

# Decontamination of SAE Surface: An *In Vitro* Study

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## Abstract

**Aim:** To investigate the impact of different treatments used to detoxify dental implants on the titanium oxide layer ( $\text{TiO}_2$ ) roughness and chemical composition and how these changes may impact in the re-osseointegration of an implant.

**Materials and methods:** 25 titanium discs (Ti6Al4V) coated with a SAE surface treatment (Sandblasting and Acid-etching) were subjected to a series of mechanical and chemical treatments simulating surface decontamination of dental implant affected by peri-implantitis.

The morphology and roughness (mainly  $S_a$ ,  $S_q$ ,  $S_{ku}$ ,  $S_{sk}$ ,  $S_{dr}\%$ ) of the surface layer was investigated with scanning electron microscope (SEM) and confocal interferometer respectively, while the chemical composition was analyzed with X-ray photoelectron spectroscopy (XPS). All samples were analyzed before and after treatment. Chemical and mechanical treatments employed for detoxification of the implant surface included tetracycline hydrochloride (TC), phototherapy in conjunction with toluidine blue gel (L), air-powder (OH) and ultrasonic device (US). 5 discs were used for each treatment group.

**Results:** US treatment delaminates the titanium oxide layer ( $\text{TiO}_2$ ), decreasing roughness, principally by crashing the highest peaks of the surface layer, leaving the  $\text{TiO}_2$  layer a roughness similar to a turned, machined surface. TC treatment is not completely removed by the physiologic serum irrigation and remains in the deep of the valleys of the surface. The result of this deposition is translated with a decrease of the roughness parameters in general. Bicarbonate jet polishing air powder OH leaves a similar roughness but also leaves rests of powder on the surface. Phototherapy in conjunction with toluidine blue enhances the surface exposure by modifying the texture complexity and thus increases the roughness.

**Conclusion:** In order to achieve the re-osseointegration of an implant affected by peri-implantitis, the decontamination treatment should leave at least a similar surface as the original SAE surface treatment. In terms of roughness parameters, this study shows that the phototherapy treatment not only has similar parameters of roughness comparing to the original surface, but also enhances the texture complexity of the surface that may improve the chances for re-osseointegration.

**Keywords:** Dental implants; Titanium alloy; Titanium oxide ( $\text{TiO}_2$ ); Osteointegration; Re-osseointegration; Confocal microscopy; Peri-implantitis; Surface roughness; Surface chemistry

## Introduction

Titanium is the preferred material for dental implants because of its mechanical strength and protective oxide layer, which is naturally formed and regenerated immediately in presence of air and/or aqueous media, providing protection against corrosion. Due to these characteristics, in terms of roughness and porosity in the microscopic range (depending on the treatment surface), commercially pure titanium (CP Ti) or the alloy TiAl4V are unique for osteointegration providing stability of the implant to survive the mechanical requirements of the oral environment [1-5].

Although, maintenance has been suggested after placement of the implant to ensure a favorable environment for osteointegration to occur and continue [6,7]. Such procedures are designed to diagnose and treat inflammatory responses as known as peri-implantitis, an inflammatory process around an implant, characterized by soft tissue inflammation and loss of supporting bone [8] in the peri-implant area. The presence of bacterial biofilm and its metabolic activity alters the oxide layer properties in terms of roughness and chemical composition. The infection progressively spreads among the implant surface and lead to a failing implant. Thus, the clinician has the option to either remove the infected implant or perform debridement and decontamination of the implant surface to remove such biofilms [9] to claim a further re-osseointegration process.

Re-osseointegration can be defined as the establishment of *de*

*novo* bone formation and *de novo* osteointegration to a portion of an implant that during the development of peri-implantitis suffered loss of bone-to-implant contact (BIC) and became exposed to microbial colonization [10]. When peri-implantitis occurs, several treatment strategies (mechanical, chemical, biochemical, physicochemical, etc.) can be employed for removal of attached biofilms on the surface of the implant [11]. Chemical treatments typically employed for debridement of contaminated surfaces include citric acid, tetracycline, doxycycline, saline, chlorhexidine and hydrogen peroxide [12]. These chemicals may be applied in conjunction with various mechanical treatments in order to facilitate biofilms removal. They include cures, ultrasounds, and air-powder blasting, Er:YAG and  $\text{CO}_2$  laser [13-15]. Recently, diode laser and phototherapy have been employed to remove biofilms with promising results [16,17]. The key factors for re-osseointegration [10,18] of an implant affected by peri-implantitis is not only to remove the bacterial biofilm, but also to regain the original implant surface roughness properties. Surface roughness analysis must be defined

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at least with different amplitude parameters ( $S_a$ ,  $S_q$ ,  $S_{sk}$  or  $S_{ku}$ ), and completed with a hybrid ( $S_{dr}\%$ ) or a spatial parameter ( $S_{tr}$ ) [19,20]. For this purpose, optical instruments as Atomic Force Microscopy (AFM) or Confocal interferometer are preferred for a practical 3D measurement of the surface roughness [21]. When an implant surface is studied, the extracted surface could be divided in three patterns, form, ondulation and roughness, all these three are separately studied using filters. The filtering of the roughness separates the macroroughness from the microroughness.

