Cellular investigations on electrochemically deposited calcium phosphate composites

PETRA BECKER*, HANS-GEORG NEUMANN
DOT GmbH, Charles-Darwin-Ring 1a, 18059 Rostock, Germany
E-mail: becker@dot-coating.de

BARBARA NEBE, FRANK LÜTHEN, JOACHIM RYCHLY
Department of Internal Medicine, University of Rostock, Ernst-Heydemann-Str. 6, 18057 Rostock, Germany

Electrochemically deposited calcium phosphate (CaP) coatings are fast resorbable and existent only during the first period of osseointegration. In the present study, composite coatings with varying solubility (hydroxyapatite (HA), brushite with less HA and monetite (M) with less HA) were prepared and the influence of the degradation and the reprecipitation of CaP on osteoblastic cells were investigated. On the brushite composite coating a new precipitated, finely structured CaP phase was observed during immersion in cell culture medium with or without osteoblastic cells. The surface morphology of monetite and HA coatings were entirely unmodified under the same conditions. So it could be assumed that electrochemically deposited brushite with less HA acts as a precursor for new precipitated CaP. On this surface osteoblastic cells revealed a well-spread morphology with pronounced actin cytoskeleton and demonstrated good proliferation behaviour. Thus we suggest that brushite seems to be especially suitable for coating of implants as a matrix for nucleation and growth of new bone.

1. Introduction

Bioactive surface coatings utilising calcium phosphate (CaP) are widely used in coatings for both orthopaedic and dental implants [1, 2]. Generally, these coatings are only necessary during the first healing period of osseointegration as a matrix for the early immobilisation of osteoblast-like cells and development of vascularised bone tissue on the implant [3]. Finally, the CaP coatings have to be resorbed and replaced by new bone tissue [4]. Electrochemically deposited CaP coatings match such efforts [5, 6]. In the present study, coatings with varying solubilities were prepared and the influence of the degradation and the reprecipitation of CaP on osteoblast like cells were investigated.

2. Materials and methods

2.1. Titanium modification

Disc-shaped samples of titanium were electrochemically coated with a composite of CaP-phases brushite (B) with less hydroxyapatite (HA) (BONIT®, thickness 20 µm). Brushite phase was transformed to monetite (M) by heat treatment or to HA by chemical treatment (0.5 M NaOH at 37 °C). The CaP phases were determined by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD).

2.2. Scanning electron microscopy (SEM)

The material surfaces were investigated with the scanning electron microscope DSM 960A (Carl Zeiss, Jena). For cell analyses, cells were grown on discs for 24 h, fixed with 4% glutaraldehyde (1 h), postfixed with 0.5% OsO_4, dehydrated through a graded series of alcohol, dried in a critical point dryer (K 850, EMITECH), sputtered with a coater (SCD 004, BAL-TEC) and examined.

2.3. Cell culture of osteoblastic cells

Osteoblastic cells of the osteosarcoma cell line MG-63 (ATCC, LGC Promochem, Wesel, Germany) were seeded with a density of 3×10^4 cells/cm^2 onto the material plates and were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS) and 1% gentamycin at 37 °C and in a 5% CO_2 atmosphere.

2.4. Analysis of the actin cytoskeleton

MG-63 cells were cultured for 48 h and stained accordingly [7]. Briefly, cells were fixed with 4% PFA (10 min), permeabilised with 0.1% Triton X-100 (10 min) (Merck), incubated with phalloidine-TRITC (Sigma;

*Author to whom all correspondence should be addressed.

