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The Microstructure of Mineralized Tissue on Titanium Implant Interface

H. K. Nakamura[1], W.-A. Chiou[2], L. Saruwatari[1], H. Aita[1] and T. Ogawa[1]

[1.] The Jane & Jerry Weintraub Center for Reconstructive Biotechnology, University of California School of Dentistry, Los Angeles, CA 90095-1668
[2.] Materials Characterization Center, University of California, Irvine, CA 92697-2575

Titanium implant therapy has been applied for several decades in oral/maxillofacial and orthopedic reconstruction. Osseointegration and bone regeneration are essential for clinical success, and have recently been investigated extensively in the realm of biomedical engineering. Although abundant research has focused on the tissue-titanium interface, findings regarding the structure at this level are still controversial [1, 2]. The process of bone-titanium integration and biomechanical performance is not yet fully understood. While a recent study [3] revealed that osteoblasts generate harder and more delamination-resistant mineralized tissue on titanium than on polystyrene, understanding the microstructure and chemistry at the interfaces is of utmost importance. The purpose of this study is to characterize the mineralization behavior at the interfaces with different substrates such as titanium/tissue and polystyrene/tissue using cross-section SEM and TEM techniques.

Polystyrene culture dishes (Corning) were coated with Ti (250-500nm) using electron-beam physical vapor deposition technology (in the SLOAN e-beam evaporator). Bone marrow cells were isolated from the femurs of 8-week-old male Sprague-Dawley rats and cultured in osteoblastic-induced media. At a sub-confluency, the cells were seeded on both the original/untreated polystyrene dishes or Ti-coated polystyrene dishes. After culturing for 28days, the samples were washed with 1xPBS and prepared for EM observation. The samples were trimmed and embedded in a BEEM capsule with LR White embedding medium. Ultra-thin cross sections were microtomed with a diamond knife and collected on a thin carbon film to preserve the integrity of the ultra-thin sections. The internal and interfacial structures of the Ti-bone interface were also fractured and examined with a SEM.

SEM images show that cultured tissue on the untreated polystyrene has a fibrous network with small globular structures (Figs.1a and 1b). On the other hand, cultured tissue on Ti-coated polystyrene is relatively uniform and solid (Figs. 2a and 2b). Numerous crystallized aggregates/globules were observed on the Ti coated tissue. A close-up view of these globules highlights well-developed/defined nanocrystals composed mainly of Ca, P and O, indicating calcium phosphate mineral. The cross-sectional BSE (backscattered electron) image reveals a mineralized tissue layer on the surface of Ti (about 400nm) (Fig. 3). The cross-sectional TEM image (Fig. 4a) also illustrates a layer of well-mineralized tissues developed on the Ti substrate. A high magnification TEM image reveals the layer consists of fibrous and some platy minerals that have the same diffraction pattern of a typical hydroxyapatite (Fig. 4b). Although difficult to ultramicrotome, the cross-sectional TEM image of a tissue layer cultured on polystyrene also depicts very high contrast (between arrows in Fig. 5a). High magnification TEM shows that there are poorly defined nanocrystallites hidden in the amorphous-like tissues on polystyrene (Fig. 5b). A small amount of fibrous materials, presumably hydroxyapatite, were also seen at the tissue/polystyrene interface. TEM observation illustrates that the onset of mineralization is gradual but with slower speed in the tissues cultured on polystyrene.

