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Role of biphasic calcium phosphate ceramic-mediated secretion of signaling molecules by macrophages in migration and osteoblastic differentiation of MSCs

Jing Wang [a], Dan Liu [a], Bo Guo [b,*], Xiao Yang [a], Xuening Chen [a], Xiangdong Zhu [a,*], Yujiang Fan [a], Xingdong Zhang [a]

[a] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China
[b] Department of Ophthalmology, West China Hospital of Sichuan University, Chengdu 610041, China

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ABSTRACT

The inflammatory reaction initiates fracture healing and could play a role in the osteoinductive effect of calcium phosphate (CaP) ceramics, which has been widely confirmed; however, the underlying mechanism has not been fully elucidated. In this study, various signaling molecules from macrophages under the stimulation of osteoinductive biphasic calcium phosphate (BCP) ceramic and its degradation products were examined and evaluated for their influence on the migration and osteoblastic differentiation of mesenchymal stem cells (MSCs). The results of cellular experiments confirmed that the gene expression of most inflammatory factors (IL-1, IL-6 and MCP-1) and growth factors (VEGF, PDGF and EGF) by macrophages were up-regulated to varying degrees by BCP ceramic and its degradation products. Cell migration tests demonstrated that the conditioned media (CMs), which contained abundant signaling molecules secreted by macrophages cultured on BCP ceramic and its degradation products, promoted the migration of MSCs. qRT-PCR analysis indicated that CMs promoted the gene expression of osteogenic markers (ALP, COL-I, OSX, BSP and OPN) in MSCs. ALP activity and mineralization staining further confirmed that CMs promoted the osteoblastic differentiation of MSCs. The present study confirmed the correlation between the inflammatory reaction and osteoinductive capacity of BCP ceramic. The ceramic itself and its degradation products can induce macrophages to express and secrete various signaling molecules, which then recruit and promote the MSCs to differentiate into osteoblasts. Compared with BCP conditioned media, degradation particles played a more substantial role in this process. Thus, inflammation initiated by BCP ceramic and its degradation products could be necessary for osteoinduction by the ceramic.

Statement of Significance

It is known that the inflammatory reaction initiates fracture healing. The aim of this study was to examine whether osteoinductive BCP ceramics could cause macrophages to change their secretion patterns and whether the secreted cytokines could affect migration and osteoblastic differentiation of MSCs. Moreover, the duration of inflammation could be influenced by the local ionic environment and the degradation products of the implant. Our experimental results revealed the correlation between the inflammatory reaction and osteoinductive capacity of BCP ceramic. The ceramic itself and its degradation products can induce macrophages to express and secrete various signaling molecules, which then recruit and promote the MSCs to differentiate into osteoblasts. Compared with ionic microenvironment, degradation particles played a more substantial role in this process. Therefore, the appropriate inflammation initiated by BCP ceramic and its degradation products could be essential for osteoinduction by the ceramic. We believe that the present study improves the understanding of the effect of biomaterial-mediated inflammation on MSC migration and differentiation and established a preliminary correlation between the immune system and osteoinduction by biomaterials.

* Corresponding authors.
E-mail addresses: guobohx@163.com (B. Guo), zxd7303@163.com (X. Zhu).

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Abbreviations

IL-1	Interleukin 1
IL-6	Interleukin 6
IL-12	Interleukin 12
IL-17A	Interleukin 17A
TNF-α	Tumour necrosis factor alpha
MCP-1	Macrophage chemotactic protein 1
MIP-1	Macrophage inflammatory protein 1
RANTES	Regulated on activation, normal T cell expressed and secreted
Eotaxin	Eosinophil chemotactic protein
MDC	Macrophage Derived Chemokine
TGF-β	Transforming growth factors beta
VEGF	Vascular Endothelial Growth Factor
EGF	Epidermal Growth Factor
PGDF	Platelet derived growth factor
IGF	Insulinlike Growth Factor
FGF	Fibroblast Growth Factor
ALP	Alkaline phosphatase
COL-I	Collagen I
RUNX2	Runt-related transcription factor
OSX	Osterix
OPN	Osteopontin
BSP	Bone sialoprotein
OCN	Osteocalcin

1. Introduction

CaP ceramics, principally including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and a mixture of HA and β-TCP (called BCP), are frequently used as bone substitute materials in orthopaedic and dental clinics due to their chemical similarity to bone mineral and their desirable characteristics, such as high bioactivity, osteoconduction and even osteoinduction [1–3]. In particular, osteoinduction has attracted a great deal of interest in recent years because if CaP ceramics are osteoinductive, they could directly replace autogenous bone grafts for repairing bone defects without the pre-addition of exogenous MSCs and growth factors inside the ceramics. Consequently, since osteoinductive phenomena were observed and confirmed in the 1990s [4–6], efforts to understand the underlying osteoinductive mechanism and develop more effective CaP ceramics have been ongoing.

