

Effect of Non-thermal Atmospheric Pressure Plasma Activated Water Against *Candida albicans* Biofilms on Acrylic Surface

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Abstract

Denture stomatitis, often caused by *C. albicans* biofilm formation on acrylic surfaces, presents a challenge in dental care. This study investigated the optimal conditions for generating non-thermal atmospheric pressure plasma-activated water (PAW) and its antifungal efficacy. Through a series of optimization experiments, the optimal parameters for yielding the highest concentrations of hydrogen peroxide and nitrate were found to be a 500 W of power input and a reduced distilled water volume of 500 mL. In subsequent analysis of PAW's effect on planktonic *C. albicans*, a 12-minutes PAW demonstrated yielded the highest percentage of *C. albicans* inhibition, although it was not significantly different from the inhibition achieved at the 10-minutes PAW. Furthermore, immersing *C. albicans* biofilms on an acrylic surface for 10 min in 10 and 12-minutes PAW resulted in comparable mean *C. albicans* inhibition percentages of $82.0 \pm 1.61\%$ and $82.0 \pm 3.50\%$, respectively. While the anti-biofilm efficacy of PAW was lower than that of 1% sodium hypochlorite (NaOCl), which was $98.6 \pm 1.82\%$ ($p < 0.005$). The 10 and 12-minutes PAW both markedly reduced biofilm viability across all immersion times with no significant differences observed between them at shorter durations (10, 30 min and 1 h). However, after 8 h of immersion, the 12-minutes PAW demonstrated a significant difference in biofilm inhibition from the 10-minutes PAW ($p < 0.05$). PAW is a chemically gentler alternative and indicates a promising, non-corrosive approach for future denture cleaning agents. Subsequent investigations should concentrate on augmenting PAW's anti-biofilm characteristics and examining its viability for clinical implementation in the prevention and management of denture stomatitis.

Keywords: Non-thermal atmospheric pressure plasma, Plasma activated water, *Candida albicans* biofilms on acrylic surface

Introduction

Denture stomatitis presents a significant concern in oral health, with global prevalence rates ranging from 20% to 80% [1]. This inflammatory condition, characterized by erythematous tissue under removable dentures, has a complex aetiology, including inadequate denture fit, poor oral hygiene, an immunocompromised host, and colonization by *Candida albicans*. Current

prevalent management methods for denture stomatitis include adjusting the dentures, patient education on proper oral hygiene, topical antifungal agents, and antimicrobial disinfection of the dentures [2].

Heat-cured acrylic resin, or polymethyl methacrylate (PMMA), is a commonly used denture base material due to its inherent porosity and surface

roughness. Research has shown that dental biofilms accumulate more readily on rough denture surfaces than on smooth ones. A study found that acrylic sheets polished with 600-grit sandpaper, which a surface roughness of approximately 0.65 μm , exhibited the highest *C. albicans* adherence [3]. Sodium hypochlorite is a prevalent disinfectant for dentures immersion due to its broad-spectrum pathogen elimination efficacy. It has demonstrated that a 10-minutes immersion in 1% NaOCl can remove over 90% of biofilm-forming cells [4]. Nevertheless, significant drawbacks such as the corrosion of metallic components in removable partial dentures and potential tissue toxicity necessitate the exploration of alternative antimicrobial treatments. Consequently, research has expanded to include alternative therapeutic modalities for example natural extracts, microwave irradiation, lasers and photodynamic therapy [5-8], and increasingly, non-thermal atmospheric pressure plasma (NTAPP) for microbial decontamination [9-11].

Discovered in 1879, NTAPP is an ionized gas referred to as the fourth state of matter. Unlike the conventional three states of matter (solid, liquid, and gas), plasma is created when a neutral gas is supplied with sufficient energy, such as high thermal energy or intense electromagnetic fields, leading to the ionization of atoms and the liberation of electrons. This produces an ionized gas containing both positive and negative charges [12]. In medicine, plasma can be applied in two ways: Direct exposure, where the plasma plume directly contacts cells and tissues, or indirect exposure. The latter involves generating a liquid medium in front of the plasma plume, resulting in PAW. PAW offers numerous advantages, including flexibility in application, capacity for mass production, and suitability for long-term storage [13]. During PAW production, the plasma plume emits free electrons and energized gas molecules. As these species traverse the atmosphere and interact with water, they induce the formation of various reactive oxygen and nitrogen species (RONS). This includes short-lived, highly reactive species such as hydroxyl radicals ($\cdot\text{OH}$), nitric oxide radicals ($\cdot\text{NO}$), peroxyxynitrite (ONOO^-), peroxyxynitrous acid (ONOOH), nitrous acid (HNO_2), nitric acid (HNO), and ozone (O_3). While many reactive species possess extremely short half-lives, typically ranging from milliseconds to several seconds,

more persistent and stable RONS, referred to long-lived RONS, such as hydrogen peroxide (H_2O_2), nitrate (NO_3^-), and nitrite (NO_2^-), remain in the PAW. The generation and concentration of these RONS are significantly correlated with parameters such as power input, water volume, working gas type, gas flow rate, liquid type, the distance between the plasma plume and the liquid, and activation time. These stable RONS eliminate microorganisms by compromising their cell membranes, altering or obliterating their DNA structure, and disrupting the extracellular matrix within microbial biofilms [14,15].

Numerous studies have demonstrated NTAPP's efficacy against *C. albicans*, with both direct and indirect exposure [16-22]. *C. albicans* yeast in water appeared to be less susceptible to direct plasma, requiring 30 min for complete inactivation [23], whereas PAW could achieve complete inhibition within 1 h when used immediately after production [24]. Currently, research has not concentrated on the effectiveness of PAW against *C. albicans* biofilms specifically formed on acrylic substrates, which serve as the relevant model for its proposed use as a denture cleansing agent. This study hypothesizes that PAW effectively inhibits *C. albicans* biofilm on acrylic surfaces, with the potential to alter existing denture maintenance protocols. The implications extend beyond scientific inquiry to clinical application, potentially offering a novel, efficient, and safer approach to preventing and treating denture stomatitis, thereby improving oral health outcomes for the global denture-wearing population.

