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Synergetic effects of atmospheric pressure plasma and UVA treatment for contact lens disinfection

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Abstract: Atmospheric pressure plasmas have been proved to have huge potential on biomedical field for rich of reactive oxygen and nitrogen species (RONS). This work presents synergetic effects of low-temperature plasma and UVA-LED emitting in antimicrobial actions, showing much better antimicrobial effect in buffered solution with 4 kinds of eye infection pathogens. It was found that material properties of contact lens have no significant change under this treatment.

Keywords: atmospheric pressure plasma, UVA, contact lenses, disinfection

1. Introduction

Wearing a contact lens is risking great challenge of infection by microorganism contaminated lens and lens case. The conventional sterilizing methods by using daily multipurpose solution, UVA cleaning system, enzymatic cleaner, etc., couldn't satisfy all the aspects we concern. Ultraviolet (UV) has been proposed to be an effective alternative to contact lens disinfection techniques. However, for certain kinds of pathogen, like UV-resistant microorganism, its effect may be not good enough. And also, high dose and long-time UV-emitting could change the physical properties of contact lens material [1]. Finding a proper way for contact lens disinfection is always a challenging field people chasing for.

In recent years, plasma medicine has attracted more and more attention all over the world. It was reported that RONS created by atmospheric pressure plasma have strong disinfection effect on microorganisms both on solid surface and liquid solutions [2, 3]. However, in buffered solutions, disinfection effect by RNS has been largely discounted. Pavlovich *et al.* found that short time UVA treatment of plasma activated buffered solution with E. coli would get greater antimicrobial effect than UVA and plasma treated alone [4].

In this work, the synergetic antibacterial effects of 4 kinds of eye infection pathogens are investigated by using atmospheric pressure plasma and UVA-LED emitting. Material properties such as Young's modulus, water contact angle, surface morphology, and UV transparency, after treatment are also measured.

2. Materials and Methods

Fig. 1 shows the configuration of the experimental setup. A surface micro-discharge (SMD) device driven by a neon sign power supply (V_{max} = 2.5 kV, frequency = 25 kHz) is used to create atmospheric pressure plasma. This device is operated in 'indirect mode', and a small vial with 150 μL bacteria suspension or PBS is placed in the bottom center of the chamber. After plasma treatment, the solution is well mixed for 10 s with a cap and immediately sent for UVA exposure. The UVA-emitting LED lamp was situated about 8mm below the bottom of the glass vial during UVA treatment. In this condition, SMD device is working in NOx dominated mode. The active species created in the gas phase is mainly RNS.

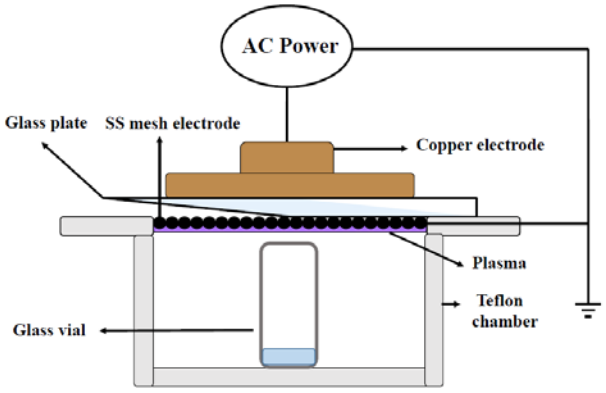

Fig. 1. Schematic of the SMD device.

For material properties testing experiments, 3 mL PBS with 5 mM nitrite and 100 μM H_2O_2, which has the similar antimicrobial effect with the plasma activated water (PAW) in our previous research [4], was used instead of actual plasma treatment for convenience. Contact lens is placed inside a glass vial with the same size of contact lenses case when UVA treated, and the UVA-LED radiation directly pass through the center of the contact lens. 4 different brands of contact lens were tested from 2 min to 6min UVA exposure, and three times repetition. The applied voltage for UVA emitting is approximately 15.87 V. More details about the experimental setup could be found in Ref. [4].

Nitrite concentration in aqueous phase as a function of time was measured by using Griess reagent. Buffered

solution (PBS) was used to avoid strong acidic environment which may cause fatal damage of contact lenses.

4 kinds of eye infection pathogenic bacteria were chosen for test in the experiments: *Pseudomonas putida* ATCC 12633, *Serratia marcescens* ATCC 13880. *Staphylococcus epidermidis* ATCC 35984, and *Staphylococcus warneri* ATCC 27836. Cells were grown in LB medium to OD_{600} 1.0, and diluted with PBS to an initial concentration around 10^6 ~10^7 cfu/mL before treatment. The antimicrobial experiments were divided into 3 groups: 1) UVA exposure only; 2) 5 min plasma treatment only; 3) 5 min plasma treatment followed by 1 or 2 min UVA exposure. After these treatments, the bacteria suspension was diluted in PBS and plated on LB agar. Colonies were counted after 72 h for calculating the log reduction.