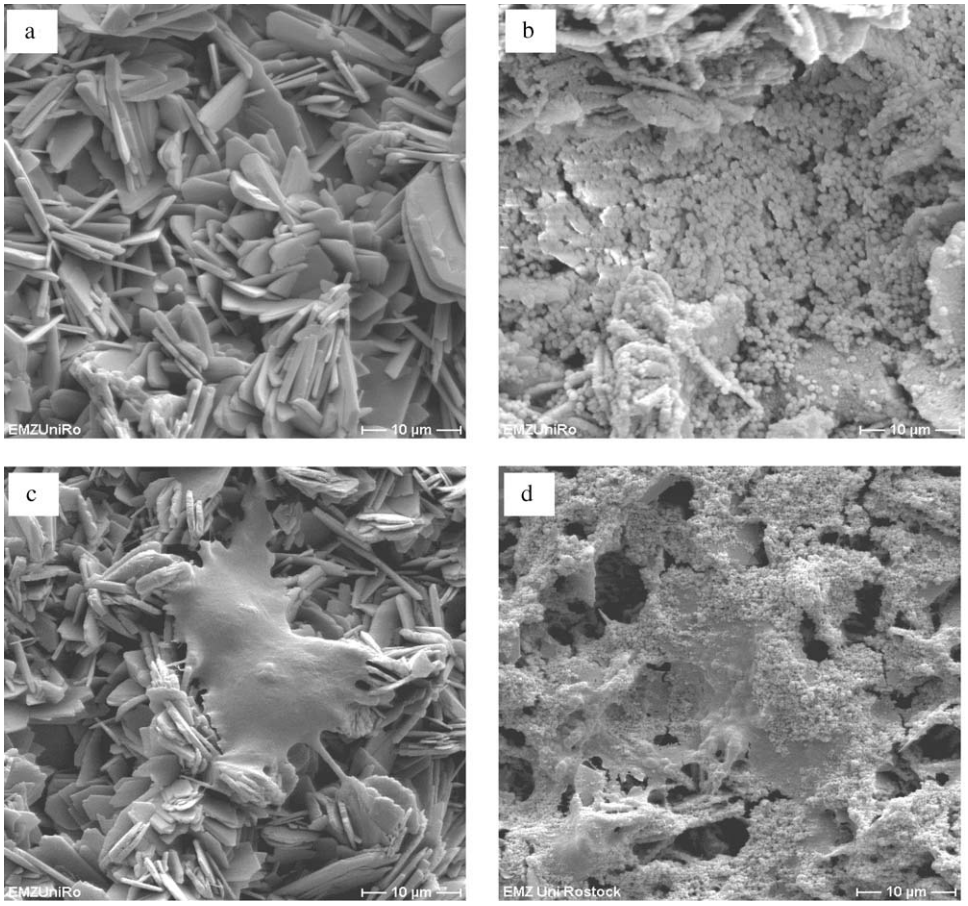

Figure 1 SEM micrographs of (a) a B/HA composite surface, and (b) after immersion in DMEM for 30 h. In (c), B/HA composite surface cultivated with MG-63 cells for 8 h, and in (d) for 30 h. Note the new precipitated, finely structured CaP on the surface of B/HA composite (b, d).

diluted 1 : 100, 30 min), washed, embedded and analysed using an inverted confocal scanning microscope LSM 410 (Carl Zeiss). For all experiments a 63 × oil immersion objective Plan-Neofluar (1.25 oil/0.17) was used. The size of the images was 512 × 512 pixels.

3. Results

The initial electrochemically deposited coating is a composite of mainly brushite with less hydroxyapatite (< 5%). The structure of the coating is fine crystalline whereby CaP crystallites are fixed to the surface in the shape of platelets or pins that are in near vertical alignment (Fig. 1(a)). After immersion in cell culture medium at 37 °C for 48 h without cells on B/HA composite coating a newly precipitated, finely structured CaP phase could be observed (Fig. 1(b)). The morphology of crystals of M/HA composite or pure HA coating under the same condition were entirely unmodified. In contrast to M/HA or pure HA coatings MG-63 cells grown on the B/HA surface are partly covered by newly precipitated, finely structured crystals after 30 h of cell culture (Fig. 1(d)). EDX analyses showed that these new crystals are reprecipitations of calcium phosphate (Fig. 2). MG-63 cells demonstrated a well-spread morphology (Fig. 1(c)) and the actin cytoskeleton of these osteoblastic cells revealed pronounced stress fibres on all surfaces tested (Fig. 3). Proliferation of MG-63 cells after seven days of cell culture on B/HA coating as well as on pure HA was high comparable to cells grown on collagen I as positive control (data not shown).

