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Changes in surface characteristics of titanium and zirconia after surface treatment with ultraviolet light or non-thermal plasma

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Positive effects of irradiation with ultraviolet (UV) light or treatment with non-thermal plasma on titanium and zirconia surfaces have been described in various studies. The aim of this study was to assess and compare the changes in the physicochemical surface conditions of titanium and zirconia surfaces after a short treatment with UV light or with non-thermal plasmas of argon or oxygen. Titanium and zirconia samples with moderately rough surfaces were treated for 12 min either in a UV-light oven or in a non-thermal plasma reactor that generates non-thermal plasmas of oxygen or argon. Changes in surface conditions were assessed by confocal microscopy, dynamic contact angle measurement, and X-ray photoelectron spectroscopy (XPS). No changes in roughness occurred. Ultraviolet irradiation and non-thermal plasma significantly increased the wettability of the titanium and zirconia surfaces. X-ray photoelectron spectroscopy showed an increase of oxygen and a significant decrease of carbon after treatment with either method. Thus, ultraviolet light and non-thermal plasma were found to be able to improve the chemical surface conditions of titanium and zirconia following a short exposure time. However, further in vitro and in vivo studies are needed to determine the relevance of the results.

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Since the discovery of 'osseointegration' by BRÅNEMARK *et al.* in the 1970s (1), many attempts have been made to improve the osseointegrative properties of dental implants. Osseointegration of implants is basically determined by the physical, topographical, biological, and chemical surface conditions (2). The dental implants used currently are topographically optimized (e.g. by a combination of sandblasting and acid-etching of the implant surface) and some have been chemically modified (e.g. by coating with drugs, proteins, or growth factors) (3, 4). More recent approaches are biological or pharmaceutical modifications, which are the subject of current research (5, 6). Generally, titanium and zirconia are the materials favoured for dental implants. As a result of exposure to the intra-oral environment and the high masticatory forces, dental implants are required to possess a higher resistance to corrosion and bacterial contamination, in addition to a solid implant-to-bone connection, than orthopaedic implants. Bone-to-implant contact (BIC) is widely used in research to assess osseointegration, and the BIC value depends on the implant material, surface conditions (roughness, topography, chemistry), time of healing, the magnification used in assessments, and last, but not least, on the operator. Typical BIC values range between 52% and 78% but do not reach the ideal 100% (7). One factor that could account for the less-than-ideal osseointegration could be biological aging of the implant during the period from manufacture to insertion as a result of carbonization and organic contamination of the implant surface from the atmosphere, leading to a time-dependent surface aging of the implant (8). It is assumed that after 4 wk of storage in customary packing, the surfaces of titanium and zirconia are saturated with carbon compounds (9). Four-week-old titanium surfaces showed only half the strength of the bone–implant integration compared with newly prepared titanium surfaces after 2 wk of healing in vivo and the BIC was reduced by more than 30% (10).

It is plausible that treatment of the implant surface by ultraviolet (UV) light or non-thermal plasma just before implant placement could lead to removal of the organic contamination and increase the wettability of the implant surface in order to attract proteins and cells without changing the surface properties. Several studies have shown that UV light and non-thermal plasma treatments are able to decrease the amount of carbon compounds on titanium. DANNA *et al.* (11) observed decreased levels of carbon and increased levels of titanium and oxygen on pure titanium discs after surface treatment with a non-thermal plasma device. In a recent study by ROY *et al.* (12), carbon present at the surface of pure titanium discs decreased substantially after UV-C irradiation (UV light spectra 200–280 nm). They also found that UV-C light was able to increase the amount of titanium hydroxide and decrease the amount of water. They proposed a new model of the effects of UV light on titanium surfaces saturated with carboxyl groups: when high-energy photons are applied to the surface, they may be able to break the relatively weak bonds between carboxyl groups and titanium, thereby enhancing the chance of bonding with the oxygen, nitrogen, and sulphur atoms on proteins, whereby the attachment of cells may subsequently be enhanced.

Besides the surface chemistry, the microscopic structure, surface roughness, and wettability are factors to consider for the best osseointegration of biomaterials. Alteration of the roughness of the implant surfaces can influence attachment of cells and BIC. However, any post-manufacture alteration of the surface structure would lead to loss of the manufacturer's warranty. Plasma treatment is usually a subtractive process and an increase in surface roughness and alteration of the surface structure after applying non-thermal plasma is possible (13). The surface characteristics modulate the wettability, which is an additional factor determining the bio-integrative capability of biomaterials (14). Ultraviolet light and non-thermal plasma are able to hydrophilize titanium and zirconia surfaces (13, 15, 16). Both methods are able to improve the early response of cells to implant surfaces and increase the speed of osseointegration (17, 18). HAYASHI *et al.* (19) found significantly decreased albumin adsorption, cell attachment, cell spreading, and differentiation of murine osteoblast cells on experimentally induced carbon-contaminated titanium surfaces. In vivo, AITA *et al.* (20) was able to achieve a BIC of almost 100% after 48 h of surface treatment with UV light after 4 wk of healing.

