Optical and Biological Properties of Plasma-Treated *Neurospora crassa* Spores as Studied by Absorption, Circular Dichroism, and Raman Spectroscopy

Geon Joon Lee,*† Gyungsoon Park‡ and Eun Ha Choi

Department of Electrical and Biological Physics /Plasma Bioscience Research Center, Kwangwoon University, Seoul 01897, Korea

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We studied the effect of plasma treatment on the optical, structural and biological properties of *Neurospora crassa* (*N. crassa*) spores. An atmospheric-pressure plasma jet (APPJ) was used to generate reactive oxygen and nitrogen species in aqueous solution. The APPJ treatment of *N. crassa* spores in water significantly reduced the viability of spores. The reduction in the spore viability can be attributed to the reactive species from the plasma itself and those derived from the reaction of plasma radicals with aqueous solution. These structural modifications were contingent on the medium in which *N. crassa* spores were suspended; plasma treatment of *N. crassa* spores in PBS did not significantly affect the viability of spores as compared with *N. crassa* spores in water. Scanning electron microscopy images and circular dichroism spectra indicated that the spore cell wall was damaged by plasma treatment. The optical absorption spectrum of untreated *N. crassa* spores exhibited two resonance absorption bands at approximately $\lambda_1 \approx 260$ nm and $\lambda_2 \approx 472$ nm, originating from deoxyribonucleic acid (DNA) and $\beta$-carotene. The Raman spectrum of untreated *N. crassa* spores exhibited three main peaks at 1519, 1157 and 1006 cm$^{-1}$, attributed to $\beta$-carotene inside the cell wall. The Raman spectra showed that the APPJ treatment of *N. crassa* spores in water caused degradation of $\beta$-carotene, affecting the viability of spores.

Keywords: Atmospheric-pressure plasma jet, Reactive oxygen species, *Neurospora crassa*, $\beta$-carotene, Raman spectroscopy, Circular dichroism spectroscopy

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**I. INTRODUCTION**

Plasma is used for sterilization, inactivation/removal of microorganisms, wound healing, tooth bleaching, cancer treatment, surface modification and plasma polymerization [1–6]. Therefore, there has been much interest in the effects of plasma treatment on biological materials. Among various biomaterials, microbial cells have received considerable attention in multidisciplinary research groups. *Neurospora crassa* (*N. crassa*) is known as a good eukaryotic model and is a biological source for industrially and medically useful enzymes and biomolecules [7,8]. Several research groups have explored the biological, structural, optical, and biomedical properties of *N. crassa*. Yang et al. investigated heat and oxidative stress in *N. crassa* [9]. Park et al. studied the cellular responses of *N. crassa* to non-thermal plasma [10]. Hickey et al. demonstrated live cell imaging in hypode of *N. crassa* [11]. Fabo et al. described the photoinduction of carotenoid biosynthesis in *N. crassa* [12]. Linden et al. explored ultraviolet (UV)-induced mutants in *N. crassa* [13]. Riquelme et al. studied the effects of *ropy-1* mutation on cytoplasmic organization and organelle motility in mature hyphae of *N. crassa* using laser scanning confocal microscopy and transmission electron microscopy [14]. Generally, plasma jet can generate reactive oxygen and nitrogen species (RONS) [1,3,15]. Therefore, plasma treatment can induce structural and biochemical alterations in *N. crassa* [16–18], which could impact the use of fungal spores in industry and medicine.

In this research, we studied the effects of plasma treatment on the optical, structural, and biological properties of *N. crassa* spores. An atmospheric pressure plasma jet (APPJ) was used to treat *N. crassa* spores in aqueous solution. The biological properties of the plasma-treated *N. crassa* spores were explored by measuring the viability of the APPJ-treated and -untreated *N. crassa* spores. The optical and structural properties of plasma-treated *N. crassa* spores were measured by UV-visible absorption, circular dichroism (CD), and Raman spectroscopy. The absorption and Raman spectra of all-trans $\beta$-carotene were also measured to determine the nature of the cellular response of *N. crassa* spores to plasma.

*E-mail: gjlee@kw.ac.kr
†These authors equally contributed to this work
The reactive species from the plasma itself and those derived in aqueous solution under the influence of plasma radicals were investigated by various spectroscopic methods.

II. EXPERIMENTAL METHODS

1. Plasma device and plasma treatment conditions.

The effect of plasma treatment on *N. crassa* spores was investigated using the APPJ. The plasma device used in this study is a non-thermal plasma jet operating at atmospheric pressure and consists of a dielectric glass tube with a medical needle and copper tape attached to the bottom of the microtiter plate. The needle-shaped powered electrode is made of a stainless steel tube whose inner diameter is 0.8 mm and thickness is 0.2 mm. The needle electrode is inside the cylindrical glass tube with an outer diameter of 5.0 mm, and the needle tip is 7 mm upward from the end of glass tube. The grounded copper electrode is located 20 mm away from the end of the glass tube tip, which is attached to the rear bottom surface of the microtiter plate containing the bio-solutions. The distance between the powered electrode and water surface is set to 12 mm. The two electrodes of the plasma device have been connected to a sinusoidal power supply, a commercial transformer of neon light, and the driving frequency of this power supply is approximately 22 kHz.

To study the effect of plasma treatment on the *N. crassa* spores, *N. crassa* (wild type strain: ORS-SL6a) spores were harvested from a two-week-old culture flask and suspended in water or phosphate-buffered saline (PBS) at the concentration of 2.0 × 10^7 spores per milliliter. One milliliter of spore suspension (2.0 × 10^7 spores) was placed in each well of the 48-well culture plates (SPL Lifescience, Korea). The sample was treated by the APPJ with a power of about 9 W. The Ar plasma jet was applied to each well for 3 min. The Ar flow rate was approximately 150 sccm (standard cubic centimeters per minute). After plasma treatment, the *N. crassa* spores were washed with water and then used for further analysis. In the spectroscopic measurement of *N. crassa* spores, the water or PBS solution of *N. crassa* spores was used for measuring UV-visible absorption, CD, and Raman spectra. To find the reactive species of the plasma, the optical emission spectra of the APPJ were measured using a fiber optic spectrometer (Ocean Optics, HR4000CG-UV-NIR).

III. RESULTS AND DISCUSSION

1. Viability and morphology of the plasma-treated *N. crassa* spores

Figure 1 shows the effect of plasma treatment on the viability of *N. crassa* spores at a concentration of 2.0 × 10^7 spores per milliliter. The viability of *N. crassa* spores was assessed by counting the number of germinated (viable) spores. The number of germinated spores dramatically decreased after plasma treatment in water with treatment time-dependent manner, whereas no reduction in germinated spore number was observed.