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Atmospheric plasma enhances wettability and cell spreading on dental implant metals

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Abstract

Objectives: Treatment regimens, which predictably support re-osseointegration of implants with peri-implantitis, are needed. Increased wettability may be an important factor for re-osseointegration. In this study, a cold atmospheric pressure gas-discharge plasma was applied to reduce water contact angles on titanium discs with different surface topography and to improve the spreading of osteoblastic cells.

Material and Methods: An argon plasma jet with different oxygen admixtures was used to treat titanium discs with different topologies, i.e. machined, SLA®, SLActive®, diamond bur-treated or Airflow®-treated. Water contact angles were measured before and after plasma treatment. The spreading behaviour of human osteoblastic cells was investigated.

Results: Contact angle of titanium discs (baseline values: 68°–117°) were significantly reduced close to 0° irrespective of surface topography after the application of argon plasma with 1.0% oxygen admixture for 60 s or 120 s. The cell size of osteoblastic cells grown on argon-oxygen-plasma-treated titanium discs was significantly larger than on non-treated surfaces ($p < 0.001$) irrespective of surface topography.

Conclusions: Plasma treatment reduced contact angle and supported spreading of osteoblastic cells. The application of cold plasma may be supportive in the treatment of peri-implant lesions and may improve the process of re-osseointegration.

*K.S. passed away on 08/12/2011. This article is in memoriam to his endeavor to find applications of plasma technology in dentistry.

Key words: biomaterials; dental implants; implant surface; osteoblasts MG-63; plasma technology; surface preparation

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Conflict of interest and source of funding statement

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The number of dental implants is steadily increasing, and peri-implantitis becomes a problem in dentistry. Ten years after implant installation, 16–28% of the patients develop peri-implant lesions (Roos-Jansaker et al. 2003). Various treatment methods have been described, but the problems, including cleaning of contaminated implant surfaces without destroying the elaborate surface geometry and to re-establish surface characteristics which are promotive for bone regeneration, yet await solutions (Claffey et al. 2008).

Plasma is an electrically neutral, ionized gas composed of ions, electrons, neutral particles, vacuum ultraviolet and ultraviolet irradiation, free radicals and chemically reactive neutral particles, whereas under normal pressure conditions, no vacuum ultraviolet radiation is produced. Currently, plasma treat-

ment is used to clean titanium (Ti) surfaces (Swart et al. 1992, Aronsson et al. 1997). Appropriate plasma processes render surfaces hydrophilic, and modify the oxide layer that interacts with proteins and cells of surrounding tissue. Thus, plasma application can lead to an improved adhesion of tissue (Zhao et al. 2005, Schwarz et al. 2007). Atmospheric plasma works at atmospheric pressure in contrast to high-energy vacuum plasma, which needs a complex reactor (Fridman 2008). Only a plasma device that does not need a vacuum and works at an atmospheric pressure may be used in a patient's mouth. The kINPen, developed by the INP Greifswald, is a plasma device, which works at atmospheric pressure, and its temperature range is biocompatible. Depending on distance of the plasma flame and the input power temperature ranged between 37°C and 62°C (Weltmann et al. 2009). This technique is cost-efficient, and safe and relatively simple in handling . Atmospheric plasma treatments are able to render surfaces hydrophilic (Vogelsang et al. 2010, Teraoka et al. 2006) and can lead to an improved cell adhesion (Teraoka et al. 2006, D'Sa et al. 2010). Previously, this technique has been used successfully for deactivation of bacteria (Fridman et al. 2007, Rupf et al. 2010) even in biofilms (Koban et al. 2011).

In the context of peri-implantitis treatment, two problems have to be solved: intra-oral cleaning of the bacterially contaminated implant surface and re-establishment of surface characteristics which then promote bone regeneration (Claffey et al. 2008). For a long-term success of peri-implantitis treatment, the establishment of a cell-friendly surface is important. With the kINPen, we are able to make both. It renders a "decontaminated" Ti surface hydrophilic and thus, it may facilitate reossification at the implant interface with peri-implantitis.

