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ORIGINAL ARTICLE

Photofunctionalization and non-thermal plasma activation of titanium surfaces

Anders Henningsen[1,2] · Ralf Smeets[1] · Philip Hartjen[1] · Oliver Heinrich[1] · Roman Heuberger[3] · Max Heiland[4] · Clarissa Precht[1] · Claudio Cacaci[5]

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Abstract
Objective The aim of this study was to compare UV light and non-thermal plasma (NTP) treatment regarding the improvement of physical material characteristics and cell reaction on titanium surfaces in vitro after short-term functionalization.
Materials and methods Moderately rough (Ra 1.8–2.0 μm) sandblasted and acid-etched titanium disks were treated by UV light (0.05 mW/cm^2 at λ = 360 nm and 2 mW/cm^2 at λ = 250 nm) or by NTP (24 W, -0.5 mbar) of argon or oxygen for 12 min each. Surface structure was investigated by scanning electron microscopy, confocal microscopy and X-ray photoelectron spectroscopy (XPS). Hydrophilicity was assessed by dynamic contact angle measurement. Cell attachment, viability, cell proliferation and cytotoxicity were assessed in vitro using murine osteoblast-like cells.
Results UV irradiation or NTP treatment of titanium surfaces did not alter the surface structure. XPS analysis revealed a significantly increased oxidation of the surface and a decrease of carbon after the use of either method. NTP and UV light led to a significant better cell attachment of murine osteoblasts; significantly more osteoblasts grew on the treated surfaces at each time point ($p < 0.001$).
Conclusions UV light as well as NTP modified the surface of titanium and significantly improved the conditions for murine osteoblast cells in vitro. However, results indicate a slight advantage for NTP of argon and oxygen in a short time interval of surface functionalization compared to UV.
Clinical relevance UV light and NTP are able to improve surface conditions of dental implants made of titanium.

Keywords Dental implants · Ultraviolet rays · Plasma gases · Titanium · Cell adhesion · Osteoblasts

Clarissa Precht and Claudio Cacaci contributed equally.

✉ Anders Henningsen
a.henningsen@uke.de

[1] Department of Oral and Maxillofacial Surgery, University Hospital Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany

[2] Department of Oral and Maxillofacial Surgery, German Armed Forces Hospital, Lesserstrasse 180, 22049 Hamburg, Germany

[3] RMS Foundation, Bischmattstraße 12, 2544 Bettlach, Switzerland

[4] Department of Oral and Maxillofacial Surgery, Charité University Hospital, Augustenburger Platz 1, 13353 Berlin, Germany

[5] Implant Competence Centrum, Weinstr. 4, 80333 Munich, Germany

Introduction

In 1981, Albrektsson et al. showed that surface conditions of titanium implants are a fundamental factor for successful osseointegration [1]. Topographical, biological and chemical conditions determine the surface characteristics of dental implants. Despite a low specific weight, titanium has excellent mechanical properties and a good corrosion resistance due to the dense oxide layer. The oxide layer (mainly TiO_2) is an essential precondition for successful osseointegration [2]. Present-day dental implants have an optimized surface topography and some have an optimized surface chemistry. Biologically and pharmaceutically modified surfaces are still subjects of recent research [3]. Until now, no evidence has been shown for any particular type of dental implant to be superior concerning long-term success [4]. However, higher bone-implant-contact (BIC) values and better bone apposition were demonstrated on implants with rough surfaces compared to smooth surfaces, including stimulation of cell migration and proliferation [5]. BIC values in modern implants normally vary between 65 and 73% but do not reach the ideal 100% [6].

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Many modifications have been introduced to enhance surface hydrophilicity, raise surface functionality, improve surface chemistry and decrease surface contaminations [7–9]. Among them, surface functionalization by ultraviolet (UV) light or non-thermal plasma (NTP) just prior to implant placement seems to be a very promising method to improve interactions between the proteins, cells and titanium surface, to enhance bioactivity, the speed of osseointegration and peri-implant bone apposition. UV light or NTP functionalization of titanium implant material led to increasing wettability and attractiveness for cells on titanium surfaces [10–15] and to an early osseointegration [10, 15–19].

From a clinical point of view, both methods are easy to apply. Due to technical progress and size reduction of the devices, both methods can easily be integrated into the daily routine of a dental practice. However, if these methods are equal in improving surface conditions of titanium in a short and practicable period of time is currently unknown. Therefore, the aim of this study was to compare the effects of UV or NTP functionalization on the surface properties (topography, roughness, chemistry, wettability) and cell reaction (cell attachment and morphology, cell viability and proliferation, cytotoxicity) on sandblasted and acid-etched titanium surfaces in vitro.

Materials and methods

Titanium samples

Sandblasted and acid-etched titanium disks (titanium grade 4, 15 mm in diameter, 1.5 mm in thickness) were used (Promote® surface, Camlog, Basel, Switzerland). They were γ-sterilized and stored in commercially available packages in darkness for 4 weeks after manufacturing.

UV and NTP treatments

UV light treatment was performed using an UV light oven (Therabeam® Superosseo, Ushio, Tokyo, Japan; Fig. 1a). The oven generates UV light as a mixture of spectra; intensity was about 0.05 mW/cm^2 (λ = 360 nm) and 2 mW/cm^2 (λ = 250 nm). All samples in the UV light group were treated for 12 min.

