Time-related surface modification of denture base acrylic resin treated by atmospheric pressure cold plasma

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INTRODUCTION

Chronic atrophic candidiasis, also known as Candida-associated denture stomatitis, is a very common disease affecting 15% to over 70% of the denture wearers1-6. Candida albicans has been known as the most frequently isolated Candida species responsible for this inflammatory pathology2,4-6. The adherence of C. albicans to acrylic resin denture base (polymethyl methacrylate —PMMA) has been thought to be the first step for successful colonization before subsequent plaque formation and development of pathogenesis7-9. Surface characteristics, such as surface roughness, hydrophilicity, and surface charge have all been reported to involve in the adherence of Candida7-11. Therefore, the development of methods that could modify these denture base surface properties to prevent the adherence of C. albicans would be a significant advancement in the prevention of Candida-associated denture stomatitis8,9,12.

Atmospheric pressure cold plasma is a complex mixture composed of ions, energetic free radicals, electrons, atoms, and molecules generated by gas discharge. It has been used in various applications, such as tooth whitening13,14, sterilization15-17, blood coagulation18, and inducing tumor cells apoptosis19. This technique has also been considered a potential method for surface modification of PMMA without inducing bulk alteration7,11,12,20-23. Some authors have demonstrated that the plasma treatment can improve the hydrophilicity7,11,12,20,21,23 and modify the chemical composition of the PMMA11,20-23. However, information on the adherence of C. albicans to atmospheric pressure cold plasma modified denture base acrylic resin is scarce and there is no agreement until now7,11,12. Moreover, the relationship between surface modification of denture base acrylic resins and plasma treatment time remains to be investigated.

The main purpose of the present study was to investigate the potential of different atmospheric pressure cold plasma treatment time to modify the surface physical and chemical properties of denture base acrylic resin, as well as its effect on C. albicans adherence. The durability of material modification was also evaluated. Therefore, the null hypotheses were that: (1) different cold plasma treatment time has no effect on the surface physical and chemical properties of denture base acrylic resin; (2) different cold plasma treatment time has no effect on C. albicans adherence.

MATERIALS AND METHODS

Acrylic resin specimens

The polymethyl methacrylate (PMMA) specimens (n=104) were prepared with an acrylic resin denture base material (Vertex Rapid Simplified, Vertex-Dental BV, Zeist, the Netherlands) by using heat-compression mold technique. Powder and liquid acrylic resin denture base material were mixed and processed according to the manufacturer’s recommendations. Specimens were made as disk-shaped, measuring 12 mm in diameter and 1 mm thickness. One side of the PMMA specimen was ground by silicon carbide paper up to grain size 1000. Then the specimens were immersed in distilled water at 37ºC for 48 h for residual monomer release and dried in air before experiments.

Keywords: Cold plasma, Acrylic resin, Dentures, Hydrophilicity, Candida albicans
Cold plasma treatment
A single electrode non-thermal atmospheric pressure plasma jet was used to treat the acrylic resin specimens. Figure 1 shows a schematic diagram of the experimental arrangement. The system consists of a Teflon tube (Daxiang, Beijing, China), a 1 MΩ resistor, and an outer copper foil that surrounds the Teflon tube. The outer and inner diameters of Teflon tube are 10 mm and 8 mm, respectively. The outer copper foil is connected to a 10 kHz sinusoidal high voltage source (CTP-2000K, Suman, Nanjing, China) with an 18 kV peak-to-peak voltage. Argon/oxygen (98% Ar and 2% O₂ per volume) at a flow rate of 5 L/min is used as working gas. The plasma is generated inside the Teflon tube near the powered outer electrode, and then propagated to generate a continuous plasma jet with a length of 5 cm outside the Teflon tube in the surrounding atmospheric pressure air. The distance between the tip of the plasma jet and the specimen was 10 mm. The gas temperature near the specimen ranged from 34–38°C.

Measurements of water contact angle
To determine the hydrophilicity of the acrylic resin specimens, contact angles of ultrapure water were measured using the sessile drop method with an automated goniometer system (OCA 15 plus, Dataphysics Instruments, Filderstadt, Germany) in combination with SCA-20 software. An image of the water droplet on the surface of the specimen was taken immediately after the contact. A single droplet (2.0 μL) was assessed with two measurements (right and left contact angle) for each droplet on the specimen. Forty five Specimens were selected to measure contact angles. They were randomly divided into five groups (n=9): group 1 was control group (untreated); groups 2 to 5 were experimental groups (plasma treated for 30 s, 60 s, 90 s, and 120 s, respectively). For each group, water contact angles of specimens were measured immediately after plasma treatment and at periods of 48 h, 15 days, and 30 days with storage in deionized water at 25°C and air humidity of 45%. These measurements were made to evaluate the treatment time-related wettability modification of acrylic resin surfaces and the durability of plasma treatment.