The aims of the study were as follows: (i) to determine whether surface preparation of Ti discs with atmospheric pressure plasma lowers the contact angle dependent on exposure time and gas composition and (ii) to assess if plasma treatment increases the spreading capacity of osteoblastic cells.

Material and Methods

Titanium materials

We used sandblast-etched (SLA), sandblast-etched Ti discs with a hydrophilic surface (SLActive®) and machined Ti discs (grade 4, diameter 15 mm, thickness 1 mm). All discs were kindly provided by Straumann company, Freiburg, Germany

Instrumentation modalities

We imitated two instrumentation modalities – Ti was treated (i) with a diamond bur or (ii) with airborne powder. Machined discs were treated for 60 sec with a coarse diamond bur, oriented parallel to the disc and mounted in a contra-angle handpiece (D, grain size 175 μm, Komet, Lemgo, Germany, water cooling 60 ml/min.) or with an air abrasion device for 60 sec, perpendicular to the disc, sample distance 5 mm (POW, Airflow® handy 2^+ with sodium bicarbonate powder, pressure 4.4 bar; EMS, Nyon, Switzerland; particle size 76 μm). Both instrumentations were performed freehand; the operator guided the instruments in monotonic, rectangular strokes over the discs (about 20 mm/sec). After instrumentation D and POW discs were cleaned with water. Machined (M), sandblast-etched (SLA) as well as discs with a hydrophilic SLActive® surface (ACT) served as controls.

Plasma device and surface modification

Plasma treatment was performed with an atmospheric pressure plasma jet (INP Greifswald, Greifswald, Germany). It consisted of a nozzle made of ceramics and a centred needle electrode. It was operated with a frequency of applied voltage of 1.82 MHz with an input power of 2–3 W. The gas flowed through the nozzle, and a high RF-voltage (2–6 kV) was coupled to the needle electrode. Temperature measurement yielded 42°C at the tip of the plasma jet (thermal output of 150 mW) (Fig. 1). The distance between the plasma pen (end of discharge capillary) and disc was set to 5 mm. The length of the free-burning plasma plume was 11 mm. Thus, the discs were always emerged in the plasma plume. In general, Argon (Ar)

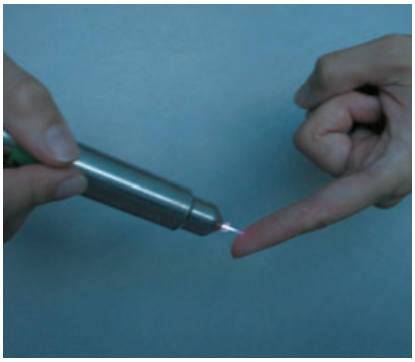

Fig. 1. Atmospheric pressure plasma jet (1.0% O_2/Ar-plasma) with a visible, cold flame.

(99.999% Ar, Alpha Gaz 1 Ar, Air Liquide, Düsseldorf, Germany) is the working gas for maintaining the discharge, and small amounts of oxygen (O_2 med., Praxair Deutschland, Düsseldorf, Germany) can be admixed for surface treatment (Weltmann et al. 2008, Foest et al. 2005). Ar gas flow was set to 5 slm (standard litres per min.) for all treatments.

Three different gas-plasma compositions (Ar-plasma, 0.2%O_2/Ar-plasma and 1.0%O_2/Ar-plasma) and three different treatment intervals (30, 60 and 120 s) were used on four different surfaces (M, D, POW and SLA).

Contact angle measurement

The contact angle was measured in the sessile drop mode on a plane surface with distilled water (0.5 μl drop-volume) immediately after plasma treatment (Digidrop; GBX, Bourg de Peage, France). SLActive® discs were dried before measurement. Altogether, 360 discs were treated (four surfaces × three gas admixtures × three treatment intervals × 10 repetitions). Each disc was measured at three spots before and after plasma treatment, and these three measurements were averaged for each disc.

