Transient Spark Coating for Dentistry

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ABSTRACT: Cold atmospheric plasma jets have started to demonstrate their potential in various medical applications. Those devices generate a plasma using noble gases or air and are used to disinfect the inflamed tissues and even trigger natural healing processes. We are developing a plasma jet device with the purpose of depositing in situ a coating on implant materials that reduces bacteria attachment. The coating is not antimicrobial which is regarded as controversial. This new treatment shall offer physicians a possibility to control reinfection caused or triggered by medical implants. In this work, we present the method as well as first evaluation results. They show how the precursor and gas carrier influence the coating properties and allow to tune their surface properties. The adhesion of the coatings to the substrate was excellent, while the adhesion of Escherichia coli on the coated substrate was significantly reduced. Those preliminary results show the potential of this atmospheric plasma jet–based coating technique.

KEY WORDS: plasma jet deposition, implant coating, transient spark, plasma polymerization, dental implant, dentistry atmospheric pressure, disinfection, infection control

I. INTRODUCTION

In an industry-independent study on periimplantitis,1 596 dental implants were tracked over 9 years. After 2 years 70% and after 3 years 81% of the inserted implants showed an inflammatory change of the surrounding bone. Half of the implants showed a peri-implant bone loss of 3 mm after 9 years. Ultimately, a bacterial infection leads to the loss of the implant.

Methods to treat periimplantitis are based on the mechanical cleaning of the affected implant with subsequent use of liquid chemicals for disinfection, often accompanied by a systemic antibiotic therapy. Those techniques often come with severe drawbacks.2–4

A better approach would be to combine a soft mechanical cleaning and the disinfection of the implant and peri-implant tissues with the aid of a cold atmospheric pressure plasma jet. First studies show promising results regarding a plasma-assisted peri-implantitis treatment5,6 and plasma-assisted disinfection treatments in dentistry in general.7,8 However, those treatments options aim at disinfection only.

Plasma treatments have also been developed to optimize the surface properties of dental implant and facilitate their osseointegration. However, those are performed in special process chambers at low pressure to obtain homogeneous results. They are
performed at the end of a manufacturing process, before implantation, and cannot be applied or transferred to human medicine at a dental chair.

Lastly, plasma jets can be used as a tool to coat surfaces at atmospheric pressure. The usual way is to add a precursor in the carrier gas, that provides building material for the coating process. Those techniques promise a comparatively simple and cheap way to produce functional, thin layers and is in early development. Parameter control and coating homogeneity are some serious challenges.\textsuperscript{9–11}

We build upon the three aforementioned research areas and develop a low power, atmospheric pressure plasma jet device to coat in situ (in the mouth of a patient) a dental implant, as an option to treat peri-implantitis. In this new approach, the treatment shall not only disinfect the implant but also prevent a bacterial re-infection by means of nanofilm coating that reduces bacterial adhesion and later the formation of biofilm to the implant. We present here the method and first results of an early, prospective work.

\section*{II. MATERIAL AND METHODS}

\subsection*{A. Samples and Sample Preparation}

The samples used for the physical and chemical analysis were wafers coated with a 70-nm titanium layer. For analysis of the contact angle and bacterial tests, square titanium plates (10 × 10 × 0.3 mm) with a purity of 99.9\% were used.

The samples were prepared via a cleaning procedure: three ultrasonic baths of three minutes each of acetone, isopropanol and deionized water. For tests of bacterial adhesion, one side of each titanium sample was mechanically treated to simulate a surface corresponding to the surfaces of dental implants or endoprostheses. This was done using corundum chips with a grain size of \( \sim 120 \) \( \mu \)m and a corundum blaster of \( \sim 6 \) bar air pressure and maximum particle addition for \( \sim 30 \) seconds. This resulted in an average surface roughness of 4 \( \mu \)m.

Methods differ to perform plasma deposition at low or atmospheric pressure. In low-pressure plasma processes, a mix of gases is preferred, such as methane and oxygen.\textsuperscript{12,13} At atmospheric pressure, the precursor is usually liquid, and a carrier gas bubbles through the precursor.\textsuperscript{14,15} The C/O ratio seems to indirectly influence the hydrophilicity of the coating and the bacterial response to the sample.\textsuperscript{16–18} In this work, we selected different liquid precursors with different C/O ratios, and with a low health hazard considering the globally harmonized system precautionary statements and lethal dose (LD50 in mg element per kg body weight). Different precursors were thus tested: ethyl-acetate, benzyl-acetone, dimethyl-sulfoxide, para-xylene (also called p-xylene) and pentyl acetate (all from Sigma-Aldrich, Germany).

