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# The combined action of UV irradiation and chemical treatment on the titanium surface of dental implants

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ABSTRACT

The purpose of this paper is to describe an innovative treatment for titanium dental implants, aimed at faster and more effective osteointegration.

The treatment has been performed with the use of hydrogen peroxide, whose action was enhanced by concomitant exposure to a source of ultraviolet light. The developed surface oxide layer was characterized from the physical and chemical points of view. Moreover osteoblast-like SaOS2 cells were cultured on treated and control titanium surfaces and cell behavior investigated by scanning electron microscope observation and gene expression measurements.

The described process produces, in only 6 min, a thin, homogeneous, not porous, free of cracks and bioactive (in vitro apatite precipitation) oxide layer. High cell density, peculiar morphology and overexpression of several genes involved with osteogenesis have been observed on modified surfaces.

The proposed process significantly improves the biological response of titanium surfaces, and is an interesting solution for the improvement of bone integration of dental implants. A clinical application of the described surfaces, with a 5 years follow-up, is reported in the paper, as an example of the effectiveness of the proposed treatment.



## 1. Introduction

Many surface treatments were implemented on titanium implants in order to improve adhesion and differentiation of the peri-implant cells [1–4], but the issue is still open, mainly because a multifunctional action is needed and a multi-purpose solution has not yet been satisfactorily achieved. The here proposed treatment leads to the growth of a layer of titanium oxide, with intention of positively interfering on the cellular metabolism and on in vivo apatite precipitation. The aim is to obtain a surface presenting a high osteointegration ability, because of these two simultaneous actions. It is of particular interest the ability of the treated surface to simultaneously induce mineralization (in vivo apatite precipitation) and high recruitment/activation of osteoblasts. In fact, if osteoblasts are favored to adhere to the surface, respect to bacteria, and new bone is formed in short times, a stable seal around the implant is obtained, avoiding future infections. The current process consists in a treatment in a bath of hydrogen peroxide, combined with exposure to a UV light source, in order to increase the peroxide action. The samples were subjected to treatments at different times. Moreover, a comparison of the results obtained with and without the exposure to an ultraviolet source has been performed. The combined action of the UV light and hydrogen peroxide is well known, concerning its use in sterilization, water treatment and bleaching [5,6], but it constitutes an innovation in the surface treatment of titanium. As shown by the laboratory tests, we can say that the treatment is able to enhance the formation of hydroxyl groups on the surface, which is of interest in order to stimulate the cell differentiation and adhesion [7–11]. In vitro studies have indicated that the specific properties noted for hydroxylated titanium surfaces have a significant influence on cell differentiation and growth factor production. A surface enrichment in calcium and phosphate groups has been observed, after immersion in simulated body fluids of the treated samples. It is widely reported in the scientific literature [12] that this effect is an evidence of the material's ability to stimulate, after implantation, the growth of the bone mineral component (hydroxyapatite). It is of interest to get bioactive behavior of the surface by introducing a thin and well adherent layer of titanium

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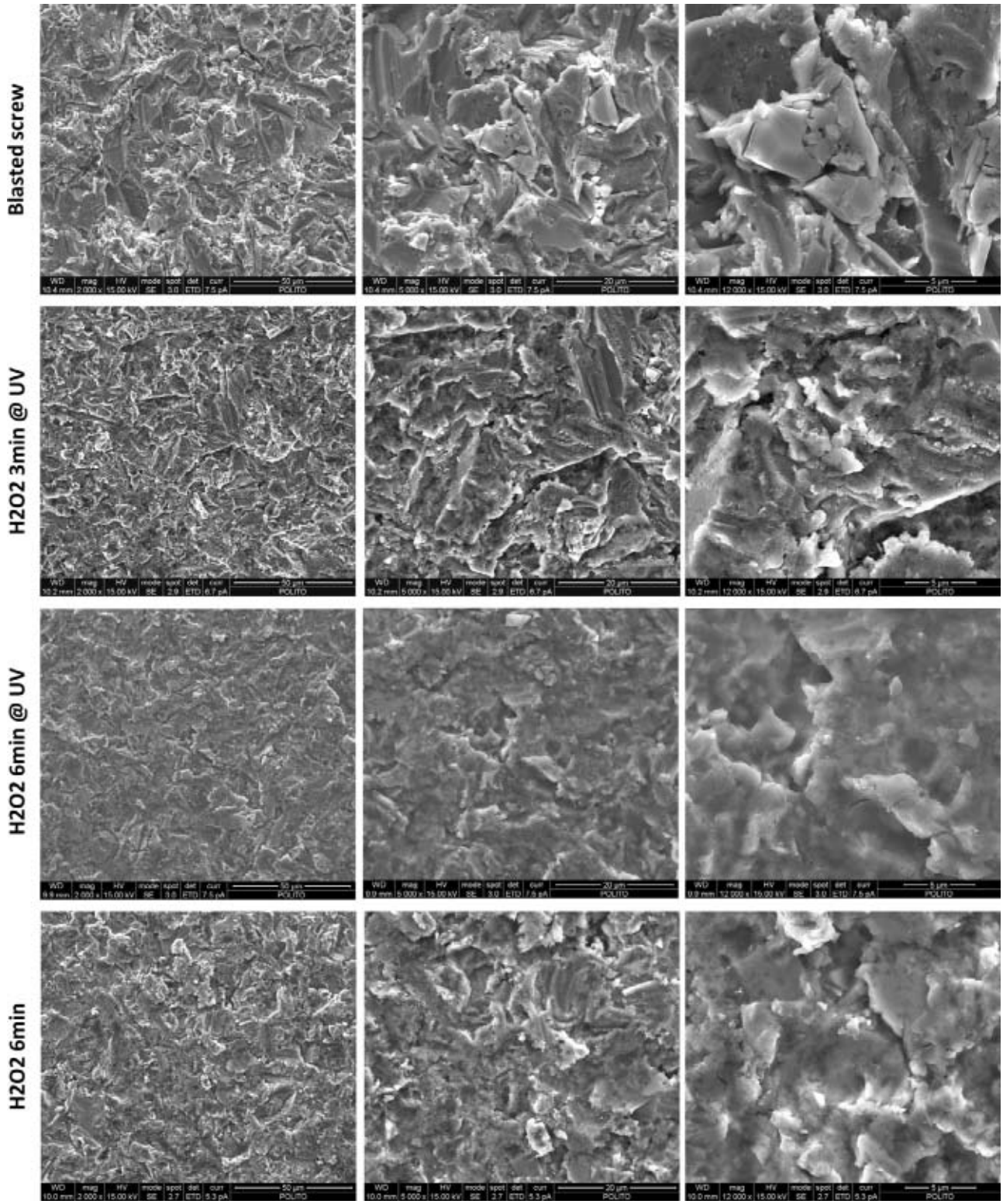


