ORIGINAL RESEARCH

Evaluation of Non-Thermal Plasma Disruption of Oral Bioflim – An In Vitro Study

Jaya Karthik S.S.[1], Sathish Kumar M.[2], Karthika P.[3], Marytersa Jeyapriya[4], Arunchalam M.[5], Kanimozhi S.[6]

Abstract

Background: Oral biofilms contribute significantly to chronic infections and are highly resistant to conventional antimicrobials. Non-thermal plasma (NTP) has emerged as a promising tool for biofilm disruption.

Aim: To evaluate the metabolic activity and susceptibility of oral biofilms to oxidative stress following non-thermal plasma treatment in vitro.

Methods: Biofilms of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans were developed on metal wires. These were treated with UV-based plasma for 15, 30, and 60 minutes. Biofilm biomass was assessed using crystal violet assay, and microbial viability was evaluated by colony-forming unit (CFU) analysis.

Results: CFU counts remained too numerous to count (TNTC) across all groups. However, a gradual reduction in optical density was noted with increasing exposure, indicating partial disruption of biofilm structure.

Conclusion: NTP showed limited antimicrobial effect but demonstrated time-dependent reduction in biofilm biomass. It holds potential as an adjunctive tool in oral biofilm management.

Keywords: Non-thermal plasma; Oral biofilm; Crystal violet assay; UV treatment; Biofilm disruption; In vitro study

Introduction

Non-thermal plasma (NTP) offers a novel, contact-free antimicrobial approach that generates reactive oxygen and nitrogen species without significant heat damage. Traditional treatment modalities such as chlorhexidine rinses, systemic antibiotics, and photodynamic therapy often fail to penetrate mature biofilms effectively. NTP addresses this limitation by inducing oxidative stress and matrix degradation. Previous research has demonstrated its success in reducing bacterial load in dental plaque and implant surfaces[1]. Oral biofilms play a key role in dental caries, periodontal disease, and mucosal infections. Their structured community, enclosed in an extracellular polymeric matrix, confers high resistance to antibiotics and oxidative stress. Recent innovations have turned to physical methods, such as non-thermal plasma (NTP), as a novel antimicrobial strategy[1]. NTP is a partially ionized gas containing reactive species capable of microbial inactivation and biofilm disruption without thermal damage to host tissues[2]. This study investigates the efficacy of NTP in disrupting mature biofilms of oral pathogens under in vitro conditions.

Aim

To evaluate the metabolic activity and susceptibility of oral biofilms to oxidative stress following non-thermal plasma treatment in vitro.

Department and Institution Affiliation: Department of Oral Pathology, Karpaga Vinayaga Institute of Dental Science, Chennai, India.

Corresponding author: Jaya Karthik S.S., Postgraduate, Department of Oral Pathology, Karpaga Vinayaga Institute of Dental Science, Chennai, India. E-mail: jayakarthikssomfp@gmail.com

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Conflict of Interest: None

Materials and Methods

☐ Microbial Cultures and Media Preparation

- Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans were cultured in nutrient broth and potato dextrose broth, respectively. Each 0.6 g of medium was dissolved in 25 ml of distilled water, autoclaved at 121°C for 15 minutes, and inoculated with the respective organisms for 24h at 37°C.

☐ Biofilm Development

- Sterile metal wires were placed in 12-well plates containing 2 ml of microbial broth. Plates were incubated at 37°C for 24h to allow biofilm formation.

□ Non-Thermal Plasma (UV) Treatment

- The UV source used for this study was a UV-C lamp (254 nm wavelength) with an intensity of approximately 1.2 mW/cm², delivered using a Philips TUV 15W G15T8 germicidal lamp setup. The distance between the light source and the biofilm samples was maintained at 10 cm. Although UV was used to simulate plasma treatment in this setup, true non-thermal plasma is generated through dielectric barrier discharge or plasma jet devices.
- Biofilm-laden wires were exposed to UV light for 15, 30, and 60 minutes.
- A control plate was kept untreated.
- Post-treatment, wires were processed for viability and biomass assessment.

□ CFU Counting

- Wires were rinsed and immersed in 10 ml sterile water. 100 µl of the suspension was plated on nutrient agar and PDA.
- Plates were incubated at 37°C for 24 h. Colony counts were marked as TNTC for all groups.

