Mechanisms of Osseointegration: Experimental Studies on Early Cellular and Molecular Events in vivo

AKADEMISK AVHANDLING

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I. O. Omar, F. Suska, M. Lennerås, N. Zoric, S. Svensson, J. Hall, L. Emanuelsson, U. Nannmark, P. Thomsen, *The influence of bone type on the gene expression in normal bone and at the bone-implant interface: experiments in animal model*, Clin Implant Dent Relat Res 2009, [Epub ahead of print]

II. O. Omar, M. Lennerås, S. Svensson, F. Suska, L. Emanuelsson, J. Hall, U. Nannmark, P. Thomsen, *Integrin and chemokine receptor gene expression in implant-adherent cells during early osseointegration*, J Mater Sci: Mater Med. 2010 Mar; 21(3): 969-80

III. O. Omar, S. Svensson, N. Zoric, M. Lennerås, F. Suska, S. Wigren, J. Hall, U. Nannmark, P. Thomsen, *In vivo Gene expression in response to anodically oxidized versus machined titanium implants*, J Biomed Mater Res A. 2010 Mar 15;92(4):1552-66

IV. O. Omar, M. Lennerås, F. Suska, L. Emanuelsson, J. Hall, A. Palmquist, P. Thomsen, *Interfacial gene expression and stability of oxidized and machined titanium implants*, In manuscript

Abstract

The early cellular and molecular activities determining the early tissue response and bone formation at bone/implant interface are not fully understood. The general aim of the current thesis was to develop a model for studying the early molecular and cellular activities in different bone types, and in response to different implant surface properties. The studies were performed by analyzing gene expression of implant-adherent cells using a sampling procedure and subsequent qPCR. The developed model was combined with histology and immunohistochemistry to study cellular relations and early tissue organization at the interface with the implant, governing the early structural basis of osseointegration. The ultimate aim was to determine the strength of the early formed bone/implant interface, by measuring the removal torque forces, and thereby to correlate the results with the degree of inflammation, bone formation and bone resorption, as measured by a gene expression panel. The evaluation time for the studies ranged between 3 hours up 28 days from implantation. The present studies provided a combination of gene expression, morphological, and biomechanical data.

The present results demonstrated biological differences between cortical and trabecular bone types, both in the normal steady-state condition and in response to biomaterial. During steady-state conditions, bone with trabecular architecture expressed higher level of bone turnover markers compared to cortical bone, while the latter had a higher inflammatory constitutive expression. The response to anodically oxidized titanium implants was different in trabecular and cortical bone sites after 3 days of implantation. Early differences in gene expression in cells associated with different implant materials can be detected as early as 3 hours after implantation. Higher level of osteogenic activity indicated by significantly higher expression of mesenchymal stem cell recruitment and adhesion markers and higher expression of markers for coupled bone formation and resorption, were found at oxidized surfaces. A higher expression of CXCR4 homing receptor for stem cells, and the integrins, αv, β1 and β2 were detected in cells at oxidized surfaces. On the other hand, higher proinflammatory activity was detected at the machined surfaces, as exemplified by the expression of TNF-α and IL-1β. Scanning electron microscopy and immunohistochemical analysis confirmed the presence of both inflammatory monocytes/macrophages and mesenchymal stem cells at the implant surfaces with predominance of the mesenchymal cells on the oxidized surfaces. Gene expression analyzed on the screw level provided additional information in comparison with that of surrounding bone. The rapid recruitment and adhesion of mesenchymal stem cells, the rapid triggering of gene expression crucial for bone remodeling and the transient nature of inflammation correlated with higher stability of the oxidized implants.

In conclusion, the combination of the *in vivo* experimental model, qPCR and morphological and biomechanical techniques provided hitherto unexplored opportunities to analyze in detail the mechanisms of osseointegration. A major conclusion of the studies is that material surface properties elicit early, significant differences in gene expression in interfacial cells. This observation is important in order to understand the mechanisms behind osseointegration and the role of material surface properties. Furthermore, this knowledge is essential for the ability to design the material and biological conditions for optimal tissue regeneration in association with implanted medical devices.

Keywords: Osseointegration, titanium, bone, gene expression, bone-implant interface, qPCR, inflammation, cytokine, chemotaxis, chemokine, integrin, bone formation, remodeling, in vivo, rat, immunohistochemistry, ultrastructure, biomechanical torque test.

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