

Surface modification of titanium based alloys with bioactive molecules utilizing electrochemically fixed oligonucleotides

[D. Scharnweber^{1\)}](#), [J. Michael^{2\)}](#), [R. Beutner^{1\)}](#), [I. Israel^{2\)}](#), [U. Hempel^{3\)}](#), [B. Schwenzer^{2\)}](#), [H. Worch^{1\)}](#)

1) Max-Bergmann-Zentrum für Biomaterialien, 2) Institut für Biochemie, 3) Institut für Physiologische Chemie; all Technische Universität Dresden, Dresden, Germany

INTRODUCTION: Though titanium based alloys are used in routine surgery, problems arise from special medical indications, for example bad local bone quality or systemic diseases. Here, a method for bio-surface engineering of implants in a modular way is presented, offering the possibility to adapt biochemical surface properties specifically prior to implantation.

In a first step an oligonucleotide that acts as anchor group (AON) is fixed via one terminus (regiospecifically) by partial electrochemical entrapment into anodic oxide layers. This AON is subsequently hybridized with a complementary oligonucleotide (CON) conjugated to bioactive molecules. This staged process enables flexibility and modularity of the system based on the hybridization ability of nucleic acids.

METHODS: Samples of Ti6Al7Nb (Synthes Inc.) were grinded and etched (HF/HNO₃). Oligonucleotides (Thermo Electron Corp.) were partially ³²P-labelled by Hartmann Analytic GmbH. Amounts of ON on the surface were determined using a PIPS spectrometer system (Canberra / Ortec).

Immobilization of a 60mer, 5'-phosphorylated anchor strand (AS) was performed in acetate buffer (pH = 4.0) up to potentials of 15 V_{SCE}. Stability of fixation was tested by immersion in 50 mM TRIS-HCl (pH = 7.5) for up to 24 h. Hybridization experiments with 31mer complementary (CS) and non-complementary (NS) strands to AS, respectively, were carried out in the same electrolyte. Hybridization was performed in two steps of 30 min each (without/with 10 mM MgCl₂).

Conjugates were synthesized from a cell adhesion peptide (GRGDSP, Bachem) and CS according to Ruth [1]. Bioactivity of the conjugates was tested by blocking the integrine receptors of rat-calvaria osteoblasts (³H-labelled) with GRGDSP alone and with its conjugate, respectively, before seeding on polystyrene cell-culture plates.

RESULTS: During immersion of AS coated samples for up to 24 h merely adsorbed ON desorb to a large extent (fig. 1). Anodic oxidation with potentials of at least 4 V_{SCE} leads to stable fixation. After desorption up to 43 % (14.5 V_{SCE}) of the amount of ON detected after immobilization was present at the surface, hence fixed. A maximum surface density of 4 pmol/cm² after desorption

could be achieved by immobilization from 400 nM AS solution at a potential of 8 V_{SCE}.

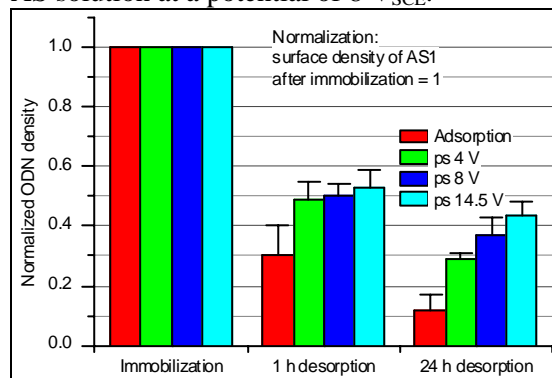


Figure 1: Normalized AS density on the surface after immersion in 50 mM TRIS-HCl (pH = 7.5)

Hybridization efficiency depends on the surface density of immobilized anchor strand and reaches values of up to 1.0. Addition of MgCl₂ increased hybridization rates.

Similar hybridization behaviour was exhibited by the conjugates. The successful binding of the conjugates to integrins of osteoblasts could be demonstrated.

DISCUSSION & CONCLUSIONS: Oligonucleotides can be fixed stably in anodic oxide layers on titanium based alloys and remain accessible for hybridization with complementary strands. Hybridization of a conjugate of complementary strand and RGD peptide with the fixed anchor strands is possible to the same extent as for the non-conjugated strand. Furthermore, the cell adhesion peptide preserves its binding capabilities to integrine receptors of osteoblasts if conjugated to ON. All results clearly indicate that modular implant adaptation as introduced above is possible.

REFERENCES: [1] Ruth JL. Conjugation of enzymes to linker arm oligodeoxynucleotides. In: Eckstein F, editor. Oligonucleotides and Analogues. IRL Press, 1991. p. 270 et sqq.

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