Implant surface has been classified by their average surface roughness value ( $S_a$ ), the mean height of the peaks and mean depth of the valleys on the surface (Table 1) [5]. The surface exposure, defined with a hybrid parameter  $S_{dr}\%$ , represents the developed surface area of a rough surface (3D) in comparison to a perfectly flat, smooth surface (2D). Both  $S_a$  and  $S_{dr}\%$  parameters have been identified to have a strong bone response in animal studies when the surface was moderately rough and  $S_{dr}\%$  of 50% [1,22-36].

Rougher surface instead, like the old plasma-spray surface, reports an impaired bone response [1,22-24]. Wennerberg et al. presented an overview of surface roughness characteristics ( $S_a$  and  $S_{dr}\%$  parameters) of the four most oral implant systems (Table 2) [20]. It concludes that stronger bone response cannot be fully explained by differences in microroughness and suggest the possibility of an altered nanoroughness pattern and physiochemical effects behind the demonstrated strong bone response [27]. Recent studies supports that nanometer-sized particles may play a major role in the protein adhesion and the subsequent cellular response during healing [28,29].

The goal of this study was to do a quantitative and qualitative analysis of the decontamination treatments effects on the roughness and the chemical composition of the titanium oxide ( $TiO_2$ ) layer of original SAE (Sandblasting and Acid-etching) surface. The rationale for this investigation was based on the hypothesis that the synergy between chemical and mechanical forces employed in these procedures may cause permanent deterioration of the original SAE surface. In order to claim a further re-osseointegration process, it is critical to investigate which treatment for decontamination could result in greater incidence of implant failure. In this study, the effects of a series of decontamination procedures are investigated on alloy Ti6Al4V, using confocal interferometry, microscopy and X-ray photoelectron spectroscopy.

Roughness	Smooth	Minimally rough	Moderately rough	Rough
$S_a$	$S_a < 0.5 \mu m$	$0.5 \mu m < S_a < 1.0 \mu m$	$1.0 \mu m < S_a < 2.0 \mu m$	$S_a > 2.0 \mu m$

Table 1: Classification of surface roughness [5].

Implant	$S_a$ ( $\mu m$ )	$S_{dr}$ (%)
Turned, Machined (Branemark)	0.9	34
Osseotite (Biomet 3i)	0.68	27
Nanotite (Biomet 3i)	0.5	40
Prevail Ti-6Al-4V (Biomet 3i)	0.3	24
TiOblast (Astra Tech)	1.1	31
OsseoSpeed (Astra Tech)	1.4	37
Tiunite (Nobel Biocare)	1.1	37
SLa old batch (Strauman)	1.5	34
SLa new batch (Strauman)	1.78	97
SLActive (Strauman)	1.75	143

Table 2: Surface topography of implants from the four major companies [20].

## Materials and Methods

### Materials

A series of treatments typically employed in the treatment of peri-implantitis were investigated in this study. These include tetracycline hydrochloride (TC), phototherapy in conjunction with toluidine blue gel (L), air-powder (OH), and ultrasonic device (US).

25 titanium discs SAE surface treatment (Grade 5 (Ti6Al4V), sandblasted with large grits of alumina ( $Al_2O_3$ ) of 0.25-0.5 mm and acid etched with HCL/  $H_2SO_4$  ( $S_a$ :  $1.857 \pm 0.067 \mu m$ ),  $\varnothing$  10 mm, thickness: 5 mm, and rinsed with deionized water in ultrasonic cleaning for 15 min, dried in the air and then packaged in a polymer sterilizing bag before Gamma irradiation (MIS institute, Savion, Israel) were analyzed before and after different chemical and mechanical treatments employed for detoxification of the dental implant surface. 5 discs were used for each group, TC, L, OH, US and control group (Q). The samples were stored in their original packing, a polymer sterilizing bag, before and after each procedure. As all the procedures during the study could potentially contaminate the surface with organic and inorganic residues, in the present study, special attention was taken in the manipulation of the samples discs with titanium clamps.

### Further sample preparation

**Bicarbonate jet polishing:** The Ti alloy samples were jet polished (Turbodent, Mectron, Carasco GE, Italy) with bicarbonate powder (particle size  $150 \mu m$ ) with saline as irrigant for 1 minute, and then cleaned with saline. Finally the discs were dried and packed in their original package.

**Tetracycline:** Tetracycline hydrochloride (TC) is an antibiotic and acts as a bacteriostatic but can, at certain concentrations, be highly bactericidal. Samples were exposed to a Tetracycline hydrochloride/saline (Sigma-Aldrich) solution (TCH=50 mg/ml) for 1 minute. The discs were rinsed with saline and finally dried and store in their original package.