Although dental implants are sold as medical devices in sterile packages with a shelf life typically of up to 5 yr, there is no information available about the durability of saturation of the implant surfaces with carbon compounds, and thus their bioactivity. Only limited attention has been paid to the stability of the bioactivity of the implant surfaces over time.

Although several studies have indicated that UV light and non-thermal plasma are able to improve the surface chemistry and increase the wettability of titanium and zirconia surfaces, it is currently not known whether UV irradiation and different non-thermal plasma treatments are comparable in improving the physicochemical surface conditions of titanium and zirconia after a defined short time interval of surface treatment. Therefore, in the present study we investigated the changes in surface-material characteristics after one cycle (12 min) of treatment either with UV light or non-thermal plasma of argon or oxygen, as evidenced by the electron-microscopical structure and measures of roughness, hydrophilization, and surface chemistry of both materials.

Material and methods

Samples and treatment

Titanium discs made from titanium grade IV (15 mm in diameter, 1.5 mm in thickness; Camlog, Basel, Switzerland) and zirconia discs made from tetragonal zirconia polycrystal (ZrO_2 95%, Y_2O_3 5%, 12 mm in diameter, 1.4 mm in thickness; Swiss Dental Solutions, Kreuzlingen, Switzerland) were used. The surfaces of the titanium discs were sandblasted and acid-etched (Camlog Promote Surface; Camlog), while the surfaces of the zirconia discs were blasted with zirconium dioxide. After manufacturing, all samples were γ-sterilized with a cobalt 60 source and a measured dose at the predefined measuring time point of 30.8 kGy (specified dose range 24–45 kGy). All samples were stored in Tyvek blister packs (DuPont, Wilmington, DE, USA) at 20°C and at a humidity of 65% in darkness for 4 wk. All measurements were performed in a clean room under controlled conditions of 20°C and 46% relative humidity.

In total, 32 titanium and 32 zirconia samples were used. Titanium and zirconia samples were divided randomly into one control and three experimental groups in equal parts (eight per group). For each material, the discs allocated to the control group ($n = 8$) were untreated. The discs allocated to the three experimental groups ($n = 8$ each) were treated either with UV light using an UV light oven (Therabeam Superosseo; Ushio, Tokyo, Japan) emitting UV light at $\lambda = 360$ nm (0.05 mW cm^{-2}) and at $\lambda = 250$ nm (2 mW cm^{-2}) or with non-thermal plasma of either argon or oxygen using a non-thermal plasma reactor (Yocto III; Diener Electronic, Ebhausen, Germany). Non-thermal plasma treatment conditions were 24 Watts and −0.5 mbar. All samples in the experimental groups were treated for 12 min.

Assessment of surface structure and roughness

Scanning electron microscopy, topography, and roughness measurements were carried out on one disc from each group immediately after X-ray photoelectron spectroscopy (XPS). Scanning electron microscopy images were obtained with an Evo MA25 (Zeiss, Oberkochen, Germany). The topography of the discs was determined by confocal microscopy (S neox; Sensofar, Barcelona, Spain) with 20× and 50× objectives. Roughness values according to ISO 4287:1997-04 and ISO 13565-2:1996-12 were determined on 20, 4.8-mm-long profiles extracted from the topographies applying a Gaussian filter with a cut-off distance, λ_c, of 0.8 mm according to ISO 4288:1996-08 (MountainsMap, Release 6.2.7487; Digital Surf, Besançon, France). In addition to the arithmetic mean deviation of the roughness profile (R_a), the kernel roughness parameters (R_k, R_{pk}, R_{vk}) were analysed to assess the roughness of the titanium and zirconia discs in detail. The kernel roughness, R_k, is the general roughness without taking the peaks and the valleys into account. The peaks described by R_{pk}, combined with the kernel roughness, R_k, are relevant for the primary stability of an implant, while the valleys described by R_{vk} become important after the bone in growth. For assessment of the kernel roughness values a double Gaussian filter was applied.

Wettability

Dynamic contact angles as outcome variables were measured on advancing water droplets, according to DIN 55660-2, using a contact angle meter (Surftens Universal; OEG, Frankfurt, Germany). For each material, measurements were carried out on five discs from each experimental or control group and repeated on five spots per disc. Results for the five spots were averaged to obtain an estimate for each disc. SURFTENS software (Release 4.3; OEG), which is an image processing software for measurement of contact angles of liquids on solids, was used for measurement and data processing.

