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Inhibition of *C. albicans* Formation by Non-thermal Atmospheric Pressure Plasma Jet (NTAPPJ) on Acrylic Resin Surface

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Abstract

The aim of this study was to investigate the antibacterial effects of non-thermal atmospheric pressure plasma jet (NTAPPJ) against C. albicans on denture resin. Self-cured acrylic resin was used for a denture base, and the surface was treated with NTAPPJ using either compressed air (AP) or nitrogen gas (NP) or was left untreated (control). To analyze the surface characteristics, surface roughness and wettability, as well as chemical composition were observed. We also evaluated the antibacterial effect of NTAPPJ against C. albicans. Average surface roughness values were similar in all three experimental groups, and the NTAPPJ-treated resin surfaces exhibited hydrophilicity. On XPS analysis, the hydrocarbon peak was decreased while the hydroxyl group (O–H) peak was increased on the NTAPPJ-treated resin surfaces. The number of C. albicans colony-forming units was decreased in response to NTAPPJ treatment. These results suggested that NTAPPJ treatment could improve surface wettability as well as exhibit a good antibacterial effect. Therefore, NTAPPJ treatment is a promising method for preventing denture stomatitis or infection.

***Keywords**: Acrylic resin, Antibacterial effect, Candida albicans, Non-thermal atmospheric pressure plasma jet*

1. Introduction

The growing elderly population has resulted in older patients requiring dentures, which are the most common treatment for loss of dentition [1]. Denture plates and resins, especially polymethyl methacrylate (PMMA) and 4-methacryloxyethyl trimellitic anhydride (4-META) resin, have long been used in the field of prosthodontia. Acrylic resin is aesthetically favorable, and its solubility and absorptiveness are low, with comparably precise reproducibility and ease of operation and repair. On the other hand, the polymerizing process produces bubbles that result in pockets where plaque can accumulate, leading to unhygienic oral conditions in denture wearers [2-5].

Attachment of microorganisms to the denture surface causes denture-related stomatitis. This disorder (also known as denture sore mouth) is characterized by inflammation and erythema of oral mucosa that is covered by the denture [6-8]. *C. albicans*, the most

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common bacteria causing denture stomatitis, can be present on the surface of a denture, attach to the prosthetic materials, and remain there as a biofilm rather than floating freely as in normal, denture-free circumstances [9-10]. Therefore, it is necessary to remove the bacteria either mechanically (by brushing) or chemically (using a denture-cleansing agent) to prevent both the formation of a biofilm and the attachment of *C. albicans* to the resin, leading to denture sore mouth. However, tooth brushing is ineffective against microbial activity in denture biofilms and removes only more substantive debris. In addition, inappropriate brushing techniques can affect the morphology and physical properties of the denture material. Some chemical agents used for denture cleaning are reported to damage acrylic resin [11-12].

Recently, the use of plasma has attracted increasing attention in the biomedical field. Non-thermal atmospheric pressure plasma (NTAPP) in particular is an innovative technology that has the potential to destroy microorganisms and offers an original alternative to sterilization. NTAPP has many advantages, foremost of which are its bactericidal effects at room temperature. Moreover, it is harmless to the human body and can be applied directly to exposed areas without causing cell damage [13]. It has been reported that low-temperature plasma can inactivate bacteria, biofilm, yeast, and spores [14].

Thus, the aim of this study was to evaluate the antibacterial effects of an NTAPPJ jet against C. *albicans* present on denture resin.

2. Experimental Materials and Methods

2.1. Preparation of Specimens

A self-curing denture base resin (Jet Denture Repair Powder, Lang Dental, and Wheeling, IL, USA) was mixed, according to the manufacturer's instructions, and then poured into 12 mm × 2 mm Teflon molds. A glass slide was placed onto each specimen within the mold to flatten its surface. After one hour of curing time, the specimens were separated from the molds. All specimens were sterilized with ethylene oxide (EO) gas before each experiment. The specimens were fabricated for testing the effects of NTAPPJ (n=5) and in two experimental groups, one treated with nitrogen gas (NP) (n=5) and one treated with compressed air (AP) (n=5), and in an untreated control group.

2.2. NTAPPJ Treatment

The NTAPP jet device was manufactured at the Plasma Bioscience Research Center at Kwangwoon University in Seoul, Korea. The gases used were either compressed air (AP) or nitrogen (NP), delivered at a flow rate of 1 L/min (maximal output voltage = 2.24 kV, current = 1.08 mA), and the distance between the plasma pen and the specimen was set to 3 mm. The NTAPPJ treatment was applied for 2 minutes in the AP and NP groups.