Materials and methods

Experimental setup for plasma activated water treatment

NTAPP employed in this schematic diagram was designed by the Faculty of Engineering, Rajamangala University of Technology Lanna is shown in **Figure 1**. The plasma generator consisted of a custom-designed high-voltage (HV) power supply connected to a high voltage electrode in a glass tube, which together generated a plasma discharge directly under the water. The HV power supply was configured with a controlled current of 500 mA to generate an output voltage up to 4 kVp. A stainless-steel disk with a diameter of 50 mm served as the ground electrode and was placed at the

bottom of a 1,000 mL beaker. The vertical spacing between the high-voltage electrode and the water surface was precisely maintained at 5 cm. Ambient air, introduced into the glass tube at a flow rate of 3 liter per minute, served as the working gas for PAW production.

The study followed a multi-phase optimization approach. Initially, PAW was generated from 900 mL of distilled water using power input of 400, 500 and 700 W over various activation times (2, 4, 6, 8 and 10 min) to identify the optimal power setting for RONS production.

The setting that produced the highest RONS concentration was then selected for next phase. Subsequently, the volume of distilled water was varied to 700 and 500 mL, while maintaining the optimal power setting constant. This was done to determine the water volume that resulted in the highest RONS production. Finally, once both the optimal power input and water volume were identified, extended plasma activation time were explored to further enhance RONS concentrations in the PAW.

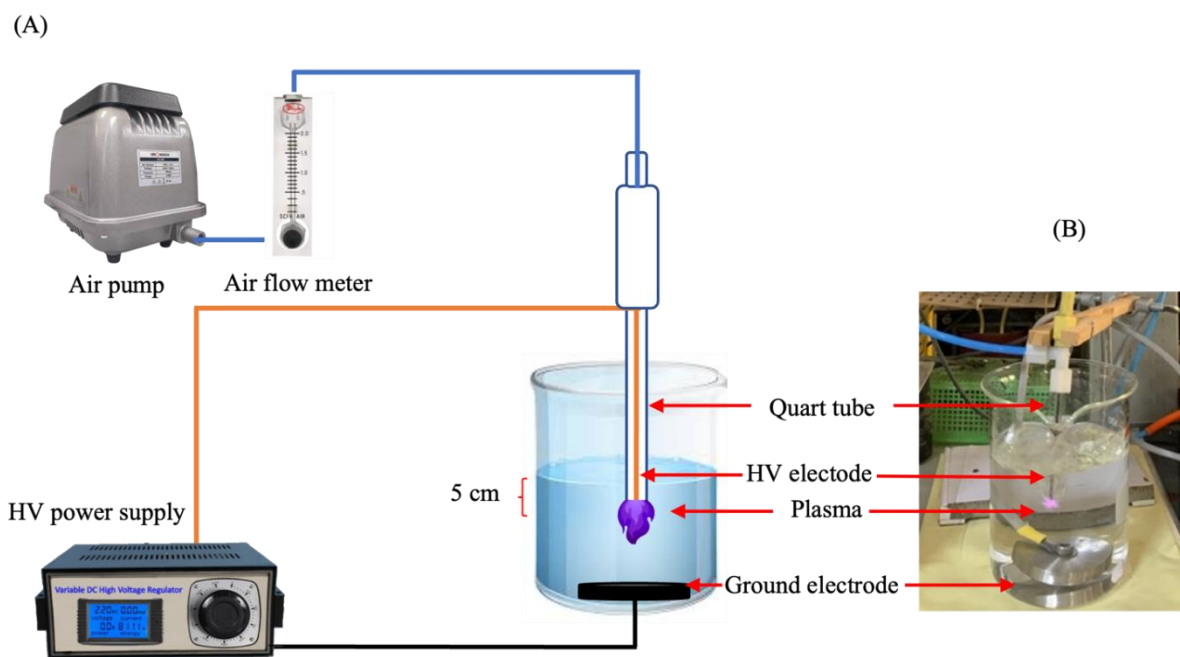


Figure 1 Schematic diagram showing the non-thermal atmospheric pressure plasma configuration (A) and (B) Illustration of PAW generators.

Reactive species concentration measurements

The concentration of long-lived RONS in PAW was determined using analytical methods. H_2O_2 concentrations were quantified using titanium oxysulfate colorimetry [25] (Sigma-Aldrich, Germany), where the reaction of H_2O_2 with titanium oxysulfate forms a yellow peroxotitanium complex. The absorbance of this complex was then measured spectrophotometrically at 407 nm, and concentrations were estimated using a standard calibration curve. NO_3^- concentrations were determined using a calibrated portable nitrate photometer [26] (HI 96786C, Hanna instrument, USA) via cadmium reduction method, followed by a Griess reaction. Meanwhile, NO_2^-

concentrations were assessed using the Griess reagent method [27,28] (Thermo Fisher Scientific, USA) with the resulting azo compound measured spectrophotometrically at 540 nm for concentrations calculation using a standard calibration curve. The pH was measured using a pH meter (Hanna instrument, USA).

Effect of PAW on planktonic *C. albicans*

A reference strain of *C. albicans* ATCC 10231 (National Science and Technology Development Agency, Thailand) was cultured in Sabouraud Dextrose Broth (SDB; Merck, Germany) at 37 °C for 24 h. The PAW was assessed against *C. albicans* using microplate

assay. The culture turbidity was then adjusted to 0.5 McFarland standard (approximately 1×10^6 CFU/mL) using spectrophotometer (sunrise TECAN, Austria) at 540 nm. A 10^{-2} dilution of the *C. albicans* suspension was prepared, and 100 μ L of this diluted suspension was added to each well of 96-well microplates. The experiment included six groups in triplicate: a no-treatment distilled water group (control) and five groups receiving of 200 μ L 2, 4, 6, 8, and 10-minutes PAW, respectively. After incubation at 37 °C for 16 h, cell density was quantified by measuring optical density using spectrophotometer. The reduction of fungal concentration suspension was calculated using the formula [29]:

$$\% \text{ Reduction} = \left(\frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treatment}}}{\text{OD}_{\text{control}}} \right) \times 100 \quad (1)$$

where $\text{OD}_{\text{control}}$ represents the optical density of the a no-treatment distilled water group $\text{OD}_{\text{treatment}}$ represents the optical density of PAW groups.