For plasma and UVA damage test of contact lens' material, 4 kinds of commercial contact lenses (Acuvue2, Acuvue Oasys, Air Optix, and PureVision2) were used to detect the damage after plasma and UVA-emitting treatment. Young's modulus, water contact angle, surface morphology, and UV transparency properties were measured at CooperVision after treatment.

3. Results

Fig. 2 shows the NO_2^- concentration in PBS vs. plasma treatment time. Around 5 min, the NO_2^- in PBS reaches 1.2 mM, and ~2 mM within 8min. This result is very similar to previous work in our group [5].

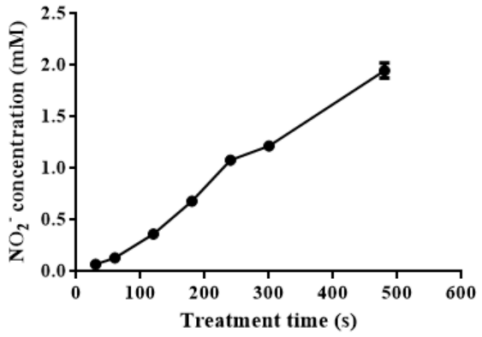

Fig.2. Nitrite concentration in buffered solution as a function of time.

For all the bacteria tested in this experiment, *Serratia marcescens* is one kind of UV-resistant bacteria, and one typical biofilm forming bacteria on contact lenses case. UVA only treatment could get 4-6 log reduction of non-UV resistant bacteria within 6 min exposure. However, for *Serratia marcescens*, it only gets about 2 log reduction for 6 min treatment. And as we know, nitrite is one of the primary species generated by high power plasma treatment of water, but has low antimicrobial effect in buffered solution. 5 min plasma treatment only get less than 1 log reduction for each bacteria strain. In the 5 min plasma plus 1 or 2 min UVA-emitting group, another 2-4 log reduction has been obtained compared to UVA treatment only. Fig. 3 shows the combined antibacterial effect of plasma with UVA-emitting for *Serratia marcescens*. As shown in this figure, short exposure (1~2 min) of UVA-emitting has little effect of *Serratia marcescens*, and 5 min plasma treatment could get ~1 log reduction. When combine plasma treatment with UVA-emitting, the antibacterial effect sharply increased (another 4 log reduction compared to 2 min UVA treatment only).

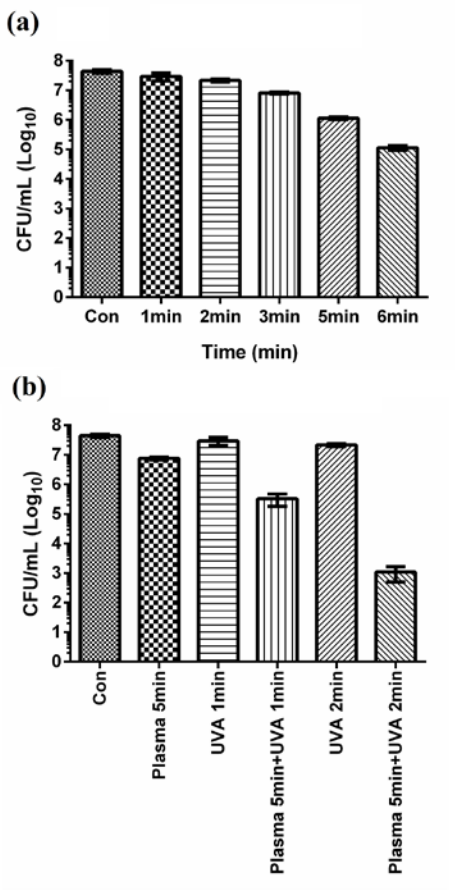

Fig. 3. (a) *Serratia marcescens* inactivation as a function of time by UVA-LED treatment only; (b) Anti- *Serratia marcescens* effect comparison of 5 min. plasma treatment, UVA-LED treatment and 5 min. plasma plus UVA-LED treatment.

The effect of plasma and UVA-LED treatment on material properties is another important factor has to concern. Young's modulus, water contact angle, surface morphology, and UV transparency properties were measured for each kind of contact lenses. Results show that no significant changes have been found after up-to 6min UVA-LED exposure.

The most likely explanation for the remarkable synergetic effects of plasma and UVA treatment is that a strong oxidant and antimicrobial agent peroxynitrite ($ONOO^-$) was formed in plasma treated buffered solution

by following UVA exposure [4].

4. Conclusion

Antibacterial effect of UVA-LED and plasma effect of contact lenses with 4 kinds of contamination bacteria strains were tested, and contact lenses material properties after plasma and UVA treatment were measured as well. It was found that plasma plus UVA treatment has fast and highest antibacterial effect, especially for UV-resistant bacteria. Material properties of contact lenses have no significant change before and after these treatments. This study provides an efficiency and high potential clinic use for contact lenses disinfection.

5. References

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