**Fig. 1.** SEM images of a blasted screw (first row) and of blasted screws treated for 3 min (second row) or 6 min (third row) in $H_2O_2$ under UV irradiation or 6 min without UV irradiation (last row).

oxide, avoiding the use of thick coatings of a foreigner material. In fact, it is widely reported in literature that bioactive apatite coatings led to numerous failures in the dental applications, both because of delamination and bio-resorption [13–16]. Considering the specific application of this approach to titanium dental implants surfaces, it must be considered that presently peri-implant cell/material interaction are controlled by topography modification of implant surfaces. Stimulation of osteogenic cells differentiation by surface topography has been a major achievement of present day surface engineering of titanium bone contacting devices [17–19]. The combination of finely tuned chemistry of the surface oxide layer, such as obtained by the present process, with topography offers

interesting perspectives on the control of the peri-implant environment. It is known that the interplay of surface chemistry and topography affects cell response [20], and proper control of both set of properties could lead to better and enhanced implant surfaces.

## 2. Materials and methods

### 2.1. Sample preparation

The proposed surface modification process involves immersion of the samples (discs or implants) in hydrogen peroxide (130 vol) under UV irradiation (CLIP TECNOGAZ lamp) for 3 or 6 min at room temperature. A characterization of treated dental implant was performed. A set of analyses were also performed on some plane samples (discs), in order to avoid the instrumental artefacts due to the complex geometry of the dental implants. The plane samples were obtained by cutting commercially pure titanium bars (ASTM B348, Gr2, Titanium Consulting and Trading) with an automatic cutting machine equipped with an alumina blade. The discs (10 mm in diameter and 2 mm in thickness) were polished by SiC abrasive papers. Some discs were sand-blasted with the same process condition of the commercial dental implants.

In brief the following samples were considered for analyses:

Sandblasted commercial dental screws (S-DS).
Sandblasted and $H_2O_2$–UV treated dental screws (SUV-DS).
Polished plane samples (P).
Polished and $H_2O_2$–UV treated plane samples (PUV).
Sandblasted and $H_2O_2$–UV treated plane samples (SUV).
Dental screws (S-DS and SUV-DS) and also plane samples (P, PUV and SUV) were characterized from the physical and chemical point of view.

For biological characterizations plane samples were employed. SUV surfaces were compared with polished ones (P) and also with sandblasted acid etched (SA) ones, obtained by large grit blasting (alumina particles in the size range 250–400 μm), followed by a double acid etching step involving hydrofluoric acid and hydrochloric/sulfuric acid mixtures, as most of the commercial dental implants.

### 2.2. Chemical and physical characterization

The surface morphology and chemistry were investigated by means of scanning electron microscopy (SEM – FEI, QUANTA INSPECT 200), equipped with energy dispersive spectroscopy (EDS – EDAX PV 9900). The chemical composition of the outermost surface layer and chemical state of the surface elements were investigated by means of X-rays photoelectron spectroscopy (XPS – PHI 5000 VERSA PROBE, PHYSICAL ELECTRONICS). XPS technique was employed also for the determination of the thickness of the surface oxide layer on plane samples (depth profile analysis). The surface oxide was progressively etched by means of an Ar gun and surface elements were monitored in function of the etching time in order to detect the transition point between metallic substrate and surface oxide layer. The surface roughness was determined on the plane samples by profilometry (optical profilometer contactless Talysurf CCI 3000 Å). The surface wettability was investigated by static contact angle measurements. A drop of double distilled water was deposited on the samples surface by a syringe and its shape recorded by a heating microscope equipment (Expert System Solution) and analyzed by the Image J software.

a)

| Element | Untreated screw [% at] | Treated screw [%at] |
|---|---|---|
| C | 49.3 | 53.9 |
| O | 32.9 | 34.5 |
| Ti | 8.0 | 7.4 |
| Al | 5.3 | - |
| Si | 2.8 | 1.9 |
| N | 1.8 | 2.4 |

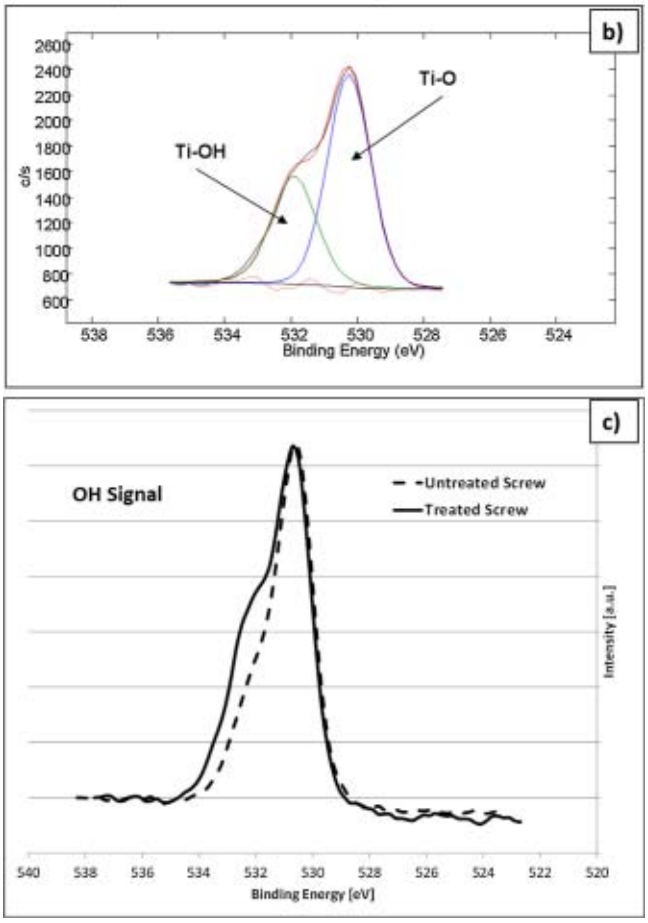


**Fig. 2.** XPS analyses for a untreated and treated (6 min UV irradiation) screws. (a) Comparison of the chemical surface composition of the two screws; (b) detailed analysis of the oxygen region of the treated screw (c) Comparison between the oxygen region of the two screws.