**Ultrasound:** An ultrasonic scaler with steel tips (Sirosonic, Sirona, Bensheim, Germany) (30 KHz) was applied on the samples with water as irrigant during 1 minute. Finally the discs were dried and packed in their original package. By cavitation, a phenomenon that releases hydrogen peroxide, and the vibratory motion of the tip, the ultrasonic scaler decontaminates the surface in contact.

**Photodynamic therapy:** Toluidine Blue (TB) (Sigma-Aldrich), a blue photosensitizer in gel, was applied on the samples during 1 minute, then activated with the illumination (Application FotoSan Lamp at 570 nm) for 1 minute and finally rinsed with saline. The discs were dried and packed in their original package. The toluidine blue gel, when activated, release free oxygen radicals. These are very reactive and generate a cytotoxic effect.

### Confocal interferometry

Confocal interferometry (Leica, Leica DCM, Barcelona, Spain) was used to measure the surface topography and calculate the surface roughness parameters. Images were taken with a confocal objective with a magnification of 20X and a numerical aperture (NA) of 0.50. The measured area was  $636 \times 477 \mu m^2$ . A Gaussian filter with a size of  $50 \times 50 \mu m^2$  was applied before parameter calculation. A selection of five different parameters was made to characterize the surface topography.

- $S_a$ : Average height deviation from a mean plane measured in  $\mu m$  and represents a pure amplitude parameter.

- $S_q$ : Root mean square roughness.  $S_q$  which is insensitive in differentiating peaks or valleys, is an amplitude parameter used to specify the surface.
- Ssk: Skewness, an amplitude parameter, describes the asymmetry of the height distribution, is used to distinguish if the height deviation is mainly due to dominating peaks or valleys. A positive value indicates predominance of peaks, a negative indicates predominance of valleys.
- Sku: Kurtosis, an amplitude parameter, describes the “peakedness” of the surface topography, is used to specify the distribution of peaks and valleys. A Sku of 3 represents a Gaussian distribution of peaks and valleys.
- Sdr%: Developed surface area, measured in percentage enlargement compared to a totally plane reference area, is a hybrid parameter.

### Microscopy

The surface of each sample was microscopically surveyed at multiples points, pre and post-treatment. SEM Images were taken at high magnification using a Scanning Electron Microscopy (JEOL JSM-7100F, Barcelona, Spain). The operating conditions were acceleration voltage (15 kV), magnification of 200X and a working distance of 10 mm.

### X-ray photoelectron spectroscopy

Chemical composition of the surface was investigated with X-ray photoelectron spectroscopy (XPS) PHI5500 Multitechnique System (Physical Electronics, Barcelona, Spain) with a monochromatic X-ray source (Aluminium Kalfa line of 1486.6 eV energy and 350 W), placed perpendicular to the analyzer axis and calibrated using the 3d5/2 line of Ag with a full width at half maximum (FWHM) of 0.8 eV. The analyzed area was a circle of 0.8 mm diameter, and the selected resolution for the spectra was 187.85 eV of Pass Energy and 0.8 eV/step for the general spectra and 23.5 eV of Pass Energy and 0.1 eV/step for the spectra of the different elements. In depth measurements for composition depth profiles were obtained by sputtering the surface with an Ar<sup>+</sup> ion source (4 keV energy). A low energy electron gun (less than 10 eV) was used in order to discharge the surface when necessary. All Measurements

were made in a ultra-high vacuum (UHV) chamber pressure between  $5 \times 10^{-9}$  and  $2 \times 10^{-8}$  torr.

## Results

### Confocal interferometry

The Original SAE surface (Q) presented a moderately rough surface as the L, OH and TC group, according to the definition suggested by Wennerberg et al. (Table 3, Figure 1) [5]. Instead, the US group presented a minimally rough surface. The US group demonstrated a significant lower  $S_a$  and  $S_q$  value compared to the rest of the groups (Q, L, OH and TC). L group showed the highest  $S_a$  and  $S_q$  value followed by the control group (Q), the air powder abrasive group (OH) and finally the tetracycline hydrochloride group (TC). Thus, the following relation for the roughness  $S_a$  and  $S_q$  was obtained:

$$(Q) \approx (L) > (OH) > (TC) \gg (US)$$

In terms of distribution of peaks and valleys, the Ssk parameter, was similar for all groups except for the TC group and particularly the US group. While the US treatment reduces significantly the peaks of the surface (negative Ssk), the TC group reduces the deep of the valleys (positive Ssk) in comparison with the original surface (Q). The Sku parameters demonstrated a similar Gaussian distribution for all the groups, except for the US treatment group where the Sku was greater than 3, indicating that the effects of the treatment was mainly on the peaks of the surface.