Cell culture, spreading, metabolic activity and SEM of human osteoblastic cells (MG-63)

The culture of human osteoblastic cells (MG-63, ATCC, CRL-1427; LGC Promochem, Wesel, Germany) as well as the preparation for spreading and scanning electron

microscopy (SEM) were performed according to Matschegewski et al. (2010). Cells were cultured in DMEM without fetal calf serum and gentamicin. The cell area of 40 cells on each disc was quantified after cultivation of 30 min., 60 min. and 24 h, with an inverted confocal laser scanning microscope LSM 410 (Carl Zeiss, Jena, Germany). Metabolic activity was measured after 24 h of cultivation, and the CellTiter-96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, USA) was used according to the manufacture instruction. For SEM, cells grew for 60 min. and 24 h on the surfaces, and preparations were prepared according to standard protocol. Imaging was performed with 10 kV and 3.3 Ampere.

In a pilot study, we examined the influence of incubation time (30 min., 60 min., 24 h) on cell area on three replicates. In comparison to the respective 30-min. value, an increase of about 45–75% at the 60-min. value has been measured. Cell area reached a plateau after 24 h of cultivation. On the basis of these findings, we restricted our further investigation to a cultivation time of 60 min. with 1.0%O_2/Ar-plasma application. The main experiment was performed on six replicates (M, D, POW and SLA; each with and without plasma application (−P, +P) and ACT).

Statistics

Data on continuous variables were expressed as mean and standard deviation (SD). For continuous data, comparisons between groups were done applying Mann–Whitney *U*-tests or *t*-tests as appropriate. Pairwise tests were applied to find differences between different surface topographies (with or without plasma application) and ACT, which served as a control. To counteract the problem of multiple comparisons, appropriate *p*-values were corrected using the Bonferroni correction.

Multilevel linear regression models were used to assess effect of plasma treatment and time on measured values (contact angle, spreading and MTS) for varying surface topographies (instrumentations). Random effects were incorporated to account for clustering within trials. Fixed effects included trial, plasma treatment (−P, +P), time (30, 60 and 120 s) and their interaction (plasma × time). *Post hoc* linear combinations of estimators were evaluated.

Furthermore, linear hypotheses were tested after estimation using *post hoc* Wald tests, i.e. differences in measured values (spreading and MTS) within the four topographies between samples with and without plasma application.

Analyses were conducted using STATA/SE 12.0 (StataCorp 2011).

Results

Instrumentation and topography

The SEM images (Fig. 2) illustrate the differences between the different surface topographies. The original M surface exhibited pronounced machining marks, which were no longer visible after instrumentation with D and POW. D caused a very irregular topography with sharp craters and edges. POW modified the topography only slightly in comparison to M. Some areas differed little from M, and others displayed holes and grooves, and even powder residues could be detected. SLA and ACT did not differ in terms of topography, and they were very rough due to deep macro cavities and porosities covered with micro gaps.

Plasma treatment and contact angle

Plasma treatment with all gas compositions decreased the contact angles of Ti discs (Table 1). Plasma application (baseline) was effective on all surfaces ($p < 0.001$) *versus* ACT. Treatment with 1.0%O_2/

