

# Formation of osteoclast-like cells on HA and TCP ceramics

R. Detsch<sup>a,\*</sup>, H. Mayr<sup>b</sup>, G. Ziegler<sup>a,b</sup>

<sup>a</sup> *BioCer Entwicklungs-GmbH, 95447 Bayreuth, Germany*

<sup>b</sup> *Friedrich-Baur-Research Institute for Biomaterials, 95447 Bayreuth, Germany*

Received 9 November 2006; received in revised form 9 March 2007; accepted 16 March 2007

Available online 15 July 2007

## Abstract

An essential property of bone substitute materials is that they are integrated into the natural bone remodelling process, which involves the resorption by osteoclast cells and the formation by osteoblast cells. If monocyte cells adhere to a calcium phosphate surface (bone or bone substitute material), they can fuse together and form multinucleated osteoclast cells. In this study we show that osteoclast-like cells derived from a human leukoma monocytic lineage responded in a different way to tricalciumphosphate (TCP) than to hydroxyapatite (HA) ceramics. Both ceramics were degraded by resorbing cells; however, HA enhanced the formation of giant cells. The osteoclast-like cells on HA formed a more pronounced actin ring, and larger lacunas could be observed. TCP ceramics are medically used as bone substitute materials because of their high dissolution rate. On the other hand, highly soluble calcium phosphate ceramics like TCP seem to be inappropriate for osteoclast resorption because they produce a high calcium concentration in the osteoclast interface and in the environment.

© 2007 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

**Keywords:** Calcium phosphate; Monocyte; Osteoclast; Degradation; Bioresorption

## 1. Introduction

Reconstructive orthopaedic surgery methods involving autogenous and allogeneous bone grafting are currently used to treat large bone defects. Due to their serious limitations, severe clinical problems have arisen and new approaches for bone tissue repair are essential [1,2]. The main advantage of using bone allografts or autografts is their osteoconduction. However, allografts can lead to an immune response, which requires long-term medication. Another major problem is the risk of transferring diseases (e.g. AIDS or hepatitis). Currently, autografts are better alternatives for repairing bone defects caused by tumours and serious trauma. However, the use of these substitutes is also restrictive. In the autograft procedure not only is the operation time increased, but morbidity at the donor site is more frequent, which poses a high risk to the

patient's health. Therefore, synthetic calcium phosphate ceramics are becoming more widely used as bone substitutes in orthopaedic and oral surgery [3]. Synthetic bone substitutes can solve certain problems associated with bone transplantation. An ideal synthetic material should provide a framework for continuous bone resorption and bone deposition [4,5].

Adult human bone maintains a dynamic state, which continually undergoes a process termed remodelling through the coordinated actions of bone resorption and synthesis, through osteoclasts and osteoblasts, respectively. Osteoclasts originate from haematopoietic precursor cells, which are possibly present in bone marrow, and differentiate into multinucleated cells by the fusion of mononuclear progenitors [6]. The developing multinuclear cells are responsible for the resorption of bone matrix (a process involving attachment to the bone surface), establishment of cell polarity, migration and subsequent degradation of bone matrix components [7]. Calcium phosphate resorption depends on the ability of the osteoclast to generate

\* Corresponding author. Tel.: +49 921 555591; fax: +49 921 555589.  
E-mail address: [rainer.detsch@biocer-gmbh.de](mailto:rainer.detsch@biocer-gmbh.de) (R. Detsch).

an acidic extracellular compartment between the cell and the bone surface. An acidic pH value is essential for bone mineral dissolution. The primary cellular mechanism responsible for this acidification is the active release of protons into the extracellular space. These protons allow the solubilization of hydroxyapatite (HA) crystals [8–10]. The other component of the bone, the organic bone matrix, which consists of substances such as collagen, is digested by acid lysosomal enzymes secreted by osteoclasts [11]. The activity of bone-resorbing cells is highly regulated and stimulated by hormones and cytokines [12]. Steroid hormones act on various cell types to regulate development, cell proliferation and cell differentiation. All steroid hormones act by binding to intracellular receptors, which interact with associated gene expressions. The active metabolite of vitamin D,  $1\alpha,25(\text{OH})_2\text{D}_3$  ( $\text{VD}_3$ ), regulates cell proliferation and consequently osteoclastogenesis. Addition of phorbol esters such as phorbol-12,13-dibutyrate (PDBu) can stimulate and induce the differentiation of human leukaemia U-937 cells into monocyte/macrophage cells and finally into apoptosis [13–15].