NTP functionalization was performed using a Yocto III NTP plasma reactor (Diener Electronic GmbH, Ebhausen, Germany; Fig. 1b). The generator frequency is 100 kHz. The vacuum chamber is made of borosilicate glass. Several treatment cycles are possible; the treatment conditions that were used in this study were 24 W, −0.5 mbar and 12 min. The temperature ranges between 36 and 40 °C during treatment. Additionally, UV light is produced with peaks at λ = 320 nm using argon plasma and λ = 240 nm using oxygen plasma. One group of titanium disks was treated by pure oxygen plasma; the other group was treated by pure argon plasma.

Scanning electron microscopy

The surface structure was investigated using an Evo MA25 (Zeiss, Oberkochen, Germany).

Surface roughness measurements

The roughness of the samples was investigated by confocal microscopy (S neox, Sensofar, Barcelona, Spain) with a ×20 and ×50 objective. The analysed surface was 4.8 × 0.66 mm, length according to ISO 4288:1996. Data was processed by MountainsMap Software (Release 6.2.7487, Digital Surf, Besançon, France) applying a Gaussian filter with a cut-off distance lambda c of 0.8 mm according to ISO 4288:1996. The microroughness was removed by applying a Gaussian filter with a cut-off distance lambda s of 2.5 μm according to ISO 3274:1998.

Wettability

The advancing dynamic contact angle of a water droplet was measured according to DIN 55660-2 using a contact angle meter (Surftens Universal, OEG®, Frankfurt, Germany). Measurements were performed on five different areas of the titanium surfaces and averaged. In case of spreading of water droplets over the whole titanium surface, repeated measurements were not possible. Data was processed by Surftens Software (Release 4.3, OEG®, Frankfurt, Germany).

XPS analysis

XPS measurements were performed with a Kratos Axis Nova (Kratos Analytical, Manchester, UK) using monochromatic AlKα-irradiation (1486.7 eV), 225 W and angle of incidence 54.6°. Three disks per treatment were analysed in the centre of the disk. The spectra were analysed using CasaXPS Software (Version 2.3.14, Casa Software Ltd., Devon, UK). Peakshifting was corrected by referencing aliphatic carbon to 285 eV. The areas of the peaks were determined after subtraction of an iterated Shirley background, corrected by the sensitivity factors given by Kratos and herewith the composition was calculated assuming a homogenous compound. The thickness of the oxide layer was calculated based on the concentrations of the metallic titanium and of the oxidized titanium applying the Hill equation. The inelastic mean free path of photoelectrons from the Ti2p orbital in a titanium dioxide compound was calculated with the formula of Tanuma et al. to be 2.17 nm [20]. The thickness of the samples was corrected with a factor of 0.67 in order to take into account the roughness of the samples [21].

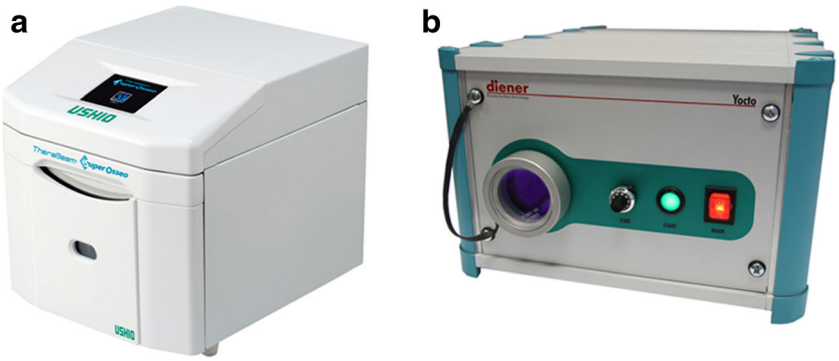

Fig. 1 **a** Ushio Therabeam® Superosseo (Tokyo, Japan) and **b** Diener Yocto III (Ebhausen, Germany). Both pictures were kindly provided by the manufacturers

Osteoblastic cell culture

For all in vitro experiments, murine osteoblast-like cells MC3T3-E1 (C57BL/6, Sigma-Aldrich®, Munich, Germany) were used. Cells were cultured in α-modified minimum essential medium with nucleosides (MEM α Gibco™, Invitrogen™, Paisley, UK) supplemented with 10% foetal bovine serum (FBS Gibco™, Invitrogen™, Paisley, UK) and 100 units/mL penicillin/100 μg/mL streptomycin (Gibco™, Invitrogen™, Paisley, UK). Cells were incubated in a humified atmosphere of 95% air and 5% CO_2 at 37 °C. At 80% confluency, cells were detached using 0.05% trypsin-EDTA (Gibco™, Invitrogen™, Paisley, UK) and seeded onto the treated or non-treated disks at a density of $2.4 \times 10^5/cm^2$ (cell attachment) or $0.5 \times 10^5/cm^2$ in culture wells.

Reference material (positive control)

RM-A, a polyurethane film containing 0.1% zincdiethyldithiocarbamate (Hatano Research Institute, Food and Drug Safety Center, Hadano, Kanagawa, Japan), was used as positive control reference material.

