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Titanium implants with modified surfaces: Meta-analysis of *in vivo* osteointegration

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ABSTRACT

Titanium-based implants are widely used in modern clinical practice, but their "optimal" properties in terms of porosity and topology, roughness and hydrophilic parameters are being a subject of intensive discussions. Recent *in vitro* results have shown a possibility to optimize the surface of an implant with maximal repelling of bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and improvement in human osteogenic and endothelial cell adhesion, proliferation and differentiation. In this work, these different grades titanium implants were tested *in vivo* using the same analytical methodology. In addition to material parameters, key histomorphometrical parameters such a regeneration area, bone adaptation area and bone-to-implant contact were determined after 2 and 4 weeks of implantation in rabbit animal model. Porous implants have more clear differences than non-porous ones, with the best optimum values obtained on hydrothermally treated electrophoretically deposited titanium. These *in vivo* data correlate well with the optimal prediction made by *in vitro* tests.

1. Introduction

Titanium implants are widely used in modern clinical practice especially in load-bearing applications such as orthopedic and dental implants. Many producers are developing various grades of implants, with the modern trend on surface modification. All titanium implants might be roughly divided into ones with porous surface (normally coating such as vacuum plasma sprayed (VPS) titanium) and without (polished, sandblasted, etched or otherwise treated). Furthermore, any of this type may be additionally coated with an external layer (hydroxyapatite, bioactive glass, etc.) and it is of a general knowledge that such modification would affect surface roughness, porosity, wettability and consequently cell and bacterial adhesion to the implant. Together with biomechanical factors, these set conditions for bone ingrowth and osteointegration. Although the process is generally well understood, many links between input (surface characteristics, coatings) and output (bone to implant contact, etc.) parameters are only qualitatively known. It is difficult to make a vast number of specific surfaces with different features being independently varied to evaluate their specific effect *in vivo*. It was recently demonstrated *in vitro* to be possible to adjust titanium coating parameters to reduce bacterial adhesion and to enhance cell proliferation and differentiation at the same time vs. non-optimized plasma-sprayed Ti [1]. These data, however, might not be directly extrapolated to *in vivo* host conditions, as there are many other variables, most of them are beyond control (such as specific animal mobility or its health conditions). Thus, *in vivo* results are more difficult to quantify and whether such quantification is made, the scatter in the data is rather significant to accept or decline a particular test specimen.

Such post-analysis of the data is often limited to very simple (ANOVA) methods for single hypotheses. This is difficult to apply to scattered data with linked variables (and when their error distribution is unknown). The FDA guidelines recommend Bayesian methods for planning and evaluation (docket No. 2006D-0191) instead of simple statistics, which might affect the likelihood function and bias the output. However, it is still quite common to see conclusions based just on p-values in many reports [2,3]. Taking into account the variety of the factors, it is not straightforward to find out the "real" links between the implanted specimen properties and in vivo output. For example, what is the relevance of *in vivo* studies using a proximal tibia where the extracted implant (cylinder) is mechanically loaded and the push-out force is recorded [4–7]. Despite its simplicity, this test often produces inadequate results: the knowledge on the implant push-out resilience of example ~370 J makes little sense when the standard deviation on this value is ±480 J. When tibia/femur being histologically examined, every implant commonly displays differences in bone in-growth. The cylinder main axis is not always perpendicular to the bone surface

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Table 1
Key parameters of the various implant materials. Values represent average ± standard deviations, except for IPC where minimum, mean and maximum sizes are given.

Sample	S_a	S_z	S_{tr}	S_{dr}	Wetting angle	Porosity	IPC (low)	IPC (high)	IPC (mean)
	(μm)	(μm)	(−)	(%)	(°)	(%)	(μm)	(μm)	(μm)
Etched cp Ti	0.32 ± 0.03	4.00 ± 0.78	0.56 ± 0.10	0.39 ± 0.04	73.0 ± 8.1	0	0	0	0
Anodised cp Ti	0.33 ± 0.05	4.12 ± 0.84	0.53 ± 0.12	0.40 ± 0.04	46.5 ± 10.8	0	0	0	0
Anodised cp Ti → MBAG	3.18 ± 0.43	32.73 ± 8.00	0.73 ± 0.16	94.01 ± 25.22	0	0	0	0	0
EPD Ti (P)	4.48 ± 0.37	50.73 ± 1.83	0.64 ± 0.13	61.71 ± 15.41	102.2 ± 3.4	51.2 ± 3.9	2	50	7
EPD Ti (P_Vm)	7.95 ± 0.68	103.80 ± 12.46	0.70 ± 0.07	218.96 ± 41.39	105.7 ± 7.2	65.2 ± 3.2	2	150	10
EPD Ti (P) → HT	4.93 ± 1.95	67.24 ± 22.08	0.67 ± 0.14	56.87 ± 27.20	0	45.9 ± 1.0	2	50	7
EPD Ti (P) → MAO	4.30 ± 0.24	51.67 ± 7.73	0.75 ± 0.12	35.59 ± 9.85	0	28.0 ± 3.8	0.2	15	5
EPD Ti (P) → SGBAG	4.26 ± 1.62	60.75 ± 11.46	0.79 ± 0.06	40.49 ± 11.11	0	51.3 ± 3.4	1	30	5

S_a — arithmetical mean height of the surface; S_z — ten point height, average height of the 5 highest and 5 lowest points; S_{tr} — texture aspect ratio: >0.5 means isotropic, <0.3 indicates directionality; S_{dr} — developed area ratio: percentage of additional surface area as compared to an ideal plane the size of the sampling area.