\subsection*{B. Atmospheric Plasma Jet Device and Method}

The plasma jet device comprises two electrodes, one being inside the nozzle and the second being the metal implant itself. This arrangement allows a guided treatment and a selective coating on the implant surface without harming the surrounding tissue.
The carrier gas first passes through the liquid precursor contained in a gas washing bottle. The enriched gas is transported to the plasma nozzle (Fig. 1). The nozzle is a polypropylene syringe tip (Optimum® plastic dosing needle “precision tips,” Nordson EFD, USA) with an inner diameter of 0.84 mm. The inner electrode is an insulated copper wire. The copper wire is 0.5 mm thick and has an ~ 50-µm insulation layer. The end of the wire has no insulation and ends with the nozzle tip.

The voltage source is a PNC 3500–50 ump (Heinzinger Electronic GmbH, Germany), which provides DC voltage to a voltage switch (GHTS 80, Behlke GmbH, Germany). A pulse generator (AFG 2021, Tektronix GmbH, Germany) provides the voltage function (pulse shape) that drives the voltage switch. A series resistor (HS5, RS-Components GmbH, Germany) limits the final voltage of the plasma. The electrical power delivered to the plasma is 5.6 W (rms).

The precursors are filled into the wash bottles and attached to the gas feedline. A separate bottle was used for each precursor to minimize cross-contamination.

The nozzle is mounted in place of the printing head of a 3D printer (Renkforce RF 100, RS-Components GmbH, Germany) and can be moved in the X, Y, and Z directions at different velocities. This allows a constant distance between nozzle outlet and substrate surface and a constant nozzle speed.

The voltage (2.2 kV) is pulsed at a frequency of 1 kHz with 1% duty cycle and a pulse width of 10 µs. Unless explicitly stated otherwise, the polarity of the inner electrode is positive while the substrate is grounded. The waveform is a square wave voltage with 18-ns edge steepness. The influence of the polarity on the shape of the plasma is shown in Fig. 2. With the positively polarized discharge, a plate-shaped glow can be observed on the ground electrode, while the area covered by visible plasma is much smaller with negative polarity.

C. Physical Characteristics of the Coatings

The growth rates were calculated from the coating thickness deposited on coated wafers. The thickness of the coatings was measured with a mechanical profilometer (P11, KLA-Tencor, USA) using the following parameters: 200 µm/s needle tip speed, 100 Hz sampling rate, 2 mg needle tip contact value and 1 µm needle tip radius.

The plasma polymers were analyzed via Fourier-transform infrared spectroscopy (BioRad Excalibur FTS 3000, Varian, USA) delivering information about the chemical bonds and functional groups. The measurements were performed over a wavenumber
range of 4000–400 cm\(^{-1}\), with a resolution of 4 cm\(^{-1}\) and a sensitivity of 16 (64 scans) after a background measurement. The plasma nanofilms were deposited on bare double side polished silicon wafers (525 μm).

The atomic compositions of the coatings were obtained by X-ray photoelectron spectroscopy (Perkin Elmer PHI 5600 ESCA System with MgK\(_a\) radiation, rbd Instruments, USA). The plasma coatings were analyzed on silicon wafer coated with a 70 nm layer of titanium. The overview spectra were measured at 800 eV with a step size of 0.8 eV, a dwell time of 20 ms and a pass energy of 187.85 eV. The analyzer angle relative to the sample surface was 45° for all spectra.

The water contact angle of the coating was measured using the sessile-drop method (advancing contact angle) with the Drop Shape Analysis System (DSA 10 MK2, Krüss GmbH, Germany). This indirectly delivers information about the surface energy of the nanofilm.

To evaluate the adhesion of the plasma nanofilm on titanium coated wafers, we performed peel tests along the D3359-09 method\(^1\) using the tape Tesa® “crystal clear” (tesa SE, Germany). All coatings were deposited to have the same thickness of 180 nm. The adhesion of the p-xylene coating was further tested after scribing the coating along a cross pattern grating (d = 1 mm). The tape was peeled off with a fast movement parallel to the substrate surface; this was performed twice per sample. The surface of a dental implant is rough to increase its osseointegration. Thus, the third test series was performed to investigate the adhesion of the coatings on surfaces that were roughened prior to coating deposition.

