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PHYSICO-CHEMICAL PROPERTIES AND HEALING CAPACITY OF POTENTIALLY BIOACTIVE TITANIUM SURFACE

J. Strnad[1*], *Z. Strnad*[1] *and J. Šesták*[2]

[1]LASAK – Laboratory for Glass and Ceramics, Papírenská 25, 16200 Praha 6, Czech Republic
[2]Institute of Physics, Academy of Sciences of the Czech Republic, Cukrovarnická 10, 162 53 Prague 6, Czech Republic

The aim of this study was to evaluate physico-chemical properties and the healing capacity of surface treated titanium. Surface treatment combining sand-blasting, acid etching and alkaline etching (BIO surface) was evaluated together with machined titanium as a reference surface. Hydration, wetting angle, surface area and roughness parameters were evaluated for both surfaces. Stability of dental implants with both surfaces implanted in the tibia of dog was measured during the healing of twelve weeks. BIO surface exhibited lower wetting angle, larger surface area, higher degree of hydration and higher average roughness compared to machined titanium. Implants with the BIO surface maintained their stability during the whole healing period in contrast to those with machined titanium surface, which showed a statistically significant decrease in stability three and nine weeks after implantation.

Keywords: *alkali-etched surface, bioactive surface, dental implants, hydroxyapatite, osseointegration, resonance-frequency analysis, secondary stability, titanium, wetting angle*

Introduction

More than a quarter century ago two material groups have been found to be able to form a mechanically stable and functional interface with bone. One group consisted of certain soda-lime-silica glasses, with or without addition of phosphorus oxide, and the first glass exhibiting the bone-bonding ability was discovered by Hench [1–3] and registered under the name 'Bioglass'. The glasses exhibiting the bone bonding ability were designated as bioactive, with the following definition: 'the bioactivity is the characteristics of an implant material which allows it to form a bond with living tissues'.

Another material found to exhibit the bone-bonding ability was machined titanium. This characteristic of titanium was first described by Branemark and coworkers [4] and the phenomenon of attachment to bone was named osseointegration with the following definition: 'osseointegration represents the formation of a direct contact of a material with bone without intermediate fibrous tissue layer, when observed using light microscope'.

The major difference between the two material groups was represented by the materials reactivity and related kinetics of the bone-material interface formation. Very reactive bioactive glasses formed a stable interface with bone within days where as machined titanium required healing periods of several months to reach the same bone-implant contact. Thanks to the high reactivity of bioactive glasses, the course of the glass-body environment interaction could be investigated more easily, and the bone bonding ability was described and quantified in direct relation to the glass composition [5–7]. Soon other materials like hydroxyapatite, sol/gel prepared glasses or glass-ceramics, which were found to exhibit similar bone-bonding ability, were included into the 'bioactive' group [8–10]. All these materials exhibited the precipitation of apatite mineral on their surfaces as a result of chemical interaction of the materials surface with body environment [1]. For initial assessment of potential bioactivity of a material, the apatite formation occurring in vivo was reproduced in vitro in simulated body environment [11] and a good correlation of in vivo and in vitro results was found for the above mentioned, highly bioactive materials [1, 9].

The investigation of the osseointegration observed in the titanium group was more difficult because of the low reactivity of titanium in body environment. Changes detected in the passive oxide layer during the exposure to body environment were not comparable in magnitude to those occurring in bioactive materials. With the intention to increase the implant surface available for bone in-growth and fixation and/or to increase the blood clot retention on the materials surface, machined titanium is most often modified by sand-blasting, plasma spraying or acid etching. Roughened surfaces showed higher bone/implant interface strength (removal torque values) and higher BIC (=bone/implant contact) after the same

* Author for correspondence: strnad@lasak.cz

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

healing time compared to machined titanium [12–17]. Some authors suggested that surfaces with the mean roughness parameter Sa in the interval of 1.0–1.5 μm show stronger bone response than smoother or rougher implant surfaces [18]. On the other hand it was shown that the optimal roughness value varies according to the chemical composition of the tested surfaces [19, 20]. Acceleration of the bone-implant interface and the increase of the bone-implant contact in the early phases of healing [21–23] was successfully achieved by plasma spraying of a hydroxyapatite as a bioactive material on the titanium substrate, however, hydroxyapatite plasma sprayed coatings have been the subject of many controversies regarding their long-term stability and thickness as well as low long-term success rates [24–27]. On the other hand some authors reported mid and long term clinical results showing high success rates of hydroxyapatite coated implants [28–30].

