Acta Biomaterialia 9 (2013) 8037–8045

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Review

Calcium phosphate ceramics in bone tissue engineering: A review of properties and their influence on cell behavior

Satyavrata Samavedi [a], Abby R. Whittington [a,b,c], Aaron S. Goldstein [a,c,*]

[a] Department of Chemical Engineering, Virginia Tech, Blacksburg, VA 24061, USA
[b] Department of Materials Science and Engineering, Virginia Tech, Blacksburg, VA 24061, USA
[c] School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form 14 May 2013
Accepted 11 June 2013
Available online 19 June 2013

Keywords:
Calcium phosphate
Ceramics
Osteoinduction
Protein adsorption
Cell adhesion

ABSTRACT

Calcium phosphate ceramics (CPCs) have been widely used as biomaterials for the regeneration of bone tissue because of their ability to induce osteoblastic differentiation in progenitor cells. Despite the progress made towards fabricating CPCs possessing a range of surface features and chemistries, the influence of material properties in orchestrating cellular events such as adhesion and differentiation is still poorly understood. Specifically, questions such as why certain CPCs may be more osteoinductive than others, and how material properties contribute to osteoinductivity/osteoconductivity remain unanswered. Therefore, this review article systematically discusses the effects of the physical (e.g. surface roughness) and chemical properties (e.g. solubility) of CPCs on protein adsorption, cell adhesion and osteoblastic differentiation in vitro. The review also provides a summary of possible signaling pathways involved in osteoblastic differentiation in the presence of CPCs. In summary, these insights on the contribution of material properties towards osteoinductivity and the role of signaling molecules involved in osteoblastic differentiation can potentially aid the design of CPC-based biomaterials that support bone regeneration without the need for additional biochemical supplements.

1. Introduction

Calcium phosphate ceramics (CPCs) are a class of tunable bioactive materials that have been widely used for bone tissue repair and augmentation [1]. They possess surface properties that support osteoblast adhesion/proliferation (i.e. osteoconduction) and stimulate new bone formation (i.e. osteoinduction) [2,3]. More significantly, CPCs have been shown to promote bone growth in vivo [4], and recruit bone marrow stromal cells (BMSCs) to ectopic sites to induce bone formation [5]. However, not all types of CPCs have the same biological effect in vivo [6–8]; while most are osteoconductive, only certain types are osteoinductive [9]. Such differences in their ability to induce osteoblastic differentiation are related to subtle differences in the physical and chemical properties of CPCs. For example, chemical properties such as surface chemistry and charge can influence biological phenomena like protein adsorption [10], which can subsequently drive osteoblastic differentiation via cell–extracellular matrix (ECM) interactions [11,12]. Likewise, physical properties such as surface roughness can also aid cell differentiation by influencing cell adhesion [13]. Furthermore, certain surface features can facilitate the adsorption of cell-adhesive proteins and thus provide conditions conducive to the formation of stable focal adhesive complexes. Therefore, understanding the roles of specific material properties in modulating cell behavior is critical towards designing osteoinductive CPCs.

This review discusses the influence of the physical and chemical properties of CPCs on the adhesion and osteoblastic differentiation of cells in the context of bone tissue engineering (BTE). In particular, it seeks to explain how the differences in CPC properties, such as surface roughness, chemistry, solubility and crystallinity, correlate to differences in osteoconductivity and osteoinductivity (Fig. 1). Although this review is intended to provide an understanding of cell-level interactions with CPCs, it discusses only a limited number of important studies that are relevant. The review begins by highlighting the osteoinductive effects of CPCs in vitro (Sec. 2), and then summarizes important chemical properties of four types of CPCs commonly used in BTE, namely hydroxyapatite (HAP), tricalcium phosphate (TCP), amorphous calcium phosphates (ACPs) and biphasic calcium phosphates (BCPs) (Sec. 3). Since osteoblastic differentiation is also regulated via other biological phenomena, Sec. 4 provides an analysis of how CPC properties affect two key precursor events: protein adsorption (which occurs immediately upon CPC implantation within the body) and cell adhesion (which follows protein adsorption). Here, the review does

* Corresponding author. Address: Department of Chemical Engineering, Virginia Tech, 133 Randolph Hall, Blacksburg, VA 24061-0211, USA. Tel.: +1 540 231 3674; fax: +1 540 231 5022.
E-mail address: aaron.goldstein@vt.edu (A.S. Goldstein).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.06.014