The main objective of this study was to cultivate human leukemia monocytes (cell line U-937) and to generate multinuclear cells from these cultures to serve as a model for bone resorption in studies of synthetic bone substitute materials. This cell line can adhere to calcium phosphate surfaces and resorb dentine slices [16]. We have investigated the effect of  $\text{VD}_3$  and PDBu on the differentiation of U-937 cells to macrophage/osteoclast-like cells. Typical parameters, including tartrate-resistant acid phosphatase (TRAP), actin-ring formation and cell morphology, were analysed after 8 and 21 days. The two calcium phosphates, HA and tricalciumphosphate (TCP), used in this work were prepared by conventional ceramic processing and characterized physicochemically. In addition, lacunas resulting from osteoclast activity were examined. For comparison, the degradation of HA and TCP (as dissolution rate in buffered media) was measured.

## 2. Materials and methods

### 2.1. Materials processing

The samples used in this study were prepared from commercially available HA and TCP powders. The respective powder was mixed with organic additives in a solvent and, after mixing, the granules were dried and sieved. The granules were uniaxially pressed, and the resulting green bodies were sintered at 1300 °C for 1 h in air to produce dense ceramic discs. The relative densities of the samples, as measured by Archimedes' method, were 99% (HA) and 98% (TCP). The surface roughness,  $R_a$ , of the two ceramics ranged from 0.2 to 0.5  $\mu\text{m}$ . The ceramic samples were examined by various characterization methods, including X-ray diffraction (XRD, Seifert 3000P, Germany) and scanning electron microscopy (SEM, Fei Quanta 200, The Netherlands). All samples had the same

diameter of 15 mm, corresponding to the size of a 24-well cell culture plate.

The chemical dissolution of HA and TCP was analysed in a degradation experiment immersing HA, TCP and Thermanox<sup>®</sup> in 1 ml of a Tris-buffered saline solution at 37 °C for 21 days. The calcium content was quantified by the *o*-cresolphthalein complexone method (Sigma, Germany). This technique is based on the formation of a coloured complex with *o*-cresolphthalein complexone in an alkaline medium. The intensity of the colour was measured at 540 nm (DU-460 Beckman, Germany) and quantified using a standard solution. The analysed calcium content was related to the sample surface of 175 mm<sup>2</sup>.

### 2.2. Cell culture

The human monocyte-like cell line U-937 (DSMZ Deutsche Sammlung für Mikroorganismen und Zellkultur, Germany) was cultured in RPMI-1640 (Sigma, Germany) supplemented with 10 vol.% fetal bovine serum (FBS, Sigma, Germany) and 1 vol.% penicillin/streptomycin (Invitrogen, Germany) at 37 °C in an atmosphere of 5%  $\text{CO}_2$ . Cells were grown as a suspension culture in 25 cm<sup>2</sup> culture flasks (Nunc, Denmark). After the cell culture became dense, cell suspension was centrifuged at 250 g for 5 min. The supernatant was removed and the cell pellet was dissolved in a fresh culture medium. Before cell seeding, the calcium phosphate material samples (HA and TCP) were cleaned by soaking in Extran (Merck, Germany) and sodium dodecyl sulphate (SDS, Sigma, Germany) solutions. Afterwards they were sterilized at 134 °C in an autoclave (Systec, Germany) and placed in a 24-well cell culture plate (Greiner, Germany). Thermanox<sup>®</sup> plastic coverslips (Nunc, Denmark) were used as standard materials.

U-937 cells were seeded at a concentration of 100,000 cells ml<sup>-1</sup> in the presence or absence of 10<sup>-7</sup> M of  $\text{VD}_3$  and 10<sup>-7</sup> M PDBu for 21 days. After 10 days the supernatant was discarded to remove the non-adherent cells. The adherent cells were cultured up to 21 days.

### 2.3. Cell counting

To determine the number of the cells in cultures, the suspended cells were counted with a Coulter Counter Z2 (Beckman, Germany). The adherent cells were detached from the surface by trypsin/EDTA (Sigma, Germany) and counted in the same way as the suspended cells.

### 2.4. Cell viability

Cell viability was measured by mitochondrial activity. The WST-1 assay (Roche, Germany) is a colorimetric assay for cellular dehydrogenase activity in which the absorbance at 450 nm is proportional to the activity of succinate dehydrogenase. Cells were cultured for 21 days and then washed with phosphate-buffered saline solution (PBS). Then

ID	Title	Pages
1979	Formation of osteoclast-like cells on HA and TCP ceramics	10

**Download Full-Text Now**



<http://fulltext.study/article/1979>



-  **Categorized Journals**  
Thousands of scientific journals broken down into different categories to simplify your search
-  **Full-Text Access**  
The full-text version of all the articles are available for you to purchase at the lowest price
-  **Free Downloadable Articles**  
In each journal some of the articles are available to download for free
-  **Free PDF Preview**  
A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>