Titanium Oxide and Bone Anchorage

Role of the Complement System, and Delivery of Osteoporosis Drugs from Mesoporous TiO_2

Akademisk avhandling

som för avläggning av medicine doktorsexamen vid Sahlgrenska akademin 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

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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.

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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 (TiO_2) layer. Several advancements have been done on TiO_2 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 TiO_2 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 TiO_2 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 TiO_2 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 TiO_2 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
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