Surface chemistry

The exact chemical composition of the outer 10 nm of the disc surfaces was assessed by XPS (Kratos Axis Nova; Kratos Analytical, Manchester, UK). Measurements were performed with monochromatic AlKα-irradiation (1,486.7 eV) at 225 W and using an angle of incidence of 54.6° with respect to the surface normal. CASAXPS software (Version 2.3.14; Casa Software, Devon, UK) was used to process data and analyse the spectra. Peak shifting as a result of charging was corrected by referencing aliphatic carbon to 285 eV. Detailed spectra as well as survey spectra were analysed following subtraction of an iterated Shirley background and were corrected by sensitivity factors given by the manufacturer (21). The composition of the samples was assumed to be homogeneous. For each material, three discs per experimental/control group were analysed in a single spot in the centre of the disc.

Statistical analysis

Roughness parameters were analysed descriptively. Statistical analysis was performed with SPSS 20 (IBM, Armonk, NY, USA). A Kruskal–Wallis test with post hoc Bonferroni correction was used to test whether the dynamic contact angle measurements differed between the four groups. The data from the XPS were analysed statistically by a two-sided Student's *t*-test. Our hypotheses were: (i) UV light and non-thermal plasma are able to decrease significantly the contact angles of water droplets on titanium and zirconia surfaces compared with untreated surfaces; (ii) UV light and non-thermal plasma are able to decrease significantly the amount of carbon compounds on titanium and zirconia surfaces compared with untreated surfaces; and (iii) UV light and non-thermal plasma are able to increase significantly the amount of oxides on titanium and zirconia surfaces compared with untreated surfaces.

For all outcomes, statistical significance was declared for $P < 0.05$.

Results

Surface structure

The scanning electron microscopy images of the untreated titanium and zirconia samples showed rough surfaces (Fig. 1). The sandblasted and acid-etched titanium samples additionally showed a superimposed microroughness (Fig. 1A). The microstructure of the zirconia samples consisted of rounded (ceramic) grains with a diameter in the range of 0.65 ± 0.15 μm (Fig. 1B). No differences in the surface structure were observed between the experimental and control groups. The R_a was 1.8 μm on the titanium samples and 1.9 μm on the zirconia samples in the control groups (Table 1). Kernel roughness (R_k) values ranged between 5.7 and 6.2 μm for the titanium samples and between 6.1 and 7.2 μm for the zirconia samples. However, the peaks were more pronounced on the titanium samples with reduced peak height values (R_{pk}) ranging from 2.4 to 2.6 μm compared with the range from 1.8 to 2.2 μm on zirconia samples. The valleys (R_{vk}, reduced valley depth) were similar in both types of material, with values ranging from 2.6 to 3.0 μm on the titanium samples and from 2.7 to 3.0 μm on the zirconia samples. There were no relevant differences in surface roughness parameters between the control and any of the experimental groups or between the experimental groups themselves.

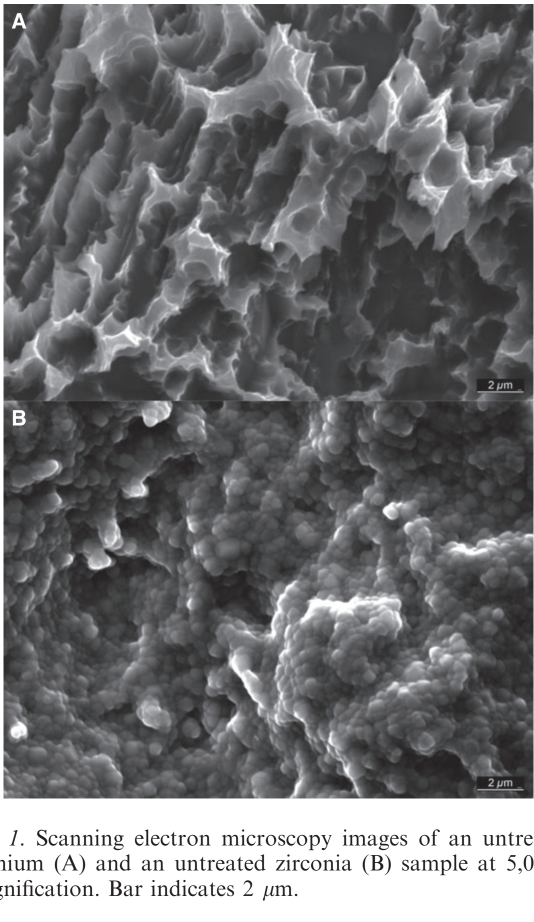

Fig. 1. Scanning electron microscopy images of an untreated titanium (A) and an untreated zirconia (B) sample at 5,000× magnification. Bar indicates 2 μm.