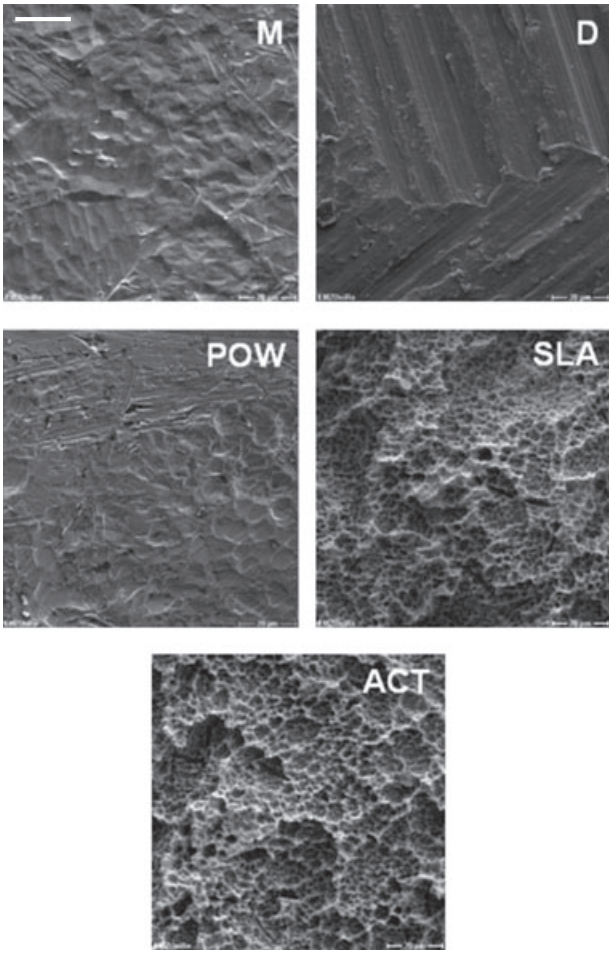

Fig. 2. SEM images of the used Ti surfaces. Magnification: ×1000, M: machined, D: coarse diamond grit, POW: Airflow® – treated, SLA: SLA®, ACT: SLActive®. (bar = 20 μm, magnification: ×1000).

Table 1. Mean contact angles in ° (baseline and after plasma application) for different titanium discs in relation to the plasma application time and the gas composition. ACT served as control surface. $N = 10$ per group

	Baseline	Exposure time	Ar-plasma	0.2%O_2/Ar-plasma	1.0%O_2/Ar-plasma	*p*-value **
M	88.07 ± 1.75*	30 s	5.36 ± 0.51	5.15 ± 0.44	0.30 ± 0.48	(ref.)
		60 s	1.75 ± 0.59	0.90 ± 0.74	0.20 ± 0.42	<0.001
		120 s	1.60 ± 0.66	0.30 ± 0.48	0.0 ± 0	<0.001
		p-value**	–	0.37	<0.001	*P* for interaction < 0.001
D	67.84 ± 2.29*	30 s	12.70 ± 0.55	7.50 ± 0.22	6.54 ± 0.10	(ref.)
		60 s	9.81 ± 0.17	6.43 ± 0.24	2.50 ± 0.85	<0.001
		120 s	8.93 ± 0.12	5.52 ± 0.27	0.80 ± 0.79	<0.001
		p-value**	–	<0.001	<0.001	*P* for interaction < 0.001
POW	72.22 ± 2.20*	30 s	21.84 ± 0.66	13.22 ± 0.41	10.51 ± 0.60	(ref.)
		60 s	11.61 ± 0.79	8.69 ± 0.83	4.87 ± 0.27	<0.001
		120 s	6.96 ± 0.42	3.86 ± 0.59	0.0 ± 0	<0.001
		p-value**	–	<0.001	<0.001	*P* for interaction < 0.001
SLA	117.18 ± 0.07*	30 s	4.20 ± 0.44	0.0 ± 0	0.0 ± 0	NA
		60 s	0.0 ± 0	0.0 ± 0	0.0 ± 0	NA
		120 s	0.0 ± 0	0.0 ± 0	0.0 ± 0	NA
		p-value†	NA	NA	NA	NA
ACT	5.48 ± 0.97	Contact angle without plasma treatment 5.48 ± 0.97				

Data are presented as mean ± standard deviation.
M, machined; D, coarse diamond grit; POW, Airflow®-treated; SLA, SLA®; ACT, SLActive®.
*$p < 0.001$ *versus* ACT, *t*-tests.
***p*-values were based on linear regression models including time, plasma type and the interaction of both as independent variables. Models were separately evaluated for each material.
†No regression model performed due to non-variability in measured value.

Ar-plasma reduced contact angles significantly ($p < 0.001$) independent of the time interval. 0.2%O_2/Ar-plasma and pure Ar-plasma were less effective when applied for 30 s. The use of plasma for 60 s or 120 s significantly reduced contact angles to nearly 0° ($p < 0.001$). Plasma application was effective on all surfaces. Irrespective of topology, the 1.0%O_2/Ar-plasma resulted in a significantly higher reduction of contact angles than 0.2%O_2/Ar-plasma or pure Ar-plasma. With increasing exposure time, contact angles decreased significantly ($p < 0.001$). According to linear regression analyses, time and plasma type were significantly related to contact angles for different surface topographies. The twofold interaction between time and plasma type was significant for all three materials ($p < 0.001$). Application of Ar-plasma on M discs caused a gradual decrease in contact angle over the 120 s, whereas application of 1.0%0_2/Ar-plasma immediately reduced the contact angle nearly to 0° (already after 30 s).

