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Protecting ultra- and superhydrophilic implant surfaces in dry state from loss of wettability

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Abstract: Ultrahydrophilic titanium miniplates with sandblasted and acid etched (SLA) surfaces were protected from loss of hydrophilicity by an exsiccation layer of salt and stored in a dry state. Various salts in different concentrations were tested in respect to their conservation capacity and optical appearance. Potassium phosphate buffer in a specified composition appeared to be optimal. This optimal system was applied in a long time storage experiment showing no loss of hydrophilicity over years. It was also transferred with success to superhydrophilic dental implants.

Keywords: dental implants; dry conservation; exsiccation layer; hydrophilic; superhydrophilic; imaginary contact angles; sandblasted and acid etched (SLA); Titanium; ultrahydrophilic.

1 Introduction

Hydrophilic surfaces are very sensitive and lose their wetting properties by storing in air [1]. So a conservation of the hydrophilicity is necessary. As early as 2000 [2, 3] Jennissen reported the preparation of ultrahydrophilic surfaces ($\theta < 10^\circ$) on titanium by treatment with chromosulphuric acid and its conservation in dry methanol (for review see [4]). The accelerated and improved osseointegration of this surface has been demonstrated in the mandible of dogs [5]. Later Steinemann and Simpson reported that hydrophilic but not ultrahydrophilic sandblasted and acid etched (SLA) surfaces could be stored and conserved in certain salt solutions (e.g. NaCl) [6], now employed by Straumann GmbH known under the name SLActive®. Its benefits have been demonstrated over the years in preclinical as well as clinical studies [7].

Here we report the preservation of surfaces with ultra- and superhydrophilic properties in the dry state by an exsiccation layer of salt ions, which protects the surface from loss of wettability [4, 8, 9].

2 Material and methods

SLA (type 1): Titanium miniplates ($14 \times 14 \times 1.5$ mm) were manufactured from a titanium rod (grade 4, ASTM F67) and sandblasted with corundum. The sandblasted plates were etched with 50% H_2SO_4 at evaluated temperature by a proprietary method developed by Morpho-plant GmbH leading to an ultrahydrophilic and microstructured surface containing 1–3% corundum [9]. SLA (type 2): Titanium sheets ($300 \times 300 \times 1$ mm, grade 4, ASTM F67) were sandblasted with corundum and sandblasted miniplates ($14 \times 14 \times 1.0$ mm) were stamped from these sheets. The sandblasted miniplates were etched by a combined process, which is developed and proprietary by Morpho-plant GmbH. The first step is etching in diluted HF at room temperature and the second step etching in 50% H_2SO_4 at evaluated temperature leading to an ultrahydrophilic, microstructured and nearly corundum free surface.

Dry Conservation of the SLA surface by an exsiccation layer of salt: The test samples with the initial SLA surfaces were incubated in the specified salt solution for several hours at room temperature. Afterwards they were taken out from the salt solution and air dried for 2 h under clean conditions [4, 9] in two variants: (1) open storage: in air under ambient conditions; (2) in closed glass vials.

The following abbreviations are used for the tested buffers (in parenthesis the molar ratio is given).

- PBS: phosphate buffered saline: 136.8 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 and 8.1 mM Na_2HPO_4
- PBS(+): phosphate buffered saline with Mg and Ca: 136.8 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8.1 mM Na_2HPO_4 , 1.0 mM CaCl_2 , 0.5 mM MgCl_2 .
- NaPB: sodium phosphate buffer: Na_2HPO_4 , NaH_2PO_4 (5.4/1)
- KPB: potassium phosphate buffer: K_2HPO_4 , KH_2PO_4 (5.4/1)

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– MgPB: magnesium phosphate buffer: The specified amount of MgHPO_4 was suspended in water and the minimum amount for solubility of H_3PO_4 was added.