4. Discussion and conclusion

Brushite is a CaP-phase which is postulated as an intermediary phase in bone mineralisation *in vivo* [8]. Our investigation demonstrated, that electrochemically deposited brushite with less HA acts as a precursor for newly precipitated CaP in *in vitro* experiments independent on cellular activities. On the B/HA surface osteoblastic cells in the cell culture were covered by newly mineralised CaP crystals within 48 h. On these surfaces MG-63 cells demonstrated well-developed actin filaments. Thus, we suggest that these electrochemically deposited coatings, brushite composite as well as the pure HA coating seem to be especially suitable for the surface treatment of orthopaedic and dental implants as a matrix for nucleation and growth of new bone.

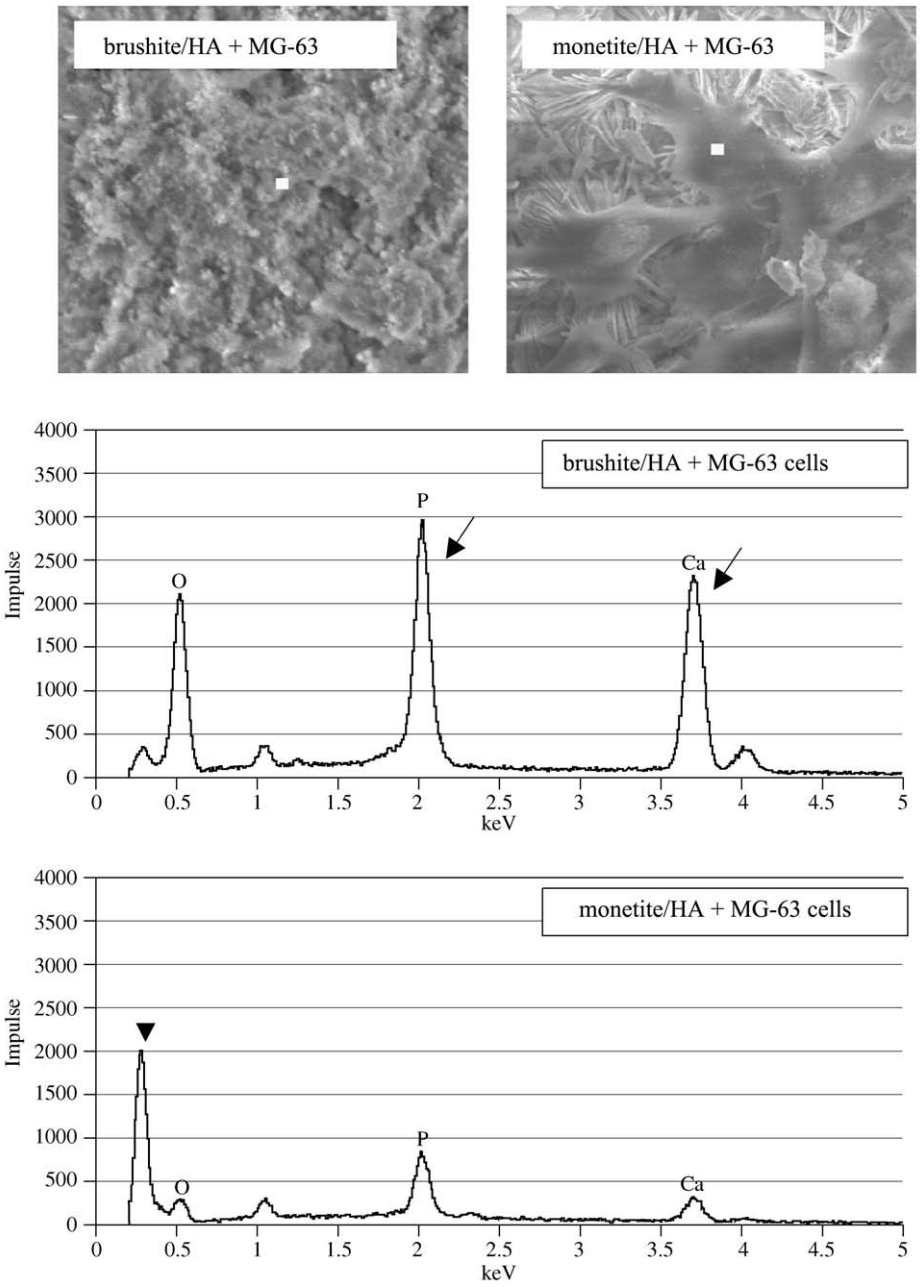