Irrespective of exposure time and plasma composition, the largest contact angle reduction was found on M. Mean values varied between 5.36° (Ar-plasma after 30 s) and 0° (1.0%O_2/Ar-plasma after 120 s), indicating an ultra-hydrophilic surface ($p < 0.001$ compared with Ar-plasma). POW surfaces showed a similar reduction after plasma treatment. The 1.0%O_2/Ar-plasma application for 120 s reduced contact angles to 0°. D discs, increased treatment times and plasma compositions ($p < 0.001$) effectively reduced water contact angles. After application of 1.0%O_2/Ar-plasma for 120 s, the contact angle decreased to 0°. Considering contact angles among all materials, POW surfaces showed lowest contact angles reduction for treatment time of 30 and 60 s. However, the 1.0%O_2/Ar-plasma application for 120 s reduced contact angles to 0°. After Ar-plasma treatment for 30 s, the contact angle was higher (21.84°), but still hydrophilic. For SLA, contact angles were throughout reduced to 0.0° (except of Ar-plasma for 30 s). However, no regression analyses were performed due to non-variability of measured values within groups. On the basis of these results, we used only 1.0% O_2/Ar-plasma for the cell spreading experiments.

Plasma application and cell area

Surface topography had a big influence on cell area: the largest cells were displayed on machined surfaces (934 μm^2) and the smallest on SLA (425 μm^2) (Fig. 3). Irrespective of surface topography (M, D, POW and SLA), plasma application facilitates cell growth and the cell area increased significantly ($p < 0.001$) by about more than 57%. As cells adapted very intimately into the three-dimensional surface structure, the actual area was underestimated in two-dimensional image. Cells grown on the commercially available SLActive® surface (ACT) showed the smallest cell area.

Plasma application and cell morphology and metabolic cell activity

Cells on the untreated discs M-P, D-P, POW-P demonstrated a hemispherical shape with delicate filopodia and cell areas within 553–934 μm^2, whereas cells on plasma treated discs (M + P, D + P, POW + P) demonstrated increased cell areas of up to 57–86%. Cells on the SLA surface (SLA-P) behaved differently: they were flat, rhomboid or spindle-shaped and exhibited no filopodia. Cells on the plasma-treated SLA surface (SLA + P) were closely adapted into the macro- and microtopography, whereas cells on the industrial produced hydrophilic surface (ACT-P) spread over the microstructure. After 24 h of cultivation (Fig. 4), the cells transformed from a

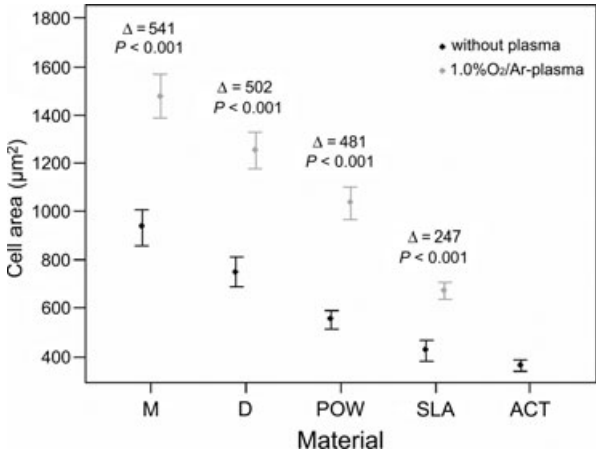

Fig. 3. Observed spreading values of cells after 60 min. according to material and 1.0%O_2/Ar-plasma application. Δ, difference in observed spreading values between −P and +P. *p*-values were based on *post hoc* tests comparing spreading values between −P and +P groups for each material after linear regression models including material and Ar-plasma application as independent factors. M: machined, D: coarse diamond grit, POW: Airflow® – treated, SLA: SLA®, ACT: SLActive®.

thick, hemispherical shape into a flat, spindle form irrespective of plasma treatment. On M, D, POW discs without plasma application, the cell walls could be clearly delineated against the surface, whereas cells on treated plasma surfaces seemed to be fused into the disc, the cell border was difficult to discern and the disc topology was shining through the cells. Cells on SLA surfaces behaved differently. While cells on untreated SLA surfaces did not sink into the rough macrostructure, cells on plasma-treated SLA and ACT discs followed the macro and the microstructure, and the microtopology could be anticipated through the cell.