Contact Angle Measurements: Before measurement the exsiccation layer of salt was washed off [8] the sample by dipping in water and MeOH and dried in air. Classical contact angles were measured by the method of Wilhelmy in ultrapure water on a DCAT 11 EC tensiometer (Data-physics GmbH, Filderstadt, Germany) at constant 20°C with the Dataphysic's SCAT software (vers. 3.2.2.86). In this software there is no base line correction for imbibition [10] yielding contact angles (θ) from 0° to 180°, whereby the mathematically incorrect definition $\theta = 0$ for $\cos(\theta) > 1$ is reported [11–14]. Imaginary contact angles after base line correction were calculated by a custom made algorithm in the program MatLab (vers. 7.14, The MathWorks Inc., Natick, MA, USA). Herewith for $-1 < \cos(\theta) < 1$ the value of the contact angle lays between 180° and 0° as above, whereas for $\cos(\theta) > 1$ contact angles in imaginary number space, i.e. imaginary contact angles, are obtained [15–17].

Nomenclature: Classical and real contact angles are denoted by a small letter theta (θ) and imaginary contact angles by a capital theta (Θ) [14]. Advancing and receding angles are identified by the indices a and r, respectively. Hybrid dynamic contact angles denote the combination of an advancing contact angle in real with a receding contact angle in imaginary number space [10]. For surfaces $\theta < 10^\circ$ the term “ultrahydrophilic” and for surfaces with pure imaginary contact angles the term “hyperhydrophilic” is employed [10].

Long Term Stability: For long term stability testing 200 miniplates with SLA (type 2) surface were prepared and contact angles were measured immediately after etching with the mean classical values: $\theta_a = 1.1 \pm 3.2^\circ$ and $\theta_r = 0.0 \pm 0.0^\circ$. For the storage experiment only samples with classical contact angles of 0° were chosen (87% of the samples). These samples were conserved by an exsiccation layer of 96 mM KPB, fixed in alumina tubes and packaged in closed glass vessels and sterilized by gamma irradiation with a dose of minimum 25 kGy by BBF Sterilisationservice GmbH (Kernen, Germany). One part of the sterile samples were stored at ambient temperature (*real time ageing*), whereas the other part was stored at 60°C (*accelerated ageing*) in an oven under recording of the temperature according to ASTM F1980 [18]. Since this ASTM is valid for sterile packaging and the activation energy for the chemistry involved in dry salt conservation is unknown the statement “Real time aging programs provide the best data” [18] should be noted. After defined storage times contact angle were measured.

Table 1: Influence of the exsiccation layer on the dynamic contact angles (classical evaluation) of SLA surfaces after 3–5 days storage under open conditions.

Initial medium	Conc. (mM)	pH	S*	n	$\theta_a \pm \text{SD}$	$\theta_r \pm \text{SD}$
Initial surface			1	5	0.0 ± 0.0	0.0 ± 0.0
Air			1	5	72.0 ± 17.0	0.0 ± 0.0
PBS	149	7.4	1	5	4.6 ± 10.2	1.3 ± 3.0
NaPB	960	7.3	1	5	0.0 ± 0.0	0.0 ± 0.0
NaPB	96	7.3	1	5	0.6 ± 1.3	0.0 ± 0.0
NaPB	9.6	7.3	1	5	0.0 ± 0.0	0.0 ± 0.0
KPB	960	7.6	1	5	0.0 ± 0.0	0.0 ± 0.0
KPB	96	7.6	1	5	0.0 ± 0.0	0.0 ± 0.0
KPB	9.6	7.6	1	5	6.0 ± 7.4	3.6 ± 6.5
Initial surface			2	40	0.3 ± 1.5	0.0 ± 0.0
Air			2	5	84.2 ± 6.7	0.0 ± 0.0
PBS(+)	151	7.4	2	5	0.0 ± 0.0	0.0 ± 0.0
KPB	96	7.6	2	5	2.8 ± 3.9	0.0 ± 0.0
MgPB	100	2.7	2	5	0.0 ± 0.0	0.0 ± 0.0
MgPB	10	3.5	2	5	0.0 ± 0.0	0.0 ± 0.0
$(\text{NH}_4)_2(\text{HPO}_4)$	100	7.9	2	5	3.5 ± 7.8	0.0 ± 0.0

*S, Surface; 1, SLA (type 1); 2, SLA (type 2).