As lately as in the late nineties specific surface modifications of machined titanium have been developed with the intention to modify the reactivity of titanium chemically so that it gains the best defined characteristics of well proven bioactive materials - the ability to induce apatite mineral formation in vitro. Until now, to the knowledge of the authors, only two surface modifications of titanium supporting the precipitation of bone mineral apatite have been developed and clinically introduced. In 1999, alkali treatment [31] was used in combination with sand-blasting and acid etching on LASAK implants (BIO surface) [32, 33] and in 2000 fluoridated titanium surface (Osseospeed) was introduced by ASTRATECH [34, 35].

From the present scientific literature it seem necessary to evaluate both surface roughness and chemistry in order to be able to draw reliable conclusions regarding the effect of the surface treatment on the bone-implant interface formation. A method of quantitative evaluation of the clinical benefit of a surface treatment has not yet been established. Machined surface with defined surface roughness is often used as a reference when evaluating the effect of a specific surface treatment. The aim of this study is to evaluate the physicochemical properties and the stability time dependence during healing of potentially bioactive titanium surface and to compare it with the machined surface as a reference.

Experimental

Sample preparation

This study was designed as a comparative study of two surfaces: turned machined titanium surface (Ti-M) and potentially bioactive titanium surface (BIO). The BIO surface is created by sand-blasting, acid etching and a final treatment in an alkaline solution. Ti-M and BIO surfaces were prepared on implants used for the stability time dependence measurement.

Methods of surface characterization

Surface characterization was performed using a scanning electron microscope (SEM, Hitachi, Japan) with an accelerating voltage of 15–30 kV. Surface roughness measurement was carried out using a Talysurf 6 profilometer (Taylor Hobson, Leicester, United Kingdom). The mean roughness (Ra) was measured and recorded at a traverse speed of 0.5 mm s^{-1} on a traverse length of 2 mm with a diamond-tipped stylus. The specific surface area was calculated by the BET method from the results of the krypton gas absorption study. The surface area is expressed in relation to unit geometric surface area of the implant. The determination was performed by absorption of krypton on a ASAP 2010 M instrument (Micromeritics, USA). Diffuse.

Reflectance Infrared Fourier Transformed (DRIFT) Spectroscopy was used to determine the degree of surface hydration. The measurement was performed on a Nicolet 740 instrument (Nicolet Madison, USA) with resolution of 4 cm^{-1}. The presence of hydroxyl groups on the sample surface was quantified using the absorption band height at 3400 cm^{-1} [36]. Dynamic contact angle measurement was performed using the Wilhelmy plate method, using Tensiometer K15 (Kruss GmbH , Germany). The wetting angle values in water were determined from the dependence of the wetting force on the immersion depth. The mean values of the wetting angle were calculated from four repeated measurements.

To evaluate the stability time dependence for implants with TI-M and BIO surfaces the resonance frequency analysis (RFA, Integration Diagnostics, Gothenburg, Sweden) was performed using an animal model and a commercially available F37 L5 transducer attached to the implant. The Implant stability measurement was performed in the 1st, 3rd, 9th and 12th week of healing time. A total of 6 implants per time point and per treatment group were used for the measurement. Implants were placed in a dog tibia with the same average primary stability for both groups. The experimental procedure was described earlier [37].

Results and discussion

BIO surface treatment alters the initial, low-roughness surface of the machined titanium (Ra=0.828±0.024 μm) to a rough surface (Ra=2.264±0.311 μm) (Fig. 1, Table 1)) with micro- and nano-porous gradient structure.