Cell attachment and morphology

Cell attachment was assessed by measuring the quantity of living cells attached to the titanium disks (live-dead staining, LDS) after 2, 24 and 72 h of incubation. After rinsing with PBS (Gibco™, Invitrogen™, Paisley, UK) cells were stained with fluorescin diacetate/propium iodide and fluorescence microscopy was carried out. Cells were counted automatically by ImageJ software (Release 1.5 h, U.S. National Institutes of Health, Bethesda, MD, USA). Cell morphology was evaluated.

Viability

Viability was assessed by measuring 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT, Cell Proliferation Kit II, Roche Diagnostics, Mannheim, Germany) as a function of redox potential. After 48 h of incubation, 50 μL of XTT labelling solution was added to each well followed by incubation for another 4 h. Extinction was measured in a multi-well spectrophotometer (ELISA reader) with 450- and 690-nm (reference wavelength) filters.

Cell proliferation

To assess proliferation, the "Cell Proliferation ELISA, BrDU (colorimetric) test kit" (Roche Diagnostics, Mannheim, Germany) was used. After 24, 48 and 72 h of incubation, cells were labelled with BrdU labelling solution and incubated for another 2 h followed by fixation by FixDenat reagent for 30 min. The fixed cells were incubated for 1 h with anti-BrdU-POD antibody and washed three times for 5 min with PBS. Substrate reaction was initiated by tetramethylbenzidine (TMB) for 20 min followed by termination by 25 μL 1 M H_2SO_4. Extinction was measured in a multi-well spectrophotometer (ELISA reader) with filters 450 and 690 nm (reference wavelength).

Cytotoxicity

Cytotoxicity was assessed by a lactate dehydrogenase cytotoxicity assay (LDH-Cytotoxicity Assay Kit II, BioVision, Milpitas, CA, USA). After 24, 48 and 72 h of incubation, 10 μL of the culture supernatant was incubated with 100 μL LDH reaction mix for 30 min. After addition of stop solution, the absorbance was measured using a multi-well spectrophotometer (ELISA reader) with 450- and 690-nm (reference wavelength) filters.

Statistical analysis

All statistical analyses were performed using SPSS 20 (IBM, Armonk, NY, USA). Cell culture experiments were performed in groups of six in three independent experiments. To compare differences of the test groups versus the control groups, a Kruskal-Wallis test with a post hoc Bonferroni correction was used. p values <0.0083 were regarded as statistically significant. Surface parameters of each material were compared

for statistically significant differences using two-sided Student's *t* test. Level of significance was set at 5% ($p \leq 0.05$).

Results

Scanning electron microscopy

The scanning electron microscopy (SEM) images of the disks showed a surface with both a macroroughness and a superimposed microroughness (Fig. 2a and b), as it is typical for sandblasted and acid-etched surfaces. Only very few particles from the grit blasting remained on the surface of the disks despite the subsequent cleaning/etching process. No difference of the surface structure was observed between the non-treated disks and the disks after the plasma or the UV treatment.

Surface roughness measurements

In agreement with the SEM images, the surfaces showed pronounced narrow peaks and valleys in the titanium disks. The mean arithmetic roughness (Ra) was between 1.8 and 2.0 μm. There were no significant differences between the disks after the different surface treatments.

Contact angle

Prior to surface treatment, the contact angles were high (113° ± 10°), signalizing hydrophobicity (Fig. 3). The dynamic contact angles dropped to 12° ± 3° after UV treatment. After surface plasma treatment, the water droplet spread and no contact angle could be determined. Differences between non-treated and UV- and NTP-treated titanium disks were statistically significant ($p < 0.001$) also between UV and plasma treatments ($p < 0.001$), but not between O_2 and Ar plasma treatments.

XPS analysis

The survey spectra of the titanium disks showed prominent signals of oxygen, titanium and carbon. Depending on the analysed disk, there were additionally traces of nitrogen, fluorine, magnesium, silicon, sulphur and/or calcium.

The contribution of metallic titanium decreased with the treatments, indicating that the oxide layer did grow due to the plasma treatment. The thickness of the oxide layer on the metallic substrate was estimated based on the Hill equation. It was 5.6 ± 0.1 nm on the non-treated disks. After the plasma treatments, it grew to a thickness of 15 to 16% to 6.6 ± 0.2 nm while with the UV treatment the growth was marginal (4 to 6% to 5.9 ± 0.1 nm), but still statistically significant ($p = 0.01$) compared to that of non-treatment. No statistically significant differences were found between the plasma treatments. The atomic ratio of carbon to titanium decreased from 0.98 ± 0.03 on the non-treated disks to 0.72 ± 0.03 after the UV treatment, 0.69 ± 0.02 after the O_2 plasma and to 0.65 ± 0.01 after the Ar plasma treatment. The decrease was statistically significant for all treatments compared to that of non-treatment ($p < 0.002$), between UV and Ar plasma treatments ($p = 0.03$) but not between UV and O_2 plasma and between the NTP treatments.