Evaluations of surface roughness and surface topography
The surface roughness and topography were evaluated with a 3-D laser scanning microscope (LSM) (VK-X200, KEYENCE, Osaka, Japan). Nine specimens were selected. For each specimen, surface roughness and topography were measured before and after plasma treated for 30 s, 60 s, 90 s, and 120 s, respectively. Four measurements were made for each specimen to get the average surface roughness value (Ra, μm). The surface topography images of specimens were obtained at ×3,000 magnification.

Measurements of adherent C. albicans
C. albicans strain ATCC 10231 was used. The yeasts were grown in an agar YPD culture medium (1% yeast extract, 1% peptone, 4% dextrose, and 2% agar) for 48 h at 37°C. One loopful of this young culture was transferred to 30 mL of YPD liquid medium (1% yeast extract, 2% peptone, and 4% dextrose) and incubated at 37°C for 10 h. The yeasts were harvested, washed twice with phosphate-buffered saline (PBS, pH=7.2) and centrifugation at 3,000 g for 5 min. Candida suspension was spectrophotometrically standardized to a concentration of 1×10⁷ cells/mL.

Forty five Specimens were randomly selected and divided into five groups. For each group, 9 specimens were treated as described in water contact angle measurement. The acrylic resin specimens were placed into the 24-well microtiter plates and immersed in 1.5 mL of the Candida suspension for 90 min at 37°C to promote adherence and colonization. Subsequently, non-adherent cells were removed from the specimens by gently washing with 2 mL PBS for 1 min. The specimens were transferred to 15 mL centrifuge tubes and shaken for 2 min. The amount of the C. albicans cells adhering to specimen surfaces was evaluated by colony-forming units (CFUs) analysis.

All the procedures were done by the same operator.

X-Ray photoelectron spectroscopy
The effect of the plasma treatment on the elemental composition and chemical bond of the acrylic resin surface was monitored by X-ray photoelectron spectroscopy (XPS) analysis (Axis Ultra, Kratos Analytical, Manchester, UK). Five specimens were selected. One specimen was untreated, other 4 specimens were plasma treated for 30 s, 60 s, 90 s, and 120 s, respectively. The XPS peak position was calibrated using the C1s peak at 284.8 eV to compensate for residual charging effects. Data for percent elemental composition, elemental ratios, and peak fit analysis parameters were calculated using software supplied by Kratos with the XPS.
Statistical analysis
Comparisons of mean contact angle, surface roughness and C. albicans adherence data were analyzed with One-way analysis of variance (ANOVA) in SPSS 16.0. Surface roughness was analyzed with repeated measures ANOVA. Statistically significant differences were determined at a significance level of $p<0.05$. As significant differences ($p<0.05$) were found, the comparisons of the differences between the experimental groups were analyzed by the post hoc test of L-S-D.

RESULTS

Water contact angle
Figure 2 shows the water contact angle changes of cold plasma treated denture base acrylic resin. The water contact angle of denture base acrylic resin decreased from 77° to 52° after cold plasma treatment for 30 s (Fig. 3), which indicated that the surface became more hydrophilic after short treatment. Statistically significant differences were observed between the control and each of the plasma treatment groups ($p<0.05$). But the experimental groups (group 2–5) did not differ from each other.

After 48 h of immersion in deionized water, there was significant increased in water contact angle for experimental groups ($p<0.05$). No significant difference was observed between the control (group 1) and group 2 ($p>0.05$), whereas the lower contact angles were observed in groups 3, 4 and 5 ($p<0.05$). After immersion in deionized water for 15 days, groups 4 and 5 exhibited lower contact angles ($p<0.05$), whereas groups 1, 2 and 3 did not differ from each other ($p>0.05$). There were no significant differences ($p>0.05$) in the water contact angle values between control and experimental groups after immersion in deionized water for 30 days.

Surface roughness and surface topography
Surface roughness values of all groups were presented in Table 1. There were no significant differences among all groups evaluated ($p>0.05$). The 3-D LSM images of the

Table 1  Means and standard deviations (SD) of roughness values (Ra, μm) for groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Roughness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>30 s</td>
<td>0.23±0.02</td>
</tr>
<tr>
<td>60 s</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>90 s</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>120 s</td>
<td>0.22±0.02</td>
</tr>
</tbody>
</table>

No significant differences were found among all groups evaluated ($p>0.05$).
same specimen were obtained before and after plasma treatment (Fig. 4). The surface topography of untreated specimen showed irregular lines or cracks on the surface resulting from the polishing process (Fig. 4a). It became slightly smoother with few cracks after treatment. With the plasma treatment time increasing, the specimen surface was smoother gradually (Figs. 4b–e).