2.3. Surface Characterization

The surface roughness before and after the NTAPPJ treatment was analyzed with the use of an optical microscope (ContourGT) (Bruker, Billerica, MA, USA). Using the vertical scanning interferometry mode and a green light source, roughness parameters were quantified at a magnification of 10×, with a scanning area of 63 mm × 47 mm. The surface wettability before and after the NTAPPJ treatment was measured through contact angle analysis. After one drop (4 μL) of distilled water was applied, the surface of the specimen was examined after 10 s with the use of a video contact angle measurement system (Phoenix 300, Surface Electro Optics, Gyeonggi-do, Korea). The chemical composition of the specimen surface was analyzed by means of X-ray photoelectron spectrometer (XPS) (K-Alpha, Thermo Scientific VG Systems, East grinstead, UK).

Photoelectrons were generated by a monochromatic Al Kα line (1486.6 eV) The X-ray sources and beam were powered with 12 kV and 3 mA, with a beam diameter of 400 µm.

2.4. Evaluation of Antibacterial Activity

Candida albicans cultures (*C. albicans*; ATCC 796) were acquired from the Korean Collection for Type Cultures and were cultured using brain–heart infusion agar at 37°C. The microorganism-containing solution was diluted to a concentration of 1×10^5 colony-forming units (CFUs)/mL with selective media for *C. albicans*. To test for antibiotic efficacy, 100 µL of bacterial solution was pipetted onto each specimen. The specimen were then treated with NTAPPT, as described earlier. After each plasma treatment, the specimens were transferred to a 24-well plate, and 1 mL of culture medium was inserted in each well. The specimens were incubated for 24 h at 37°C. After 24 h, the specimens were rinsed using an ultrasonic cleaner (Ultrasonic Cleaner SH-2100, Saehan, Seoul, Korea) for 3 minutes, and 100 µL of each rinse was placed on a solid agar plate. Plates were incubated for 48 h at 37°C to determine the number of viable organisms *C. albicans* (expressed in CFUs).

2.5. Statistical Analysis

SPSS and PASW statistical software version 19.0 (IBM, Armonk, NY, USA) was used for analyses. To determine statistically significant differences between groups, the data were subjected to a one-way analysis of variance (ANOVA) and Tukey's honest significance difference test. P-values of <0.05 were considered statistically significant.

3. Results

3.1. Surface Characteristics

Table 1 shows the surface roughness values before and after treatment with NTAPPJ. The mean surface roughness values determined on 3D optical microscopy were 0.31 µm for the control group, 0.24 µm for the AP group, and 0.23 µm for the NP group. However, there were no significant differences in these values among the three groups (p=0.117).

Table 1. Surface Roughness (Ra) of Specimens with and without NTAPPJ Treatment

Group	Mean Ra (±SD) (µm)	p-value
Control (n=5)	0.31(±0.04)[a]	0.117
Compressed air (AP) (n=5)	0.24(±0.04)[a]	
Nitrogen gas (NP) (n=5)	0.23(±0.07)[a]	

[a] Values with the same letter showed no significant differences (p>0.05). NTAPPJ = non-thermal atmospheric pressure plasma jet

Table 2 shows the results of the contact angle measurements. The mean (±SD) contact angles for the NTAPPJ-treated surfaces were significantly smaller than those in the untreated control group: 75.83±3.46 degrees for the control specimens, 55.64±2.38 for the AP specimens, and 44.03±4.87 for the NP specimens (p<0.05). When compared with the other two experimental groups, the NP group was the most hydrophilic because of its significantly smaller contact angle ($p<0.05$).

Table 2. Contact Angle of Specimens with and without NTAPPJ Treatment

Group	Mean angle (±SD) (degrees)	p-value
Control	75.83(±3.46)[a]	<0.001
Compressed air (AP)	55.64(±2.38)[b]	
Nitrogen gas (NP)	44.03(±4.87)[c]	

a, b, and c Values with the same letters showed no significant differences ($p>0.05$). NTAPPJ = non-thermal atmospheric pressure plasma jet.