### 2.3. Bioactivity

The inorganic bioactivity was evaluated in vitro by soaking the samples in simulated body fluid (SBF) at 37 °C for 15 days. The SBF solution was prepared according to the Kokubo protocol [12]. The samples were gently washed in double-distilled water at the end of the soaking period and observed by means of SEM–EDS, looking for hydroxyapatite precipitation.

### 2.4. Cell culture

Osteoblast like SaOS2 cells were used in the cell growth experiments. Experimental cell culture medium (BIOCHROM KG, Berlin) consisted of Minimum Eagle's Medium without L-glutamine, 10% fetal bovine serum, streptomycin (100 g/L), penicillin (100 U/mL), and 2 mmoles/L L-glutamine in 250-mL plastic culture flask (Corning TM). Cells were cultured at 37 °C in a humidified incubator equilibrated with 5% $CO_2$. Cells were harvested prior to confluence by means of a sterile trypsin-EDTA solution (0.5 trypsin g/L, 0.2 g/L EDTA in normal Phosphate Buffered Saline, pH 7.4),

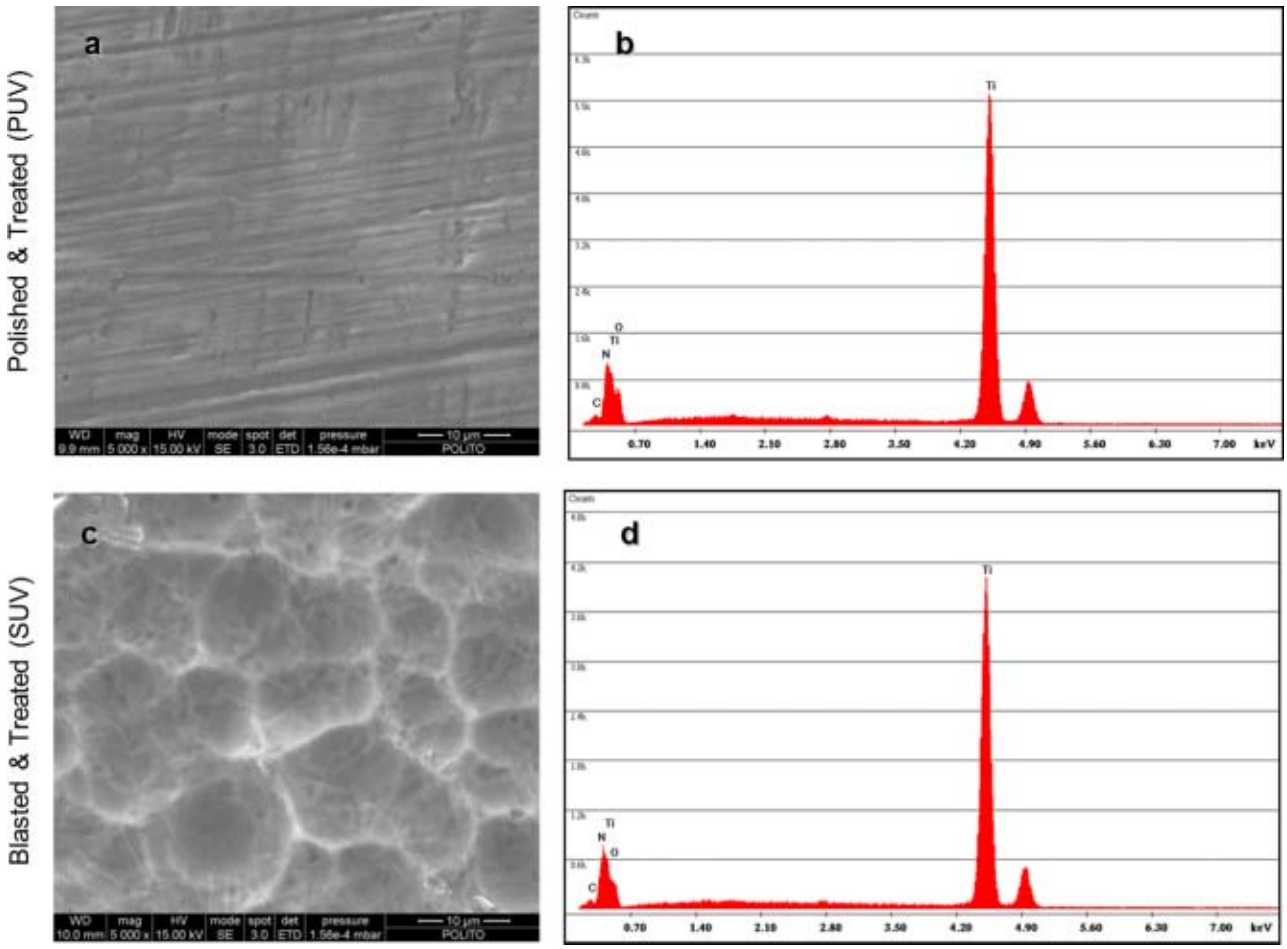


**Fig. 3.** SEM image (a) and EDS analysis of the area (b) for a polished and treated plane sample (PUV) and SEM image (c) and EDS analysis of the area (d) for a blasted and treated plane sample (SUV).

resuspended in the experimental cell culture medium, and diluted to $1 \times 10^5$ cells/mL. For experiments, 5 mL of the cell suspension were seeded into 6-well tissue culture polystyrene plates (9.6 $cm^2$ of growth area; FalconTM), containing the samples. Experiments were performed in triplicate.