Figure 2 EDX analysis of the B/HA surface cultivated with MG-63 osteoblastic cells after 48 h of culture time compared to M/HA with MG-63 cells. Note that cells on B/HA are covered by reprecipitated CaP (arrows at the peaks of phosphate, P and calcium, Ca). Cells on the surface of M/HA are uncovered demonstrating smaller P and Ca peaks but pronounced carbon peaks (arrowhead). In the SEM images (above) the position of the EDX analysis is indicated (white spot).

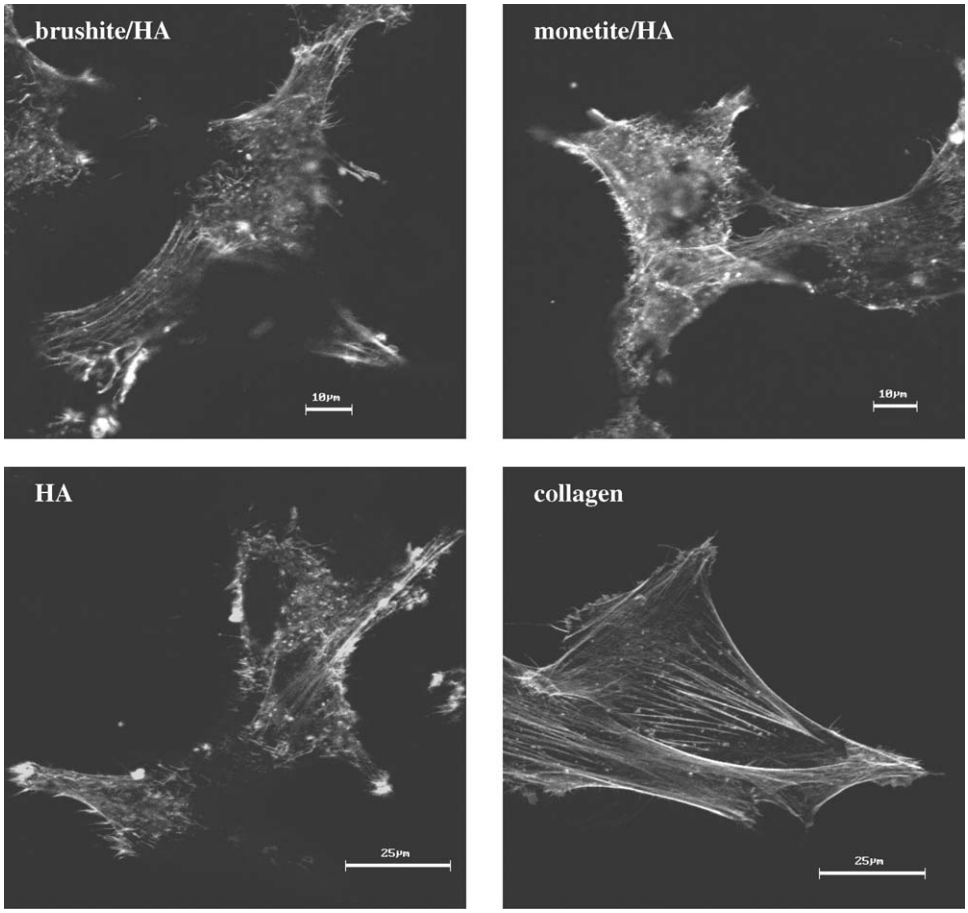

Figure 3 LSM images of the actin cytoskeleton of MG-63 cells on brushite composite and monetite composite as well as pure HA, compared to collagen I (Col). Note that cells reveal a spread morphology and actin stress fibres on all modified surfaces. LSM 410 (Carl Zeiss).

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