Biofunctionalisation of titanium implants with streptavidine and biotinylated BMP-2

Volker Ettelt[(1)], Matthias Epple[(2)], and Michael Veith[(1)]

[(1)] Laboratory of Biophysics,
Faculty of Electrical Engineering and Applied Natural Sciences, University of Applied Sciences,
45665 Recklinghausen, Germany

[(2)] Laboratory for Inorganic Chemistry,
Faculty of Chemistry, University of Duisburg-Essen,
45141 Essen, Germany

E-Mail: volker.ettelt@w-hs.de
Web: http://www.bio.w-hs.de/OFC/
https://www.uni-due.de/chemie/ak_epple/

Abstract

Bone morphogenetic protein (BMP-2) biofunctionalized titanium surfaces are widely used in medical implant science. BMPs exert important functions in the regulation of bone repair. Even though titanium has a high biocompatibility and mechanical strength, specific cellular adhesion and stimulation are diminished by non-specific protein adhesion in vivo [1, 2]. Common approaches for the biofunctionalization of titanium with BMP-2 try to avoid this problem by immobilization of unphysiologically high amounts of BMP-2, resulting in undesired side effects (ectopic bone formation, inflammation, burst effect) [3–5]. Therefore, biologically inert surfaces which reduce non-specific protein binding to a minimum and allow a defined amount of protein immobilization at the same time are attractive especially for medical applications [6, 7]. TiO_2 surfaces biofunctionalized with streptavidin show a suppression of non-specific protein adsorption [8]. Here, a biotinylated TiO_2 model surface was biofunctionalized via self-assembly of streptavidin [9] for strong non-covalent immobilization of biotinylated BMP-2 (bBMP-2), shown by surface plasmon resonance (SPR) and infrared reflection absorption spectroscopy (IRRAS). Non-biotinylated BMP-2 did not bind to the biofunctionalized surface, enabling only immobilization of defined amounts of bBMP-2. Mass spectrometry (MALDI-TOF) showed an N-terminal biotinylation of BMP-2, which does not interfere with receptor interaction [10–12]. The surface coverage with bBMP-2 was 70 µg/cm^2, determined by SPR and ELISA technique.

The presented approach offers a promising alternative to the common biofunctionalization of titanium with BMP-2, avoiding side effects caused by BMP-2 overdosage and desorption by a tunable immobilization of bBMP-2. Furthermore, it bypasses the problem of non-specific protein adsorption. However, further studies are necessary to determine the osteogenic potential of immobilized bBMP-2 on titanium.

Furthermore, the approach presented here also offers the possibility to be transposed to calcium phosphate.