### 2.5. Observation of cell morphology by SEM

At selected time interval, as reported in the paper, samples were dehydrated using water–ethanol solutions, with increasing concentrations of ethanol (up to 100%). Hexamethyldisilazane (HMDS, Aldrich) was employed for the final dehydration step. Cell morphology was investigated by means of scanning electron microscopy (SEM, LEO 420 SEM, LEO Electron Microscopy Ltd.), by using an accelerating potential between 15 and 25 kV. Relevant instrumental parameters are reported on the micrographs. Dehydrated samples were gold sputter-coated (AGAR Auto Sputter Coater) before SEM observations.

### 2.6. Gene expression by RT-PCR

Real time reverse transcription polymerase chain reaction (qRT-PCR) was used in order to assess the expression of cell differentiation markers. Cells were cultured on the described materials and total RNA was extracted at selected time points using MagMax Total RNA Isolation Kit (Applied Biosystems) according to the manufacturer's instructions. RNA quality was assessed by checking the A260/A280 ratio (1.6–2.0). Then total RNA was used as a template for cDNA synthesis using random hexamers as primer and Multiscribe Reverse Transcriptase (High Capacity cDNA RT Kit from Applied Biosystems). Taq Man probe and primers from Applied Biosystems (Hs 00266705_g1, GAPDH; Hs 00164004_m1, Coll I; Hs 01029144_m1, ALP; Hs 01047976_m1, RunX2; Hs 00609452_g1, OCN; Hs 00960641_m1, OPN) was used for cDNA amplification and relative gene quantification. A Step-One instrument (Applied Biosystems) with the software Step-One, version 2.1, was employed for Real time PCR. Experiments were performed in duplicate for all samples and targets. PCRs were carried out in a total volume of 20 μl and the amplification was performed as follows: after an initial denaturation at 95 °C for 10 min, the PCR was run for 40 cycles at 95 °C for 15 s and at 60 °C for 1 min. The comparative threshold (Ct) cycle method, consisting on the normalization of the number of target gene copies versus the endogenous reference gene GAPDH, was used in order to normalize the content of cDNA samples. The Ct is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold baseline when amplification of the PCR product is first detected. For comparative analysis of gene expression, data were obtained using the $\Delta$Ct method.

### 2.7. Clinical outcome

Macro and X-rays radiographic images of a clinical case with 5 years follow-up is reported, as an example of the effectiveness of the proposed treatment.

## 3. Results

As first, dental implants and plane samples were treated and chemically/physically characterized. Samples in form of dental implants are useful in order to verify the effects of the treatment on a surface as close as possible to that one of practical interest, for the final application, and in order to verify if the process is feasible, uniform and effective on a device with a complex shape. On the other hand, plane samples are needed in order to perform some analyses of interest, for a complete surface characterization, such as soaking tests in SBF, wettability and roughness measurements. A detailed characterization of the plane samples is also of relevance, in order to ensure that the plane surfaces employed for the cellular tests were comparable to those of the treated implants.

### 3.1. Samples in the form of dental implants

Two different soaking times, 3 or 6 min in hydrogen peroxide solution, were initially considered and used for the treatments of some dental implants. Fig. 1 reports the SEM observations of the screws treated for 3 or 6 min in $H_2O_2$ under UV irradiation, compared with a simply blasted screw, for control purposes; moreover, the images of a surface treated for 6 min without UV irradiation are added for a comparison. The treatments resulted in a uniform surface layer, covering all the implant. It can be observed that the typical topography of the blasted surfaces is maintained, after the treatment 3 min long; sharp peaks and cutting edges can be observed. On the other side, a longer soaking time (6 min) induces the formation of a thicker surface layer, which attenuates the sharp asperities. Surface smoothing of the sharp and cutting edges is even much more evident on the sample subjected to the UV irradiation during the chemical treatment (6 min). Looking at these consideration, the 6 min long $H_2O_2$ treatment with UV irradiation has been selected for the further treatments.

The chemical composition (EDS analysis, not reported) confirmed that the surface layer consists of titanium oxide and no contamination occurs during the treatment. No residual elements from the blasting procedure can be detected.

The XPS analyses of the untreated and treated (6 min UV irradiation) screws (Fig. 2) confirm the decrement of Al and Si contaminations (blasting residues), after treatment, and the presence of a moderate oxidation (Fig. 2a). The presence of carbon on the surface can be attributed to adventitious hydrocarbon contaminants, always present on the titanium surfaces [21]. The detailed analysis of the oxygen region shows a signal characteristic of the hydroxyls groups (Fig. 2 b and c), significantly increased after the treatment.

### 3.2. Plane samples

The morphology of the plane samples (polished/blasted and treated samples) was investigated by SEM observation (Fig. 3). The treated surface is homogeneous without any cracks or discontinuity of the surface oxide layer; some polishing tracks are visible, on the polished samples, confirming that the thickness of the oxide layer is thinner than about 100 nm (see roughness and XPS data Table 1 and Fig. 4). These results confirm that the chemical process can be applied to surfaces of different initial roughness (flat or blasted), with analogous results.

**Table 1**
Roughness and wettability measurements on plane samples.

| | Ra (μm) | Contact angle (°) |
|---|---|---|
| Polished (P) | 0.18 ± 0.02 | 65.1 ± 1.8 |
| Polished & treated (PUV) | 0.13 ± 0.01 | 71.2 ± 9.4 |
| Blasted and treated (SUV) | 0.43 ± 0.01 | 82.6 ± 9.1 |