Wettability

The contact angles of the droplets in the control group exceeded 111° on the titanium discs and 51° on the zirconia discs, indicating poor wettability of the surfaces. After 12 min of surface treatment, the contact angles decreased substantially in all experimental groups (Table 2). On the non-thermal plasma-treated titanium discs, this manifested in a spread of the droplets on the surface to such an extent that contact angles could not be measured. In the zirconia experimental groups, contact angles also decreased, but were still measurable after treatment with non-thermal plasma. After treatment with UV light, the contact angles also decreased, but remained above 9° for titanium discs as well as for zirconia discs (Table 2). Differences between the control group and the experimental groups of titanium, as well as between the experimental groups of UV light and non-thermal plasma, were all statistically significant ($P < 0.001$). Differences between the control groups and all experimental groups of zirconia, as well as between all experimental groups of zirconia, were statistically significant ($P < 0.001$). Treatment with plasma of argon resulted in the best wettability of the zirconia discs, while treatment with UV light resulted in the poorest wettability.

Table 1

Roughness values of titanium and zirconia samples

Sample material	Roughness values	Control group	Experimental groups		
			UV light	O_2-Plasma	Ar-Plasma
Titanium	R_a	1.8 (0.1)	1.9 (0.1)	2.0 (0.1)	1.9 (0.1)
	R_k	5.7 (0.3)	6.0 (0.4)	6.2 (0.5)	5.9 (0.4)
	R_{pk}	2.6 (0.3)	2.5 (0.2)	2.6 (0.4)	2.4 (0.4)
	R_{vk}	2.6 (0.4)	3.0 (0.4)	2.8 (0.3)	2.8 (0.4)
Zirconia	R_a	1.9 (0.1)	2.2 (0.1)	2.1 (0.1)	2.1 (0.2)
	R_k	6.1 (0.5)	7.2 (0.7)	7.1 (0.5)	7.0 (0.6)
	R_{pk}	1.8 (0.3)	1.8 (0.4)	2.1 (0.4)	2.2 (0.4)
	R_{vk}	2.7 (0.5)	3.0 (0.6)	2.9 (0.5)	3.0 (0.6)

Values are given as mean (SD), all in micrometres (μm).
Ar, argon; R_a, arithmetic mean deviation of the roughness profile; R_k, kernel roughness (roughness not taking into account the highest peaks and lowest valleys); R_{pk}, reduced peak height; R_{vk}, reduced valley depth; UV, ultraviolet.

Table 2

Wettability of titanium and zirconia samples

Study group	Titanium samples		Zirconia samples	
	Left side	Right side	Left side	Right side
Control group	111.4 (10.0)	115.2 (9.8)	50.8 (4.7)	51.3 (3.6)
Experimental groups				
UV	12.8 (5.2)	11.7 (1.5)	9.4 (0.8)	9.3 (0.8)
O_2-Plasma	0 (–)	0 (–)	4.2 (1.0)	4.3 (1.0)
Ar-Plasma	0 (–)	0 (–)	2.9 (1.2)	2.6 (0.9)

Values represent the mean (SD) dynamic contact angle measurements in degrees.
Ar, argon; UV, ultraviolet.

X-ray photoelectron spectroscopy analysis

The XPS survey spectra of the control and experimental titanium discs showed prominent peaks for titanium, oxygen, and carbon (Fig. 2A). Argon was not detected, even on the argon-plasma-treated titanium discs. In addition, traces of fluorides, magnesium oxides, silicates, sulphates, and calcium oxides were detected.

In addition to the survey spectra, detailed spectra of titanium 2p, oxygen 1s, and carbon 1s were acquired. The total amount of carbon on the titanium discs decreased significantly in all experimental groups ($P < 0.002$), whereas between the experimental groups only the difference between UV light and non-thermal plasma of argon were statistically significant ($P = 0.03$; Table 3). Because of the different amount of organic contamination (polycarbonyls, esters, and carboxylic groups) shielding the substrate differently, the ratio of oxygen to titanium was compared (Fig. 3A). The