To estimate the resistance to mechanical abrasion we used a toothbrush (Dr. Best®, average hardness, Germany) to brush 20 times the coating in one direction under continuous water flow.

**D. Biological Effect of the Coating: Bacterial Culture**

The coating is meant to reduce the adhesion of bacteria on the sample surface. To evaluate the anti-attachment effect of the coatings, the samples are immersed in a bacterial culture and the density of adhering bacteria is evaluated. The procedure is as follows.
Transient Spark Coating for Dentistry

Bacterial strain *Escherichia coli* XL1-Blue (with transformation and expression vector pMH-33, 5.359 bp) containing the sfGFP gene was used. Five milliliters of Luria broth medium is brought in a sterile tube (0.5 g/l NaCl) with 50 mg glucose (1% w/v) and 0.5 mg Ampicillin (100 µg/ml). Then, the medium is inoculated with a single colony of the *E. coli* strain and the tube is incubated for 24 hours (37°C, 200 rpm). Subsequently, 1 ml of the culture is diluted in a 50 ml LB-Luria medium with 5 mg Ampicillin (100 µg/ml) in a sterilized 100 ml culture flask to obtain an optical density (OD) of 0.1 at a wavelength of 600 nm. The culture is incubated (37°C, 200 rpm) until it reaches an OD$_{600}$ of 0.7. 100 mg of Arabinose (0.2% w/v) is then added to the culture to induce sfGFP expression.

The samples are placed with 3 ml of the bacterial solution in one well of a microtiter plate each and incubated 24 hours at 37°C without shaking. Lastly, the samples are rinsed with deionized water before the measurement to remove non-adhering bacteria. The number of adhering bacteria on the surface is evaluated using gray values with a microscope and a 200× magnification with a GFP-Filter (Eclipse TE2000-U, Nikon, Germany).

E. Statistical Analysis

For surface energy analysis (contact angle) and bacterial adhesion, experiments were done in triplicates using three samples per condition. Measurements were performed at least 5 times per sample. Two-tailed *t*-tests were used to determine statistical differences at 0.05 level of significance. Contact angle measurements and bacterial counting inherently exhibit a homoscedastic variance, which was considered for the statistical analysis.

III. RESULTS AND DISCUSSION

A. Growth Rate

The deposition obtained using ethyl acetate, benzyl acetone, and dimethyl sulfoxide was very erratic and highly inhomogeneous. The surface of the silicon sample is also locally etched during the plasma process. Any measurement of thickness or coating effect is not possible, and those precursors are not further evaluated. For deposition of p-xylene and pentyl acetate on bare silicon substrate, using argon leads to less inhomogeneous coating than with helium. Using helium leads to very homogenous coatings (Fig. 3A). It is assumed that the thermal and electrical conductivities of helium lead to the smoother deposition process.

Using helium as carrier gas, the deposition rate of p-xylene is 1193 nm/s and of pentyl acetate 732 nm/s for one run. One explanation for the lower deposition rate of pentyl acetate is its higher oxygen content. The competitive ablation and polymer formation mechanism leads to a relatively stronger coating removal due to the increased number of oxygen atoms. This effect has already been observed many times.\textsuperscript{20,21}
A further reason for the higher deposition rate of p-xylene lies in its chemical structure, namely its three double bonds in the aromatic ring. Pentyl-acetate, on the other hand, has only one double bond at one of the two oxygen atoms. Merche et al. commented as follows: “More generally, for the synthesis of organic coatings, the deposition rate will also strongly depend on the chemical structure of the precursor. Precursors easy to polymerize and containing one or two double bonds will have very high deposition rates, while, for example, fully saturated precursors will have very small deposition rates.”

FIG. 3: (A) The lines are p-xylene coatings on a titanium-coated silicon substrate with different number of runs per line (six, nine, and 12 runs per line). The red arrows represent the measuring lines (3 × 3 measurements per plate). (B to D) Exemplary layer thickness measurements on titanium substrate for six runs [≈ 25 nm; (B)], nine runs [≈ 60 nm; (C)], and 12 runs [≈ 100 nm; (D)].
B. Chemical Analysis

The results from the XPS analysis are presented in Table 1, showing the percentage of carbon (C1s), nitrogen (N1s), oxygen (O1s) and titanium (Ti2p). The non-coated substrate displays the expected ratio 2:1 of oxygen and titanium of the titanium oxide naturally covering titanium surfaces. It also shows that the layer generated with pentyl acetate (C7H14O2) has a lower nitrogen, resp. higher oxygen concentration than the p-xylene [C6H4(CH3)2] layer, which is expected considering the chemical structure of the precursors. The presence of nitrogen can be explained by the interaction with ambient air during deposition. The high ratio of oxygen in the p-xylene layer is caused by the interaction of the plasma and the surrounding air. A binding of oxygen in the layer occurs rapidly after the process due to the many C–H dangling bonds. Their presence is shown in the Fourier transform infrared spectroscopy (FTIR) spectra shown in Fig. 4.

