A methodological platform to study molecular biocompatibility of biomaterials
Experimental and clinical studies

Akademisk avhandling
som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs universitet kommer att offentligen försvaras i sal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, 8 juni 2016, kl. 13.00

av
Maria Lennerås

Fakultetsopponent: Professor John Hunt
The Institute of Ageing and Chronic Disease, University of Liverpool, UK

Avhandlingen baseras på följande delarbeten:

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

II. Omar OM*, Lennerås ME*, Suska F, Emanuelsson L, Hall JM, Palmquist A, Thomsen P. The correlation between gene expression of proinflammatory markers and bone formation during osseointegration with titanium implants. *Biomaterials*. 2011;32(2):374-86.

III. Lennerås M, Palmquist A, Norlindh B, Thomsen P, Omar O. Oxidized titanium implants enhance osseointegration via mechanisms involving RANK-RANKL regulation. *Clin Implant Dent Relat Res*. 2015;17 Suppl 2:e486-500.

IV. Lennerås M, Ekström K, Vazirisani F, Shah FA, Junevik K, Thomsen P, Omar O. Adhesion and activation of monocytes and MSCs on titanium surfaces analysed by FACS, qPCR and protein profiling. *In manuscript*

V. Lennerås M*, Tsikandylakis G*, Trobos M, Omar O, Vazirisani F, Palmquist A, Berlin Ö, Brånemark R, Thomsen P. The clinical, radiological, microbiological and molecular profile of the skin-penetration site of transfemoral amputees treated with bone-anchored prostheses. *In manuscript*

*Equal contributions

UNIVERSITY OF GOTHENBURG

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ABSTRACT

The aim of this project was to develop a methodological platform in order to advance our scientific understanding of the mechanisms of osseointegration. Screw-shaped, titanium implants, with different surface properties, were inserted in the rat tibia, or incubated in mono- or co-culture of human monocytes and MSCs. After different time points, the implant-adherent cells or the peri-implant bone were harvested and processed for different analyses. For *in-vivo* studies, qPCR, immunohistochemistry, histomorphometry, electron microscopy and removal torque analyses were used. In the *in-vitro* study, FACS, qPCR, ELISA and protein profiling were applied. Finally, qPCR was employed in a clinical study to analyse the abutment-adherent cells of osseointegrated fixtures. At the early time points *in vivo*, a higher gene expression of MSC recruitment and adhesion factors (CXCR4 and integrin-β1) was found in cells adhering to the oxidised compared to machined implant. This was corroborated by predominance of MSCs at the oxidised surface, as judged by immunohistochemistry and SEM. At the later time points, cells adhering to oxidised implants retained a higher expression of bone formation (ALP and OC) and bone remodelling (TRAP and CatK) genes. The qPCR findings correlated with histomorphometric, electron microscopy and removal torque measurements, revealing progressively increasing bone-implant contact and bone bonding and, as a result, an increase in the biomechanical stability of the oxidised implant. The enhanced RANKL/OPG expression ratio corresponded to the remodelling phase at the bone-implant interface. The qPCR analysis of FACS-sorted cells showed that the co-existence of monocytes and MSCs on the implant surface, *in vitro*, upregulates the gene expression of some cytokines in a cell-specific manner. The clinical study showed that bacterial colonisation was frequently detected on the skin, the abutment and in the bone canal. A higher expression of TNF-α was associated with positive cultures of *S. aureus*, whereas fixture loss was associated with lower expression of OC and IL-10.

In conclusion, the present methodological platform enables detailed analyses of the events at the bone-implant interface. Employing this platform demonstrated that implant surface properties elicit a cellular and molecular cascade for rapid cell recruitment and enhanced bone formation and remodelling, which accelerates bone maturation and implant stability. Finally, the results of the thesis provide a first line of information on factors that could affect the performance of percutaneous implants.

Keywords: Osseointegration, titanium, inflammation, cell recruitment, cell adhesion, bone regeneration, bone remodelling, gene expression, immunohistochemistry, histomorphometry, removal torque, ultrastructure, FACS, protein profiling, transfemoral amputation, abutment, percutaneous, bacteria, clinical signs.

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