Antifungal activity against *C. albicans* biofilms on acrylic surface

Cylindrical heat-cured acrylic plates (10 mm diameter \times 2 mm thickness) as shown in **Figure 2** were prepared following the manufacturer's instructions, with residual monomers removed by immersion in water at 37 °C for 48 h. Surface standardization was achieved using a metallographic polisher (MoPao 160E, China) with 600-grit wet sandpaper for 1 min per side. The average surface roughness (Ra) was quantified using a profilometer (Mitutoyo, Japan), and only plates with an average Ra between 0.6 to 0.9 μ m were selected [30]. The plates were then sterilized with ethylene oxide gas [31]. *C. albicans* biofilm formation was initiated on the sterile plates. The sterilized filtered artificial saliva (Faculty of Dentistry, Chiang Mai University) was verified via culture testing. Initially, acrylic plates were exposed to 1 mL artificial saliva per well in 24-well plates at 37 °C for 2 h to facilitate pellicle formation. After washing twice with phosphate-buffered saline (PBS), a standardized *C. albicans* culture was

introduced (1 mL/well) and incubated at 37 °C for 90 min to enable initial cellular adhesion. Non-adherent cells were removed via a twice PBS wash, and specimens were transferred to new 24-well plates containing fresh culture medium. To ensure mature biofilm formation, the culture medium was replaced at 24-hour intervals throughout a 48-hours maturation period, resulting in the establishment of a robust *C. albicans* biofilm.

Following biofilm maturation, specimens were subjected to antifungal treatment. The existing culture medium in each well was removed and replaced with 1 mL of PAW, which was generated using the optimal activation time determined from prior experimental. The specimens were divided into six groups (n = 3 per group); four PAW-treated groups exposed for varying exposure durations (10, 30 min, 1 and 8 h), 10-minutes immersion in 1% sodium hypochlorite group and a no-treatment distilled water (control) in each exposure durations.

After the designated immersion periods, the specimens were processed for colony enumeration to quantify biofilm efficacy. Each acrylic plate was transferred to a microcentrifuge tube containing 1 mL of culture medium, and the biofilms were dislodged via 5-minutes ultrasonic vibration (Biosonic UC125, USA). The resulting suspension was diluted to 10^{-2} concentration, and 100 μ L was plated in triplicate on Sabouraud Dextrose Agar (SDA; Merck, Germany). The plates were incubated at 37 °C for 24 h before the colonies were counted.

The percentage of biofilm inhibition was calculated using the formula:

$$\% \text{ biofilm inhibition} = \left(\frac{\text{Colony count}_{\text{control}} - \text{Colony count}_{\text{experimental}}}{\text{Colony count}_{\text{control}}} \right) \times 100 \quad (2)$$

where $\text{Colony}_{\text{control}}$ represents the colony count from no-treatment distilled water group and $\text{Colony}_{\text{experimental}}$ represents the colony count from PAW-treated groups or sodium hypochlorite-treated group.



Figure 2 Acrylic surface after polishing.

Statistical analysis

The data were shown as mean \pm standard deviation (SD). The statistical analysis of data was performed by GraphPad Prism version 10. Normal distribution of data was tested by Shapiro-Wilk test. One-way and two-way analysis of variance (ANOVA) were used followed by Turkey's multiple comparisons at a confidence level of 0.05, 0.01, and 0.005.

Results and discussion

Reactive species concentration and pH measurements

A plasma generator was used to generate PAW from 900 mL of distilled water, using a constant current of 500 mA, a potential difference of 4 kV_p, and a gas flow rate of 3 liter per. PAW was generated with power

input settings of 400, 500 and 700 W for activation times of 2, 4, 6, 8 and 10 min. The concentrations of hydrogen peroxide, nitrate, and nitrite, along with the pH, were measured for each condition. The findings demonstrated that the RONS concentrations varied significantly with both power input and activation time. Hydrogen peroxide concentrations consistently increased over times as shown in **Figure 3(A)**, while nitrate levels initially rose, then slightly decreased before gradually increasing as shown in **Figure 3(B)**. Nitrite concentrations were initially low and became undetectable thereafter as shown in **Figure 3(C)**. Significant differences in pH were also observed at all power levels when comparing the 2-minutes PAW to the longer activation periods as shown in **Figure 3(D)**.