Cell attachment and morphology

Cells were seeded directly on the disks. Generally, cells on the treated surfaces were larger and more elongated compared to such on non-treated surfaces. Representative images after 24 h of incubation are shown in Fig. 4. During 72 h of incubation, the numbers of cells increased steadily. Numbers of cells attached to the treated surfaces were always significantly higher compared to those of the non-treated surfaces (Fig. 5a). Ar-

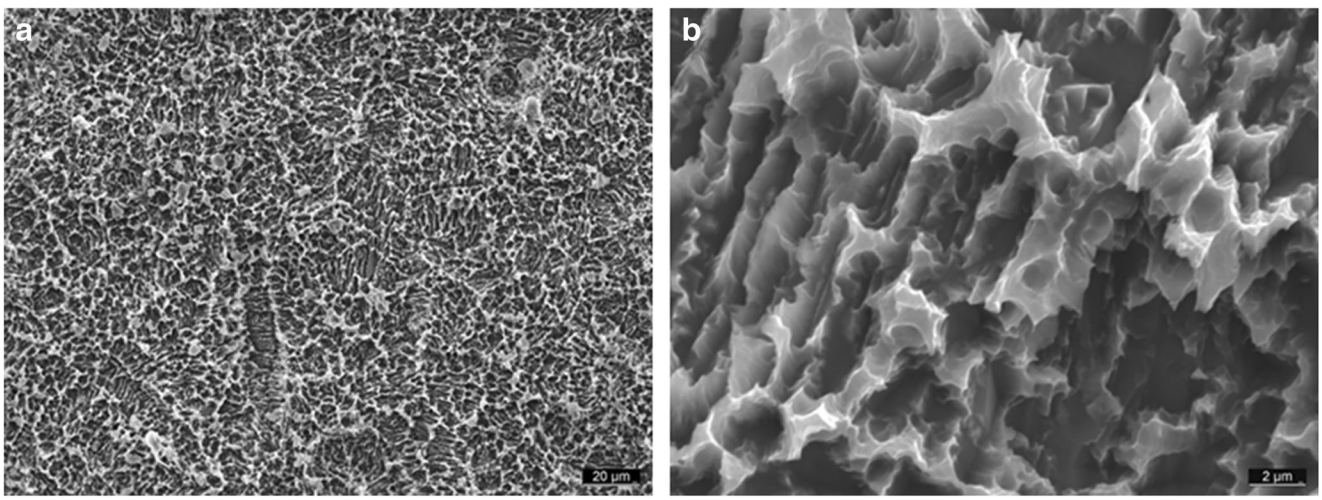

Fig. 2 SEM sample image of a non-treated titanium disk with **a** ×500 and **b** ×5000 magnifications. Source: authors