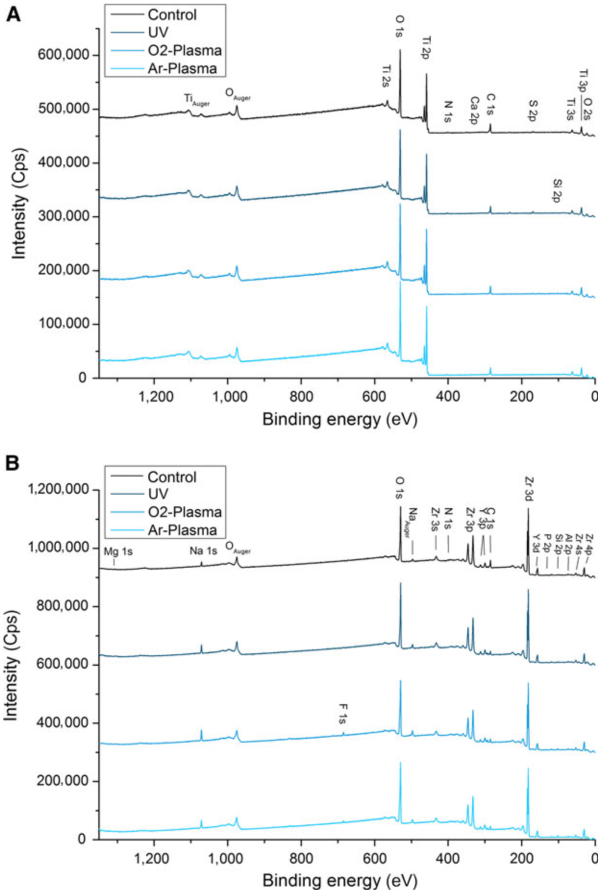

Fig. 2. X-ray photoelectron spectroscopy survey spectra of the titanium (A) and zirconia (B) samples. From top to bottom: control group and experimental groups [after treatment with ultraviolet (UV) light, oxygen plasma and argon (Ar) plasma]. Non-labelled signals are from other orbitals of the elements mentioned [e.g. oxygen 2s (O 2s) at 23 eV].

Table 3

Surface composition of the discs

Sample material	Disk	$Ti_{metallic}$	Ti_{IV}	C_{total}	$C_{C\text{-}O}$	C_{COOx}	O I	O II	O III
Titanium	Untreated	1.3 (0.1)^{2,3}	19.7 (0.6)^{1,2,3}	21.8 (0.6)^{1,2,3}	4.1 (0.3)^{1,2,3}	3.3 (0.1)^{1,2,3}	40.7 (1.1)^{2,3}	10.7 (0.6)^{2,3}	2.7 (0.6)^{1,2,3}
	UV light	1.2 (0.1)^{4,5}	21.7 (0.6)^{1}	17.4 (0.4)^{1,5}	3.0 (0.1)^{1,4,5}	1.8 (0.2)^{1}	41.0 (2.6)^{4,5}	11.3 (2.3)^{4,5}	4.0 (1.0)^{1}
	O_2 plasma	0.8 (<0.1)^{2,4}	22.3 (0.6)^{2}	16.7 (0.2)^{2}	2.7 (0.1)^{2,4}	1.0 (0.2)^{2}	44.0 (<0.1)^{2,4,6}	8.0 (<0.1)^{2,4}	4.3 (0.6)^{2}
	Ar plasma	0.7 (0.1)^{3,5}	23.0 (<0.1)^{3}	15.8 (0.1)^{3,5}	2.6 (0.1)^{3,5}	1.2 (0.1)^{3}	46.3 (0.6)^{3,5,6}	8.0 (<0.1)^{3,5}	3.7 (0.6)^{3}
	Disk	Zr	Y	C_{total}	$C_{C\text{-}O}$	C_{COOx}	O I	O II	O III
Zirconia	Untreated	17.7 (0.6)	2.6 (0.2)	23.1 (1.7)^{1,2,3}	3.8 (0.2)^{1,2,3}	3.7 (0.1)^{1,2,3}	35.7 (1.5)^{1}	11.3 (0.6)^{2,3}	3.5 (0.4)^{3}
	UV light	19.7 (0.6)^{4,5}	2.8 (<0.1)	11.2 (1.0)^{1,5}	1.2 (0.3)^{1}	2.6 (0.3)^{1,4,5}	42.0 (<0.1)^{1,4,5}	12.0 (<0.1)^{4,5}	3.6 (1.8)
	O_2 plasma	18.0 (1.0)^{4}	2.6 (0.1)	8.6 (0.4)^{2}	1.3 (0.2)^{2}	1.3 (0.3)^{2,4}	38.0 (1.0)^{4}	16.0 (<0.1)^{2,4}	5.1 (0.7)
	Ar plasma	18.0 (<0.1)^{5}	2.6 (0.1)	9.9 (1.8)^{3,5}	1.4 (0.4)^{3}	1.2 (<0.1)^{3,5}	37.0 (1.0)^{5}	15.7 (0.6)^{3,5}	6.1 (1.1)^{3}

Values are given as mean (SD) concentration of the elements in at %.

Applying a peakfitting allowed us to separate metallic titanium ($Ti_{metallic}$) from oxidized titanium with oxidation state IV ((Ti_V). The total amount of carbon (C_{total}) is presented, as well as the contributions from carbon bound to oxygen ($C_{C\text{-}O}$) and the contribution of carbon bound to multiple oxygen atoms as in ester, carboxylic or carbonate groups (C_{COOX}). Oxygen is presented as oxides (O I), as oxygen bound to OH groups (O II), and as oxygen bound to adsorbed water (O III).

Superscript numbers represent significant differences between the groups ($P < 0.05$) as follows:

^{1}Untreated vs. ultraviolet (UV) light.

^{2}Untreated vs. O_2 plasma.

^{3}Untreated vs. argon (Ar) plasma.

^{4}UV light vs. O_2 plasma.

^{5}UV light vs. Ar plasma.