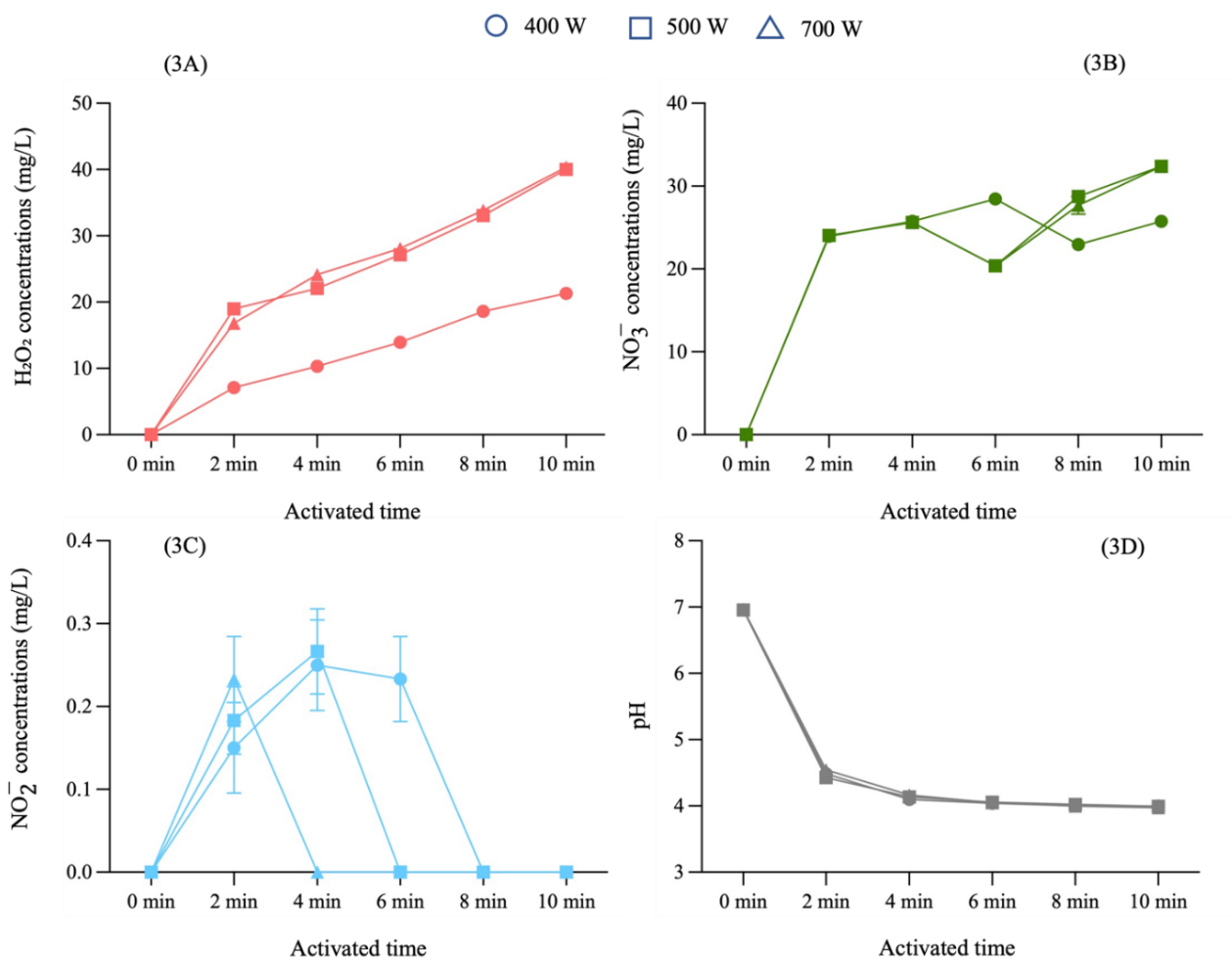


Figure 3 The concentrations of hydrogen peroxide, nitrate, nitrite and pH in PAW, with different power input settings at various activation times.

To optimize RONS production, we selected the 500 W power setting for subsequent experiments, as it yielded significant quantities of hydrogen peroxide and nitrate comparable to the 700 W setting, thus offering greater energy efficiency. We then varied the volume of distilled water to 700 and 500 mL while maintaining the optimized parameters. **Figure 4** shows that the concentrations of hydrogen peroxide, nitrate, nitrite, and pH exhibit similar trends in relation to variations in

power input. This showed that the PAW generated from 500 mL of distilled water with a 500 W power input produced the highest RONS concentrations. To further enhance RONS concentrations, the activation duration was prolonged to 12 min, which resulted in a continued rise in the mean concentrations of hydrogen peroxide and nitrate, while nitrite remained undetectable as shown in **Table 1**.