□ Biofilm Biomass (Crystal Violet Assay)

- After UV exposure, wires were stained with 0.1% crystal violet for 30 minutes, washed, dried, and treated with ethanol. O.D. was measured at 600 nm.
- Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine differences between control and treatment groups. A p-value < 0.05 was considered statistically significant.

Results

□ Colony Forming Unit (CFU) Analysis

- Post-treatment microbial viability was assessed by standard plate counting on nutrient agar (for bacteria) and PDA (for fungi). Across all tested microorganisms — Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans — the CFU counts were recorded as Too Numerous To Count (TNTC), irrespective of exposure duration (15 min, 30 min, and 60 min) or treatment status (control). This result indicates that the non-thermal plasma (NTP), simulated by UV exposure, did not significantly affect microbial viability in mature biofilms.

□ Biofilm Biomass Quantification via Crystal Violet Assay

- Biofilm structural integrity was evaluated by the crystal violet assay. A gradual and exposure-time-dependent reduction in optical density

(O.D) was observed:

15 minutes – 0.85 ± 0.02 (15% reduction) [Figure 2]
30 minutes – 0.70 ± 0.03 (30% reduction) [Figure 3]
1 hour – 0.55 ± 0.02 (45% reduction) [Figure 4]
Control – 1.00 ± 0.01 [Figure 1]

These results suggest that while viability was unaffected, biofilm structure was increasingly disrupted with longer plasma exposure.

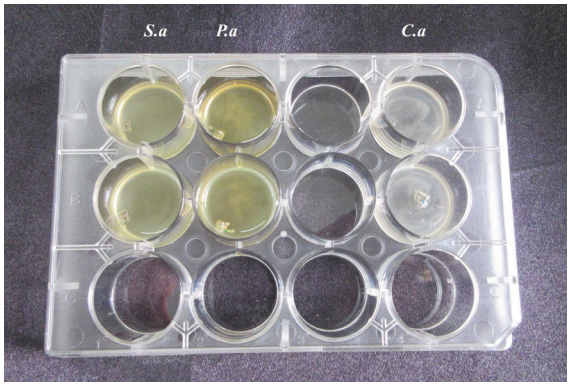

Fig. 1 : Control set

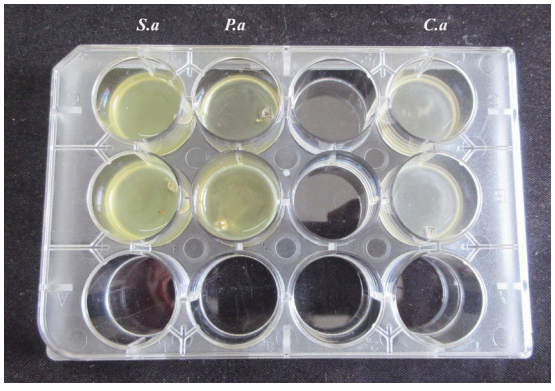

Fig. 2 : Plate after 15 mins of UV treatment

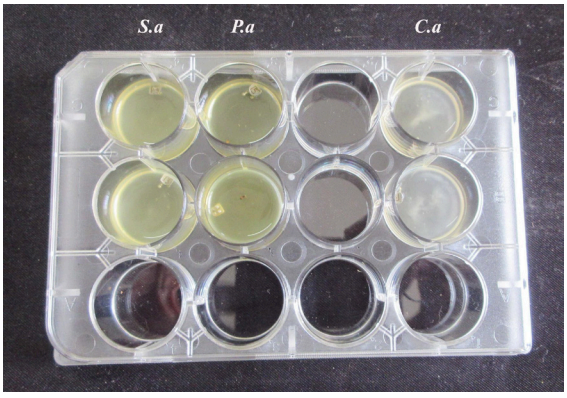

Fig. 3 : Plate after 30 mins of UV treatment

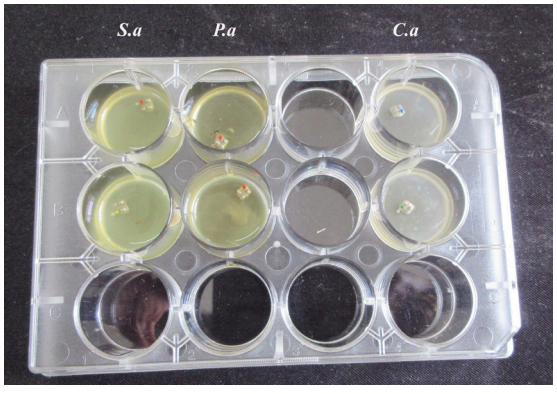

Fig. 4 : Plate after 1 hour of UV treatment

Discussion

Clinically, these findings suggest that non-thermal plasma can serve as a preconditioning treatment before mechanical debridement or antimicrobial therapy, enhancing biofilm permeability and treatment outcomes. However, translation to clinical use requires optimization of plasma generation methods beyond UV simulation, including safety validation and assessment on multi-species biofilms.