Application to Dental Implants: Screw type test implants (l = 13 mm, $\varnothing(\text{top}) = 5$ mm, for the shape of the implants see [16]) were manufactured from a titanium rod (grade 4, ASTM F67) and sandblasted with corundum. The sandblasted implants were etched under similar condition like the SLA (type 2) plates on a semiautomatic pilot construction (medSurface dent, RENA GmbH, Gütenbach, Germany) [16]. The dry conservation was also performed on this pilot construction. Therefore the etched samples were transferred in a salt bath containing 96 mM of KPB and incubated for 60 min at ambient temperature in a flow. Afterward the implants were dried at 60°C for 60 min and packed in closed glass vessels. Contact angles were measured by the method of Wilhelmy as described previously after storage for 1 month and washing off the protective layer [8, 17].

3 Results

3.1 Examination of different salts for conservation of hydrophilicity in dry state by classical analysis

Contact angles obtained by different ions contained in the exsiccation layers during storage for 3–5 days under open conditions are shown in Table 1. PBS preserves the ultrahydrophilicity quite well, but shows a blotchy pattern after drying (Figure 1B) impairing the optical appearance and homogeneity. Therefore an alternative exsiccation layer

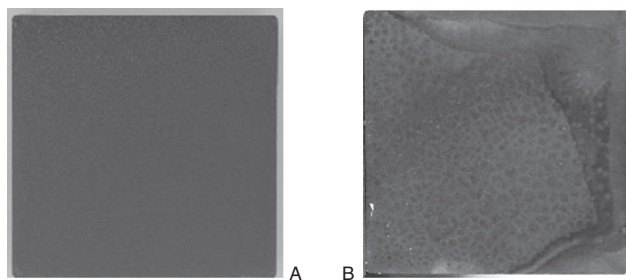


Figure 1: Optical appearance of exsiccation layers obtained from different salts. (A) 96 mM KPB, (B) 149 mM PBS.

Table 2: Influence of the exsiccation layer on the dynamic contact angles (classical evaluation) of SLA surfaces (type 2) after 14 days storage under packed conditions.

Initial medium	n	Conc. (mM)	pH	$\theta_a \pm SD$	$\theta_r \pm SD$
Initial surface	20			0.6 ± 1.8	0.0 ± 0.0
Air	5			86.9 ± 6.8	0.0 ± 0.0
KPB	5	96	7.6	0.0 ± 0.0	0.0 ± 0.0
MgCl ₂	5	100	6.3	7.9 ± 5.5	0.0 ± 0.0
CaCl ₂	5	100	6.7	3.3 ± 7.4	0.0 ± 0.0

showing the same conservation with a homogenous appearance was necessary. Our screening efforts led to PBS containing Mg and Ca (PBS(+)) and to pure phosphate buffers (Table 1).

Table 2 shows the contact angle of surfaces protected by exsiccation layers of different salts after storage in closed vessels for 14 days. Ultrahydrophilicity is not only retained by phosphate salts but also by MgCl₂ and CaCl₂. In contrast to PBS it was found out that KPB in the initial concentration of 96 mM was optimal for homogeneity (Figure 1A). In lower concentrations homogeneity is reduced and in higher a viscous layer (syrup) is formed. NaPB shows comparable behavior as KPB, but it is more difficult to obtain a homogenous film. In higher concentrations NaPB tends to form residues of white salt on the dark film additionally. However, the exsiccation layer obtained by ammonia phosphate show a quite

inhomogeneous appearance. As well, by using MgPB in low concentration (10 mM) the allocation of the salt film on the surface was poor, and in higher concentration (100 mM) large amounts of syrup like residues retain on the surface.

In conclusion, KPB in initial concentration of 96 mM is the best system for conservation of surfaces of SLA titanium in dry state in respect to preservation of hydrophilicity, optical appearance and biocompatibility.