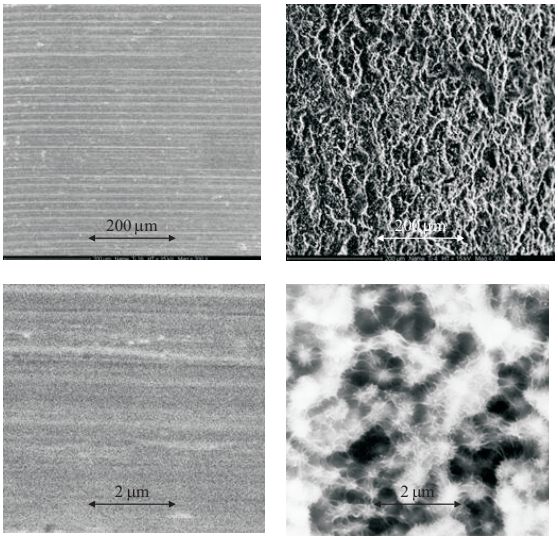

Fig. 1 Scanning electron microscopic images of the machined surface a – TI-M and the b – BIO surface

The surface area measurements showed that combination of sand-blasting and acid and alkali treatments used in the BIO surface preparation results in a structured porous surface exhibiting an approx. 100-fold increase in surface area compared to the machined surface (Table 2).

Table 2 summarizes the results of the contact angle measurements for the machined and BIO surface. The machined surface exhibited hydrophobic characteristics. On the other hand, the low contact angle of the BIO surface (27.2°) indicates an easily wettable hydrophilic surface.

The diagram in Fig. 2 shows that the number of hydroxyl groups is greater on the BIO surface compared to the machined surface. The level of hydroxylation of the BIO surface was roughly an order of magnitude greater ($1.3 \cdot 10^8$) compared to the other surfaces (Ti-Unite-$2.4 \cdot 10^7$; SLA $3.0 \cdot 10^7$).

The time dependence of the stability for implants with machined surfaces $ISQ_t(M)$ (control group) exhibits a statistically significant decrease after three (p=0.0098) and nine (p=0.0082) weeks of healing and this decrease in the stability ends in the 9th week (Fig. 3). The stability increased again after the ninth week and returned to almost the initial values in the twelfth week. In contrast to this, the $ISQ_t(B)$ values of the BIO implants (test group) did not exhibit statistically significant changes (p>0.05) throughout the measured intervals between 0 and 1, 3, 9 and 12 weeks of healing time (Fig. 3).

The time dependence (Fig. 3) indicates that the BIO surface helps to maintain implant stability during the early healing time compared to the machined surface. A statistically significant difference between the test and control groups was observed after 3 (p=0.0064) and 9 (p=0.00019) weeks.

The net contribution of the BIO surface to the implant stability (with reference to machined surfaces) during the healing time (t=1; 3; 9 weeks) was estimated as the difference between the BIO implant stability $ISQ_t(B)$ and the machined implant stability

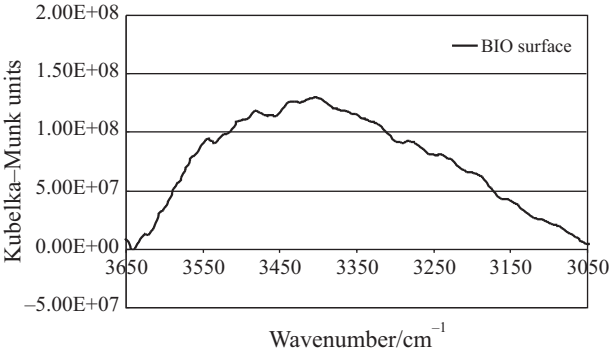

Fig. 2 DRIFT spectra of the BIO surface (machined surface as a reference)

$ISQ_t(M)$. The time dependence of the stability differences exhibits a statistically significant positive slope (0.84) of the linear regression line in the first nine weeks of follow-up (Fig. 4).