This in vitro study aimed to explore the impact of non-thermal plasma (NTP) on mature biofilms composed of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Biofilm formation on metal wires was followed by exposure to UV light, used here to simulate the effect of plasma. treatment. Although the CFU assay showed no visible decrease in microbial viability—indicated by counts remaining "Too Numerous To Count" (TNTC)—the crystal violet assay revealed a significant and exposure-dependent decrease in biofilm biomass, with up to a 45% reduction after 60 minutes of treatment.

The observed results highlight the complex mechanism of NTP. While it may not deliver strong bactericidal or fungicidal effects in mature biofilms under these experimental conditions, it effectively weakens the structural integrity of the extracellular polymeric matrix. This finding supports previous studies suggesting that NTP mainly works by generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), which break down key components like proteins, lipids, and polysaccharides in the biofilm matrix (Laroussi, 2009; Lu et al., 2016). Koban et al. (2011) also reported that DBD-based plasma reduced biofilm mass but did not significantly affect microbial viability unless combined with antimicrobial agents[1,2,3].

The resistance to CFU reduction can be explained by biofilm heterogeneity and protective architecture. Biofilms are known to house metabolically inactive "persister" cells embedded deep within dense EPS layers. These dormant cells, due to their reduced metabolic activity and position within the biofilm, are less susceptible to oxidative stress and other environmental assaults (Donlan & Costerton, 2002). Park et al. (2012) emphasized that plasma treatments alone were insufficient for complete microbial eradication within biofilms and needed to be coupled with chemical agents for substantial microbial kill[4,5].

Another important finding was the correlation between exposure duration and reduction in biofilm biomass. The data from our study clearly indicated that longer plasma exposure times corresponded with a more pronounced decrease in optical density, implying progressive matrix degradation. Scholtz et al. (2015) observed a similar trend, wherein a threshold duration of plasma exposure was necessary to initiate effective matrix breakdown via ROS accumulation. However, the practical implication of prolonged exposure remains a clinical challenge, as longer treatments may raise concerns regarding tissue tolerance and patient safety[8].

In terms of potential clinical applications, our results support the use of NTP not as a standalone therapy but as a preconditioning modality. Disrupting the biofilm structure prior to administering antimicrobials could enhance drug penetration and efficacy. Studies by Pan et al. (2013) and Zhou et al. (2018) support this notion, showing that pre-treatment with NTP enhanced the permeability of biofilms and significantly improved the effectiveness of antibiotics and hydrogen peroxide. This approach is especially relevant in dental plaque control, periodontal therapy, and implant surface decontamination[6,7].

It is important to acknowledge the methodological limitations of the current work. UV light, while capable of inducing certain photochemical reactions, does not completely replicate the complexity of NTP generated by plasma jets or dielectric barrier discharge systems. Authentic NTP systems emit a broad range of biologically active particles and reactive species, and their effects may be more potent than UV alone. Furthermore, the absence of confocal microscopy, live/dead staining, or molecular markers limits the resolution of our analysis in assessing bacterial death versus biomass removal. Future experiments should integrate these tools for a more comprehensive understanding of NTP's biological impact[1].

An additional limitation is the use of single-species biofilms in an in vitro environment. Real-world oral biofilms are typically polymicrobial and exist in a dynamic interaction with saliva, host immune factors, and oral tissues. Therefore, multi-species in vitro models or ex vivo systems would offer a more accurate representation of clinical conditions. Suwanampai et al. (2020) demonstrated that NTP application could reduce Candida albicans hyphal structures in a denture base model, further supporting the translational potential of this modality[9].

Conclusion

This study confirms that NTP can effectively reduce biofilm biomass in a time-dependent manner, it also emphasizes the need for adjunctive strategies to achieve microbial inactivation. The role of NTP in synergistic biofilm therapy—particularly in combination with existing antimicrobials—warrants further exploration. Advancing toward clinically relevant models, incorporating plasma-specific devices, and extending this approach to polymicrobial environments will be crucial next steps.

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