With an Sdr% of 23%, the phototherapy treatment presented a higher surface exposure and thus, a greater ability for fluid retention compared with the original surface (Sdr% of 20%). The OH group compared to the original surface had a similar Sdr% value. Instead, the TC group and specially the US showed a significant decrease of surface exposure comparing to the original surface.

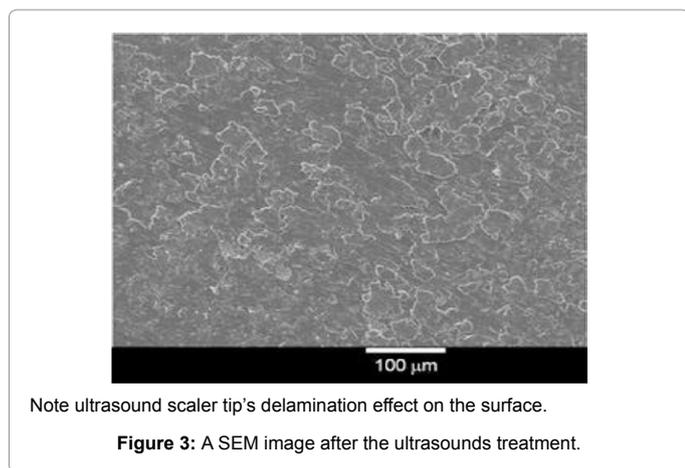
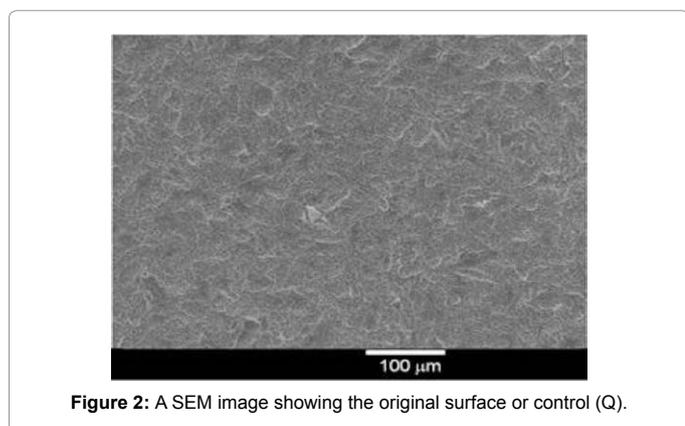
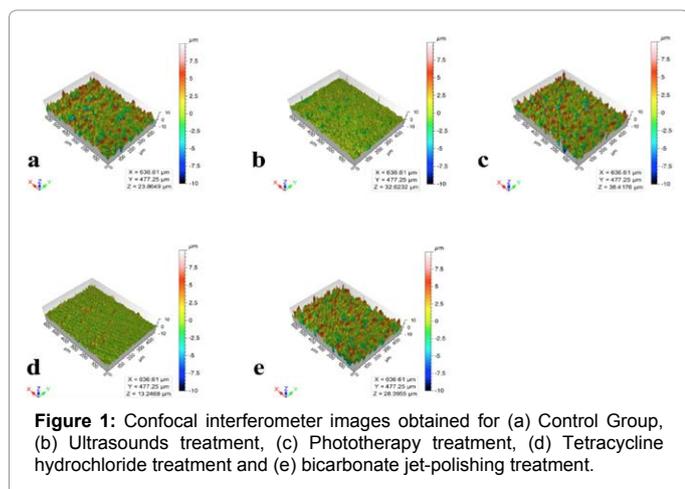
### SEM

SEM analysis confirmed that the result of the aggressive effect of the US treatment on the original SAE surface tends to a surface similar to a machined surface. L surface appears similar to the original SAE surface Q, while OH surface appears lightly smoother compared to Q surface. Presences of treatment residues (Sodium bicarbonate and

Parameter	Q	$\sigma$ (Q)	US	$\sigma$ (US)	L	$\sigma$ (L)	OH	$\sigma$ (OH)	TC	$\sigma$ (TC)
Sa ( $\mu$ m)	1.857	0.067	0.794	0.151	0.151	0.113	1.822	0.086	1.530	0.451
Sq ( $\mu$ m)	2.419	0.085	1.134	0.171	0.171	0.133	2.337	0.105	1.986	0.572
Sz ( $\mu$ m)	35.300	12.505	20.677	8.473	8.473	6.498	26.043	2.378	29.428	13.753
Ssk	0.064	0.183	-0.717	0.600	0.600	0.219	-0.152	0.135	0.103	0.429
Sku	4.409	0.974	13.758	11.314	11.314	0.397	3.554	0.175	4.354	0.813
Sv ( $\mu$ m)	17.110	5.791	9.828	0.995	0.995	5.161	11.978	2.060	13.595	9.152
Smr (%)	0.0048	0.0008	0.0317	0.0223	0.0223	0.0028	0.0014	0.0012	0.0023	0.0023
Smc ( $\mu$ m)	2.962	0.11	1.285	0.319	0.319	0.240	2.872	0.134	2.394	0.698
Sxp ( $\mu$ m)	4.870	0.27	2.403	0.210	0.210	0.239	4.867	0.270	4.050	1.346
Sdar (sq. $\mu$ m)	366126	9176	319096	559	559	11984	365985	4354	352415	15381
Smean ( $\mu$ m)	0.0077	0.0297	0.0263	0.0422	0.0422	0.0233	0.018	0.012	0.0101	0.0306
Spar (sq. $\mu$ m)	303822	0	303822	0	0	0	303822	0	303822	0
Sp ( $\mu$ m)	18.19	7.15	10.850	7.714	7.714	4.142	14.062	1.607	15.834	5.508
St ( $\mu$ m)	35.30	12.50	20.677	8.473	8.473	6.498	26.042	2.376	29.428	13.753
Sdc ( $\mu$ m)	4.82	0.18	1.972	0.490	0.490	0.372	4.745	0.273	3.936	1.147
Sdr%	20.51%		5.03%		23.10%		20.46%		15.99%	