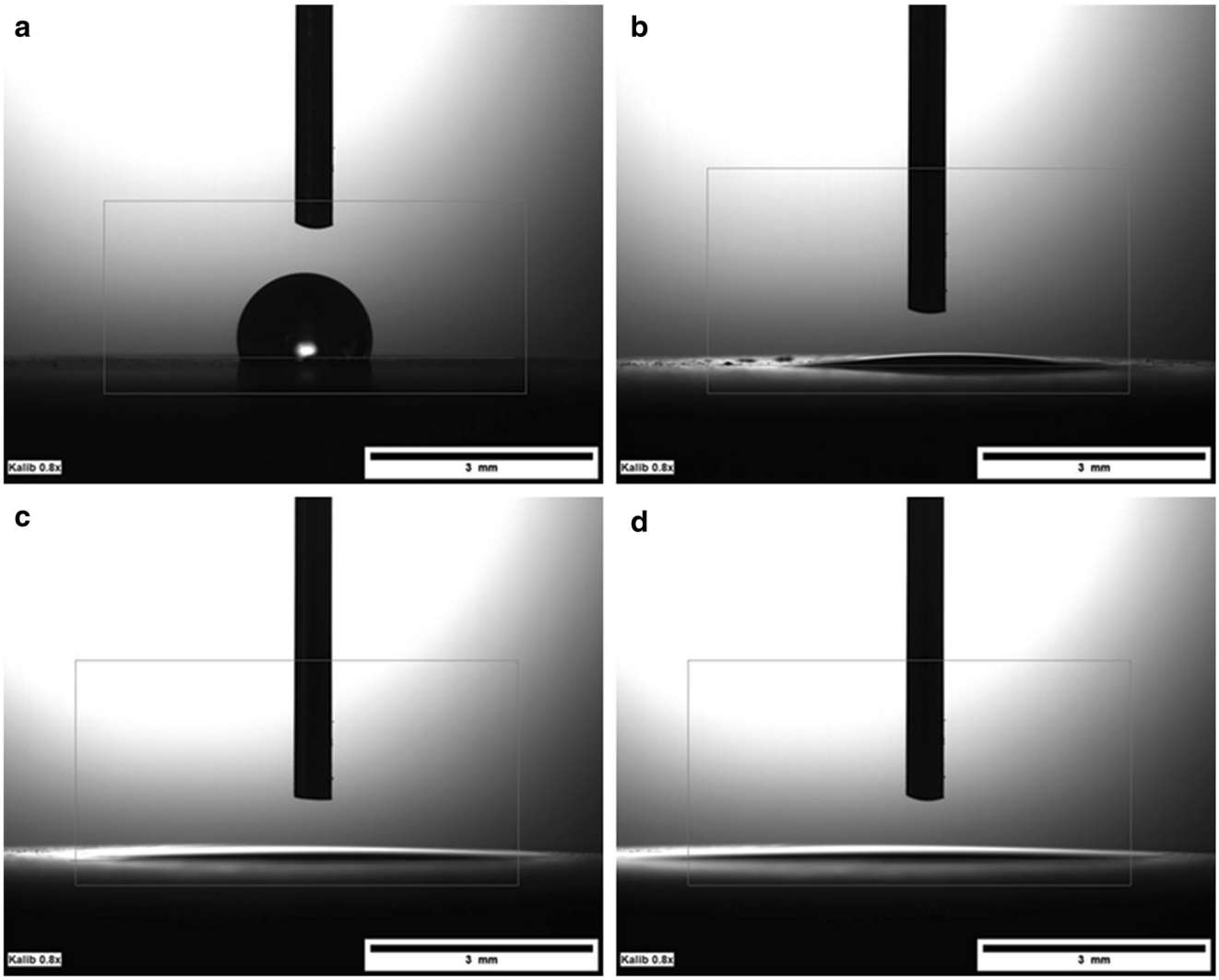

Fig. 3 Drop shapes, visualized in static drops. **a** Non-treated, **b** after 12 min of UV treatment, **c** after 12 min of O_2 plasma treatment and **d** after 12 min of Ar plasma treatment. Source: authors

plasma-treated disks showed a significantly higher cell attachment only after 2 h of incubation compared to UV-treated, O_2-plasma-treated and non-treated disks (Table 1).

Cell proliferation

BrdU incorporation was higher for plasma-treated disks compared to non-treated and UV-treated after 24 h (Fig. 5b). Results were only statistically significant after 24 h indicating a marginal advantage for cell proliferation after plasma surface treatment (Table 1).

Viability

Generally, XTT absorbance after 48 h of incubation was higher in osteoblasts on plasma-treated surfaces compared to that of non-treated and UV-treated surfaces (Fig. 5c) indicating a higher viability. The differences between the plasma-treated and non-treated as well as UV-treated surfaces were statistically significant (Table 1).

Cytotoxicity

Cytotoxicity was assessed by LDH assay after 24 h of incubation compared to RM-A samples. Neither the treated nor the non-treated disks were cytotoxic (Fig. 5d). Differences between the disks were not significant (Table 1).

Discussion

A number of studies have investigated the positive effects of UV light or NTP on titanium surfaces. This is to the best knowledge of the authors the first study to prove if these methods are comparable in changing and improving the physical material characteristics (topography, roughness, chemistry