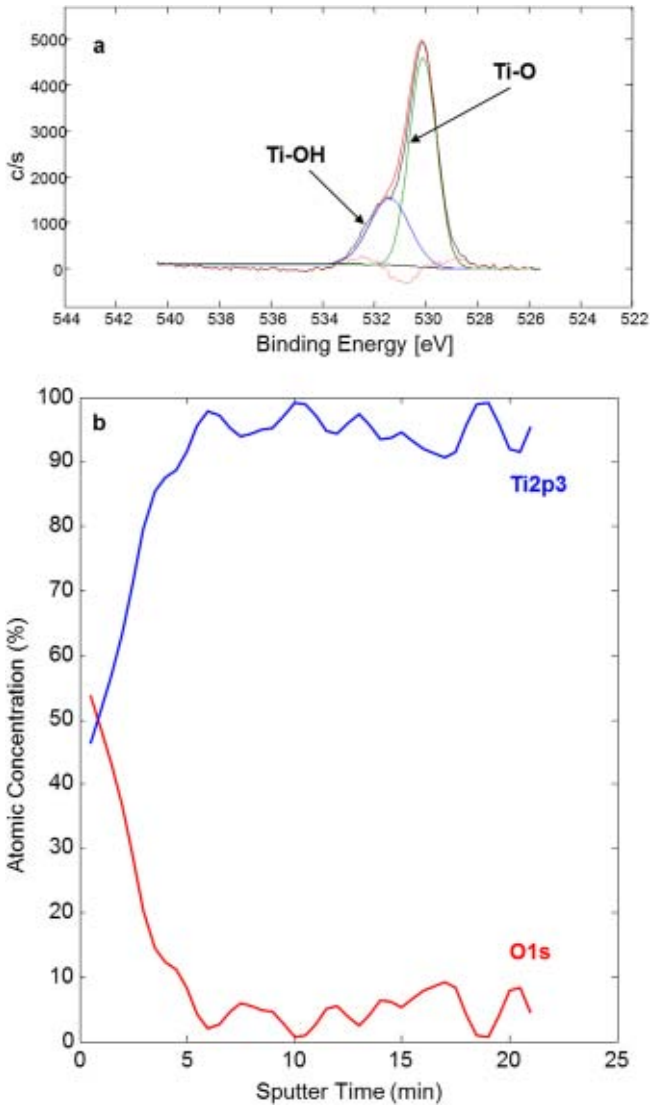


**Fig. 4.** XPS detailed analysis of the oxygen region for a blasted and treated plane sample – SUV (a) and XPS depth profile analysis of the same surface (b).

The XPS analyses confirm, on polished and blasted plane samples, the results obtained on the dental implants, with the presence of a moderate amount of hydroxyls groups (Fig. 4a), that is higher than what detected on the untreated titanium discs (data not shown).

The thickness of the oxide layer was measured by etching the surface with Ar ions, and monitoring the trend of the XPS signals of oxygen and titanium, moving from the outermost layer of the surface to the underlying material (Fig. 4b). The signal related to oxygen is almost zero after 6 min of Ar sputtering. A thickness of about 40 nm, of the surface oxide layer, can be deduced, considering the sputtering rate of titanium oxide with Ar ions.

The roughness measurements evidenced a similar surface topography for polished and polished-chemically treated surfaces, confirming that the surface oxide layer is not rough and it is thinner than 100 nm (Table 1). The blasted and treated plane samples present an Ra value that is around 400 nm (Table 1).

The wettability tests (Table 1) showed a slight increase of the contact angle values, after the treatment, and higher values for the blasted and treated surfaces in comparison with the polished ones. An increase in the contact angle for the blasted surfaces is in

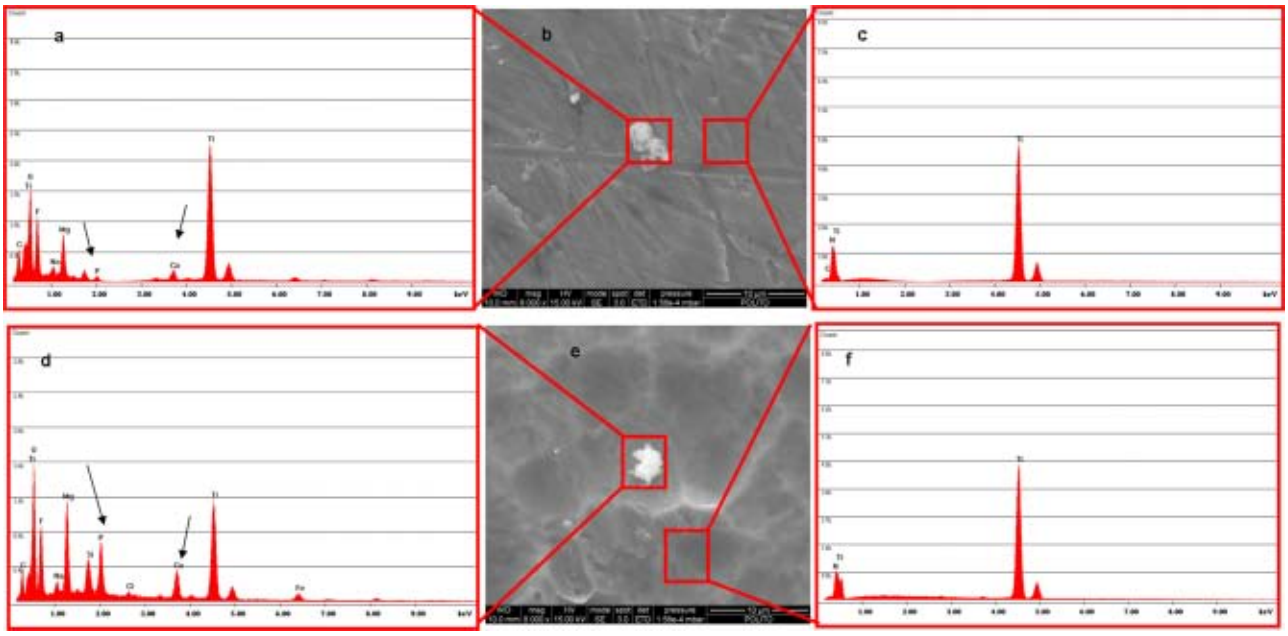


**Fig. 5.** SEM images and EDS analyses for polished and treated (a–c) and sand-blasted and treated (d–f) Ti discs after soaking in SBF for 15 days.

accordance with the literature, concerning the surface wettability of the dental implants [22].

SEM–EDS analyses performed after the soaking in SBF (Fig. 5), on both on the polished-chemically treated (PUV) and on blasted-chemically treated (SUV) samples evidence the presence of numerous particles with the typical morphology of hydroxyapatite and containing Ca and P. This result shows that the treated surfaces are bioactive and induce mineralization. The pH value of the solution remains around the physiological value of 7.4 for the whole soaking period, without abrupt changes, which could induce cytotoxic effects.

