 6 June 2011

Keywords:
Calcium phosphate cement
PLGA
Microspheres
Degradation
In vitro

ABSTRACT

Calcium phosphate cements (CPCs) are frequently used as bone substitute material. Despite their superior clinical handling and excellent biocompatibility, they exhibit poor degradability, which limits bone ingrowth into the implant. Microspheres were prepared from poly(D,L-lactic-co-glycolic acid) (PLGA) and included in injectable CPCs as porogens in order to enhance its macroporosity after the polymeric microspheres had degraded. Upon degradation of the PLGA microspheres, acid is produced that enhances the dissolution rate of the CPC. However, the effect of the characteristics of PLGA microspheres on the degradation rate of CPCs has never been studied before. Therefore, the purpose of the current study was to investigate the dependence of CPC degradation on the chemical and morphological characteristics of incorporated PLGA microspheres. With respect to the chemical characteristics of the PLGA microspheres, the effects of both PLGA molecular weight (5, 17 and 44 kDa) and end-group functionalization (acid-terminated or end-capped) were studied. In addition, two types of PLGA microspheres, differing in morphology (hollow vs. dense), were tested. The results revealed that, although both chemical parameters clearly affected the polymer degradation rate when embedded as hollow microspheres in CPC, the PLGA and CPC degradation rates were mainly dependent on the end-group functionalization. Moreover, it was concluded that dense microspheres were more efficient porogens than hollow ones by increasing the CPC macroporosity during in vitro incubation. By combining all test parameters, it was concluded that dense PLGA microspheres consisting of acid-terminated PLGA of 17 kDa exhibited the highest and fastest acid-producing capacity and correspondingly the highest and fastest amount of porosity. In conclusion, the data presented here indicate that the combination of dense, acid-terminated PLGA microspheres with CPC emerges as a successful combination to achieve enhanced apatitic CPC degradation.

1. Introduction

Calcium phosphate cements (CPCs) are widely used as bone substitutes. They exhibit several advantages compared to other materials, such as biocompatibility, excellent osteoconductivity and injectability [1,2]. However, apatitic CPC is hardly degradable, which enables this material to act as a barrier for complete regeneration of a bone defect.

CPCs can be degraded by active or passive resorption. Active resorption is a cellular process mediated by osteoclasts, while passive resorption is based on the chemical solubility of the CPC material. The introduction of an adequate (macro)porosity and pore interconnectivity into the CPC will favor fluid flow as well as cell penetration, and as a consequence will accelerate its degradation.

A plethora of strategies have already been explored to introduce macroporosity into CPCs, but the majority of these strategies can only be applied to pre-made calcium phosphate ceramics. For example, porosity generation by means of particle leaching is incompatible with CPCs since the liquid component of the CPCs dissolves these inorganic porogens prior to setting of the cement in the bone defect site [3–8]. Foaming agents, such as carbon dioxide, hydrogen peroxide and albumin, have also been studied as macroporosity inducers [9–11]. Although an interconnected pore network can be generated by this approach, the pore size distribution cannot be controlled. In addition, the foaming agents can interfere with the in vivo setting properties of the CPC [12].

A more promising approach is offered by the inclusion of polymeric microspheres from natural (e.g. gelatin) or synthetic polymers (such as poly-trimethylene carbonate (PTMC), chitosan, pectin or poly(D,L-lactic-co-glycolic acid) (PLGA)) into the CPC in order to introduce macroporosity [1,13–15]. PLGA is a widely used and approved polymer. A major advantage of PLGA is that it degrades hydrolytically, leading to the production of its monomers lactic and glycolic acid, which are then incorporated into the Krebs cycle and excreted naturally as carbon dioxide and water. The acidic nature of the resulting monomers lactic and glycolic acid is an additional advantage of PLGA in combination with poorly

* Corresponding author. Tel.: +31 24 36 14920/14006; fax: +31 24 36 14657.
E-mail address: J.Jansen@dent.umcn.nl (J.A. Jansen).

1742-7061/$ - see front matter © 2011 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2011.05.036

degradable CPCs. These by-products acidify the surrounding area of the PLGA microspheres, which can lead to accelerated dissolution of the CPC, since calcium phosphate ceramics generally dissolve under acidic conditions [16]. However, it should be realized that an acidified environment caused by acidic degradation by-products can induce inflammatory responses in vivo [17]. For this reason, PLGA is often combined with other materials that can counteract excessive acidification, thereby reducing adverse tissue responses in vivo [18–20].

Though PLGA microspheres have been previously used in combination with CPC, there is a need to fine-tune the properties of PLGA microspheres to achieve a controlled polymer degradation and concomitant bone formation. For example, it is known that several parameters, such as the chemical characteristics of the used PLGA and the morphology of the microspheres, can affect their degradation and subsequent generation of CPC porosity. Regarding chemical characteristics, the major parameters to influence polymer degradation rate are: (i) the lactic/glycolic acid (L/G-A) ratio; (ii) molecular weight; and (iii) end-group functionalization. In this study, PLGA with a fixed 50:50 L/G-A ratio was selected because of its recognized fast degradation behavior [21,22]. Considering molecular weight, high molecular weight polymers are known to degrade in a slower ratio than low molecular weight polymers [23,24]. Further, functionalization of PLGA end-groups is an important property in polymer chemistry and more specifically in PLGA degradation [25,26]. However, in the majority of the studies dealing with PLGA, this material property has been ignored [27,28]. PLGA is commercially available with two different end-group functionalizations: end-capped or acid-terminated (Fig. 1). Acid-terminated PLGA, often referred to as uncapped or hydrophilic PLGA, exhibits a free carboxylic group at the polymer terminus. End-capping of the PLGA occurs after reaction of the polymer with an alcohol group and, as a result, the last acid group of the polymer is replaced by an ester group. This ester group at the end of the polymer results in a more hydrophobic polymer, which theoretically should delay PLGA degradation.