$^{6}O_2$ plasma vs. Ar plasma.

concentrations of oxygen from titanium oxide (O I) were statistically significantly higher on the discs treated with non-thermal plasma. Non-thermal plasma of argon was statistically significantly more effective in increasing titanium oxide (O I) than all other treatments ($P < 0.02$). The total content of hydroxides and organic oxygen (O II) decreased in discs subjected to plasma treatments. These differences were statistically significant ($P < 0.02$) between non-thermal plasma treated discs and controls as well as compared with discs treated by UV light, which created no significant reduction of organic oxygen. The concentrations of the polycarbonyls ($C_{C\text{-}O}$) were significantly lower in all treatment groups compared with the untreated control group ($P < 0.02$). Differences between experimental groups of non-thermal plasma and UV light were also statistically significant ($P < 0.04$) whereas this was not the case comparing the two non-thermal plasma treatments. A higher concentration of oxygen with a double bond or adsorbed water (O III) was mainly a result of the significant increase of nitrates on the plasma-treated surfaces ($P < 0.03$) compared with the control group as well as compared with the discs treated with UV light. The amount of ester and carboxylic groups (C_{COOx}) were significantly lower in all treatment groups compared with untreated controls ($P < 0.003$), whereas differences between the treatment groups were not statistically significant.

The survey spectra of the zirconia discs showed prominent signals of sodium, oxygen, carbon, zirconium, and yttrium (Fig. 2B). In addition, traces of nitrogen, fluorine, magnesium, aluminium, silicon, phosphorus, potassium, and zinc were detected on the surfaces. Argon was not detected, even on the argon-treated samples.

The decrease of the total amount of carbon was even more pronounced compared with the titanium discs and was statistically significant for all experimental groups compared with the control group ($P < 0.01$; Table 3). Within the experimental groups, the carbon concentration was only significantly lower after applying non-thermal plasma of argon compared with UV treatment ($P = 0.04$). The concentration of the organic contamination ($C_{C\text{-}O}$) decreased significantly in every experimental group compared with the control group ($P < 0.02$) but without differences between the experimental groups. The amount of ester and carboxylic groups (C_{COOx}) decreased significantly in all experimental groups compared with the control groups ($P < 0.03$) and between non-thermal plasma and UV light ($P < 0.01$). The concentration of oxygen from oxides (O I) increased significantly in the experimental group of UV-light compared with all other groups ($P < 0.02$). Similarly to the results on the titanium samples, the atomic ratio of hydroxides (O II) to zirconia increased significantly with the non-thermal plasma treatments compared with all other groups (Fig. 3B; $P < 0.01$). The atomic ratio of oxygen with a double bond or adsorbed water (O III) to zirconia was statistically significantly higher for non-thermal plasma of argon compared with the controls ($P = 0.04$). The atomic ratio of yttrium to zirconium was not affected by the surface treatments, being 0.14:1 to 0.15:1 for all discs ($P > 0.2$).

Discussion

Most dental implants fail because of impaired osseointegration or late destructive alterations at the bone–implant

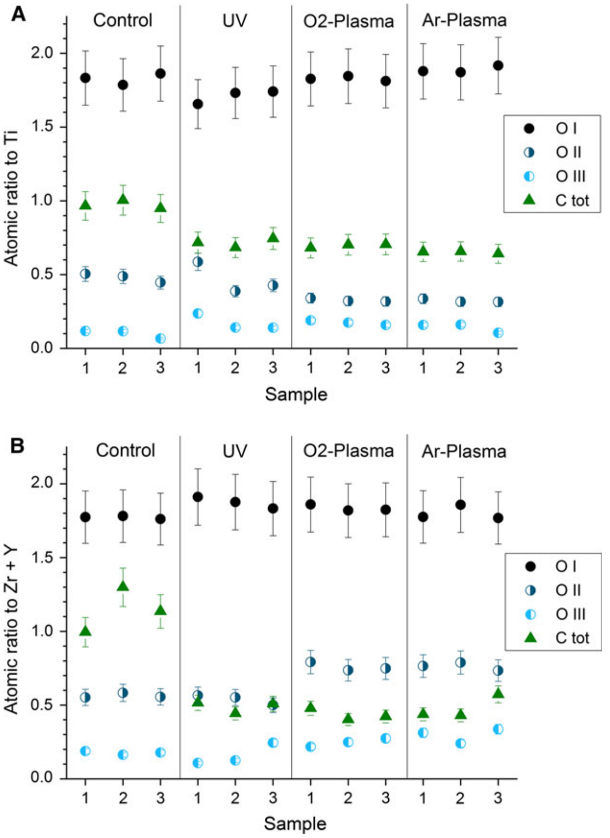

Fig. 3. Atomic ratios of titanium (Ti) (A) on the titanium samples and of zirconium (Zr) and yttrium (Y) (B) on the zirconia samples to oxygen from oxides (O I), oxygen from hydroxides (O II), oxygen with a double bond or adsorbed water (O III), and the total amount of carbon (C_{tot}), in the control and experimental groups. Ar, argon; UV, ultraviolet.

interface (8, 22–24). 'Biological aging' (i.e. the time-related absorption of carbon compounds by implant surfaces) is being discussed as one reason for reduced initial osseointegration (10, 20). To the best of the authors' knowledge, this study is the first to conduct a direct comparison of UV-light and non-thermal plasma treatments on both titanium and zirconia implant materials to evaluate changes in electron-microscopical structure, roughness, hydrophilization, and surface chemistry after 12 min of treatment each.