Plasma treatment did not induce an inhibitory effect on metabolic activity of cells (MTS-test) irrespective of surface topology after 24 h of cultivation (Table 2). Only plasma-treated SLA surfaces exhibited a significantly higher activity compared with untreated SLA surfaces ($p = 0.001$). M, D, and POW showed significantly higher values for metabolic cell activity in comparison to ACT ($p < 0.01$), irrespective of plasma application (−P or +P).

Discussion

Various chemical and physical surface modifications of Ti improve the healing process. The cell–surface interactions are fundamental for the clinical implantation machinery. Recent studies have shown that roughness induces different cell behaviour of bone cells (e.g. cell attachment, proliferation, differentiation and mineralization) (Schwartz et al. 2001, 2005, Lüthen et al. 2005). In addition to the topography, the surface chemistry has a great influence on cell behaviour. Currently, the SLActive® (ACT) surface with its hydrophilic property is regarded as one gold standard, and it leads to a shorter healing time and improved osseointegration because its bone-to-implant contact is improved in comparison to SLA during the early state of healing (Buser et al. 2004).

As expected, after plasma treatment, the contact angles of all discs were significantly reduced compared with untreated discs from 70°–120° to 0°–10°, which is comparable to the contact angle of industrially processed SLActive® surface. All plasma gas compositions and exposure times led to an increased wettability, irrespective of surface roughness, but reduction of contact angle was dependant on time and gas composition. These results are consistent with previous studies (Yoshinari et al. 2006, Wei et al. 2009).

It is known that reactive species are formed as well as hydroxyl groups are attached (Kolb et al. 2007) on the Ti surface when oxygen is admixed into the plasma and thus hydrophilic characteristics result. Hydrophilic surfaces improve cell adhesion on implant materials (Horbett et al. 1988, Jimbo et al. 2008). Studies from Swart et al. (1992) and Jansen et al. (1991) described increased cell attachment, but no alteration of fibroblast function as a result of a hydrophilic surface by plasma application. In our experiments, cell size increased by about the same value (about 500 μm^2) after plasma treatment, and the fusion of the cells into the three-dimensional surfaces is impressive, regardless of the topography and roughness (Ra values can be found in the online appendix). The physical parameters measured by us do not provide information about the reasons, whereas the very rough diamond bur (D), plasma-treated surfaces showed a similar influence on cell size compared with the much smoother machined (M) or air-blasted (POW) plasma instrumented surfaces. Obviously, the influence of the topography on human osteoblastic cells is reduced by the chemical and physical properties of the surface. In our pilot study, plasma treatment had its most pronounced effect on cell size immediately after the cells were seeded and on cell fusion into the surface after 24 h, whereas Silva et al. (2008) demonstrated that fibroblasts showed greater cell viability after more than 3 days of cultivation on chitosan-treated with Ar-plasma. Future research has to dissect the interaction between plasma and cell kinetics and investigate in depth if the promotive effect of 1.0%O_2/Ar-plasma lasts longer than 24 h.

With regard to osteoblastic reaction, the SLA surface was the only exception. On SLA surface cell size increased by only about 250 μm^2, and in contrast to our expectation, the SLActive® surface did not show a similar effect on cell spreading compared with the SLA surface. Our in vitro results seemed to be in disagreement with animal studies that showed an improved healing of ACT in comparison to SLA (Buser et al. 2004, Schwarz et al. 2007). Our SEM images of osteoblastic cells after 24 h of cultivation may provide one explanation for this putative