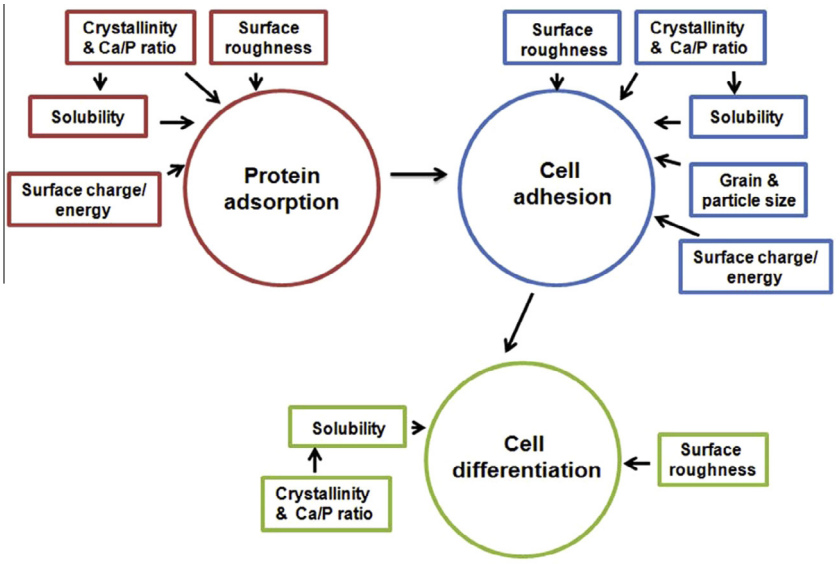

Fig. 1. Schematic of key CPC properties that affect a cascade of biological processes including protein adsorption, cell adhesion and cell differentiation.

not describe data from in vivo experiments due to the difficulties associated with discussing the complex experimental settings found in animal studies. Sec. 5 provides a summary of possible signaling pathways involved in osteoblastic differentiation in the presence of CPCs, with special focus on the roles of ions and focal-adhesion mediated changes. Finally, Sec. 6 offers concluding remarks concerning the present and future roles of CPCs in BTE.

2. Osteoinductivity of CPCs

Osteoinductivity is the ability of a material to recruit and induce progenitor or undifferentiated cells to differentiate towards the osteoblastic lineage [3]. Common markers of the osteoblastic phenotype include type I collagen, alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2), osteopontin (OPN), osteocalcin (OCN) and bone sialoprotein (BSP); the roles of these and other proteins involved in osteoblastic differentiation are summarized in Table 1 [14–23]. The osteoinductivity of CPCs in particular varies significantly depending on material properties. In addition, cell type and the presence of osteogenic supplements also influence the degree of osteoinduction. Typically, undifferentiated mesenchymal stem cells (MSCs) such as BMSCs and adipose-derived stem cells (ADSCs) offer a more rigorous test of osteoinductivity than osteoblastic cell lines such as MC3T3-E1 and MG63. However, MSCs are also commonly cultured in the presence of osteogenic supplements to direct their differentiation towards the osteoblastic lineage. While the use of osteogenic supplements can potentially marginalize the contribution of CPCs, a considerable number of studies have reported the osteoinductive effect of CPCs in their presence. Therefore, the following sections summarize the effect of CPCs on cell differentiation both in the presence and absence of osteogenic supplements.

2.1. Osteoinduction in the presence of osteogenic supplements

In vitro studies of osteoblastic maturation conducted in the presence of osteogenic supplements (e.g. dexamethasone, ascorbic acid and β-glycerolphosphate [24]) show that CPCs generally have a positive osteoinductive effect. For example, Lee et al., who examined the differentiation of human MSCs on electrospun scaffolds with and without HAP, showed elevated expression of ALP, OCN and BSP mRNA at days 14 and 21 on scaffolds loaded with HAP [25]. Further, the authors found that the expression of ALP, BSP and OCN mRNA increased with increasing HAP content, indicating a positive osteoinductive effect of HAP loading concentration. In another study, Eslaminejad et al. showed an increase in the expression of OCN and OPN mRNA from day 1 to day 21 by rat BMSCs cultured on alginate–gelatin–β-TCP scaffolds in the presence of osteogenic supplements [26]. While the studies by Lee et al. and Eslaminejad et al. indicate that the incorporation of a CPC phase into a scaffold can promote osteoblastic differentiation, comparison of the osteoinductivity of CPCs with different stoichiometries and crystallinities reveals diverse trends. In a study comparing ACP and HAP of similar particle sizes, Hu et al. showed that HAP in-

Table 1
Markers of osteoblastic differentiation [14–20].

Name	Type	Primary function during differentiation
Runx2	Transcription factor	Helps in the differentiation of MSCs into immature osteoblasts
Osterix (OSX)	Transcription factor	Acts downstream of Runx2 and promotes osteogenesis, while inhibiting chondrogenesis
Alkaline phosphatase (ALP)	Enzyme	Increases local concentration of phosphate ions that in turn initiate mineral growth
Type I collagen	ECM protein	Mineral nucleation begins at the junction between two collagen fiber bundles
Osteopontin (OPN)	ECM protein	Acts as an inhibitor towards irregular formation of mineral crystals
Bone sialoprotein (BSP)	ECM protein	Binds to Ca^{2+} via free hydroxyl groups and promotes nucleation of mineral
Osteocalcin (OCN)	ECM protein	Regulates mineral growth, direction, size and quantity during late stages of differentiation
Osteonectin (ONN)	ECM protein	Similar role to that played by osteocalcin
Bone morphogenic protein 2 (BMP2)	Growth factor	Secreted by immature osteoblasts and may trigger a variety of autocrine/paracrine pathways
Bone morphogenic protein 7 (BMP7)	Growth factor	Similar role to that played by BMP2