Mechanism of Bone Formation and Guided Tissue Growth at the Interface with Resorbable Bioactive Implant

A. El-Ghannam[*1,2], C.Q.Ning[1]
[1]Center for Biomedical Engineering, Graduate School, [2] Center for Oral Health Research, College of Dentistry
University of Kentucky, Lexington, KY 40506

Introduction: Controlled dissolution of bioactive ceramics is of prime importance for stimulation of bone cell function, graft material resorption and tissue regeneration. Silica-calcium phosphate nano composite (SCPC) is a new bone graft material characterized by superior bioactivity and resorbability compared to bioactive glass and hydroxyapatite [1,2]. In the present study we cultured rat bone marrow mesenchymal stem cells with SCPC and correlated between dissolution kinetics and the mechanism of bone mineralization. The effect of the chemical composition of SCPC on the phase composition and the dissolution kinetics in serum-containing physiological solution is described.

Materials and Methods: Compact disks (13mm diameter x 2mm height) made of SCPC samples SCPC10, SCPC30 and SCPC50 containing 3, 10 and 19 mol % SiO_2 respectively were prepared under a pressure of 250 MPa following the same protocol previously reported [2]. The disks were subjected to heat treatment at 800 °C for 1h in air. The phase composition of the compact disks was determined by X-ray diffraction analysis (XRD). The surfaces of the compact disks were polished and analyzed by SEM-EDX. Bone marrow stem cells were isolated from the femora of 7-week old 344 Fisher male rats. SCPC disks of each chemical compositions (n = 5) were seeded with 5 x 10^4 cells. The cells were allowed to attach for 15 min then were covered with 2ml complete α-MEM tissue culture medium (TCM). Control experiments using SCPC disks incubated with TCM without cells were run in parallel. The media was exchanged every 3 days and replaced with fresh medium supplemented with 3mM β-glycerophosphate and 50μg/mL ascorbic acid. At the times of media exchange (after 3, 6, 9 and 12 days), the media were collected and the ionic concentrations of Ca, P, Si and Na were measured using ICP-OES. Morphology and mineralization of cells attached to the ceramic were analyzed by SEM-EDX. Statistical analysis was performed using ANOVA with unequal variance at ($P<0.05$).

Results: The ionic concentration of media incubated with SCPC samples seeded with cells was similar to that without cells, indicating that cells have minimal effects on the dissolution behavior of the SCPC. After 3 days of culture, the Ca concentrations in the media incubated with all SCPCs were significantly lower than the original TCM concentration ($p<0.05$). Moreover, the Ca concentration in media incubated with the ceramic decreased as the silica-content in the ceramic increased. SEM morphologies of SCPC10 showed that the extracellular matrix (ECM) produced by attached cells was aligned along the silicon-rich phase (Fig.1).

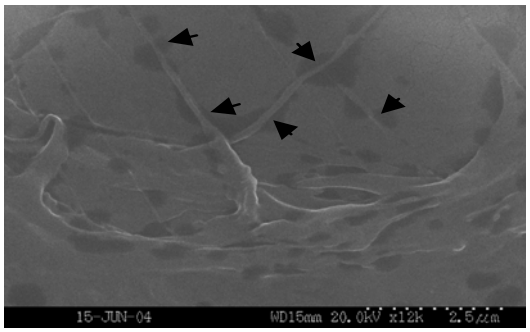

Fig. 1. The ECM produced by stem cells attached on the surface of SCPC10 was aligned along the silicon-rich phase (dark areas shown by black arrows).

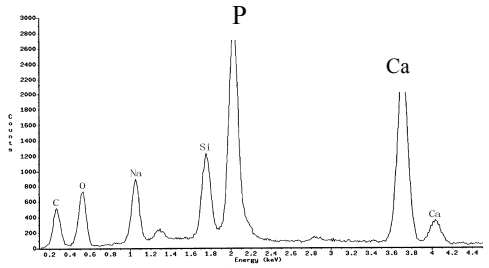

Fig. 2. EDX spectrum of the calcified nodules produced by bone marrow cells attached onto the surface of SCPC50.

No calcified nodules were observed on the surface of the cells attached to SCPC10 or SCPC30 after 3 days in culture. On the other hand, cells attached to SCPC50 produced calcium phosphate mineral which was observed only in connection with the cell layer and extracellular matrix (Fig.2).

Conclusion: Bone marrow mesenchymal stem cells did not significantly affect the dissolution behavior of SCPC in vitro. However, the cells inhibited the back precipitation of a calcium phosphate layer on the material surface. Bone marrow stem cells attached to the surface of SCPC absorbed Ca and phosphorus from the media. In conjunction with the absorption of high calcium contents, differentiated bone marrow cells produced calcified nodules and mineralized bone tissue. These data strongly indicate that bone mineralization at the interface with bioactive ceramic implant is mainly cell mediated and is enhanced by the cellular absorption of critical concentrations of dissolved Ca. Results of our study also demonstrate that silicon-rich phase(s) can provide guided cell adhesion and tissue growth.

References: 1. El-Ghannam A. JBMR,2004,69A:490-501
2. El-Ghannam A. JBMR, 2004. 71A: 377-390

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