The principles parameters Sa, Sq, Ssk, Sku and Sdr%.

**Table 3:** 12 Roughness parameters of the original surface or control group (Q), Ultrasound treatment (US), Phototherapy treatment (L), Bicarbonate jet polishing treatment (OH) and tetracycline hydrochloride treatment (TC).



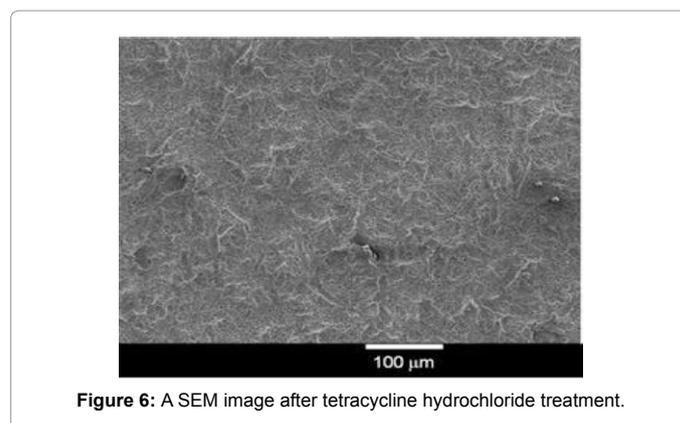
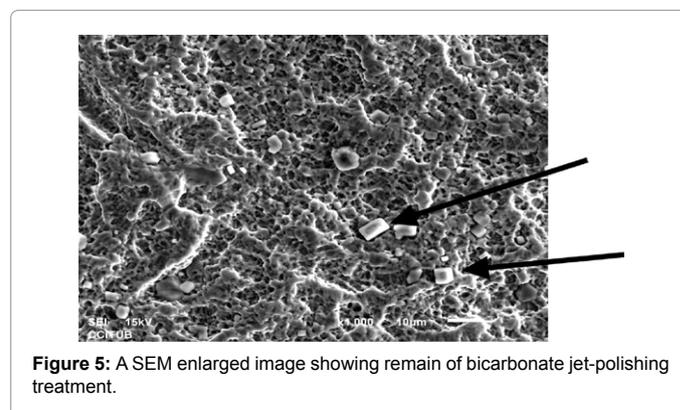
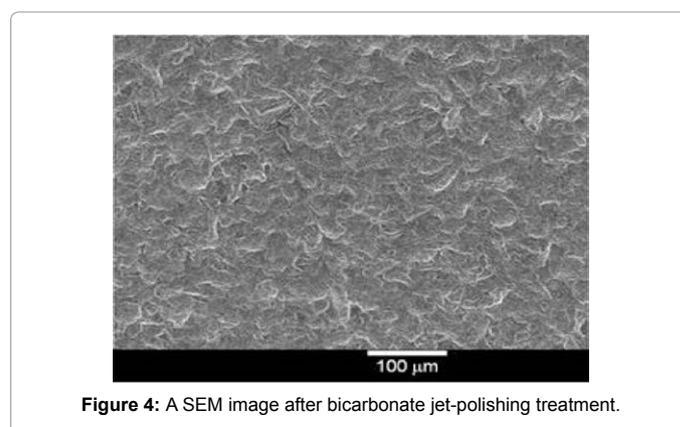
tetracycline hydrochloride) were observed on the OH and TC surface respectively (Figures 2-7).