Figure 4 shows the measured FTIR spectra of both coatings. It shows the O–H, C–H, C–O and C=O peaks. When comparing the spectra in Fig. 4, it is noticeable that they are of a similar nature, whereby the spectrum of the p-xylene layer has a slightly lower intensity. This is shown, for example, by the wider O–H peak around 3400 cm⁻¹.

<table>
<thead>
<tr>
<th>TABLE 1: Chemical composition of the coatings with non-coated substrate</th>
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<tr>
<td>Carbon (C1s)</td>
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<tr>
<td>---------------</td>
</tr>
<tr>
<td>No coating (blank)</td>
</tr>
<tr>
<td>p-xylene coating</td>
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<tr>
<td>Pentyl acetate coating</td>
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FIG. 4: Comparison of the FTIR spectra of the two investigated coatings. Except for the fingerprint region between ~ 1000–1400 cm⁻¹, the spectra are very similar in that pentyl acetate shows more oxygen-related peaks.
or the narrower C=O peak around 1700 cm\(^{-1}\). Clear differences can be found in the fingerprint region (between \(\sim 1000\) cm\(^{-1}\) to 1400 cm\(^{-1}\)), where a peak occurs in the pentyl acetate layer at 1236 cm\(^{-1}\), which does not occur in p-xylene. Generally, oxygen-related peaks are more pronounced in the pentyl acetate layer than in the p-xylene layer. This corroborates the results obtained by XPS because pentyl acetate contains two oxygen atoms and p-xylene contain no oxygen atoms in their initial chemical formula.

### C. Surface Energy

The results from the contact angle measurements are presented in Fig. 5. The addition of polar groups on a surface induces a lower contact angle and a higher surface energy.\(^{25-27}\)

1. **Effect of Air Storage**

Right after the process, uncoated titanium surface shows a contact angle of 68°, which slightly increases with time to a value of 84° after 1 month \((p < 0.05)\). The lower contact angle at the beginning is caused by the ultrasonic bath cleaning process “refreshing” the native titanium oxide layer. The contact angle values and time frame are in accordance with data shown by Sousa et al.\(^{28}\) and Feng et al.\(^{29}\)

Compared with untreated samples, the contact angles of the samples are reduced when treated with helium plasma \((p < 0.05)\) or argon plasma \((p < 0.05)\). This phenomenon has been largely described in the literature. Plasma activated oxygen from the

![FIG. 5: Results of contact angle measurement of all samples. Left part of the diagram (exponential time scale): Samples were stored in air and measurements were performed right after the deposition process 1 hour, 1 day, 1 week, and 1 month after coating. In the right part of the diagram (linear time scale 4 months later), the samples were then placed in water and measurements were performed after 1 day, 2 days, 1 week, and 4 weeks showing a contact angle recovery when stored in water. (All measurements showed a standard deviation of 6% on average; not shown here for the purpose of readability).](image)
surrounding air adhere to the surface.\textsuperscript{30–33} This effect is temporary and when stored in air, the contact angle increases back again ($p < 0.05$ each).

The pentyl acetate coatings show a lower contact angle than coatings obtained with $p$-xylene, regardless of the used carrier gas (helium or argon) ($p < 0.05$ each). This is due to the higher concentration of oxygen-based polar groups and in accordance with the XPS results. The polar groups can move into the coating layer or to its surface, which also changes the polarity. This so-called hydrophobic recovery has also been described in the literature.\textsuperscript{34,35}

### 2. Effect of Water Storage

In their work, Mantel et al. showed that hydrophilic surfaces remain hydrophilic in a hydrophilic medium.\textsuperscript{36} Figure 5 shows that the contact angle of the coatings comes back to their original “plasma treatment” value when stored in water. The “plasma treatment” value for the $p$-xylene coating is $60.4^\circ$ and the “recovered” value is $63.5^\circ$ ($p = 0.067$); the “plasma treatment” value for the pentyl acetate coating is $37.2^\circ$ and the “recovered” value is $44.4^\circ$ ($p < 0.05$). The opposite phenomenon to the above-mentioned hydrophobic effect takes place. The samples become hydrophile again when immersed in deionized water. This effect does not happen on uncoated titanium. For the coated samples, the polar groups have a rotational freedom and can strongly influence the surface properties of the coating.\textsuperscript{37} This effect was already seen with nanofilms produced with vacuum plasma polymerization.\textsuperscript{35,38} Therefore change in contact angle over time is a reversible process and can be influenced by the storage condition.