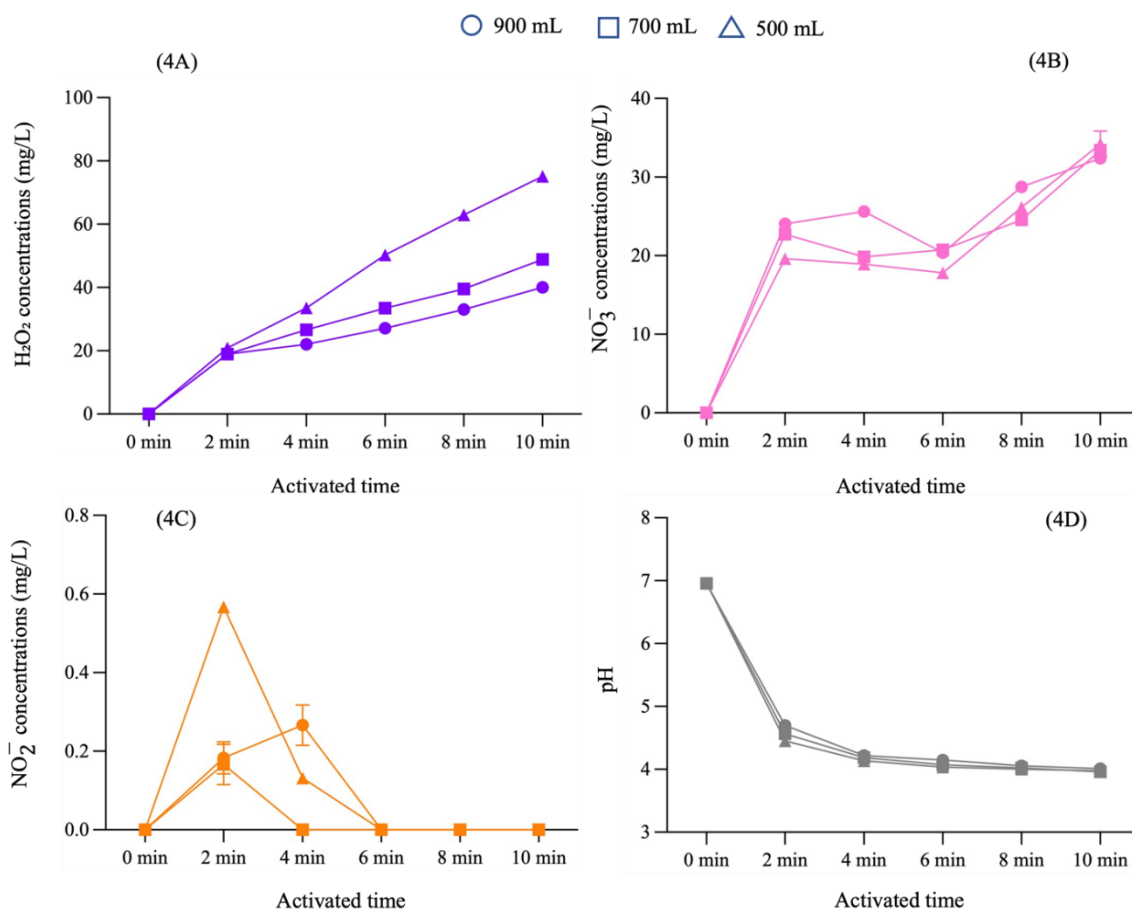


Figure 4 The concentrations of hydrogen peroxide, nitrate, nitrite and pH in PAW, with different volumes of distilled water at various activation times.

Table 1 Reactive species concentration in PAW generated from 500 mL of distilled water with a 500 W power input.

| Activation time (min) | RONS concentrations (mg/L) | | | pH |
|--------------------------|-------------------------------|------------------------------|------------------------------|------------|
| | H ₂ O ₂ | NO ₃ ⁻ | NO ₂ ⁻ | |
| 2 | 20.9 ± 0.25 | 9.6 ± 0.39 | 0.6 ± 0.01 | 4.5 ± 0.15 |
| 4 | 33.6 ± 0.81 | 18.9 ± 0.51 | 0 | 4.1 ± 0.04 |
| 6 | 50.3 ± 0.72 | 17.8 ± 0.35 | 0 | 4.0 ± 0.02 |
| 8 | 62.9 ± 1.02 | 26.2 ± 0.44 | 0 | 4.1 ± 0.04 |
| 10 | 75.1 ± 1.76 | 34.2 ± 1.46 | 0 | 4.0 ± 0.03 |
| 12 | 89.7 ± 1.48 | 38.8 ± 1.73 | 0 | 4.0 ± 0.03 |

Experimental results demonstrated the direct correlation between NTAPP activation time, power input, and the concentrations of hydrogen peroxide and nitrate in PAW. Conversely, a decrease the water volume led to an increase in hydrogen peroxide and nitrate concentrations, which is attributable to a higher concentration of RONS per unit volume. These

findings align with previous studies [32]. RONS primarily originate from the dissociation and excitation of electrons in ambient air molecules, notably hydroxyl radicals ($\cdot\text{OH}$). Upon contact with water, these molecules recombine to form hydrogen peroxide through reactions such as $\cdot\text{OH} + \cdot\text{OH} \rightarrow \text{H}_2\text{O}_2$

and $\cdot\text{OH} + \text{O}^* \rightarrow \text{H}_2\text{O}_2 + \cdot\text{H}$. Furthermore, the ultraviolet (UV) radiation emitted by NTAPP also stimulates increased hydrogen peroxide production. In our study has shown nitrite concentrations peaked at 2 min of activation and subsequently decreased to undetectable levels, indicating a dynamic equilibrium with other nitrogen species, particularly nitrate. This conversion of nitrite to nitrate in PAW is an oxidation process mediated by reactive oxygen species, including hydroxyl radicals ($\cdot\text{OH}$) and atomic oxygen (O), which act as strong oxidizers, converting less stable nitrite into more stable nitrate. As NTAPP activation time increases, the accumulation of these reactive oxygen species enhances this conversion, leading to a decrease in nitrite and an increase in nitrate. This observation corresponds to research that similarly identified this pattern in their investigation on RONS analysis and the fungicidal effects of PAW against *C. albicans* using a dielectric barrier discharge plasma device with air as the working gas [33]. Consequently, in our research, the optimization of PAW production primarily focused on maximizing hydrogen peroxide and nitrate concentrations due to their greater stability compared to nitrite, which showed only a transient increase at 2 - 4 min before becoming undetectable. The plasma activation time in this study was limited to 12 min. Due to the limitations of this investigation, increasing the activation time to 14 min caused uneven plasma plume and burning damage to the high voltage electrode.

Effect of PAW on planktonic *C. albicans*

Initial findings indicated that varying the power input and activation time of PAW had a dose-dependent effect on *C. albicans* concentration reduction. At a power input of 400 W, no reduction was observed of across all tested activation times. The percentage concentration reduction of *C. albicans* by 10-minutes PAW at 500 W was found to be the highest and was not

significantly different from that achieved 8-minutes PAW at 500 W or 10-minutes PAW at 700 W as shown in **Figure 5**. Based on these investigations, the power input was set at 500 W, and the volume of distilled water was varied to 900, 700, and 500 mL. PAW generated from 500 mL of distilled water for a duration of 10 min demonstrated the highest mean percentage concentration reduction of *C. albicans* as shown in **Figure 6**. Following the optimization of power input and water volume, extended plasma activation times were considered to further enhance RONS concentrations. The finding from this final phase showed that a 12-minutes PAW yielded the highest percentage of *C. albicans* concentration reduction, although it was not significantly different from the concentration reduction achieved at the 10-minutes PAW as shown in **Figure 7**.