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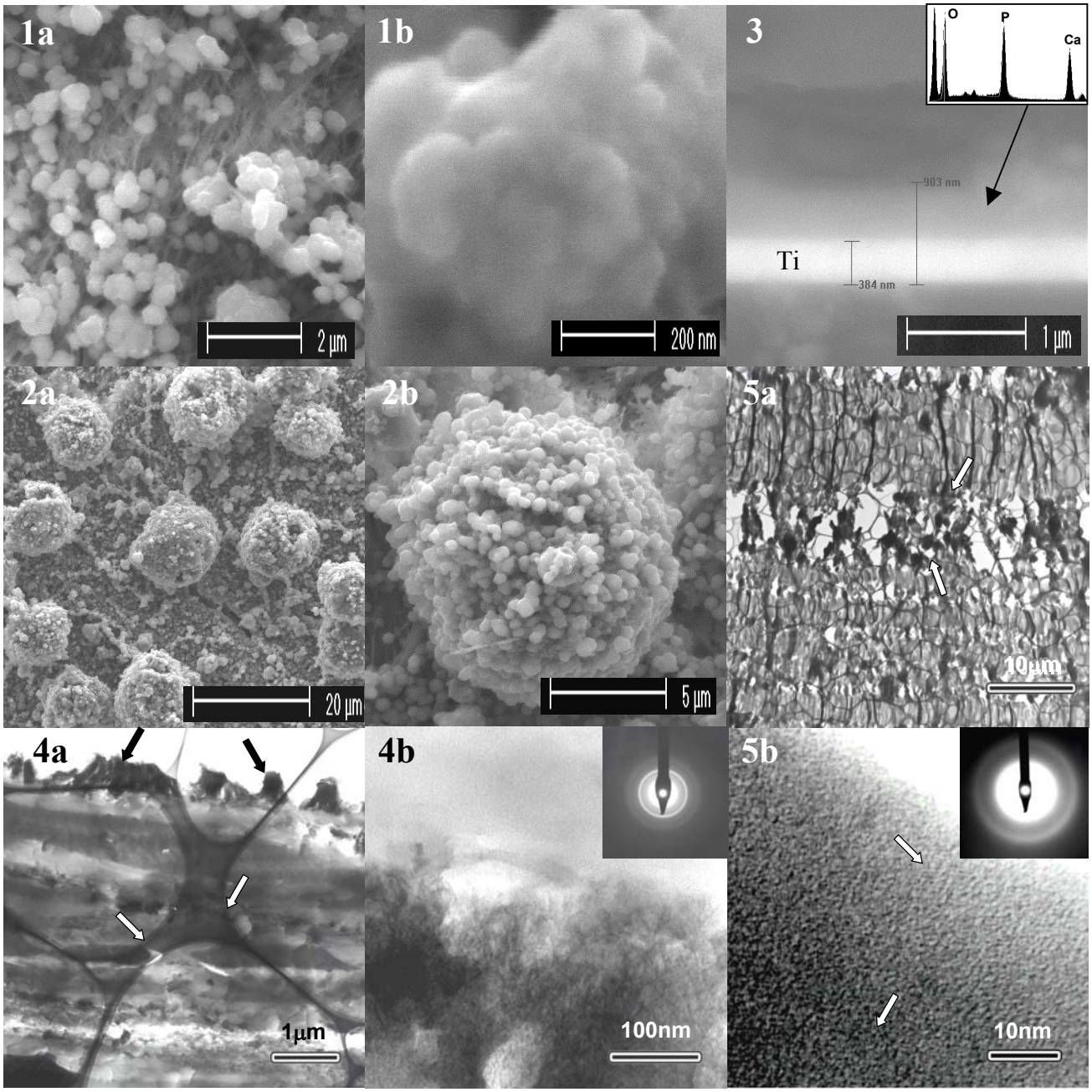

Fig. 1. Low (a) and high (b) mag. SEM images of cultured tissue on untreated polystyrene substrate.
Fig. 2. Low (a) and high (b) mag. SEM images of cultured tissue on Ti-coated polystyrene substrate.
Fig. 3. The cross-sectional BSE image reveals a well-mineralized tissue layer on the surface of Ti. EDS shows the major chemical constituents of a typical hydroxyapatite.
Fig. 4 (a). TEM image showing mineralized layer (arrows) on Ti-coated substrate. Most of Ti was torn away while ultramicrotoming. Dark lines (open arrows) are holey carbon film.
(b). High magnification TEM view and electron diffraction pattern of the mineralized tissue.
Fig. 5 (a). TEM micrograph of cultured tissue layer (arrows) on the untreated polystyrene substrate.
(b). HRTEM and ED pattern revealing the onset of crystallization/mineralization (arrows) in tissues cultured on polystyrene substrate.

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