The results of the present study suggest that UV light, as well as non-thermal plasma, may be able to improve the surface characteristics of titanium and zirconia implant materials, regarding wettability and surface chemistry, without influencing the surface structure. Non-thermal plasma of argon seemed to be more effective than non-thermal plasma of oxygen and UV irradiation in increasing hydrophilization. Although the concentrations of oxygen were significantly increased and the concentrations of polycarbonyls and carboxylic groups were significantly decreased by non-thermal plasma as well as UV light on both materials, non-thermal plasma was more effective in generating these effects.

The main limitation of the study is clearly that it is only an in vitro characterization and the clinical implication of the determined effects has to be evaluated in further studies. For practical reasons only one cycle of treatment (12 min) was used. It may be possible that the effects would be increased and demonstrated more clearly using longer time intervals. However, longer periods of treatment would also be more difficult to integrate into the daily clinical routine. The comparability of the results could be limited because different plasma tools were used in other studies.

In the present study, there were no significant differences between untreated and surface-treated groups concerning the surface structure; even non-thermal plasma did not change the roughness parameters or alter the microstructure. The minimal differences observed in roughness parameters imply that osseointegration should be similar, irrespective of type of material and surface treatments. Moreover, these findings indicate that changes in biocompatibility after surface treatment by UV light or non-thermal plasma may primarily be the result of changes in surface chemistry and wettability. Although roughness values (R_a) of 1–2 μm seem to be ideal for osseointegration of dental implant material as well as to prevent peri-implant disease and ionic leakage, superhydrophobicity of micro- and nanorough surfaces is a well-known phenomenon (25), which may lead to delayed protein binding and decreased spreading of osteoblasts in vitro. Another factor leading to decreased protein-binding capacity is the time-dependent decrease of wettability. After 4 wk of storage, the titanium samples in this study were hydrophobic, while the zirconia samples were between hydrophilic and hydrophobic. This is attributable to the different characteristics of the materials. Irradiation with UV light and treatment with non-thermal plasma are capable of improving the wettability and surface energy (12), which was also shown in the present study as a massive decrease in the size of the contact angles of the water droplets. Although each treatment method was able to turn the non-hydrophilic surfaces of the titanium and zirconia samples into (super-)hydrophilic surfaces, non-thermal plasma in general created the best wettability on titanium, and non-thermal plasma of argon created the best wettability on zirconia, indicating a high-grade increase in surface energy on both materials after only a short period (12 min) of time. NORO *et al.* (13) reported similar results when comparing the wettability of rough zirconia discs after treatment with non-thermal plasma of oxygen, UV light, or hydrogen peroxide. They found increased hydrophilicity after applying non-thermal plasma of oxygen in comparison with UV light and hydrogen peroxide. However, they used 10 min of treatment in the plasma group vs. 24 h of treatment in the UV light group. Although a time-dependent increase in hydrophilicity was shown by AITA *et al.* for UV light up to 48 h of treatment (11, 26), a time interval above 15 min seems to be barely practicable under clinical conditions.

The wettability is also modulated by the chemical composition of the surface, which likewise determines the biological cascade and initial events at the biomaterial–bone interface (27). X-ray photoelectron spectroscopy, which was used in the present study, permits the chemical composition in the top 5–10 nm of each sample to be determined quantitatively. In this study, organic material (carbon, nitrogen, and oxygen) was found on all control samples, as were fluorides, magnesium, and silicates. Little is known about the chemical stability of titanium and zirconia surfaces in commercially available packages over time, and only limited attention has been paid to the stability of the bioactivity of these surfaces over time. However, it is known that titanium and zirconia absorb organic impurities mainly from the atmosphere, which is known as 'surface aging'. Although all carbon concentrations were relatively low (less than 50 ng cm^{-2}), non-thermal plasma, as well as UV light, significantly reduced the amount of polycarbonyls and carboxylic groups in all experimental groups. The results also showed that non-thermal plasma treatment was more effective in reducing organic materials than treatment with UV light.