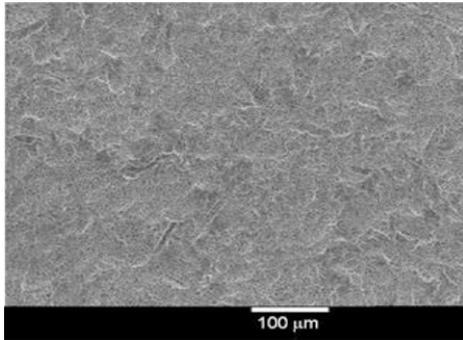
### XPS

The chemical composition is shown in Table 4. Compared to the original SAE surface (Q), while carbon level was clearly higher in all treatment groups (US, L, TC) a similar value was found in the OH group. The highest level of carbon was found in the TC group, which may be explained by tetracycline hydrochloride residues ( $C_{15}H_{16}N_3S^+Cl^-$ ) on the surface.

Instead, the oxygen level was significantly lower compared to Q in all groups; only OH treatment had similar levels. The level of titanium was significantly lower in the L and TC groups, although, similar levels were found in the US and OH groups compared to Q. The levels of nitrogen in L, US groups presented similar levels compared to Q, whereas the OH and the TC treatment showed a significantly lower and higher level respectively. The levels of silicon, decreased significantly in the OH and TC group, and insignificantly in the L group. No traces of silicon were found in the US group. The presence of aluminum in the Q and US group is explained by  $Al_2O_3$  residuals due to the blasting process. A surface free of aluminum was found in the L, OH and TC groups.

Saline residues explained a higher but insignificant level of chlorine in L, OH and TC groups on the surface. In addition, TC group





No obvious differences compared to the original surface could be detected.

**Figure 7:** A SEM image after phototherapy treatment.

revealed a significant higher level of chlorine between all groups due to  $C_{15}H_{16}N_3S^+Cl^-$  residues on the surface. The presence of sodium in the L, OH and TC group was related with the use of saline as irrigant, but especially with the sodium bicarbonate powder use in the OH group.

Traces amounts of magnesium were present in the US group explained by the deposition of the ultrasonic tip after its passage on the surface. Traces amounts of calcium were present in the US group explained by the use of water as irrigant.

## Discussion

Surface roughness and chemical composition of a determined implant surface, plays one of the major role in the osteointegration process, and such information should be considerate before peri-implantitis treatment plan.

### Original surface

The original SAE surface, showed a moderately surface roughness [5], with a  $S_a$  of 1.85  $\mu m$  and an Sdr% of 20% (Figure 2). It has been stated in several studies using various animal models that moderately rough surfaces led to faster and firmer osteointegration [1,22,30-34].

XPS analysis presented clearly titanium (Ti), oxygen (O) and carbon (C) in the original surface (Q) as reported others studies [28,35,36]. The presence of carbon (C) and nitrogen (N) is related to the atmospheric adsorption during the manipulation or during packaging. The storage of the samples in an atmospheric ambient may explain the increased level of carbon, which indirectly shadows the underneath layer of  $TiO_2$ . Although, the presence of carbon on the surface of dental

implants is not necessarily considered by the ASTM-F67 normative as a contaminant, Larsson et al. observed that the high carbon levels on CP Ti discs (machined, electropolish or anodized treatment) might be related with the samples storage during the study [35]. Effectively, while the carbon levels ranged from 35-75% in their previous study when samples were placed bare in the polymer sterilizing bag, the carbon levels decreased from 20 to 40% when samples were placed in a titanium container and then in the polymer sterilizing bag before autoclave procedure. It was stated that the package material during autoclave procedure could transfer contaminants from the polymer to the implant surfaces. Although, Wever et al. also found high carbon levels (60%) on machined titanium alloy (Ti6Al4V), but those samples were packed in aluminium foil and sterilized with autoclave [37]. Others studies registered lower carbon levels, ranging from 35-42% on titanium alloy discs treated with SAE and packed in aluminium foil [28] or ion  $CO^+$  implantation treatment with no specific storage [38] respectively. Lu et al. also found lower carbon levels ranging from 31-35% in CP Ti discs with an AE treatment (acid etched) surface only (no packaging, no autoclave) [36]. Even if this contamination is considered inevitable by other coworker, it seems that the carbon level is mainly explained by the atmospheric deposition and adsorption, but also sensible to autoclave procedure more than the treatment surface or the package material itself [37]. It has been stated that such inclusions of carbon in the dioxide layer play a hydrophobic role, and could decrease the surface energy preventing the protein adhesion and the subsequent cellular response during healing [38].

In the present study, the SAE treatment surface showed 41% carbon, 8% titanium and 43% oxygen. A strong relation seems to exist between these levels and the sample storage. Effectively, carbon level drops significantly when titanium AE or SAE surface are stored during a time (14 days) in an aqueous or NaOH (24h) [36] or NaCl solution [28]. Instead, titanium and oxygen levels increased significantly up to 27% and 61% respectively. Wennerberg et al. described that such alteration is related to spontaneous nanostructures formation on the outermost titanium oxide layer, when titanium SAE or AE surfaces are stored in aqueous solution after 14 days [28]. The decrease of the carbon level may explain the switch to hydrophilic properties of these surfaces. It was reported that nanoscale modification of titanium endosseous implant surfaces altered cellular and tissue responses, which would potentially benefit osseointegration and dental implant therapy [39]. Lu et al. also described that AE surface with an alkali treatment (NaOH solution 24h at 60°C) enhance the ability of calcium phosphate formation and thus the bond to bone ability [36].