A wealth of knowledge regarding osteoinduction by CaP ceramics has been accumulated in the past few decades. Previous investigations have clearly shown that the potential osteoinductive capacity of CaP ceramics is strongly dependent on the property of the ceramics, including the surface chemistry, surface topography, 3D architecture, degradation properties and ionic microenvironment [7–14]. Thus, not all types of CaP ceramics can successfully realize an osteoinductive outcome. Although the exact underlying mechanism of the osteoinductive phenomenon remains to be further addressed, CaP ceramics are believed to be directly involved in recruiting MSCs and promoting osteogenic differentiation of MSCs because it is widely accepted that MSCs are the first osteogenic cells recruited to the surface of CaP ceramics implanted into bone or non-osseous (ectopic) sites. Thus, to date, the understanding of CaP ceramics' osteoinductive mechanism has been reasonably restricted to an awareness of how the implanted CaP ceramics affect MSCs. However, the accepted ideas have been challenged by recent experimental evidence showing that macrophages also play important roles in recruiting MSCs and secreting growth factors in the course of new bone formation [15–20].

It is well known that an object implanted into a living body will elicit a cascade of biological events, such as vascular exudation, cellular infiltration and inflammatory reaction, in which macrophages, which are one of the most important inflammatory cell types, modulate a series of biological reactions by secreting a variety of cytokines, such as inflammatory cytokines (IL-1β, IL-6, TNF-α, IL-10, etc.) and growth factors (VEGF, PDGF, FGF, TGF-β, etc.). These signaling molecules play a peculiar role in cellular processes that are related not only to wound healing but also to osteogenesis [16,19,21–23]. For instance, both TNF-α and IL-1β have been shown to increase the migration of MSCs [24,25]. TNF-α can also promote the osteogenic differentiation of MSCs [26].

Many previous reports have indicated that macrophage cytokine secretion is predominantly dependent on the properties of the implant, including chemical components [27,28], dimensions [29,30], substrate stiffness [31], surface topography [32–34] and degradability [35]. Therefore, CaP ceramics, as common bone grafts, once implanted would trigger macrophages to directly respond to the ceramic itself and then to its degradation products, including the degradation particles and ions released under physiological conditions. Obviously, examining the exact effects of CaP ceramics on the secreted products of macrophages is essential for a deep understanding of the osteoinductive mechanism of CaP ceramics and for achieving the optimal design solution for osteoinductive biomaterials.

Recent progress in macrophage/biomaterial co-culture experiments has improved our understanding of material-dependent response of macrophage [17,36–38]. However, limited information can be found considering the co-culture of MSCs and macrophages with osteoinductive ceramics. The aim of the current study was to examine whether osteoinductive porous BCP ceramic could induce a change in cytokine secretion pattern of macrophages and subsequently have an effect on migration and osteoblastic differentiation of MSCs. Furthermore, besides material itself, we suspected that BCP-induced response of macrophages could be attributed to either local ionic environment created by BCP or the ceramic's degradation particles. Therefore, the effect of the ionic microenvironment and the degradation particles from BCP were investigated respectively. Through this study, we hope to find out the key material factor which mainly caused the possible change in secretion of cytokines from macrophages, and the correspondence of the change with the ceramic's osteoinduction.

2. Materials and methods

2.1. Material preparation and characterization

The porous BCP ceramic used in this study was fabricated by H_2O_2 foaming according to our previous study [14]. The ceramic was composed of HA and β-TCP phases, and the ratio of them was about 30/70. Its porosity was about 70% [14]. Given that the change in local environment around the implant resulted from the possible degradation of the material, an in vitro degradation experiment was designed to examine the degradation particles of BCP ceramic. A citric acid buffer was used to speed the degradation of the ceramic. In short, BCP ceramic disc ($\Phi 14 \times 2$ mm) was immersed in 0.1 M citric acid buffer (pH = 3) recirculated by a peri-