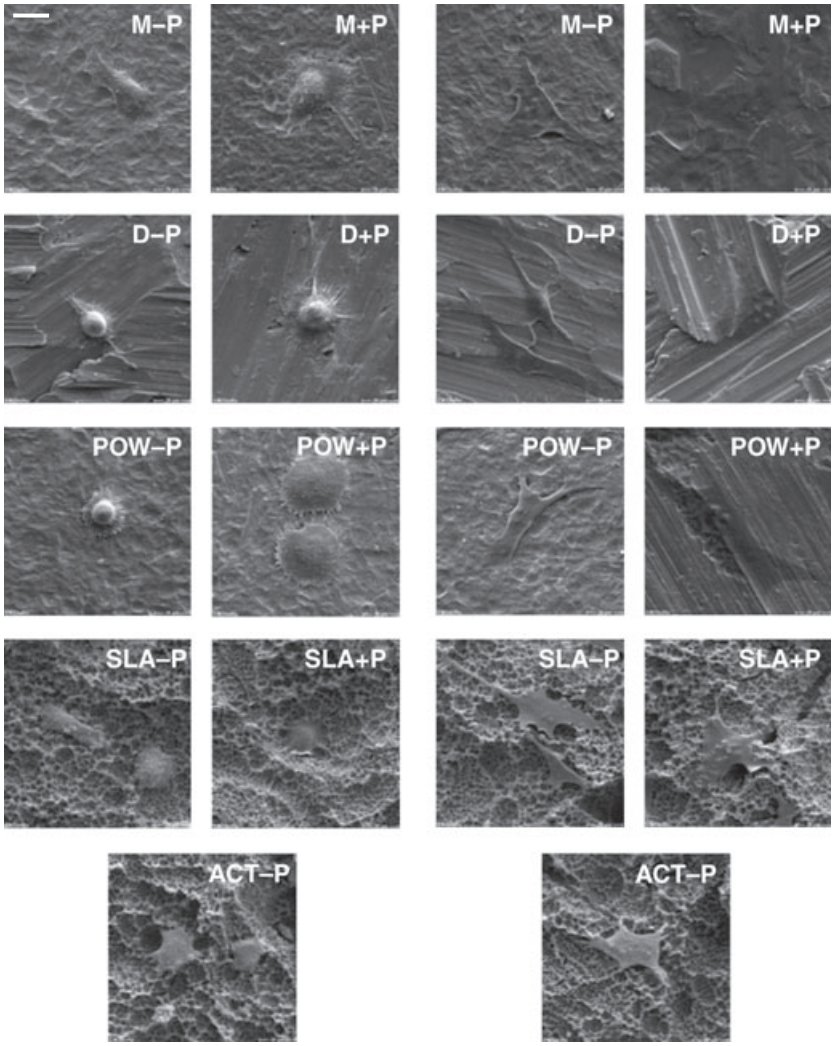

Fig. 4. SEM images of osteoblastic cells MG-63 grown for 60 min. (left side) and 24 h (right side) on machined (M), diamond bur treated (D), airflow treated (POW), sandblast-larged-etched (SLA) and hydrophilic sandblast-larged-etched discs (ACT). 1.0% O_2/Ar-plasma treatment is marked with +P. (bar = 20 μm, magnification: $\times$1000).

Table 2. Results of the metabolic activity according to the material and Ar-plasma application. The mean of ACT was set to 100%

Material	Absorbance (490 nm)		
	Without plasma	1.0%Ar/O_2-plasma	$p^{\dagger}$
M	122.5 ± 5.9**	123.4 ± 6.7**	0.78
D	127.3 ± 8.6**	127.5 ± 6.0**	0.96
POW	115.4 ± 3.7**	113.5 ± 3.3**	0.27
SLA	94.9 ± 10.2	103.0 ± 2.5	0.04
ACT	100.0 ± 7.7	–	–

Data are presented as mean ± standard deviation.
M, machined; D, coarse diamond grit; POW, Airflow®-treated; SLA, SLA®; ACT, SLActive®.
*$p < 0.01$.
**$p < 0.001$ *versus* ACT -P, unpaired *t*-test.
$^{\dagger}p$-values comparing values between −P and +P groups, based on *post hoc* Wald test after linear regression including plasma, material, the interaction of both and trial as independent variables.