### 3.3. Cell adhesion

Evaluation of the morphology of adherent cells was performed after 6 h, 24 h and 5 days. The 5 days datapoint is very indicative of how surface properties direct cell morphology and representative images were obtained for each of the tested samples. Examples of the obtained results are shown in Fig. 6 (1000×), Fig. 7 (3000×) and Fig. 8 (5000×). Concerning P and SA samples, these images confirm the classical and well know effect of surface topography on cell morphology obtained through surface roughening. In particular, cells show a flat and aligned morphology on P surfaces that bear no micrometer-range topographic cues able to stimulate cell behavior. SaOS2 cells adopt thus a flat morphology, which has been associated with minimal differentiation and scarce osteogenic activity. On the contrary, the microrough topography typical of SA treatment promotes a cuboidal morphology of cells, as clearly observed in the relevant figures. Note that the underlying topography is still clearly visible, even after 5 days culturing, that is cell density is comparatively low as compared to the P surface. This is another well-known effect of SA topography on cell behavior, in particular it is known that microroughness stimulates cell differentiation [23]. It is reported that acid-etched surface topography prevents cell spreading, keeping cells in a more active, less "fibroblast-like" phenotype [9,24,25]. As for many other cell systems, spreading and proliferation is opposed to round morphology and differentiation: cells on acid-etched surfaces are less spread, show reduced proliferation, but they are more efficient as to osteogenic activity, according to the literature. This is well reflected by the comparison of cells on P and SA surfaces. The observation of cells on SUV samples yields significant differences with observed cell morphology of both reference samples. Cell density is very high, to the point that the underlying surface topography is no longer observable. Also, cells grow in multilayers, they do not show the alignment of cells on P surface, nor the cuboidal morphology and low density of cells grown on SA. From these observations, it is clear that the surface properties of the three different samples affect cell behavior, or, more properly, cell morphology and proliferation, in a different way, directing cell growth, on the different samples, towards different directions. The implications of these different pathways on cell function, as related to osteogenesis, was investigated by RT-PCR. Measurements were performed at 5 and 10 days datapoint, in order to follow

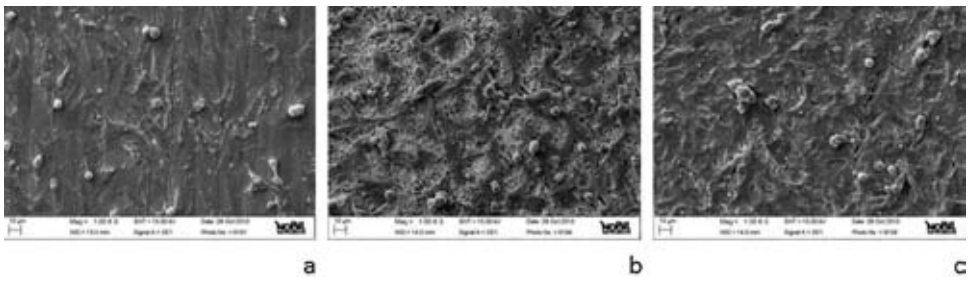


**Fig. 6.** Representative SEM images (1000×) of SaOS2 cells after 5 days culturing on P, SA and SUV samples.

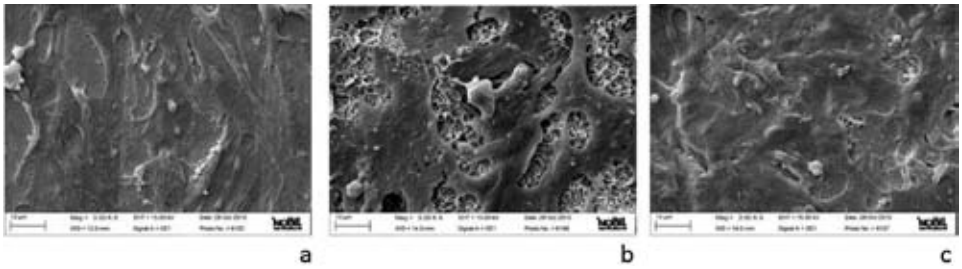


**Fig. 7.** Representative SEM images (3000×) of SaOS2 cells after 5 days culturing on P, SA and SUV samples.

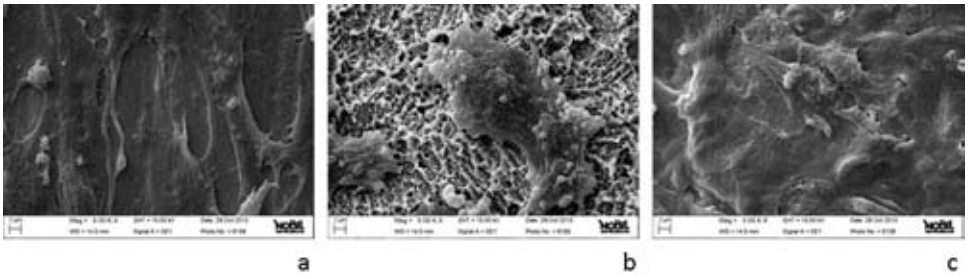


**Fig. 8.** Representative SEM images (5000×) of SaOS2 cells after 5 days culturing on P, SA and SUV samples.

evolution of gene expression after colonization of the samples surface by SaOS2 cells. Results are shown in Fig. 9. The bar-graph shows, at 5 and 10 days datapoint, the fold expression of a given gene over that recorded on sample P, which is taken as reference. At 5 days, comparison between P and SA samples shows that cells grown on the latter sustain enhanced expression of BMP-2 and BSP genes. BSP is a protein typical of the cementum layer, that is of the a-collagen proteinaceous layer, that is found at the interface between implants and newly formed bone. This is confirmed also by the 10 days datapoint, which further shows enhanced expression of osteopontin and Alp on SA surfaces. Taken together, these data indicate a greater osteogenic activity by cells cultured on SA samples and confirm the literature data at the basis of the clinical success of SA surfaces (widely confirmed at short times) and general interpretation of the SEM findings previously described. As to the SUV samples, both data points suggest a very high overexpression of most genes as compared to both reference surfaces. In particular, beside BSP, BMP-2, ALP also ColII, an early marker of osteogenesis, osteocalcin, RUNX-2 (a gene related to differentiation), osteopontin and cMyc a gene involved with proliferation. Interestingly, also RANKL encoding for a protein involved with bone remodelling and resorption, is significantly overexpressed at both datapoint. Its fold expression over control P samples however shows a decreasing trend with time, contrary to what observed on most of the other tested genes.