The investigation assessed the turbidity of microbial cultures to evaluate PAW's efficacy against *C. albicans*. The findings indicated a clear correlation between the PAW activation time and the reduction in *C. albicans*, with concentration reduction effects directly linked to elevated concentrations of RONS. This investigation demonstrates that PAW exhibits significant efficacy against *C. albicans*. The proposed mechanism of concentration reduction suggests that RONS generated by NTAPP disrupt the fungal cell wall, which is primarily composed of chitin, beta-(1,3)-glucan, and beta-(1,6)-glucan. The three substances are interconnected through hydrogen bonds, resulting in a robust and dense chitin polymer network. The reactive species have the capability to disrupt hydrogen bonds by extracting hydrogen atoms from the chitin polymer, subsequently converting them into stable water molecules [34]. In contrast, the pH of the PAW did not serve as a primary inhibitory factor, as this fungus is known to thrive in a wide pH range from below 2 to above 10 [35].

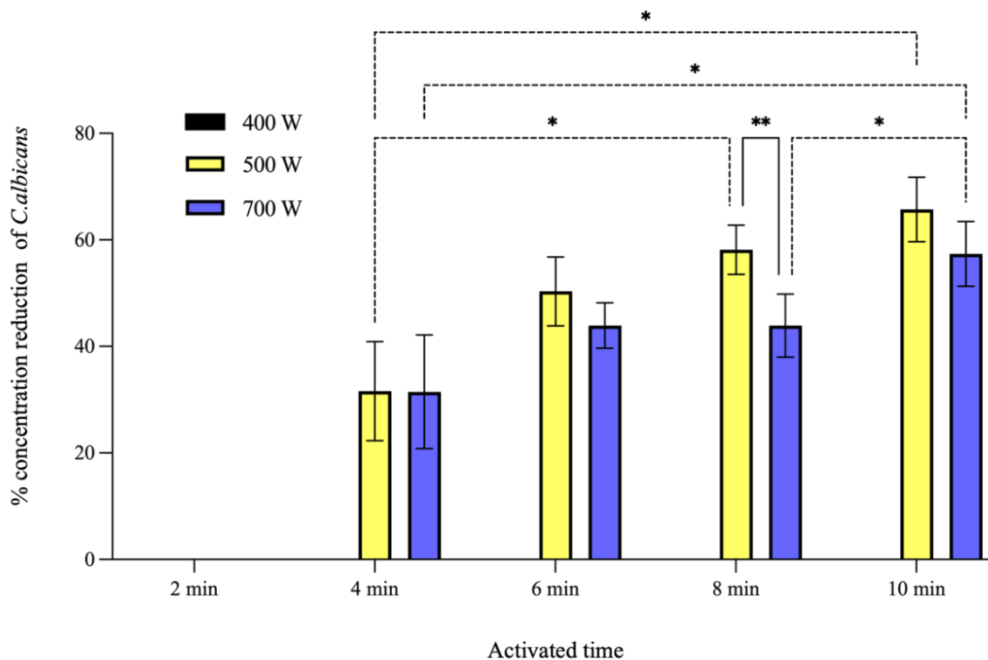


Figure 5 The percentage concentration reduction of *C. albicans* by PAW prepared from 900 mL of distilled water. PAW was generated at various activation times and with different power input settings. Symbols * and **: Indicate that the mean values are statistically significantly different at $p < 0.05$ and $p < 0.01$, respectively.

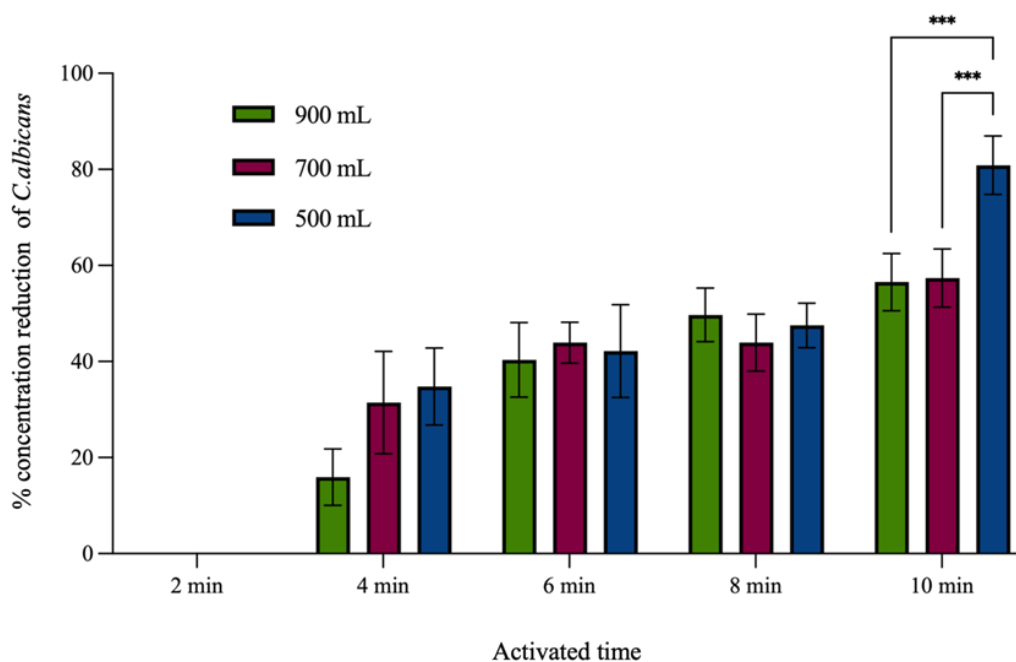


Figure 6 The percentage concentration reduction of *C. albicans* by PAW generated 500 W of power input at various activation times and with different distilled water volumes. Symbols ***: Indicate that the mean values are statistically significantly different at $p < 0.005$.

