Nano-Osseointegration of Titanium Implants: Characterization by High-Resolution Electron Microscopies

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Introduction:

Osseointegration plays a defining role in the outcome of bone-interfacing implants, with improved osseointegration translating directly to a more stable implant scenario and improved success. Owing to the inhomogeneity of biological tissues at an interface, in particular bone tissue, two-dimensional images often lack detail on the interfacial morphological complexity. Furthermore, the increasing prevalence of nano-structured surface features on implant materials calls for characterization techniques on a similar length scale. The recent application of electron tomography to the study of biomaterial interfaces with bone[1] has brought about an awareness of nano-osseointegration and, by extension, demanded increasingly advanced characterization methodologies. The objective of the present study is to set forth a complementary collection of techniques to simplify the characterization of nano-osseointegration with sufficient resolution and dimensionality.

Materials and Methods:

To demonstrate the characterization capabilities of various microscopies, laser-modified titanium implants[2], exhibiting both a micro- and nano-topography, were studied in contact with bone after 6 months implantation in New Zealand White rabbits. The implants, which have a diameter of 3.75 mm, length of 5.0 mm with a thread pitch of 0.6 mm, were laser-modified in their thread valleys and up 30% on each flank using a Q-switched Nd:YAG laser (Rofin-Sinar Technologies, Plymouth, MA) operated at a wavelength of 1064 nm. Approval for animal studies was received from the Local Animal Ethics Committee at the University of Gothenburg, Gothenburg, Sweden.

Samples were prepared for a series of transmission electron microscopy studies via a focused ion beam (FIB) in-situ lift out method. Elemental analysis, such as energy-filtered imaging (EFTEM) and energy dispersive X-ray spectroscopy (EDXS), and high-resolution transmission electron microscopy (HRTEM) were carried out on FEI's Tecnai G^2 F20. Z-contrast electron tomography was acquired over a ± 60° angular range on FEI's Titan 80-300 with Explore3D software, while 3D volumes were reconstructed in Inspect3D. Reconstructed volumes were visualized via volume rendering and segmented using Amira (Visage Imaging GmbH, Germany).

Results:

HRTEM indicated the formation of crystalline hydroxyapatite in the immediate vicinity of the implant, suggesting the implant surface is bioactive. Analytical studies suggested the presence of a functionally graded interface at the implant–bone boundary, characterized by the gradual intermixing of bone with oxide layer (Figure 1). The use of Z-contrast imaging to create electron tomograms enabled segmentation via grayscale thresholding (Figure 2) to identify bone penetration into the nanoscale surface features of the implant.

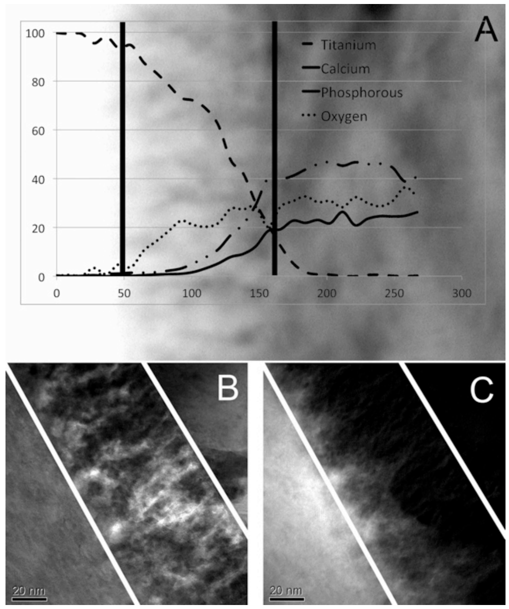

Figure 1. EDXS elemental line profile in **(a)** across the implant-oxide-bone interface, revealing a concentration gradient of indicative of Ca and P incorporation into the oxide coating. This result is further confirmed by EFTEM imaging, where a zero-loss image is shown in **(b)** and a Ca-filtered image **(c)** demonstrates the inclusion of Ca into the surface oxide, indicating nano-osseointegration.

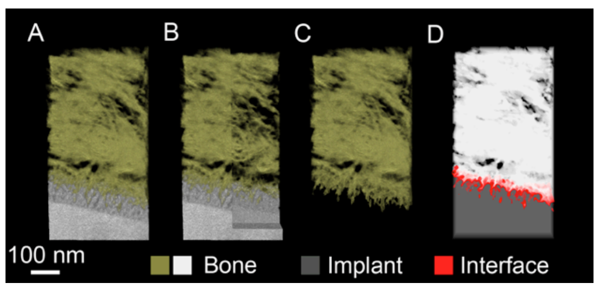

Figure 2. Segmentation of an electron tomographic reconstruction enabling identification of nano-osseointegration (A,B,C) and the precise interface region (D).

Discussion:

This work has demonstrated that a variety of complementary transmission electron microscopy techniques can be employed to fully comprehend and visualize osseointegration of titanium implants on a nanoscale level. Of particular value is the ability to see bone interdigitation with the nano-topographical features of the implant surface. Such three-dimensional segmentation techniques present promise for future characterization of nano-osseointegration on the basis that segmentation may lead to quantification. This work has demonstrated a suitable set of methods to identify the nano-osseointegration of laser-modified titanium implants and thereby supports the extension of these characterization methods to other nano-featured bio-interfaces.

References:

1. A. Palmquist, K. Grandfield, B. Norlindh, T. Mattsson, R. Brånemark, P. Thomsen, J Royal Society Interface (2012), 9:396-400.
2. A. Palmquist, L. Emmanuelsson, R. Brånemark, P. Thomsen, JBMR B (2011), 97:289-98.