### 3.4. Clinical outcome

As an example, Fig. 10 reports macro and X-ray radiographic images of a patient, before and after 5 years implantation of dental screws, treated with the surface treatment described in the present paper. It can be observed that implants have been welded to titanium rods by means of intraoral electric resistance welding, as described by the authors in [26], in order to increase the primary stability of implants. No signs of bone absorption after 5 years can be detected.

## 4. Discussion

The morphological analysis showed that the surface oxide layer is homogeneous, continuous and crack free, on the treated screws and plane samples; this means that no detrimental effect on corrosion resistance and fatigue behavior can be expected, as reported in other cases [27,28], when the treatment in hydrogen peroxide was too aggressive. It is thick enough to slightly smooth the blasted surface, avoiding the presence of cutting edges. It is reported [29] that rough surfaces, with sharp and cutting

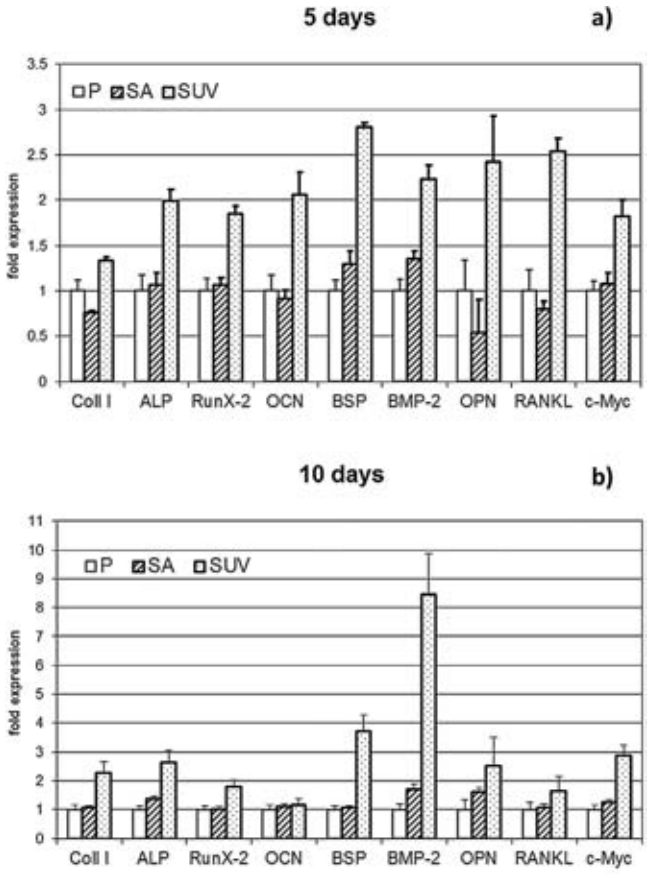


**Fig. 9.** Expression of several osteogenesis related genes by SaOS2 cells after 5 and 10 days culturing on P, SA and SUV surfaces. For each gene data are expressed as fold expression over the value recorded for the P sample.

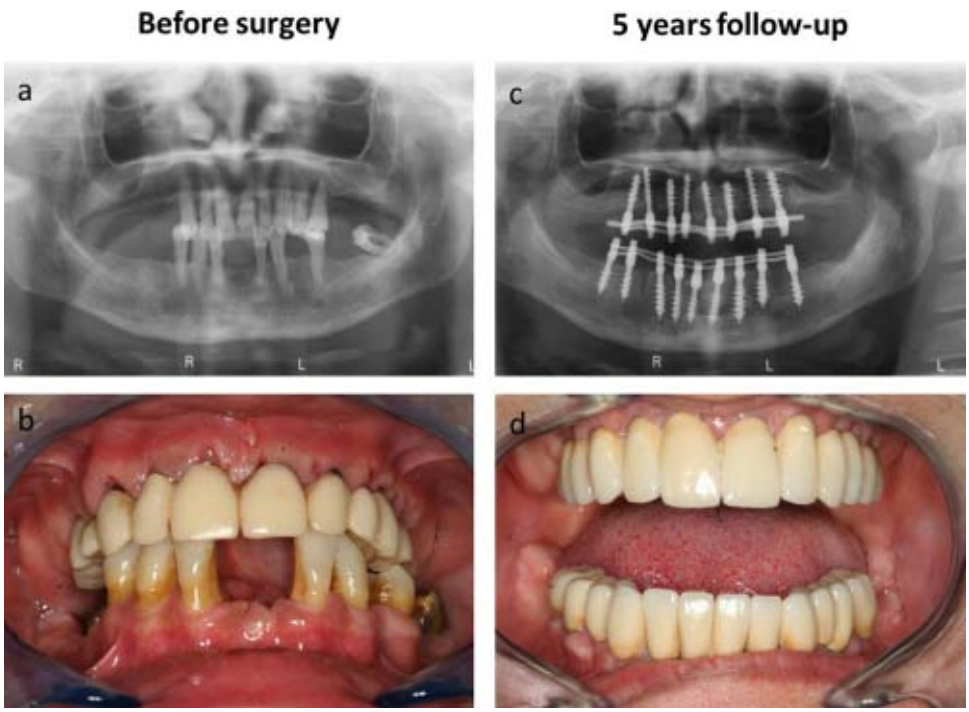


**Fig. 10.** X-rays radiographic (a) and macro image (b) of patient before surgery. X-rays radiographic (c) and macro image (d) of patient after 5 years follow-up.

edges, revealed a reduced mineralization activity of osteoblasts. This chemical treatment does not introduce a further porosity or roughness respect to the blasted surface. The roughness of the treated samples is lower than the typical values for the blasted and SLA (sand-blasted and acid etched) surfaces (at about 1–1.5 μm) [4]. This is a relevant difference, respect to the current commercial chemical processes, employed for SLA dental implants; in this case an evident porosity on the micro scale is added by the chemical treatment. Several studies [30] report that SLA implants considerable reduced failure rates, if short-term response is monitored. On the other side, it was recently reported [31] that a not negligible long-term implant failure rate still occurs, as well as infections and peri-implantitis. It is not well known which factor is crucial for a long-term response of the bone to a foreign body. It was supposed that a high level of marginal bone contact, at short time, was the crucial factor for bone integration. Actually, it is not known the ideal percentage of bone to implant contact, at short times; a stronger initial bone response could not be coupled to improved long-term clinical outcome, instead it may indicate a stronger foreign body reaction [31]. The good cell response of the here reported surface, in absence of porosity on the micrometer scale, can be of interest in this regard. It must be also considered that rough surfaces can result in an higher bacterial contamination and formation of biofilm could be more rapid [32].