The XPS analysis was used to confirm the chemical changes on the plasma-treated surfaces (Fig. 1). The spectra showed peaks at C 1s and O 1s. XPS analysis showed that the hydrocarbon (C-H) peak (at 284.7 eV) was decreased (Fig. 1a) while the hydroxyl group (O–H) peak (at 532.4 eV) was increased (Fig. 1b) with the NP and AP treatments. The peaks between 288.0 eV and 290.5 eV were identified as being caused by C–O and/or C=O bonds (Fig. 1a). In addition, the O 1s spectra in Figure 1b show the peaks between 532.4 eV and 530.7 eV, corresponding to the hydroxyl group (O–H). The O 1s peaks were significantly shifted to higher binding energies after treatment with NTAPPJ, as compared with the untreated controls.

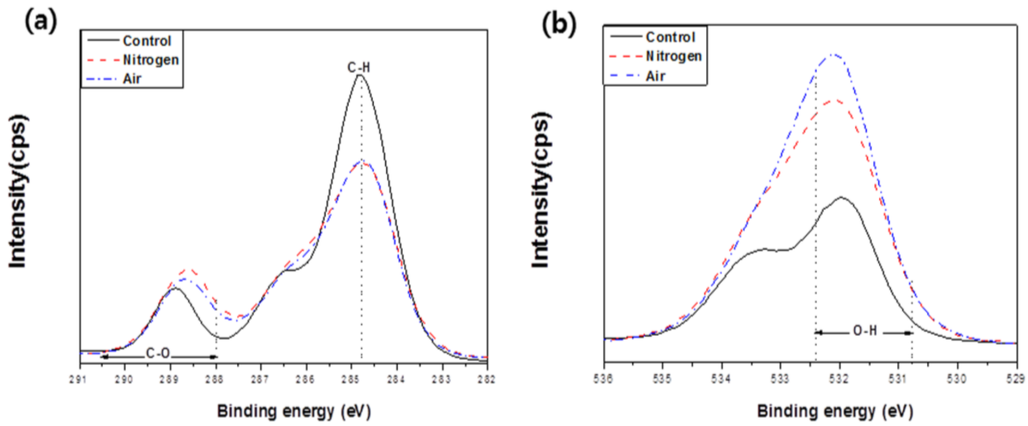

Figure 1. Chemical Composition of Surfaces analyzed with an X-ray Photoelectron Spectrometer. Spectra at C 1 s (a) and O 1s (b)

3.2. Antibacterial Activity

Results of the antibacterial activity evaluation are shown in Figure 2. The CFUs of *C. albicans* decreased in the following order: control>NP>AP. When compared with the control group, the NP group showed significantly fewer *C. albicans* CFUs ($p<0.05$); however, there was no statistically significant difference between the NP and AP groups ($p>0.05$). Figure 3 shows agar plates containing *C. albicans* CFUs.

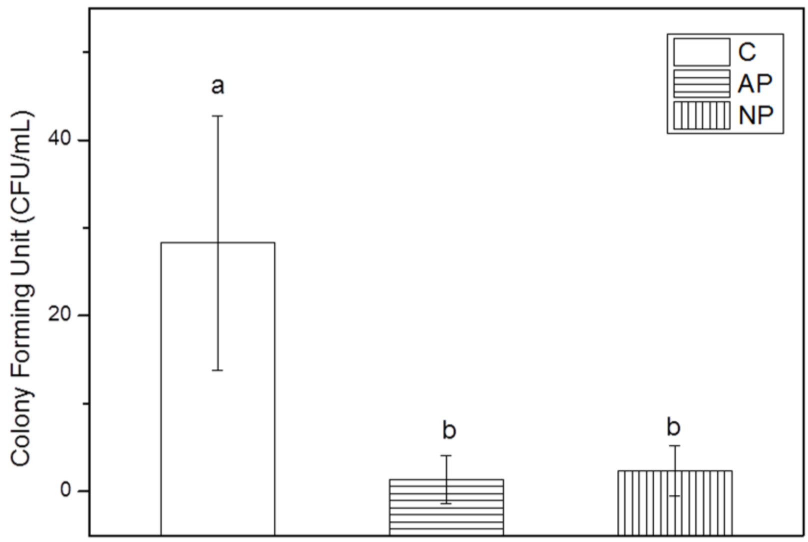

Figure 2. CFUs of *C. albicans* in the antibacterial activity test. AP = compressed gas; NP = nitrogen gas. Bars labeled with the same letters (a and b) showed no significant differences (p>0.05)