C. albicans adherence
As presented in Fig. 5, plasma treatment groups showed significantly lower C. albicans adherence than the control group ($p<0.05$). When the results were analyzed with regarding to treatment time, lowest yeast cells were counted on 90 s group.

X-Ray photoelectron spectroscopy
X-ray photoelectron spectroscopy analysis was carried out to determine the chemical modifications on specimen surfaces. The XPS wide energy spectra of control and treatment groups were presented in Fig. 6. The spectra were similar, except for the C 1s peak (binding energy around 285 eV) and O 1s peak (binding energy around 532 eV). The intensity of C 1s peak was higher than the O 1s peak before plasma treatment, but it was reversal after plasma applications. As a result of the plasma treatment, the intensity and proportion of C 1s peak decreased gradually whereas the percentage of O 1s peak increased significantly at the same time.

As shown in Table 2, the plasma treatment reduced the ratio of C/O, and this reduction was affected by treatment time. The peak area ratio of C 1s peak calculated from the XPS data were also shown in Table 2. It is obvious that plasma treatment reduced the ratio of hydrocarbon bonds (C 1s(1) peak). Increasing treatment time caused more reduction. On the other hand, the amount of methoxy group carbon (C 1s(3) peak) and carbon in the ester group (C 1s(4) peak) increased gradually.
Table2 Relative elemental proportion and peak area ratio of C1s peak determined by X-ray Photoelectron Spectroscopy (XPS)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Elemental proportion (%)</th>
<th>Peak area ratio of C1s peak (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>80.01</td>
<td>18.11</td>
</tr>
<tr>
<td>30 s</td>
<td>71.46</td>
<td>25.91</td>
</tr>
<tr>
<td>60 s</td>
<td>71.98</td>
<td>25.65</td>
</tr>
<tr>
<td>90 s</td>
<td>71.75</td>
<td>26.51</td>
</tr>
<tr>
<td>120 s</td>
<td>70.74</td>
<td>28.38</td>
</tr>
</tbody>
</table>

DISCUSSION

Acrylic resin, especially polymethyl methacrylate (PMMA) is widely used materials in dentistry. In prosthodontics, acrylic resin is most commonly used for making denture base of removable partial denture and complete denture. Surface characteristics of denture base acrylic resin, such as surface roughness, hydrophilicity, surface topography, and surface charge have all been reported to involved in the adherence of Candida, which is the essential step for development of Candida-associated denture stomatitis7-11). There has been a large body of evidence indicating that atmospheric pressure cold plasma technique may affect these surface characteristics on the surface layer without affecting their bulk properties7,11,12,20-23).

The results of the present study revealed that cold plasma treatment of acrylic resin specimens significantly increased the surface hydrophilicity when comparing with the control group thus confirmed the findings of published investigations7,11,12,21,23). The water contact angle of control group (77°) was immediately reduced to 52° after plasma treatment. At the same time, the XPS analysis, which demonstrated a decrease in the ratio of C/O. The spectra of the plasma treated specimen differed in the C 1s region with a wider and lower peak that mean the intensities of C=O bonds and C=O–O bonds increased. These were consistent with previous investigations, which indicated that atmospheric pressure cold plasma technique may increase the oxygen-containing functional groups on the surface of the polymer7,11,21). Plasma treatment could result in C–H (hydrocarbon) bonds breakage, then created free radicals onto the polymer surface. Subsequently, chemical reactions that occurred between these free radicals and oxygen from the environment incorporated polar hydrophilic groups (oxygen-containing-groups) to the polymer surfaces, mainly C=O7,11,21). Similar results were appeared in our study that the proportion of the C=O bonds (C 1s(4) peak) and C=O–O bonds (C 1s(3) peak) were increased when the specimen was treated with plasma, whereas the proportion of the hydrocarbon bonds (C 1s(1) peak) was decreased relevantly.

However, there was no further hydrophilic improvement of the surface in association with increasing plasma treatment time. On the contrary, XPS analysis suggested that increasing treatment time created more polar functional groups (C=O bonds), and caused more reduction of C/O atomic ratio. Therefore, the null hypothesis (1) is rejected. The atmospheric pressure cold plasma treatment increased the intensity of O 1s peak, which is positive correlated with the treatment time. The intensity of C 1s peak was higher than the O 1s peak in the untreated specimen, but it was reversal after plasma applications (Fig. 6). According to these results, a certain ‘threshold’ was presented in our study. It can be suspected that the improvement in the hydrophilicity of acrylic resin surface might be in association with the duration of application time before reaching this threshold. While, once the amount of polar oxygen-containing groups exceeded the threshold, prolonged treatment time couldn’t cause further hydrophilic modification anymore.

