

Titanium Oxide and Bone Anchorage

Role of the Complement System, and Delivery of Osteoporosis Drugs from Mesoporous TiO₂

Akademisk avhandling

som för avläggning av medicine doktorsexamen vid Sahlgrenska akademien vid Göteborgs universitet kommer att offentligen försvaras i Lecture Hall, Biotech Center, 5:e våning, Arvid Wallgrens Backe 20, tisdagen d. 28:e januari 2014, kl 13.00

av

Necati Harmankaya

Department of Biomaterials, Institute of Clinical Sciences,
Sahlgrenska Academy at University of Gothenburg

Fakultetsopponent: **Professor Thomas Lars Andresen**, Department of Micro- and Nanotechnology, Technical University of Denmark

Avhandlingen baseras på följande delarbeten:

- I. Paula Linderback, Necati Harmankaya, Agneta Askendal, Sami Areva, Jukka Lausmaa, Pentti Tengvall, *The effect of heat- or ultra violet ozone-treatment of titanium on complement deposition from human blood plasma*, *Biomaterials* **2010**; 31 (18):4795-801.
- II. Necati Harmankaya, Kazuyo Igawa, Patrik Stenlund, Anders Palmquist, Pentti Tengvall, *Healing of complement activating Ti implants compared with non-activating Ti in rat tibia*, *Acta Biomaterialia* **2012**; 8 (9):3532-3540.
- III. Necati Harmankaya, Johan Karlsson, Anders Palmquist, Mats Halvarsson, Kazuyo Igawa, Martin Andersson, Pentti Tengvall, *Raloxifene and alendronate containing thin mesoporous titanium oxide films improve implant fixation to bone*, *Acta Biomaterialia* **2013**; 9 (6): 7064-7073.
- IV. Johan Karlsson, Necati Harmankaya, Stefan Allard, Anders Palmquist, Mats Halvarsson, Pentti Tengvall, Martin Andersson, *In vivo drug localization at the implant/bone interface – alendronate delivered from mesoporous titania*, Submitted.
- V. Necati Harmankaya, Johan Karlsson, Anders Palmquist, Mats Halvarsson, Martin Andersson, Pentti Tengvall, *Titanium integration in ovariectomized rat tibia following systemic or local delivery of alendronate*, In manuscript.



Titanium Oxide and Bone Anchorage

Role of the Complement System, and Delivery of Osteoporosis Drugs from Mesoporous TiO₂

Necati Harmankaya

Department of Biomaterials, Institute of Clinical Sciences,
Sahlgrenska Academy at University of Gothenburg

ABSTRACT

The clinical success of bone implants of titanium (Ti) is largely ascribed to the biological performance and the physicochemical properties of the outermost titanium(IV)dioxide (TiO₂) layer. Several advancements have been done on TiO₂ in order to optimize its healing and anchorage to bone, and there is a need for further understanding and control of the molecular reactions preceding long-term osseointegration. Next generation of implants advance with their ability to target specific molecular mechanisms.

In this thesis we performed mild surface treatments of TiO₂ with improved oxide properties and bone-implant anchorage in mind. First, we exposed Ti to (UV) illumination or mild heat treatment to control the complement activation ability of the surfaces. Secondly, we evaluated *in vivo* a mild heat treated mesoporous TiO₂ drug-delivery system on Ti implants.

Ti surfaces were heated or exposed for up to 96 hours to UV-light in combination with ozone (UVO) and tested for inflammatory activity *in situ* and *in vivo*. Surfaces were immersed in blood plasma for up to 60 minutes and the deposition of complement factor C3 was evaluated by ellipsometry. The *in vivo* bone response to UVO-treated Ti relative to complement activating control surface was evaluated by histology, histomorphometry, and biomechanics.

The mesoporous coating was prepared on Ti screws (L=2.3 mm, Ø=2.0 mm) using the Evaporation Induced Self-Assembly (EISA) method. The coating was highly-ordered mesoporous TiO₂ with a thickness of 200 nm and possessed a narrow pore-size distribution. Two osteoporosis drugs, alendronate or raloxifene, were absorbed into the pores and the implants were evaluated *in vivo* in male and ovariectomized rat models.

The present results show that adsorption of complement factor C3 *in situ* can be strongly suppressed by mild heat treatment at 300°C or UVO-treatment for 12 hours or longer. A significantly lower gene expression of inflammatory markers was noted *ex vivo* on UVO-treated implants compared to complement-activating controls. Although UVO-treatment did attenuate the early inflammatory response on Ti, the bone-anchorage did not significantly benefit from this effect.

Mesoporous Ti implants loaded with a bisphosphonate, alendronate, or an oestrogen receptor antagonist, raloxifene were successfully retrieved after up to 28 days post-surgery. Raloxifene promoted a significantly higher bone-anchorage in comparison to control and ALN-loaded implants, and was supported by an increased gene expression of osteoblast and osteoclast markers. The distribution of alendronate in implant-close bone was followed for up to 8 weeks and the results show that alendronate has a long residence time in the close vicinity of the implants. Also, we have shown significant differences between local vs. systemic delivery of bisphosphonates; the local delivery promoted a significantly higher bone-implant anchorage.

In summary, the osteoimmunologic properties of TiO₂ result partly from stoichiometry of the oxide, which we have showed can be altered by means of mild heat-treatment or UVO-illumination. Mesoporous coatings may provide a unique reservoir on implant surfaces into which drugs can be loaded. This may serve to a better bone-implant healing, especially for patients suffering from osteoporotic bone-deficiency, where current pharmaceutical treatments come to short or are bound with systemic side effects when given at high doses.

Keywords: titania, complement, photocatalysis, ovariectomy, alendronate, raloxifene

ISBN: 978-91-628-8883-1

Available online: <http://hdl.handle.net/2077/34402>