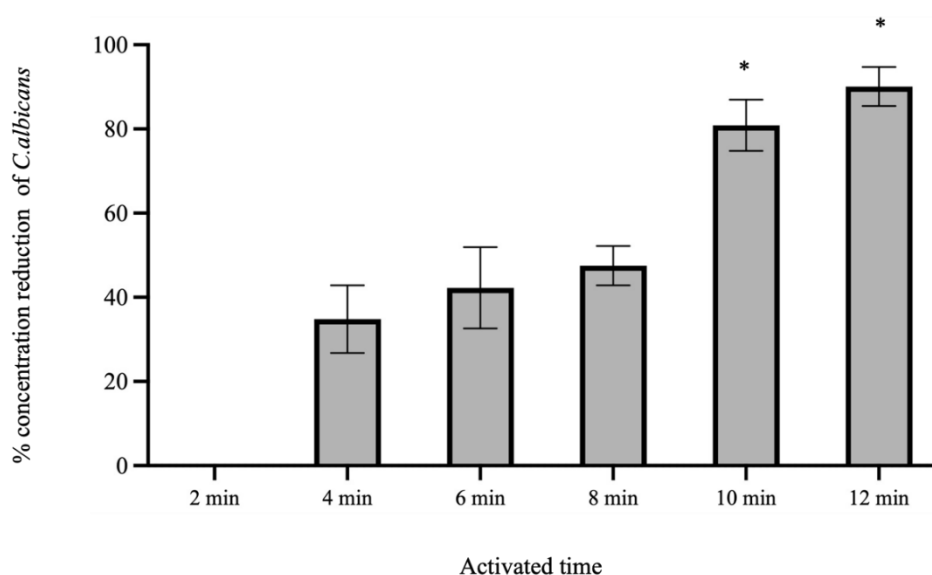


Figure 7 The percentage concentration reduction of *C. albicans* by PAW generated 500 W of power input from 500 mL of distilled water at various activation times. Symbols *: Indicates that the mean value of the group is statistically significantly different from the other groups $p < 0.05$.

Antifungal activity against *C. albicans* biofilms on acrylic surface

This study phase investigated the effect of PAW in eliminating *C. albicans* biofilms on acrylic surfaces. PAW was generated under the following parameters: 500 mL of distilled water, 500 W of power input, 500 mA current, 4 kVp potential difference, and ambient air as the working gas at a flow rate of 3 liter per minute. While 12-minutes PAW yielded higher hydrogen peroxide and nitrate concentrations than 10-minutes PAW, both were selected for this experiment due to their comparable efficacy in the prior effect of PAW on planktonic *C. albicans* test. By quantifying viable cells via colony count as shown in **Table 2** and **Figure 8**, the

findings indicated that PAW significantly diminished biofilm viability compared to the distilled water control. 10 and 12-minutes PAW both markedly reduced biofilm viability across all immersion times with no significant differences observed between them at shorter durations. However, after 8 h of immersion, 12-minutes PAW demonstrated a significant difference in biofilm inhibition from 10-minute PAW. These results imply that PAW possesses antifungal activity against *C. albicans* biofilms. Nevertheless, 10 and 12-minutes PAW was less effective than 1% sodium hypochlorite, a difference that was statistically significant ($p < 0.001$) as shown in **Figure 8**.

Table 2 Colony count of *C. albicans* biofilms at various immersion times.

| Group | Colony count ($\times 10^4$ CFU/mL) at various immersion times | | | |
|---------------------------|--|----------------|----------------|----------------|
| | 10 min | 30 min | 1 h | 8 h |
| distilled water (control) | 74.7 \pm 6.0 | 71.7 \pm 6.9 | 50.7 \pm 5.0 | 50.4 \pm 2.9 |
| 10-minute PAW | 14.1 \pm 1.6 | 14.6 \pm 1.5 | 11.1 \pm 7.4 | 11.3 \pm 2.2 |
| 12-minute PAW | 15.9 \pm 3.2 | 18.0 \pm 3.0 | 12.4 \pm 3.1 | 6.7 \pm 1.5 |
| NaOCl | 1.4 \pm 1.6 | - | - | - |

The experimental design utilized artificial saliva, composed of methylcellulose, glycerine, xylitol, and

distilled water, to develop *C. albicans* biofilms. *Candida albicans* is capable of autonomous biofilm formation.

Artificial saliva is used to increase the medium's viscosity, and this method has been adopted in several studies for the purpose of creating biofilms [36-38]. This substitution improved experimental repeatability and consistency, which is essential for controlled scientific research. However, a key limitation is the biological difference between artificial and human saliva. Human saliva contains a complex mixture of proteins, enzymes, electrolytes, immunoglobulins, and its pH and viscosity can individually varied. These differences could potentially affect the biofilm's physical structure, the initial cellular adhesion of *C. albicans*, and, most importantly, the diffusion of RONS in PAW. Accordingly, while artificial saliva enhances experimental control, this limitation makes it difficult to extrapolate the results directly to the complex human oral environment, necessitating further research in more physiologically relevant models.

The percentage inhibition of *C. albicans* of 10-minute PAW decreased slightly after 8 h of immersion, rendering it less effective than 12-minute PAW. The decreased percentage inhibition of *C. albicans* over the 8-hour period is likely attributable to the higher regrowth of *C. albicans* as well as the lower concentrations of hydrogen peroxide and nitrate in 10-minute PAW compared to 12-minute PAW. This result corresponds with a previous study that examined the effect of direct NTAPP exposure in removing clinical strains and *C. albicans* biofilms (ATCC 10231) from acrylic surfaces. It showed that biofilm levels were significantly reduced by using plasma jet for 8 min [39]. Therefore, variations in treatment duration and efficacy between studies can be attributed to differences in the experimental setup, such as the method of NTAPP application, power input setting, potential difference, working gas, and the mechanism of *C. albicans* biofilm formation.