Several studies have described the effects of UV light and non-thermal plasma on titanium and zirconia surfaces in changing the surface chemistry, attracting cells in vitro, and finally improving osseointegration in vivo (9–11, 16–18, 20, 26). Apart from examining superhydrophilicity, NORO *et al.* (13) also showed a remarkable decrease of carbon remnants and introduction of hydroxyl groups on zirconia samples that were treated by oxygen plasma, UV light, or hydrogen peroxide. Plasma of oxygen was more effective in decreasing the concentration of carbon as well as at increasing the concentration of hydroxyl groups in comparison with UV light and hydrogen peroxide. The results of the present study showed that the levels of hydrocarbons were significantly reduced on the titanium and zirconia surfaces by all methods. Non-thermal plasma was significantly more effective than UV light on the titanium surfaces but not on the zirconia surfaces. HAYASHI *et al.* (19) experimentally regulated the carbon concentration on titanium discs to achieve defined carbon/titanium ratios, as assessed by XPS. They found a concentration-dependent reduction of protein adsorption, cell attachment, and cell spreading, as well as reduced alkaline phosphatase activity and suppression of calcium mineralization of murine osteoblast-like cells by the amount of carbon on the titanium surfaces. Some studies showed positive effects for implants stored in physiological saline solution after manufacturing (3, 28). This also could prevent the surfaces from being contaminated with hydrocarbons. Another reason for the induction of (super-)hydrophilicity and increased protein binding may be the reduction of carboxyl groups combined with the reduction of hydrocarbons proposed by ROY *et al.* (12). In their model, high-energy photons that are applied to the surface may be able to break the relatively weak bonds between carboxyl groups and titanium, giving the chance to make bonds with the oxide, nitrogen, and sulphur atoms on proteins and subsequently enhance the attachment of cells. In this study, the surface treatment by UV light and non-thermal plasma led to significantly reduced amounts of carboxylic groups on the titanium and zirconia surfaces. However, non-thermal plasma was significantly more effective than UV light on the zirconia surfaces but not on the titanium surfaces.

Hydroxyl groups also play an important role in protein adsorption and can increase the chemical interactions between osteoblasts and titanium surfaces (29). Irradiation with UV-C as well as non-thermal plasma may be able to induce a one-electron oxidation with water from the atmosphere on the surface of the material to produce a hydroxyl radical and a hydrogen ion, which is subsequently able to form another hydroxyl group at the surface (30). This may explain the superhydrophilic effect on zirconia in this study that was induced by the introduction of hydroxyl groups by non-thermal plasma. Adversely, hydroxyl groups were significantly reduced by non-thermal plasma on the titanium samples in our study and non-thermal plasma of argon generated the most hydrophilic surfaces. Therefore, the reduction of hydrocarbons and carboxyl groups could be more decisive factors for the induction of (super-)hydrophilicity and consequently increase the attractiveness for proteins and cells for both materials.

The increase of oxygen on titanium and zirconia surfaces, shown in this study, combined with the formation of highly reactive radicals, which has been reported in other studies (31), may also contribute to hydrophilicity and protein adsorption. In this study, non-thermal plasma created thicker oxide layers on both materials and significantly increased the concentration of titanium oxide. Using a non-thermal plasma jet (and not a non-thermal plasma oven, as in the present study), several studies were able to show decreasing amounts of carbon remnants and increasing concentrations of oxygen on rough titanium surfaces (26, 32). In a recent study, CANULLO *et al.* (17) compared the effects of UV light and non-thermal plasma of argon on titanium surfaces. Protein adsorption and cell adhesion of murine osteoblasts after 12 min of treatment with a non-thermal plasma jet generating plasma of argon at atmospheric pressure were comparable with the results obtained with a UV oven after 3 h of treatment. They determined the surface roughness, but they did not investigate surface chemistry and wettability as in the present study, and, in the view of the authors, a treatment interval of 3 h would be very difficult, if not impossible, to integrate into the daily clinical routine.

The results of this study showed nitrates, sulphates, and calcium on the titanium samples, and showed sodium, aluminium, silicates, phosphates, potassium, and zinc on the zirconia samples. Sulphur was almost completely removed from the titanium samples by the non-thermal plasma treatment but not by UV treatment. Traces of fluorides, magnesium oxides, silicates, sulphates, and calcium oxides are commonly found as a result of the sandblasting and etching process of the implant surface (33). The presence of aluminium and silicon is possible due to the use of these materials during the blasting process (34). It is suggested that the presence of aluminium oxide may interfere negatively with the osseointegration process (35). Remnants of other elements, such as calcium, phosphorus, or fluoride, may even enhance the cell response or may increase the bone density as well as increase the binding of the bone to the implant surface (25). However, we found traces of such contaminants only on the investigated samples. Impairment of cell reactions or biocompatibility as a result of these contaminants is unlikely, but cannot be excluded.

Distinctly different protocols, different types of discs and preparations, as well as different devices complicate comparisons between studies. However, the identified effects, as well as the advantage of using non-thermal plasma for improving the wettability and the physicochemical surface conditions of titanium and zirconia surfaces in order to engineer the implant–bone interface, need to be confirmed in further in vitro studies.

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