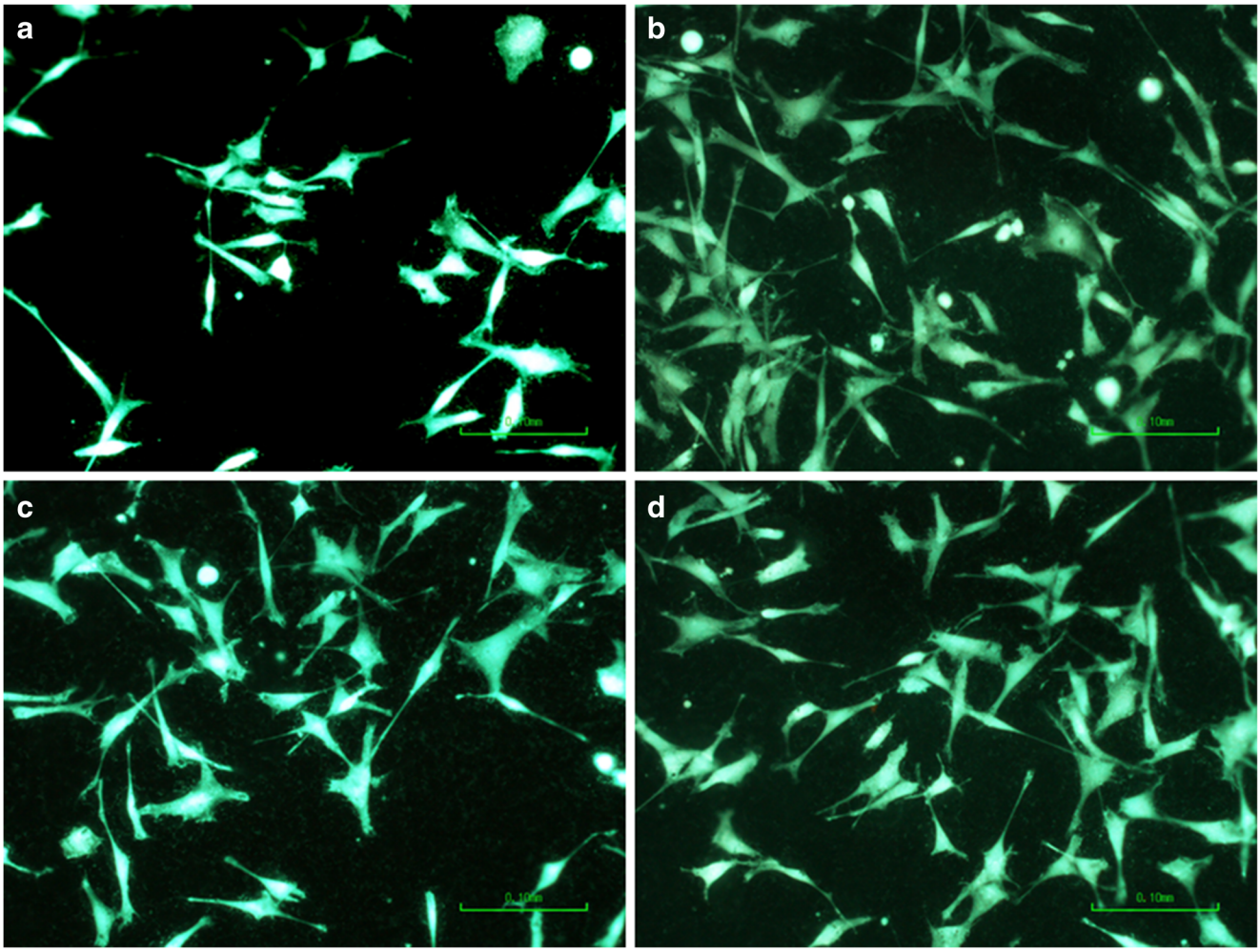

Fig. 4 Representative examples of LDS after 24 h of incubation (×20 magnification) for **a** non-treated, **b** UV-treated, **c** O_2-plasma-treated and **d** Ar-plasma-treated titanium disks. Source: authors

and wettability) and cell reaction (cell attachment and morphology, cell viability and proliferation as well as cytotoxicity) on titanium surfaces under controlled conditions.

No differences in the topography or roughness between non-treated and surface-treated disks were observed applying SEM and confocal microscopy. On the non-treated titanium disks, organic material (C, N, O) together with traces of nitrates, fluorides, magnesium, silicates, sulphates and/or calcium were detected by XPS analysis. The NTP treatments of the titanium disks led to slightly thicker oxide layers and to a reduction of organic material. The UV treatment removed the carbon partly while the increase of the oxide layer thickness was less pronounced compared to the plasma treatments. The effects of UV and NTP treatments on changing the upper surface chemistry of machined and rough titanium disks have been described in several studies. Protein adsorption and cell attachment on titanium surfaces are positively correlated with UV dose and correlates negatively with carbon remnants [13]. Similar effects of decreasing the amount of carbon and increasing the amounts of titanium and oxygen at the surface were found for surface treatments with NTP [18, 22]. In a recent study, Roy et al. found a significant decrease of carbon presented at the surface of commercially available titanium implants after irradiation treatment in a UV light oven [23]. UV light treatment was also able to increase the amount of titanium hydroxide and decrease the amount of H_2O. The authors concluded that these hydroxyl groups together with other oxide vacancies explain the superhydrophilic effect of photofunctionalization, which might be responsible for the improved interactions with cells and biological tissues that were also found in this study. Aita et al. described a time-dependent photocatalytic removal of hydrocarbons combined with an increase of the oxide layer with UV light on machined and rough titanium surfaces [10]. They also found a significant time-dependent increase in cell attachment of bone marrow cells of rats after 3 h of incubation after time intervals of UV irradiation up to 50 h. They concluded that the initial protein adsorption and cell attachment might correlate with the level of hydrocarbon remaining on the titanium. Gao et al. found that UV-A and UV-C treatments decreased the