The increment of OH groups is of interest, because OH groups are active sites for the bioactivity of the surface, they may enhance surface wettability, as well as osteointegration [8].

Wettability measurements showed a slight increase of the contact angle values, after the treatment. The role of the surface wettability on the bone integration is a widely discussed topic in literature. This parameter play a role in the interaction between the implant surface and biological fluids, as well as blood, at the early stages [22,33], and it affects the protein absorption. It has been evidenced that a high wettability can favour the absorption of the RGD sequence containing proteins and it consequently improves the osteoblast adhesion [33]. On the other hand, it is known that the osteoblasts preferentially adhere onto the moderately hydrophobic surfaces [34]. Taking into account these considerations, it can be concluded that a moderate hydrophobicity, as what observed on the modified titanium surfaces described in this paper, can be a favorable substrate for the bone cell adhesion, as observed in the biological tests presented in the present research work.

In conclusion, it must be underlined that an effective oxide layer (not porous, not cracked, thin, able to smooth the sharp edges, hydroxylated, free from blasting residues) has been obtained on the titanium surfaces by a very short treatment (6 min), through the synergistic action of hydrogen peroxide and UV irradiation on the Titanium surface.

Based on the data from cell culture, it is possible to speculate that the peculiar properties of SUV surfaces have a profound effect on SaOS2 cell activity. For both set of experiments, cells are significantly stimulated by the surface in a markedly different way as compared to P and SA. In particular, cell density (hence cell proliferation) is very high, to the point that multilayers are observed while both on P and SA density is still low and the underlying surface topography can plainly be observed. Contrary to the often observed inverse relationship between proliferation and differentiation (confirmed, in the present case, by the comparison between P and SA), most of the tested genes encoding for markers of osteogenic activity are fold-expressed many times as well. Shortly, at least based on these in vitro data, the SUV surface looks especially suitable to stimulate fast colonization and very significant osteogenic activity by osteoblast-like cells. These data are in general agreement with reports on the biological activity of hydrophylized titanium surfaces [8–11]. In fact, it is difficult to explain the observed behavior in terms of surface topography alone. Sandblasted surfaces have been deeply investigated and they do not show better properties as compared to SA, according to the literature, and, in actual practice, they have been replaced by SA in the evolution of dental implant surfaces. The fine control of the surface chemistry of SUV surfaces, in particular the high density of hydroxyl groups described in the physical and chemical characterization section of the paper, is a more convincing explanation of the observed stimulation of cell activity. Li and coworkers [35] investigated the surface characteristics and biocompatibility of sandblasted and acid-etched titanium surface modified by

ultraviolet irradiation. They performed an in vitro evaluation using MG-63 osteoblast-like cells. Similar to present findings, they concluded that UV treated surface, bearing abundant hydroxyl groups, greatly promoted the attachment, proliferation, differentiation, and mineralization of MG63 cells. Bang and coworkers [36] evaluated the activities of both osteoblastic and osteoclastic differentiation on sandblasted/acid etched and hydrophilic sandblasted/acid etched surfaces. Differentiation of osteoblasts was significantly increased on less hydrophobic surfaces, as supported also by RT-PCR evaluation for the expression of Runx2, OPN, and OCN, in agreement with our results. Interestingly, the data of the just quoted paper are obtained on surfaces that show the same topography and different chemistry, while in the present work topography of SA and SUV surfaces is different, yet the same trend of gene expression is observed. This suggests that surface chemistry, in particular the high density of hydroxyl groups, plays a major role in directing interfacial cell behavior, over and above that of topography, at least within the tested systems. Indeed, a recent interesting paper [37] supports the role of surface chemistry, in particular of surface hydroxylation, on genetic mechanisms leading to osteogenic differentiation. While previous studies investigate the role of osteogenic cells, the in vivo peri-implant environment is definitely more complicated, and interfacial interactions involve different cell types. Alfarsi and coworkers [38] evaluated macrophages response to control and hydroxylated titanium surfaces. Macrophages are important in the early inflammatory response to surgical implant placement and influence the subsequent healing response [39]. The paper reports a significant down-regulation of several pro-inflammatory genes on hydroxylated titanium surfaces, confirming that they can modulate human macrophage pro-inflammatory cytokine gene expression and protein secretion.

A clinical case confirms the in vivo effectiveness of the proposed surface treatment.

## 5. Conclusions

A novel surface treatment based on the oxidation of the titanium surfaces with hydrogen peroxide and UV irradiation has been explored in this research work. A thin, homogeneous, not porous and free of cracks oxide layer has been obtained on the titanium surfaces (both on model plane discs, polished or blasted, and on commercial blasted dental screws) by a time effective process (6 min). The treatment does not increase the roughness of the surface and, in the case of a blasted substrate, it smoothes any sharp peak and cutting edge. Moreover, it exposes hydroxyls groups. The treated metal allows the precipitation of Ca/P rich particles in simulated body fluid.

The response of osteoblast-like SaOS2 cells to a novel surface treatment based on the oxidation of the titanium surfaces with hydrogen peroxide and UV irradiation has been explored in this research work. Results show a significant effect of titanium surface chemistry on the tested cell lines. Descriptive observation by SEM shows a very high cell density and a peculiar cell morphology on SUV surfaces, clearly different from what is observed on control P and SA surfaces. Evaluation of expression of several genes related to osteogenesis indicates strongly enhanced gene expression on SUV surfaces, in agreement with recent reports on hydroxylated titanium surfaces.

The in vivo efficacy of the proposed treatment has been documented by a clinical case.

## Conflict of interest

The authors declare that they have no conflict of interest.

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