(as assumed by the test protocol), and thus stresses or energy measurements might be misleading (even negative values might be obtained due to uneven deformation, misalignment and non-uniformity of residual stresses). Some cortical bone might be delaminated or over-dried before the test and there are no data about the influence of parameters on osteointegration if it is being measured in this way. The basic simulations of this procedure [4] have confirmed these variations together with scatter in the test rig tool parameters often lead to non-interpretable test results.

The advanced meta-analytical methods and Bayesian statistics, applied to the design of experiments and output data, have an improved ability to determine the relative influence of different parameters on a given phenomenon, to find existing relationships among the considered variables, to resolve the unavoidable noise present within a vast set of numerical data. The challenge of meta-analysis application to implants testing is to overcome limitations due to a considerable amount of time needed to perform an analysis, when the number of parameters is very high and when they are linked with each other and with the output functions in an unknown or a non-quantified way [8–12]. The common definition of meta-analysis assumes it as a collection of techniques for a systematic review of the relevant literature used in reaching an overall estimate of effect size, despite some criticism due to difficulties of knowing which data should be included and to which population final results actually apply [11,12].

In this work, we are rather analyzing the effect of different titanium implants and their surface parameters linked with histomorphometrical features obtained from *in vivo* tests, for the same materials which behavior *in vitro* has been recently reported [1]. The main essential feature of advanced meta-analytical methods applied to the subject of the study is in focusing on the links between the controllable input variables (material parameters) and *in vivo* responses, without speculating about the exact mechanisms behind these interactions. For such complex tasks involving many cross-linked interactions, meta-analytical methods have proven to provide answers and correct predictions with meta-modeling and multi-variate analysis [9,13–15]. It is noteworthy that here meta-analytical methods are specifically related to meta-modeling and multi-variate approaches [8,9], and not to the conventional meta-analysis as commonly used [11,12]. Thus here no usual data search is made but rather the similar method is applied to the set of generated experimental data. Due to this, for instance, standard meta-analytical plots [11,12] are not always possible or relevant, although some results like distribution, confidence intervals and outliers still could have sense.

2. Materials and methods

2.1. Implants

Implant design was as described in [16]: commercially pure Ti sheets (grade 2, thickness 1 mm, Goodfellow, UK), were laser-cut into Ø 4 mm discs and etched in a $HF:HNO_3 = 1:5$ solution (specimens marked as "etched"). After autoclave sterilization, these discs were used both as substrate material and as the reference material for the in vivo tests. Additionally, the etched titanium surface was modified by seven different experimental coatings (Table 1).

Firstly, anodic oxidation was used to increase the thickness of the natural TiO_2 oxide layer from 15 nm to about 60 nm (this material is named "anodized"). Next, a melt-derived bioactive glass–ceramic coating (50.1 wt.% SiO_2, 25.2 CaO, 20.1 Na_2O and 4.6 P_2O_5, thickness ~ 10 μm) was applied on the anodized cp Ti substrate by dipping in a bioactive glass powder suspension followed by 30 min sintering at 800 °C in vacuum (specimens marked as "MBAG"). Porous pure Ti coatings were manufactured using electrophoretic deposition (EPD) of a TiH_2 powder suspension or a combination of a suspension and emulsion followed by dehydrogenation (500–550 °C) and vacuum sintering (850 °C), as described in [17–19]. Distinct TiH_2 powder grades of different particle sizes, all supplied by Chemetall GmbH (Germany), have been used: grade type P (average particle size 8.0 ± 2.0 μm, maximal 60 μm), U (5.0 ± 1.0 μm, maximal 45 μm) and VM (1.8 ± 0.2 μm, maximal 45 μm). Different pore size and morphologies were realized by combining various particle sizes [1].

These coatings of EPD Ti are denominated "P" and "P_Vm" respectively, hereby referring to the starting powder grades. In addition, three different functionalizing coatings have been applied to EPD Ti (P). Chemical modification without altering the roughness or pore structure was done by a hydrothermal treatment (Jožef Stefan Institute, Slovenia) to apply a nanometer thin anatase TiO_2 layer [20], specimens marked as "HT". The micro-arc oxidation (MAO, University of Bayreuth, Germany) was performed at 150 V in a 1 M H_3PO_4 solution with hydroxyapatite and $CaCl_2$ additions, was used to produce a pore-filling TiO_2 layer containing Ca^{2+} and PO_4^{3-} ions [21], specimens marked as "MAO". Finally a bioactive glass–ceramic coating (60 wt.% SiO_2, 20

Fig. 1. SEM micrograph of a histological section schematically indicating the regions of interest for histomorphometrical analysis: bone regeneration area (BRA) and bone adaptation area (BAA).