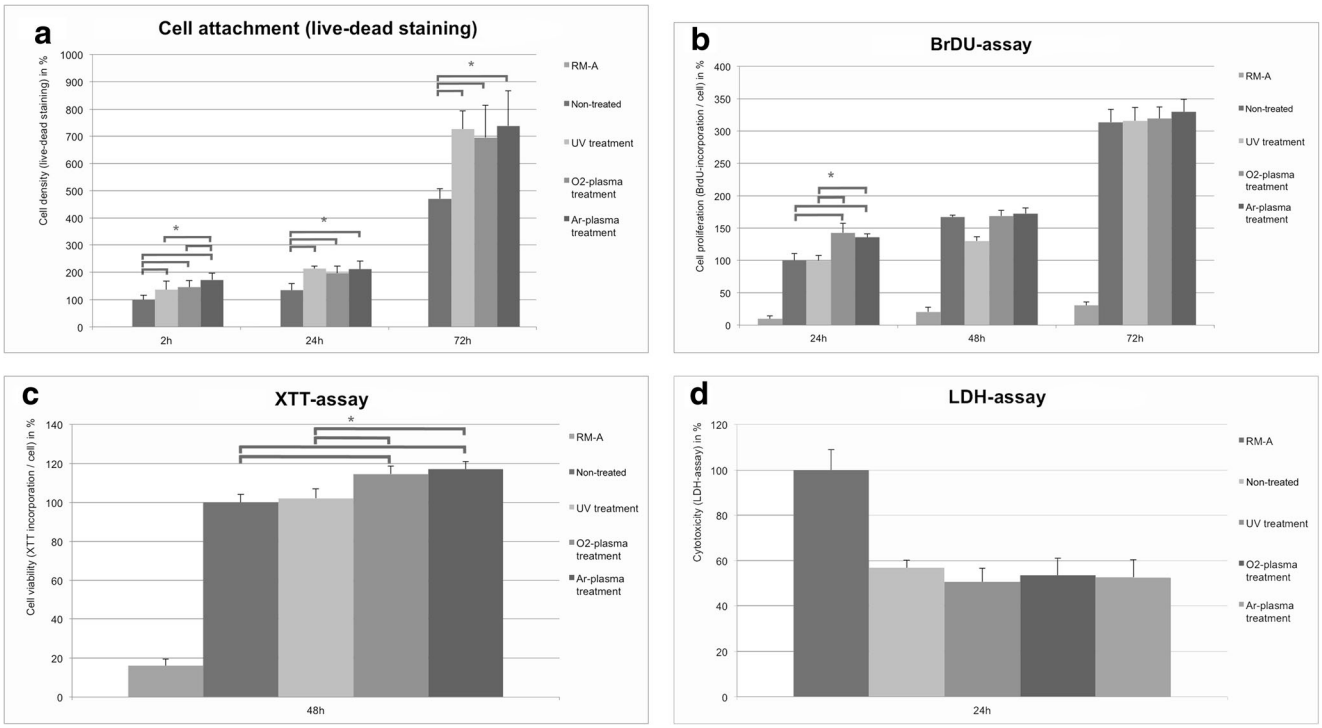

Fig. 5 **a** Cell attachment (live-dead staining) of osteoblasts 2, 24 and 72 h after seeding. **b** Cell proliferation (BrdU assay) of osteoblasts 24, 48 and 72 h after seeding. **c** Cell viability of osteoblasts (XTT assay) 48 h after seeding. **d** Cytotoxicity (LDH assay) of osteoblasts 24 h after seeding. For better visualization of differences, results are shown in percent of negative control (2-h results of the non-treated disks) ± standard deviation, $*p < 0.0083$. Measurements on RM-A were not included in the statistical analysis. Source: authors

amount of hydrocarbons, but UV-C light was more powerful to increase the oxide layer [12]. The light oven used in this study provides UV light as a mixture of UV-A and UV-C spectra. Similar positive changes of surface chemistry and the hydrophilic status including a massive decrease of carbon remnants after UV irradiation were even observed on acid-etched zirconia-based dental implant material [24].

Wettability as indicator of surface energy is improved by UV irradiation [25]. Micro- and nanostructure of the surface and the chemical composition modulate the wettability, which determines the initial events and the biological cascade at the biomaterial/host interface [26]. Implant surfaces with an intermediate roughness of 1–2 μm seem to be optimal for osteoblast proliferation and differentiation [27]. In the present study, dynamic contact angles of water droplets dropped after each surface treatment and the hydrophobic surfaces of the non-treated titanium disks were turned superhydrophilic. However, NTP led to a better wettability than UV light. Although Aita et al. showed a time-dependent increase in superhydrophilicity [10], more than 12 min of functionalization seems to be hardly practicable under clinical conditions.

Table 1 Statistical results of Kruskal-Wallis test. Level of significance was set at $p < 0.0083$ after Bonferroni correction. Results are shown for live-dead staining (LDS) after 2, 24 and 72 h, BrdU assay after 24, 48 and 72 h, XTT assay after 48 h and LDH assay after 24 h. Statistically significant results are written in italic. Positive control (RM-A) was not tested

	Non-treated vs. UV	Non-treated vs. O_2 plasma	Non-treated vs. Ar plasma	UV vs. O_2 plasma	UV vs. Ar plasma	O_2 plasma vs. Ar plasma
LDS 2 h	*$p < 0.001$*	*$p < 0.001$*	*$p < 0.001$*	$p = 0.062$	*$p < 0.001$*	*$p = 0.008$*
LDS 24 h	*$p < 0.001$*	*$p < 0.001$*	*$p < 0.001$*	$p = 0.839$	$p = 0.372$	$p = 0.465$
LDS 72 h	*$p < 0.001$*	*$p < 0.001$*	*$p < 0.001$*	$p = 0.451$	$p = 0.720$	$p = 0.327$
BrdU 24 h	$p = 0.937$	*$p = 0.002$*	*$p = 0.002$*	*$p = 0.002$*	*$p = 0.002$*	$p = 0.310$
BrdU 48 h	$p = 1.000$	$p = 0.180$	$p = 0.041$	$p = 0.132$	$p = 0.310$	$p = 0.623$
BrdU 72 h	$p = 0.818$	$p = 0.310$	$p = 0.009$	$p = 0.589$	$p = 0.039$	$p = 0.169$
XTT 48 h	$p = 0.310$	*$p = 0.002$*	*$p = 0.002$*	*$p = 0.002$*	*$p = 0.002$*	$p = 0.065$
LDH 24 h	$p = 0.010$	$p = 0.297$	$p = 0.109$	$p = 0.200$	$p = 0.522$	$p = 0.631$

