On a novel technique for preparation and analysis of the implant surface and its interface to bone

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att offentligen försvaras i Hörsal A404 vid Avdelningen för Biomaterialvetenskap, Medicinaregatan 8b, våning 4, onsdagen den 4 juni 2008, kl. 13.00 av

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Avhandlingen baseras på följande delarbeten:

I. T. Jarmar, A. Palmquist, R. Brånemark, L. Hermansson, H. Engqvist, P. Thomsen, *Technique for preparation and characterization in cross-section of oral titanium implant surfaces using focused ion beam and transmission electron microscopy*, Journal of Biomedical Materials Research, In press

II. T. Jarmar, A. Palmquist, R. Brånemark, L. Hermansson, H. Engqvist, P. Thomsen, *Characterization of the surface properties of commercially available dental implants using SEM, FIB and HRTEM*, Clinical Implant Dentistry and Related Research 2008; 10(1): 11-22

III. A. Palmquist, T. Jarmar, L. Emanuelsson, R. Brånemark, H. Engqvist, P. Thomsen, *Forearm bone anchored amputation prosthesis: A case study on the osseointegration*, Acta Orthopaedica 2008; 79(1): 78-85

IV. A. Palmquist, F. Lindberg, L. Emanuelsson, R. Brånemark, H. Engqvist, P. Thomsen, *Morphological studies on machined implants of commercially pure titanium and titanium alloy (Ti6Al4V) in the rabbit*, Submitted for publication

V. A. Palmquist, F. Lindberg, L. Emanuelsson, R. Brånemark, H. Engqvist, P. Thomsen, *Biomechanical, histological and ultrastructural analyses of micro- and nano-structured titanium alloy implants: A study in rabbit,* Submitted for publication

Abstract

The ultrastructural and biomechanical properties of the bone-implant interface are important factors for implant performance. For further understanding of the osseointegration process novel tools enabling analysis of the intact interface in high resolution is needed, preferably combined with histology and biomechanical tests. Initial studies using focused ion beam microscopy (FIB) for transmission electron microscopy analysis have shown promising results.

The general aim of the thesis was to evaluate FIB for TEM sample preparation using different lift-out techniques and protection modes applied on the implant surface and its interface to bone tissue. Further, another aim was to combine different surface analytical and biological evaluation techniques with FIB/TEM in order to correlate the ultrastructure and the biomechanics of the interface using a new implant surface with micro- and nano-scale surface features.

A combination of different techniques was used for surface analysis of commercially available and test implants made of commercially pure titanium (Ti) and titanium alloy (Ti6Al4V). Scanning electron microscopy (SEM) and interference microscopy were used for surface topographical analyses. Auger electron spectroscopy (AES) was used for surface chemical analysis and depth profiling. Morphological and structural analysis was performed using FIB/TEM. An amputation prosthesis which was retrieved after 11 years in clinical function was analyzed by histology, histomorphometry and TEM. The bone response to Ti and Ti6Al4V implants in rabbit tibia was analyzed by a combination of histology, histomorphometry, biomechanics, SEM (back-scattered mode) and TEM analysis of the intact interface prepared by FIB.

The present results showed that the FIB *in situ* lift-out technique provided a higher quality and yield of ultra-thin samples for TEM in comparison with the *ex situ* technique. In addition, *in situ* prepared samples could be re-thinned and plasma cleaned. Commercially available dental implants showed large differences in the outermost surface layer with regards to crystallinity, morphology and thickness. Osseointegrated amputation prosthesis made of machined Ti demonstrated 75% relative bone area, 85% bone-implant contact and a direct apposition of hydroxyapatite. No difference was found between machined Ti and Ti6Al4V after 8 weeks healing time in rabbit cortical bone. In contrast, laser-modified Ti6Al4V surface had a 270% increase in torque strength and altered bone fracture pattern, correlating to an ultrastructural bonding between nanocrystalline hydroxyapatite and surface features on the micro- and nano-scales.

In summary, TEM sample preparation was successfully applied on implants, giving new information on the surface morphology and crystallinity. Limitations with the technique were: sample thickness (~100 nm) casing difficulties to analyze very thin surface layers (<10 nm) and bone-implant interfaces which were not properly bonded to sustain pre-FIB preparation.

In conclusion, FIB is a new, powerful tool for sectioning ultrathin samples for subsequent TEM analysis of implant surfaces and their interfaces to bone and could be performed in combination with other techniques giving important complementary information.

Keywords: Focused Ion Beam (FIB), Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), osseointegration, rabbit, human, titanium, titanium alloy, surface analysis, bone-implant interface analysis, ultrastructure, surface modification, laser.

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