As observed in other studies, the chemical composition in the

Element%	Q	$\sigma$ (Q)	US	$\sigma$ (US)	L	$\sigma$ (L)	OH	$\sigma$ (OH)	TC	$\sigma$ (TC)
Carbon (C)	40.95	1.45	50.00	2.33	50.95	1.42	37.60	2.42	61.02	3.07
Oxygen (O)	43.27	1.23	37.64	1.03	35.27	2.05	40.02	1.65	23.97	3.62
Titanium (Ti)	7.73	1.57	6.83	1.03	4.01	0.77	7.86	0.32	1.24	1.28
Nitrogen (N)	1.41	0.27	0.99	0.09	1.97	0.34	0.57	0.29	3.90	0.53
Silicon (Si)	4.75	0.84	-	-	2.93	1.02	2.06	0.93	1.22	1.11
Aluminum (Al)	1.70	0.53	2.41	0.36	-	-	-	-	-	-
Chlorine(Cl)	0.18	0.13	0.47	0.02	2.58	0.62	1.94	1.08	5.37	1.93
Magnesium (Mg)	-	-	0.36	0.31	-	-	-	-	-	-
Calcium (Ca)	-	-	1.30	0.05	-	-	-	-	-	-
Zinc (Zn)	-	-	-	-	-	-	0.32	0.25	-	-
Sodium (Na)	-	-	-	-	2.29	0.55	9.63	3.08	3.18	1.27

**Table 4:** XPS analysis of the original surface or control group (Q), Ultrasound treatment (US), Phototherapy treatment (L), Bicarbonate jet polishing treatment (OH) and tetracycline hydrochloride treatment (TC).

original surface demonstrated the presence of others contaminants on the surface; these generally depend on manufacturing process, as machined, treatment surface, sterilization and manipulation of the implants [38,40,41]. It is known that cleansing of the thin oxide layer of titanium is an indispensable requisite to achieve osteointegration in dental implants. The presence of aluminum, as observed in others studies, was related with the blasting process with alumina ( $Al_2O_3$ ) or with the use of rotatory instruments during the manufacturing [28,38,42]. Even though, Piattelli et al. demonstrated that residual aluminum oxide particles on the implant surface, it didn't affect the osseointegration of titanium dental implants [43].

### US treatment

With the significant decrease of all the roughness parameters, the ultrasounds treatment (US) leaves a surface minimally rough [5] ( $S_a$  of  $0.79 \mu m$  and Sdr% of 5%), even smoother than a machined surface ( $S_a$  of  $0.9 \mu m$  and Sdr% of 34%) [27] (Figure 3). As observed in another study, the US treatment delaminate the original surface roughness and crash the highest peaks (Ssk negative) of the surface, leaving the valleys of the surface untreated [13]. These alterations correlate the limited potential of cleaning of ultrasound scaler tip as observed in others *in vitro* [44,45] and *in vivo* studies [46]. Interestingly, aluminum, a contaminant resulting from the blasting process, is found in the original surface and after the US treatment. The presence of alumina particles confirms again the limited cleansing of the ultrasound tip into the deep valleys of the surface. Also, it was expected the observation of traces of contaminants from the ultrasound tip and its coating after its passage on the surface. Effectively, traces of magnesium (Mg) and presence of calcium (Ca) were found on the surface. Magnesium was related to the coating of the tip and calcium was related to the use of water as irrigant. It remains unclear, which are the limits between normal or pathological levels. It has been stated that these elements could act as electrolytic cells and interfere into the osteointegration process [47-49].

### OH treatment

The bicarbonate jet polishing treatment is, as described in other *in vitro* studies, respectful with the surface treatment and leaves a moderately surface roughness [5] with a  $S_a$  of  $1.82 \mu m$  and a Sdr% of 20% [13]. While the US treatment have a partial effect on the surface, the OH treatment seems to potentially touch all the segments of the surface in concordance with other *in vitro* studies [50,51]. As observed in few studies, the OH treatment lightly smoothes the original surface by rounding the highest peaks and decreases the valleys deep 52-54]. Also, even if major parts of SAE surfaces are biocompatible, rests or traces of contaminants proceeding from the cleansing or the blasting process impede the complete osteointegration process around these contaminants. It is well known that a major part of cleaning procedure applied for removal of alumina particles doesn't leave a surface free of contaminants [51]. Interestingly, the chemical analysis in our study shows that the OH treatment leaves a surface free of aluminum, but also reduces significantly others contaminants like silicon or nitrogen. Instead, a significant amount of sodium (Na), about 10%, proceeding from the sodium bicarbonate powder is found after the OH treatment. In accordance with the significant decrease of the Sv value (deep of the valleys of the surface), it has been assumed that the remainder of the powder fulfills the valleys (Figures 4 and 5). However the effects of such remain on the cellular response during the healing process seems to be related with the particle type of the powder [54,55]. In terms of peri-implantitis, the major parts of contaminants on the surface are made of bacterial biofilm and lipopolysaccharide. Several *in vitro* studies demonstrated that the bicarbonate jet polishing treatment constitutes

an efficient therapeutic option for the debridement of implants in peri-implantitis defects [51,56,57]. Although, only two animal studies reported re-osseointegration after OH treatment [58,59].