MC3T3-E1 cells, which is the most commonly used osteoblastic cell line, were used as model in this study. Despite their murine origin, they represent a reliable and viable alternative to primary human osteoblasts but are not able to fully represent the complexity of a biological system [28]. Furthermore, the cell line is used in lots of dental implant research studies in vitro so that results are easier to interpret and discuss with other studies using the same cell line. Murine osteoblasts cultured on UV-treated titanium disks express more vinculin indicating a better ability to adjunct to the titanium surfaces and to connect to each other [29]. After NTP treatment of titanium disks, fibroblast adhesion in vitro was significantly higher on treated disks compared to that on non-treated disks [15]. Human gingival fibroblasts demonstrated a 20% increase in early cell attachment and proliferation after NTP treatment [30]. It may enhance the early attachment and proliferation of cells around titanium abutments for establishing faster soft tissue adherence. In this study, cells also appeared to be larger and more elongated on surface-treated disks indicating that the environment was favourable for culturing osteoblasts after surface treatment. Significantly more osteoblasts grew on the treated surfaces at each time point and they showed a significantly better viability on plasma-treated surfaces compared to non-treated and UV-treated surfaces after 48 h of incubation. Neither non-treated nor treated titanium disks showed signs of cytotoxicity.

In 2013, Barton et al. showed an induction of cytokine secretion and growth factor release of immortalized keratinocyte cells and concluded that wound healing could be improved by non-thermal plasma [31]. Non-thermal plasma was also able to modulate human oral mucosa ex vivo and to increase the secretion of VEGF [32]. Kwon et al. also found an increased mRNA expression of growth factors in human gingival fibroblasts after treatment with a non-thermal atmospheric pressure plasma jet and concluded that the application could be useful in gingival wound healing [33]. In another recent study, Canullo et al. investigated the effects of UV light and NTP of argon on different titanium surfaces. They found similar effects for protein adsorption and cell adhesion with plasma of argon after 12 min of treatment and UV light after 3 h of treatment [34]. Conversely to the increased cell growth, the attachment of human oral bacteria as well as biofilm formation on machined titanium surfaces after UV treatment was reduced in vitro compared to non-treated surfaces [35]. Furthermore, Daeschlein et al. proved high killing effectiveness of two different plasma devices against eight different mycobacterial species in vitro [36]. Non-thermal atmospheric pressure plasma showed the best results in terms of reduction of colony-forming bacteria (*Streptococcus mitis*) in a cortico-cancellous bone model in vitro and the authors concluded that non-thermal plasma may even be a useful tool for the treatment of medication-related osteonecrosis of the jaw [37]. Laroussi first demonstrated in 1996 that sterilization is possible by plasma at atmospheric pressure [38]. In a recent study, Annunziata et al. found a sterilizing effect for NTP of argon on contaminated titanium surfaces similar to the effects of UV light [39]. However, according to Hoffmann et al., the role of UV radiation in the sterilization process, which is naturally a part of NTP, is still unclear [40].

It has to be taken into account critically that the NTP reactor used in this study produces UV light at $\lambda = 320$ nm using argon plasma and $\lambda = 240$ nm using oxygen plasma which might additionally contribute to the superior results of NTP in improving surface chemistry, increasing wettability and improving surface conditions for cells compared to UV light treatment. The UV light oven used in this study has proven functionality in clinical studies while the results of the NTP reactor used in this study are still preclinical [41, 42]. However, there are several other atmospheric plasma devices that have proven clinical efficacy like the 2013-certified kINPen® MED (neoplas tools, Greifswald, Germany). Actually, three clinical observational studies with a total of 26 patients demonstrated positive effects for wound healing and/or reduction of bacteria [43–45]. Non-thermal plasma treatment is beneficial especially for chronic wounds, but accelerated healing was also seen with sterile wounds [46]. In a recent study, no mutagenic or genotoxic effects were found for the kINPen® MED in a hen's egg test for micronucleus induction model [47]. BioWeld1™ (IonMed Ltd., Yokneam, Israel) is another recently certified non-thermal plasma device for surgical incision closure. When compared to sutured skin closure, results of non-thermal plasma closed wounds showed comparable and favourable wound healing results macroscopically as well as histopathologically in vivo [48]. No results from clinical studies have yet been published. However, to what extent the active agents (ions, electrons, reactive oxygen and nitrogen species), UV photons and electric and magnetic fields created by non-thermal plasma each contribute to the positive effects in vitro, in vivo and clinically still remains unclear.

The results of the present study show that UV light and NTP are able to improve surface conditions on moderately rough titanium surfaces. UV light might be as effective as NTP, but probably needs a longer application time which may be difficult under clinical conditions. On the other hand, UV devices are much more affordable than NTP devices. However, the in vitro-identified effects in cell attachment, proliferation and viability need to be confirmed in vivo.

Conclusion

NTP and UV treatments result in an optimized cell environment on titanium disks compared to the non-treated control without conducting any topographical or roughness changes under laboratory conditions. However, changes of the surface

chemistry with an increased hydrophilicity led to a slight advantage in cell growth for NTP treatment when compared to UV treatment after the same time of functionalization. Due to the fact that in vitro results only have limited validity, an in vivo study is necessary to determine if and to what extent these results have effects in the complexity of a biological system.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Ethical approval No humans or animals were involved in this study. A cell line was used for in vitro experiments.

Informed consent Informed consent is not applicable for this type of study.

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