The slope value (0.84 ISQ/week) represents the difference in the rate of osseointegration between the

Table 1 Roughness parameters of BIO and machined surfaces

	R_a±SD/μm	S_m±SD/μm	R_{ku}±SD
BIO surface	2.264±0.311	86±12	3.7±1.7
Machined surface	0.828±0.024	44±3	4.2±1.3

R_a=arithmetic mean of the profile departures from the mean line; S_m=mean spacing of adjacent local peaks; R_{ku}=profile sharpness

Table 2 Contact angles and specific surface areas of BIO and machined surfaces

Surface modification	Contact angle mean±SD/°	Specific surface area mean±SD/mm^2 mm^{-2}
machined surface	79.5±4.6	1.4±0.7
BIO-surface	27.2±6.9	138.0±42.5

implants with BIO surface and those with machined surface ($\Delta ISQ(B)/\Delta t - \Delta ISQ(M)/\Delta t$), where ΔISQ corresponds to the changes in ISQ values and Δt represents the corresponding interval of the healing time of implants in the bone. Taking machined implants as a reference, the slope value can be regarded as a stability growth rate gained by the BIO surface modification.

It can be speculated that the differences in the rates of osseointegration in the initial stages of healing for the BIO and machined surface could be related to different surface reactivities [20] following from the different surface material properties, e.g. specific surface area, surface wettability, surface contact angle or surface hydroxylation/hydration [38]. In general, the surface reactivity, which is a common characteristic of bioactive materials, increases with increasing specific surface area. Therefore, the three-dimensional macro-, micro- and nano-structured BIO surface, which is more than 100× larger (Table 2) compared to the machined surface, may significantly enhance the surface reactivity with the surrounding ions, amino acids, and proteins, which determine the initial cellular events at the cell-material interface.

In addition, easily wettable hydrophilic BIO surface (Table 2) allows the establishment of the contact between the body environment (blood) and the complicated rough and porous structure of the implant, and thus contribute to cell and biomolecule migration and adhesion [39]. Moderately hydrophilic surfaces (20–40° water contact angle) were also showed to promote the highest levels of cell attachment [40].

The BIO surface, which is rich in hydroxyl groups, in contrast to the machined surface (Fig. 2), rapidly induces adsorption of calcium and phosphate ions on contact with the ions of the blood plasma [41]. The calcium phosphate-rich layer promotes adsorption and concentration of proteins [42] and constitutes a suitable substrate for the first apatite structures of the bone matrix, which are synthesized by the osteogenic cells at the beginning of the formation of the new bone tissue. This mechanism can accelerate the formation of a stable bone-implant interface [43–45], formed by fusion of the biological cement line matrix with the reactive calcium phosphate layer on the surface.

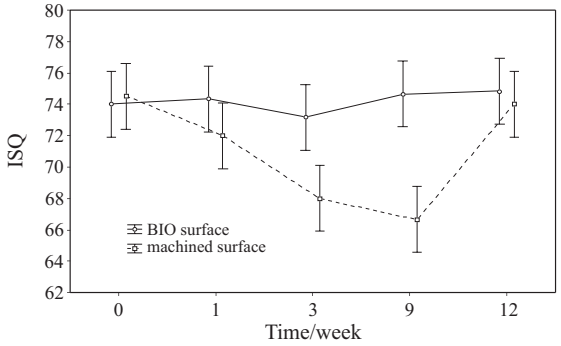

Fig. 3 The stability time dependence for the implant with — – BIO surface and - - - – machined surface TI-M (*p<0.05)

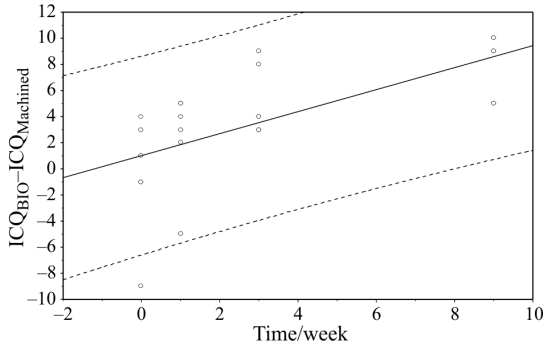

Fig. 4 Time dependence of the net contribution of the BIO surface to the implant stability with the linear regression line: $ICQ_B - ICQ_M = 0.84$ weeks$+1.01$ with its boundaries of reliability. Correlation coefficient $R=0.76$

Conclusions

Implants with the BIO surface maintained their stability during the whole healing period in contrast to those with machined titanium surface, which showed a statistically significant decrease in stability three and nine weeks after implantation. The BIO surface exhibits more favorable values of the major surface characteristics compared to the machined surface.

Acknowledgements

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