Besides the chemical characteristics of PLGA, the morphological structure of the prepared microspheres can be used to include more PLGA in a similar pore volume. Both dense and hollow PLGA microspheres have been reported in the literature, but the effect of this morphological feature has never been studied in a direct comparative study. It can be hypothesized that the larger PLGA content upon incorporation of dense PLGA microspheres will enhance the degradation of CPC matrices and consequently increase the amount of porosity formation.

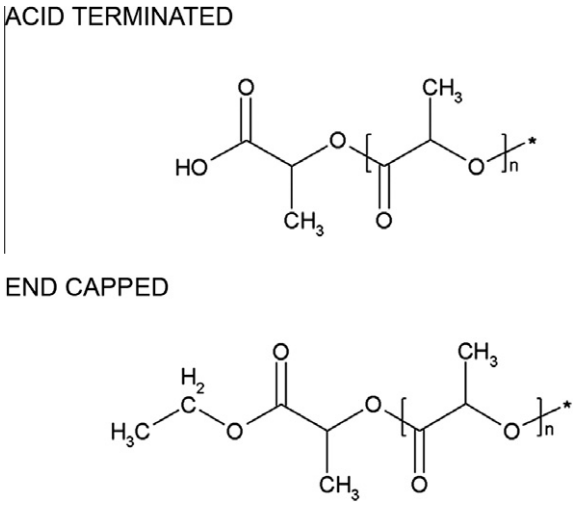

Fig. 1. PLGA end-group functionalizations.

The aim of the current study was to investigate the effect of chemical and morphological properties of PLGA microspheres on the in vitro degradation rate of these microspheres as well as the CPC–PLGA composites that contain these microspheres. Therefore, the effects of PLGA molecular weight (5, 17 and 44 kDa), PLGA end-group functionalization (acid-terminated vs. end-capped) and microsphere morphology (dense vs. hollow) on the degradation of PLGA microspheres and CPC matrices were studied. Although it is known that the particle size of polymeric microspheres can affect their degradation rate [29,30], the particle size was fixed at 40 μm in the current study to eliminate material parameters other than PLGA molecular weight, end-group functionalization and particle morphology. The particle diameter of 40 μm was selected based on the results obtained in previous in vivo experiments [31].

For this part of the study, only PLGA-17A was selected, which was based on the obtained molecular weight and end-group functionalization data. CPC and PLGA degradation were evaluated by scanning electron microscopy (SEM), microcomputed tomography (micro-CT) and reverse-phase high performance liquid chromatography (RP-HPLC).

2. Materials and methods

2.1. Materials

Poly(lactic-co-glycolic) acid (PLGA) Purasorb® materials were provided by Purac (Gorinchem, The Netherlands). Five types of polymers were used to prepare the microspheres: Purasorb® PDLG 50021A (M_w = 5 kDa, acid-terminated, L:G = 50:50); Purasorb® PDLG 5002A (M_w = 17 kDa, acid-terminated, L:G = 50:50); Purasorb® PDLG 5002 (M_w = 17 kDa, end-capped, L:G = 50:50); Purasorb® PDLG 5004A (M_w = 44 kDa, acid-terminated, L:G = 50:50) and Purasorb® PDLG 5004 (M_w = 44 kDa, end-capped, L:G = 50:50). For the preparation of the PLGA microspheres, polyvinyl alcohol (PVA) (88% hydrolyzed, M_w 22,000, Acros, Geel, Belgium), isopropanol (IPN) (analytical grade, MERCK, Darmstadt, Germany) and dichloromethane (analytical grade, MERCK) were used. CPC (85% alpha-tricalcium phosphate (α-TCP), 10% anhydrous dicalcium phosphate (DCPA) and 5% precipitated hydroxylapatite (pHA)) and 2% Na_2HPO_4 (MERCK) were the components of the CPC phase. For the RP-HPLC analysis, acetonitrile (super gradient; Lab Scan Sciences) and NaH_2PO_4 (MERCK) were used.

2.2. Preparation of PLGA microspheres

Five different types of PLGA hollow microspheres were prepared: 5 kDa acid-terminated (PLGA-5A), 17 kDa acid-terminated (PLGA-17A), 44 kDa acid-terminated (PLGA-44A), 17 kDa end-capped (PLGA-17E) and 44 kDa end-capped (PLGA-44E). We did not prepare microspheres with 5 kDa end-capped PLGA, since this material was not commercially available.

To study the effect of microsphere morphology, dense microspheres were produced from both acid-terminated and end-capped PLGA while the molecular weight was fixed at 17 kDa.

Hollow microspheres were prepared by a double emulsion technique. For this, 1.0 g of PLGA was dissolved in 4 ml of dichloromethane in a 20 ml glass tube. Then 500 μl of demineralized water (ddH_2O) was added, and the mixture was emulsified at 8000 rpm for 90 s. Subsequently, 6 ml of a 0.3% PVA solution was added and emulsified again at 8000 rpm for 90 s (ULTRA-TURRAX® high-performance disperser (IKA®)). The contents of the glass tube were transferred into a stirred 1000 ml beaker containing 394 ml of 0.3% PVA solution, and 400 ml of a 2% IPN solution was immediately added. The solution was stirred for 1 h. The spheres were allowed to settle for 15 min and the clear solution was

ID	Title	Pages
891	In vitro degradation rate of apatitic calcium phosphate cement with incorporated PLGA microspheres	10