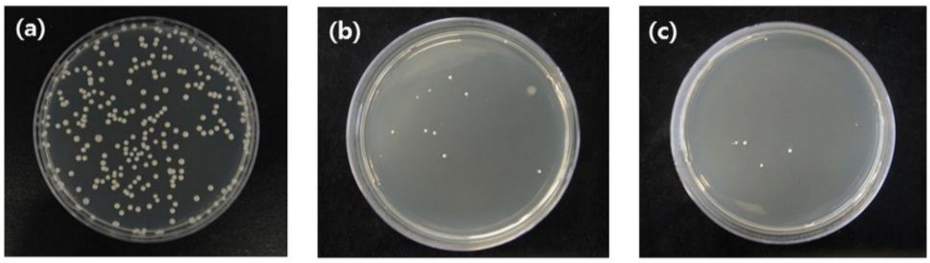

Figure. 3. Agar Plates with *C. albicans* Colonies used to determine the Antibacterial Effects of Extracts from the Control Group (a), the AP Group (b), and the NP Group (c)

4. Discussion

It is important to measure the roughness of the denture surface because this parameter influences the adhesion of microorganisms. The denture may function as a reservoir for infection, and surface irregularities increase the likelihood that bacteria will remain on the surface even after the prosthesis has been cleaned. Thus, denture cleaning techniques must not damage the denture base [15-16]. Here we showed that treatment with NTAPPJ did not increase the surface roughness of the resin specimens.

In addition, the surface in the NP group was the most hydrophilic, probably because the plasma treatments generated free radicals within the materials. Chemical reactions that occur between these free radicals and certain species, such as atomic hydrogen or oxygen, incorporate hydrophilic groups within the polymer surface, thus reducing the contact angle [17]. The hydrophobicity of the resin surface may interfere with denture retention. Our results indicated that the NTAPPJ treatment increases surface wettability, and thus improves retention, as has been demonstrated in many studies of plasma treatment [18-19].

Results of the XPS analysis confirmed the chemical changes on the plasma-treated surface (Fig. 1). The O and OH radicals might improve surface wettability in addition to having high oxidation potential that could have bactericidal effects [17, 20].

NTAPPJ treatment in our study exhibited a good antibacterial effect, with significant decreases in the numbers of CFUs in the AP and NP groups, as compared with the control group. Therefore, plasma is an innovative alternative treatment method and appears to offer some advantages over current sterilization methods. These advantages include the fact the technique effectively inactivates the required microbial load, can be performed at room temperature, and does not involve the use of toxic gases. It has the characteristics of an ideal sterilant, namely, a high degree of efficacy, fast action, penetrability, lack of toxicity, compatibility with different materials, and cost-effectiveness [21].

As mentioned earlier, the antibacterial effect of the NTAPPJ treatment in our study may have been the result of ions present in the plasma or some kind of reactive species generated when plasma reacts with oxygen and nitrogen in the surrounding air [22]. Many studies have demonstrated the antibacterial effects of plasma treatment [13, 19-22]. *C. albicans* is the pathogenic microorganism that causes denture stomatitis. These bacteria are hydrophobic and adhere well to hydrophobic materials [23]. In this study, the plasma-treated surfaces were more hydrophilic than the control surfaces. Thus, plasma treatment could interfere with the adhesion of *C. albicans*.

5. Conclusion

The aim of this study was to investigate the antibacterial effects of NTAPPJ treatment on denture resin base. From the results of surface characteristic, surface roughness values were similar in all experimental groups, and NTAPPJ treatment could improve surface wettability. These results suggested that NTAPPJ treatment could not influence surface roughness that affects the adhesion of biofilm. In addition, NTAPPJ treatment could increase the retention power of denture. From the result of antibacterial activity test, the number of *C. albicans* was decreased after NTAPPJ treatment. Therefore, these results emphasize the potential of NTAPPJ treatment for antimicrobial application in dentistry. In particular, NTAPPJ treatment is a promising method for preventing denture stomatitis or infection. However, further study is necessary for better understanding of how such chemicals are affecting on biofilm attachment and safety of NTAPPJ treatment.

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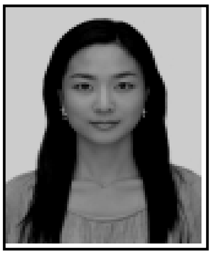

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