To assess the durability of the plasma treatment, the water contact angle of the specimens immersed in deionized water have been followed up to 30 days after plasma treatment. According to references, Zamperini et al.11,12) chose 48 h as storage period, whereas Ozden et al.23) chose 60 days. In our preliminary experiment, we found there were no significant differences in the water contact angle values between untreated and plasma treated specimen after immersion in deionized water for 30 days, so we chose 48 h, 15 days, and 30 days as storage periods in present study. The specimens had a tendency to recover their hydrophobicity, while prolonged of treatment time might offer a durable wettability (Fig. 3). Likewise, the XPS analysis suggested that more polar functional groups generated with the plasma treatment time increased. It could be suspected that although more polar oxygen-containing groups couldn’t cause wettability improvement when above the threshold, it might influence the durability of the plasma treatment.

It has been observed that the surface roughness and topography directly influences micro-organisms initial
adherence to surfaces, biofilm development, and Candida species colonization\(^\text{10,26}\). Some authors have demonstrated that materials with the roughest surface usually exhibit higher Candida adherence\(^\text{10,24,25}\). On the contrary, Hahnel et al.\(^\text{26}\) observed that there is no correlation between surface roughness and Candida adherence. Zamperini et al.\(^\text{27}\) reported that the ‘polishing effect’ of plasma treatment caused acrylic resin became much smoother with few and lower peaks. However, Yildirim et al.\(^\text{28}\) demonstrated that plasma could smoothen the cracks and lines of acrylic resin surfaces but increase the number of small hills-and-pits at the same time, which causing an increase of the roughness of the surfaces. Furthermore, Anja Liebermann et al.\(^\text{29}\) reported that plasma treatment of PMMA had no impact on the surface roughness. Despite the controversy regarding still existed, the current results revealed that the plasma treatment smoothened the cracks and lines of specimen surface (Fig. 4), while there were no significant differences in the mean roughness values among all groups. These results indicated that atmospheric pressure cold plasma treatment might affect chemical composition of acrylic resin surface without affecting their physical properties. However, measurements of adherent C. albicans in this study showed that atmospheric pressure cold plasma treatment could reduce the adherence of C. albicans to PMMA surface. This result revealed that there was no relationship between the surface roughness and Candida adherence. The surface roughness and topography of specimens were analyzed with a 3-D laser scanning microscope (LSM) in present study. Different with profilometer, the 3-D LSM was a new kind of non-contact profile and roughness measurement that could measure without damaging the target area, which was the common method to evaluate the surface roughness in previous investigations\(^\text{11,12,21}\). Non-contact design made it possible for self-control experiment. Moreover, laser beam diameter (0.4 μm) was significantly smaller than a roughness gauge stylus (2 μm), enabling more accurate measurement.

The adherence of C. albicans to acrylic resin denture base has been thought to be the first step for development of Candida-associated denture stomatitis, so inhibiting the early adherence process of C. albicans would be important for prevention of stomatitis. According to references\(^\text{11,12,21}\), immersion in Candida suspension for 90 min at 37°C was enough to promote early C. albicans adherence. However, there was no agreement about the adherence of C. albicans to plasma modified acrylic resin denture base until now. Yildirim et al.\(^\text{30}\) reported that improving the surface hydrophilicity of acrylic resin by glow-discharge plasma increased the adherence of the C. albicans. On the contrary, Zamperini et al.\(^\text{11}\) demonstrated that plasma treatment showed promising potential for reducing the adherence of C. albicans to denture base. In current study, the results revealed that atmospheric pressure cold plasma treatment creating oxygen-containing functional groups on the surface of the modified specimens improved the surface wettability and reduced the adherence of C. albicans.

A linear relationship between contact angle measurements on materials and C. albicans adherence has been demonstrated in previous investigations\(^\text{10,19}\). However, our results were different from this conclusion. Although prolonged treatment time did not cause hydrophilic improvement, the lowest adherence of C. albicans was detected on 90 s group. Therefore, the null hypothesis (2) is rejected. It can be suspected that the adherence of C. albicans on plasma-treated surface might not only relate to the hydrophilicity, but also associate with energetic species in plasma such as ions, electrons and free radicals\(^\text{30}\). But within the limitations of this study, further investigations to confirm this hypothesis are needed. Moreover, the result in vivo situation is different from that in vitro experiment. Many other factors may affect the adherence of C. albicans to denture base, such as saliva, pH of the oral cavity, oral hygiene, and so on\(^\text{2,4,10}\). We also want to investigate the adherence of C. albicans on plasma-treated acrylic resin denture base overtime. Hence, further studies are needed on this subject.

**CONCLUSION**

Atmospheric pressure cold plasma treatment could improve the surface wettability of denture base acrylic resin and reduced the adherence of C. albicans. Prolonged treatment time could not cause further wettability improvement, but might influence the durability of the modification. Moreover, plasma treatment could affect chemical composition of acrylic resin surface without affecting their physical properties. It supplies a promising way to prevent the Candida-associated denture stomatitis.

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