contradiction: the hydrophilic ACT surface also promoted the cell-to-surface contact and a closer cell adaptation into peaks and valleys in comparison to SLA. A further explanation may be that human osteoblastic cells (MG-63) cultured on hydrophilic Ti surfaces exhibited a more bone-like phenotype, because of increased alkaline phosphatase activity and osteocalcin (Zhao et al. 2005). The big difference in cell size between topographically identical plasma treated SLA and SLActive® samples suggests that factors in addition to hydrophilicity plays a major role with respect to the cell reaction. We cannot explain why, in contrast to our results, other studies showed a positive influence of hydrophobic surfaces on the behaviour of cells with contact angle of around 70° (Ikada 1994, Tamada & Ikada 1994, Lee et al. 1998). During the 24 h of cultivation on SLA, the different behaviour of cells was clearly visible. Without plasma treatment, the cells seemed to be located on the tops of the rims above valleys or porosities. These results are consistent with other studies (Kunzler et al. 2007). In agreement with Martin et al. (1995), we found that MG-63 cells showed a different behaviour on surfaces with same Ra roughness, but with different topographies (D *versus* SLA, see online appendix).

Concerning the metabolic cell activity, our data showed no inhibitory effect of 1.0%O_2/Ar-plasma, which is consistent with previous findings (Silva et al. 2008, Garcia et al. 2010). Although results from tests like MTT and MTS were used to discuss proliferation or viability of cells, these tests only describe the release of NADH by multiple reactions of dehydrogenase enzymes. Therefore, we only can speculate that cells on all used surfaces are not influenced by surface modification with respect to cell cycle phases and therefore proliferation. The safety of plasma application in the mouth with contact of living cells has to be shown in future studies.

Future studies must investigate, if plasma treatment after a "successful microbial decontamination" renders a bacterially contaminated surface into a surface promotive for bone regeneration. The impact of bacterial biofilms and their potential influence

on the chemical composition of the Ti oxide layer has to be considered, because it remains unknown as to how osteoblasts react after biofilm removal. We as well others showed that plasma treatment also has an antimicrobial effect (Koban et al. 2010, 2011, Ermolaeva et al. 2011, Rupf et al. 2011). Perhaps, the combination of antimicrobial and surface modifying actions of non-thermal plasma may be an option to treat peri-implantitis. Our in vitro study can only give a prospectus, and further in vitro and in vivo research must be conducted to understand the capability of a cold plasma application.

Our study has different limitations. Coarse diamond burs are not accepted as an appropriate method for the decontamination of Ti surfaces. Actually, we intentionally roughened machined implant surfaces, which is totally contradictory to the clinical procedure (i.e. implantoplasty to smoothen rough implant surface), but in clinical reality, it is often difficult to grind off threads, if suprastructure is cemented and overhanging. Thus, the rough surface may be regarded as a clinically undesirable, but rare outcome. The in vitro treatment with bur and Air-flow® was not standardized, but a non-standardized procedure is much closer to clinical reality.

Conclusion

We investigated the influence of atmospheric jet plasma on osteoblastic cells with regard to contact angle, cell area and metabolic activity. The contact angle was dependent both on time (30, 60 and 120 s) and gas mixture (Ar-plasma, 0.2%O_2/Ar-plasma, 1.0%O_2/Ar-plasma), but independent of surface topography. The increase of cell area after treatment with 1.0%O_2/Ar-plasma in comparison to untreated samples was nearly equal on all used samples with exception of SLA, and it was independent of surface topography. The treatment of Ti surfaces with 1.0%O_2/Ar-plasma showed no inhibitory effects on metabolic activity of cells. In summary, these results suggests that an Ar-plasma with a small oxygen admixture was effective for surface modifications resulting in favourable cell responses.

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Clinical Relevance

Scientific rationale for the study: Currently, there are no accepted treatment options to promote cell attachment after the removal of biofilm from an implant surface during a peri-implantitis treatment. We investigated the application of a cold, low pressure plasma device to re-establish surface characteristics of titanium that may promote bone regeneration.
Principal findings: Plasma treatment significantly increased wettability irrespective of surface topology. Plasma treatment increased the areas of osteoblastic cells as well a closer adaptation to the surfaces independent of topography.
Practical implications: We aim to develop a cold, low pressure plasma device for clinical application to render a titanium surface promotive for bone regeneration and to improve re-osseointegration of implants with peri-implantitis.