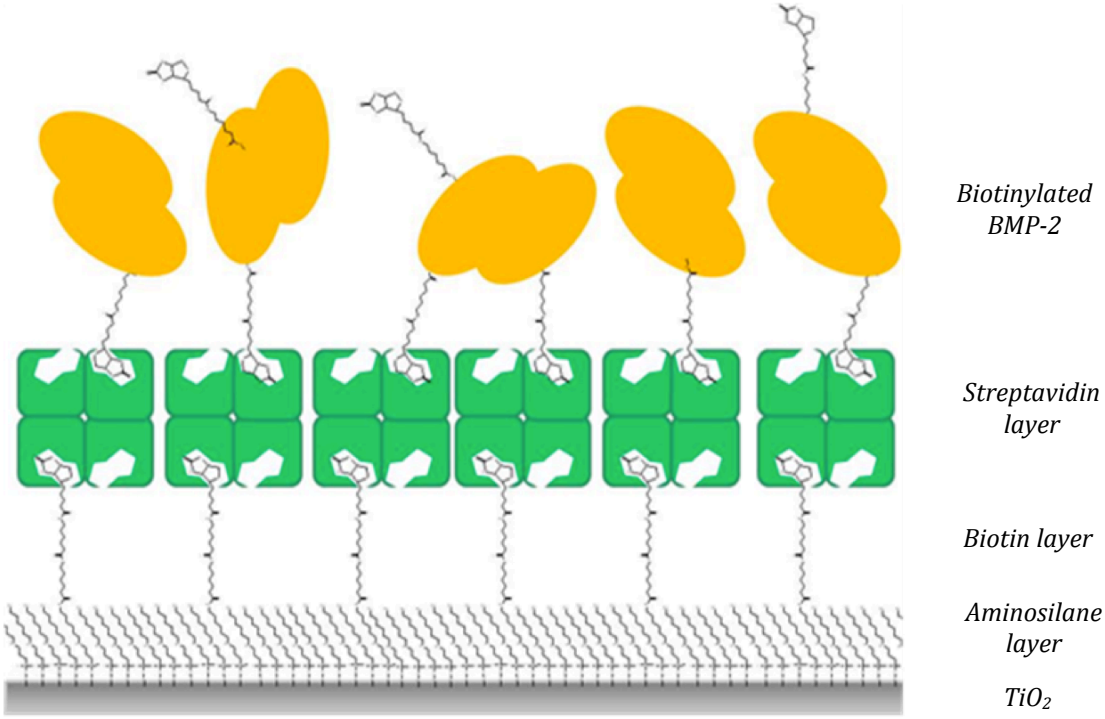

Fig.1: Biofunctionalization of a biotinylated TiO_2 model surface with biotinylated BMP-2 (bBMP-2). After silanization and biotinylation of a TiO_2 surface, an antiadhesive streptavidin layer was generated via self-assembly [9]. This layer can be biofunctionalized with bBMP-2.

References

[1] B. Kasemo, J. Gold, "Implant surfaces and interface processes", *Adv. dent. Res.*, vol. 13, pp. 8–20, 1999.

[2] S. Raghavendra, M. C. Wood, and T. D. Taylor, "Early wound healing around endosseous implants: a review of the literature", *Int J oral maxillofac implants*, vol. 20, no. 3, pp. 425–431, 2005.

[3] A. C. Carreira, F. H. Lojudice, E. Halcsik, R. D. Navarro, M. C. Sogayar, and J. M. Granjeiro, "Bone morphogenetic proteins: facts, challenges, and future perspectives", *J Dent Res*, vol. 93, no. 4, pp. 335–345, 2014.

[4] L. Shields, G. H. Raque, S. D. Glassman, M. Campbell, T. Vitaz, J. Harpring, and C. B. Shields, "Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion", *Spine*, vol. 31, no. 5, pp. 542–547, 2006.

[5] J. Even, M. Eskander, and J. Kang, "Bone Morphogenetic protein in spine surgery: current and future uses", *J Amer Acad Orthop Surg*, vol. 20, no. 9, pp. 547–552, 2012.

[6] K. S. Masters, "Covalent growth factor immobilization strategies for tissue repair and regeneration", *Macromol Biosci*, vol. 11, no. 9, pp. 1149–1163, 2011.

[7] M. Hajimiri, S. Shahverdi, G. Kamalinia, and R. Dinarvand, "Growth factor conjugation: strategies and applications", *J Biomed Mater Res A*, vol. 103, no. 2, pp. 819-838, 2014.

[8] M. Lehnert, M. Gorbahn, M. Klein, B. Al-Nawas, I. Köper, W. Knoll, and M. Veith, "Streptavidin-coated TiO_2 surfaces are biologically inert: Protein adsorption and osteoblast adhesion studies", *J. Biomed. Mater. Res. A*, vol. 100, no. 2, pp. 388–395, 2012.

[9] M. Lehnert, M. Gorbahn, C. Rosin, M. Klein, I. Köper, B. Al-Nawas, W. Knoll, and M. Veith, "Adsorption and conformation behavior of biotinylated fibronectin on streptavidin-modified TiO_X surfaces studied by SPR and AFM", *Langmuir*, vol. 27, no. 12, pp. 7743–7751, 2011.

[10] K. Hauff, C. Zambarda, M. Dietrich, M. Halbig, A. L. Grab, R. Medda, and E. A. Cavalcanti-Adam, "Matrix-immobilized BMP-2 on microcontact printed fibronectin as an in vitro tool to study BMP-mediated signaling and cell migration", *Front bioeng biotech*, vol. 3, p. 62, 2015.

[11] K. Heinecke, A. Seher, W. Schmitz, T. D. Mueller, W. Sebald, and J. Nickel, "Receptor oligomerization and beyond: a case study in bone morphogenetic proteins", *BMC Biol,* vol. 7, p. 59, 2009.

[12] T. L. M. Pohl, J. H. Boergermann, G. K. Schwaerzer, P. Knaus, and E. A. Cavalcanti-Adam, "Surface immobilization of bone morphogenetic protein 2 via a self-assembled monolayer formation induces cell differentiation", *Acta Biomater*, vol. 8, no. 2, pp. 772–780, 2012.