### D. Adhesion and Abrasion

The first tape tests showed a very strong adhesion of the coatings: no removal at all could be observed (Fig. 6B). When the coatings were scratched in a cross-pattern fashion, the

![FIG. 6: Pictures showing that the peel test had virtually no effect on the coatings obtained using helium as carrier gas (B) when compared to coating prior to peel test and toothbrushing (A). Furthermore, even after 20× brushing the surface, the coating was only partly removed (C).](image-url)
coatings were partly removed by the tape and classified in category 1B (35–65% of the surface removed) according to “D3359-Substrate-Adhesion.” The third tape tests showed the effect of surface roughening prior to deposition. Here, the tape removed about 8% of the coating. We assume that a mechanical anchoring to the surface is beneficial for the adhesion. Brushing unscratched coatings with a toothbrush did not alter the coating as can be seen in Fig. 6.

**E. Bacterial Adhesion**

Bacteria adhering on the surface were observed using a fluorescence microscope. Image analysis using mean gray values gave an indication of the density of bacteria adhering to the surface. The gray values of the different surfaces were compared after 24 hours of incubation. Figure 7 displays the results of the analysis.

On smooth titanium, both p-xylene and pentyl acetate coatings reduced the adhesion of bacteria significantly ($p < 0.05$). Roughing the surface also reduced the adhesion of bacteria ($p < 0.05$). The antiadhesive effect of the coating is reduced on the roughened surface in case of a thin coating (about 15 nm). The bacterial reduction induced by the thicker coating (about 70 nm) is significant again ($p < 0.05$). This may mean that the plasma-deposited layer is too thin to confluentely coat the rough surface.

In all cases, the antiadhesive effect obtained by pentyl acetate is stronger than with the p-xylene coating ($p$-value for pentyl acetate is much lower than that of p-xylene), which is accordance with the lower contact angle results. Those results are comparable with other deposition methods that are technically limited and cannot be adapted for an in situ treatment. 39–41

![FIG. 7: Bacteria adhesion after 24 hours on the different types of surface morphology analyzed by mean gray values (lower value means less adhesion). Inset shows examples of uncoated titanium (left) and coated titanium sample (right).](image)
IV. CONCLUSION

In this work, a novel method to use a plasma jet for the application in dentistry is described. Especially the opportunity to use a tool such as a plasma jet to deposit an anti-adhesive coating in situ may become a game-changing option for many medical fields where implants are used. In this work, a possible candidate for such an application is presented.

The coating thickness measured with a mechanical profilometer delivered information on the growth rate. We observed that using helium as a carrier gas led to more uniform coatings. The use of p-xylene led to a deposition rate of more than a micrometer per second. The XPS analysis of the coating shows a strong dependency of the precursor. The more oxygen in the precursor the more oxygen in the coating, which also led to a higher surface energy, which is explained by the introduction of oxygen-based polar groups into the coating. When stored in ambient air we observed a hydrophobic recovery. The polar groups have a rotational freedom within the coating; stored in air, the groups rotate so that the surface of the coating becomes hydrophobic. Stored in water, the groups rotate back to present their hydrophilic side, which is energetically more compatible with the surrounding. Thus, when the coating is on an implant inside the body, it will retain its original hydrophilic quality. The associated surface energy is ideal to reduce bacterial adhesion as explaining the low bacterial attachment on the coatings compared with uncoated titanium.

More work is awaiting us to further comprehend the method, optimize the technique and demonstrate its safety and efficacy.

ACKNOWLEDGMENTS

This work was supported by the German Research Foundation (DFG Grant 420539095). The authors thank Dr. Gregory Stevens for the protocol for sfGFP expression, Prof. Dr. Andreas Plückthun from the University of Zurich for the providing of the pMH-33 plasmid, and the Chair for Chemistry and Physics of Interfaces of the IMTEK, University Freiburg, for providing us with the FTIR and XPS measurement equipment. The authors declare no competing interests.

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