### TC treatment

The TC treatment leaves, with a significant decrease of all the roughness parameters, a surface moderately rough with a  $S_a$  of  $1.53 \mu m$  and a Sdr% of 16% (Figure 6). With a positive Ssk, the TC treatment alters the peaks/valleys distribution of the original surface. The diminution of the deep of the valleys, in concordance with the XPS analysis (significant increase of carbon level and decrease of oxygen level), concludes that remaining  $C_{15}H_{16}N_3S^+ Cl^-$  stays on the surface treated even after abundant rinsing with saline. Wheelis et al. reported that the  $C_{15}H_{16}N_3S^+ Cl^-$  with a pH of 2.5 is able to etch the surface, causing discoloration and pits on Ti6AlV alloy discs depending on the time of action [12]. Also, it was observed that in the presence of acidic conditions, cavities of 80 nm deep resulted from localized metal dissolution that could result in metal debris, which could potentially trigger inflammation *in vivo*. Although, it was observed that the demineralization with TC resulted in surface roughness comparable to that produced by osteoclastic activity on dentin fragments, it had beneficial effects on preosteoblast differentiation [60]. Furthermore, in accordance with the rest of TC into the valleys, some studies demonstrated that TC could also be functional in negating systemically antibiotic prophylactic treatment in the prevention of implant or biomaterial related infections [61]. Interestingly, the TC XPS analysis did not show any trace of aluminum proceeding from the SAE treatment. The lack of alumina particles might be explain with remain of TC treatment, which act as a layer that shadows the underneath particles. Further animal and human studies are needed to verify that such remain of tetracycline hydrochloride on the implant surface has advantageous benefits on re-osseointegration.

### L treatment

Phototherapy has a surface moderately rough with roughness parameters similar to the original surface, with a  $S_a$  of  $1.86 \mu m$  but a higher Sdr% of 23% (Figure 7). Ssk and Sku parameters indicate a Gaussian distribution of the surface and that the peaks predominate over the valleys as in the original surface. As described previously, Sdr% represents the surface exposure and the ability to 'expose' the surface to the proteins and the subsequent bone cells [29]. It was stated that novel surfaces, even smoother ( $S_a$  of  $0.5 \mu m$  and Sdr% of 40%) than a turned, machined implant surface ( $S_a$  of  $0.9 \mu m$  and Sdr% of 34%) had a stronger bone response. It was stated that microroughness only could explain a part of the stronger bone response to novel surface [27]. Thus, the increase of Sdr% after L treatment leads to another specific pattern of the dioxide layer, the nanoroughness, which is part of the microroughness measured with the confocal interferometry. Modification of the nanoroughness of the original SAE surface could be related with formation of nanoparticles, which may play a major role on physical and chemical properties [62-64]. Also, Toluidine Blue, the photosensitizer, when activated with the 596 nm and 630 nm light produces different oxygen radical as  $OH^\cdot$ ,  $O_2^\cdot$  and hydrogen peroxide  $H_2O_2$  [17]. It has been described that  $H_2O_2$  at 15% has the same effect of etching on the surface as tetracycline hydrochloride [12]. Thus, the XPS analysis after L treatment demonstrates deep cleansing of alumina particles, proceeding from the blasting treatment. Photoactivated disinfection is described to be effective against periodontopathic bacterial species and to reduce viability in biofilms, but was not able to completely destroy complex biofilms [17]. Instead, lethal photosensitization associated with guided bone regeneration allowed

a better re-osteointegration at the adjacent area to the peri-implant defect regardless of the implant surface [16].

## Conclusions

The clue for the re-osteointegration of a SAE surface is based on the treatment efficiency for biofilm removal, but also with the knowledge of the surface alteration in terms of roughness and chemical composition after such treatment. From the results of the study we conclude that ultrasound treatment should be avoided for the treatment of peri-implantitis due to its aggressive effects on the surface. Bicarbonate jet polishing treatment is an effective treatment even if it leaves remains of powder and smoothes the original roughness of the SAE surface. Phototherapy seems to increase the properties of surface exposure by altering the nanoroughness pattern. Tetracycline hydrochloride treatment should be used in conjunction with bicarbonate jet polishing or phototherapy for its benefits as a local antibiotic and bone preparation.

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