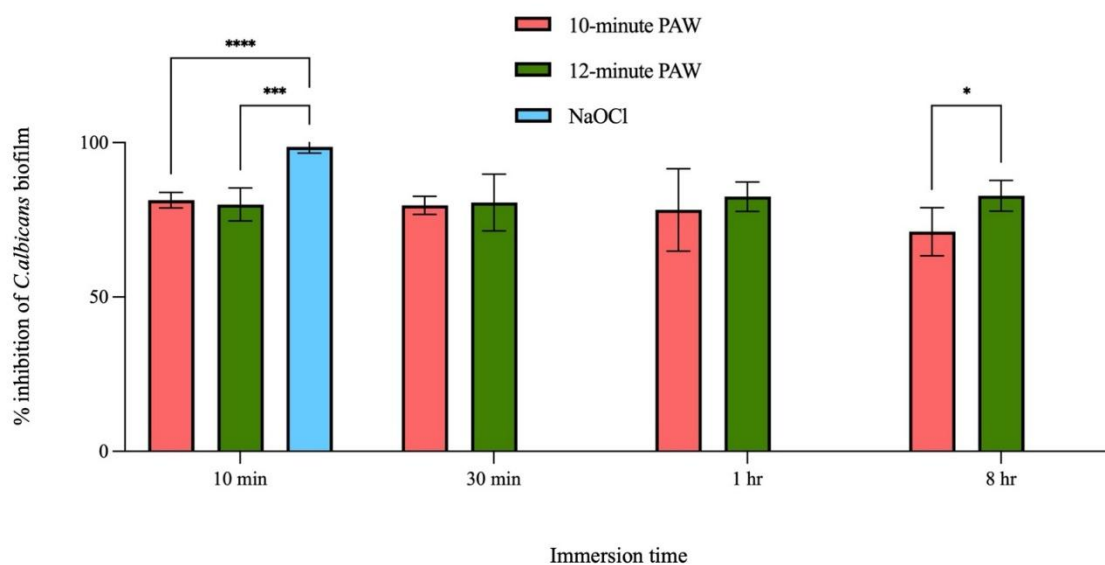


Figure 8 The percentage inhibition of *C. albicans* by 10 and 12-minutes PAW at various immersion times. * significant difference at $p < 0.05$ after comparison with treated groups, *** and **** significant difference at $p < 0.005$ and $p < 0.001$, respectively after comparison with control group.

A 10-minute immersion in 1% NaOCl in eliminating *C. albicans* biofilms compared to 10 and 12-minutes PAW can be attributed to the differing mechanisms of action and some components. NaOCl primarily eradicates pathogens by producing hypochlorous acid (HOCl), which rapidly and

efficiently penetrates the extracellular matrix of biofilms. NaOCl is highly effective at degrading organic materials, thereby deconstructing crucial components of the *C. albicans* biofilm matrix, including polysaccharides, proteins, and DNA. In contrast, PAW functions as an antibacterial by generating RONS [40].

While these RONS can also harm microbial cells and decompose biofilm matrix, total concentration and stability in PAW may be less than the concentration of produced agents in NaOCl.

Complete inhibition of *C. albicans* biofilms is hindered by the distinctive structural features and fluid transport dynamics inherent to biofilms. A previous study demonstrated that bacterial biofilms, including those of *Bacillus subtilis*, contain internal water channels. This principle applies equally to biofilms of *C. albicans*, where the thick fluid transport channels are located within the center of the biofilm and extend to the exterior, facilitating the movement of fluids from the base to the outside. Upon contact with *C. albicans* biofilms, RONS accumulate at the base and subsequently traverse the internal channels to various regions of the biofilm. This disrupts the cell membrane, allowing the cell's contents to escape [41]. This ultimately undermines cellular integrity and may lead to the disassociation of cells from the biofilm matrix. Although PAW can inhibit *C. albicans* biofilms, its efficacy against planktonic cells is comparatively diminished. This is probably attributable to the inherently complex and protective nature of biofilm construction.

Recent research indicates that NaOCl is more effective than PAW in reducing *C. albicans* biofilms. Nonetheless, PAW possesses a significant benefit over NaOCl due to its considerably lower toxicity to host tissues. The use of NaOCl presents a significant issue, particularly in medical and dental environments that must take in to account patient safety and equipment longevity are important. Future research should focus on improving the efficiency of PAW manufacturing to get higher concentrations of more stable RONS, along with devising suitable storage techniques to maintain its activity. Exploring combination therapy that incorporates PAW with additional therapies, such as ultrasonic therapy, physical debridement, or reduced doses of conventional antibacterial drugs, is also advisable. These solutions may effectively eliminate biofilms, potentially beyond the efficacy of current chemical agents, while maintaining the inherent advantages of safety and environmental sustainability.

Conclusions

PAW effectively inhibits *C. albicans* through the generation of RONS. Our findings demonstrate that 12-minute PAW prepared from 500 mL of distilled water with a power of 500 W yielded the highest concentrations of hydrogen peroxide and nitrate. Furthermore, both 10 and 12-minutes PAW demonstrated comparable efficacy in inhibiting *C. albicans* suspension. When applied to *C. albicans* biofilms on acrylic surfaces, both the 10 and 12-minutes PAW also showed similar efficacy after a 10, 30-minutes and 1-hour immersion except 8-hour immersion. While these results are promising, further clinical studies are necessary to fully explore the application of PAW against *C. albicans* on acrylic surfaces for the prevention and treatment of denture stomatitis.

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Declaration of Generative AI in Scientific Writing

The authors used Generative AI (Gemini) and Quillbot to improve the language, readability, and structural flow of the manuscript. The AI was used solely for language enhancement; all data interpretation, literature synthesis, and conclusions were performed by the authors. The authors have meticulously reviewed all AI-assisted sections for technical accuracy.

CRedit Author Statement

Pattarawan Singkharat: Conceptualization, Methodology, Formal analysis, Investigation, and Writing - Original draft preparation. **Chanchai Dechthummarong:** Methodology, Investigation; Resources, and Supervision. **Chintana Itthidecharon:** Conceptualization, Visualization, and Supervision. **Phenphichar Wanachantararak:** Conceptualization, Methodology, Data curation, Investigation, Writing - Reviewing and Editing, Visualization, and Supervision.

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