3.2 Long term wetting stability evaluated by imaginary contact angles

Long term stability of wettability conserved by a 96 mM produced KPB exsiccation layer was now controlled by baseline correction and imaginary dynamic contact angles for the first time (Table 3). Imaginary contact angles (hyperhydrophilicity) give information on wettability non amenable to measurements hitherto. After acid etching one real contact angle (5%), eight hybrid (40%) and 11 imaginary contact angles (55%) are observed. After exsiccation layer formation and γ -sterilization zero real, one hybrid (10%) and nine imaginary ($7i^\circ$ – $9i^\circ$, 90%) contact angles are observed. *Real time ageing* by storage for 30 months (ca. 900 days) at ambient temperature yields 100% imaginary contact angles of $12i^\circ$ – $13i^\circ$ for both dynamic contact angles! These studies will be extended to 5 years. In contrast *accelerated ageing* at 60°C , assumed to correspond to a storage time of 5 years, reduces the imaginary contact angles to $6i^\circ$ – $8i^\circ$ (80%) and reintroduces a hybrid contact angle (20%). However the wettability of the surfaces after accelerated ageing is still to be classed as hyperhydrophilic. These experiments also clearly show that exsiccation layer conservation of contact angles is based on chemistry and is not due to the salt layer physically protecting from putative atmospheric contaminations.

The good conservation ability of hydrophilicity as well as the appealing optical appearance of the KPB exsiccation

Table 3: Comparison of contact angles of SLA surfaces after etching and after conservation (exsiccation layer of 96 mM KPB).

Procedure	Dynamic contact angles custom computer evaluation by MatLab algorithm from wilhelmy balance weight measurements						
	Real CA		Hybrid CA		Imaginary CA		Σn
	$\theta_A \pm SD$ ($^\circ$)	$\theta_R \pm SD$ ($^\circ$)	$\theta_A \pm SD$ ($^\circ$)	$\theta_R \pm SD$ ($^\circ$)	$\theta_A \pm SD$ ($^\circ$)	$\theta_R \pm SD$ ($^\circ$)	
Acid etching	7.6 (1)	3.9 (1)	4.2 ± 2.0 (8)	$6.2i \pm 2.6i$ (8)	$6.7i \pm 2.8i$ (11)	$9.8i \pm 2.0i$ (11)	20
Exsiccation layer and γ -sterilization	–	–	1.7 (1)	$6.4i$ (1)	$7.3i \pm 2.9i$ (9)	$9.3i \pm 2.6i$ (9)	10
Storage for 30 months amb. temp.	–	–	–	–	$12.4i \pm 2.0i$ (5)	$13.4i \pm 1.8i$ (5)	5
Storage for 229 days at 60°C^b	–	–	2.2 (1)	$5.7i$ (1)	$6.2i \pm 2.4i$ (4)	$8.6i \pm 1.4i$ (4)	5

^aNo. of samples (n) in parentheses.

^bAssumed accelerated ageing corresponding to storage for 5 years at ambient temperature.

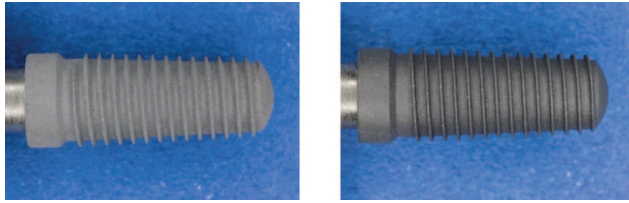


Figure 2: Pictures of dental implants with an original SLA surface (left) and after conservation with an exsiccation layer of KPB (96 mM).

layer, can be transferred to dental implants with more complicated geometry. Figure 2 shows the homogeneity of a KPB exsiccation layer on test implant with screw-type shape. Contact angle measurement by Wilhelmy of five of these KPB protected implants after storage for 31 days lead to: $\Theta_a = (19.5 \pm 2.5)^\circ$ and $\Theta_r = (27.2 \pm 1.0)^\circ$. Static pico-drop analysis yielded contact angles of 0° [17]. These values match well with the values after etching indicating stability of the contact angle [16, 17]. By classical evaluation all advancing and receding angles are wrongly classed as zero.

4 Conclusion

Ultra- and hyperhydrophilic titanium mini plates as well as dental implants with SLA surfaces can be protected from loss of hydrophilicity with an exsiccation layer of salt. KPB in an initial concentration of 96 mM appears to be optimal considering conservation capacity, biocompatibility and optical appearance. Those protected samples are suitable for gamma sterilization and could be stored for several years.

Author's Statement

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