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Plasma Biosciences Research Center for Next Generation Green and Life Science and Technology

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The Plasma Bioscience Research Center (PBRC) has been based on the plasma sciences, chemistry, electronics and biomedicines, which include creative research elements and purposes. Basic researches on the characteristic of the bioplasma sources applicable to the living cell, especially to the human body will be investigated, along with the fundamental researches of mutual interactions between the bioplasma and organic-inorganic, liquid materials. Also the general introduction for PBRC will be briefly introduced for mutual networkings and cooperations for human health care of cancer therapy and bacteria killings. We have investigated the nonthermal bioplasma sources and their interactions with microbial, fungi, yeast and living cells. Herein, we have investigated the basic interactions of nonthermal dielectric-barrier discharge plasma with the *Escherichia coli* in morphologicl and biomolecular aspects under lethal dose. This work will contribute to the understanding of the exact biological pathways of plasma interaction with living organisms.

1. Introduction

Over the past several years, many plasma jet and dielectric barrier discharge (DBD) devices that produce a cold atmospheric-pressure plasma plume have been investigated for their use for thermally sensitive materials and medical applications. We have used the micro-discharges in the porous alumina, from which a plasma jet has been ejected from the outer electrode through a 1 mm hole, showing that the temperature of the jet decreases to a value close to the room temperature. This device is capable of generating a soft plasma whose plasma potential is almost zero and plume length is in several centimeters. It exhibits low power requirements (0.5~5W) as shown by its current voltage characteristics. Soft plasmas offer attractive opportunities since non-thermal plasmas can supply chemically active species without heating and/or damaging the materials to be processed. This non-thermal atmospheric pressure soft plasma can generate the various kinds of radicals which can be important roles for the plasma interactions with microbial living cells. It is therefore, important to investigate the influence of plasma jet and DBD plasma on the *Saccaromyces Cerevisie* (yeast), *Escherichia Coli* and *T98G* human brain cancer cell lines. Also the energy band structure of these biological cells from results of secondary electron emission coefficient (γ) by gamma focused-ion beam (γ-FIB) system. Here the work-function ($\varphi(w)$), energy band width χ, center energy of the valence band $\varepsilon 0$ and any other important information of these biological cells are also accordingly analyzed.

2. Experimentals

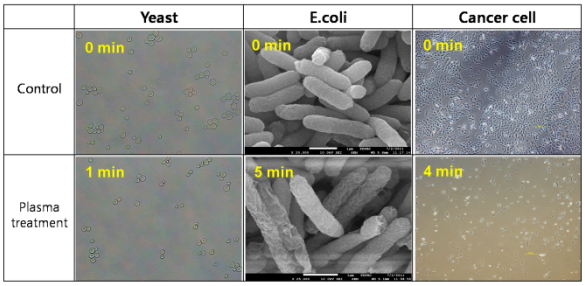
In this experiment, we have prepared three kinds of samples yeast, *E.coli* and cancer cell. We have exposed plasma to each samples. For the yeast, it has been exposed to atmospheric Ar soft plasma jet with 4 kV, 13 mA, and 22 kHz in frequency by 1 min. Yeast cells seeded in 200μl of water have been placed in each well of microtiter plate. And the atmospheric N2 soft plasma jet has been exposed to the E.coli for 5min. The discharge gap for the soft plasma is adjusted to 2 mm between the inner and outer electrode. Gas is injected into the injection needle and is then ejected through the 1 mm hole in the outer electrode via the porous alumina, whose pore diameter is 100 μm. Also the dielectric barrier discharge (DBD) plasma has been exposed to the cancer cell by 4 min in this experiment. This DBD plasma treatment system consists of a high-voltage power supply, electrodes, and dielectrics. The upper electrode is made of ITO paste electrode and lower electrode facing the sample is made of stainless steel mesh. The output power was about 17 W (2.2 kV & 11 mA with phase angle of 0.7 radians).

For observing the change in energy band structure of the biological materials, secondary electron emission coefficient (γ) has been measured by gamma focused-ion beam (γ-FIB) system[1,2]. Here the work-function ($\varphi(w)$), energy band width χ, center energy of the valence band $\varepsilon 0$ and any other important information of biological materials are also accordingly analyzed by using the slow ions of He^+[2] with energy less than 200eV. The energy band structure fe(α) function in valence

band can be measured from the electron energy distribution of the secondary electrons throughout the Fast Fourier Transform (FFT) and inverse-Fast Fourier Transform.

3. Results and Discussions

Plasma exposure to yeast has caused dramatic changes in cell morphology. After treatment with plasma for 1 min., majority of yeast cells appears to be shrunken as in Fig. 1(a). The smooth surfaces for control cells (no plasma treated) of *E. Coli* has been changed to rough and crushed surfaces after plasma treatment as in Fig. 1(b). Also it has been noted



(a) (b) (c)

Fig. 1. SEM images of each sample for yeast (a), E. Coli (b) , and cancer cells (c); (a) and (b) are treated by Ar and N2 soft plasma jet, respectively, while (c) is by DBD plasma.

that cancer cell shrinkage and cell killing by DBD plasma treatment have been observed as shown in Fig. 2(c), which are also reported by other many groups [3].

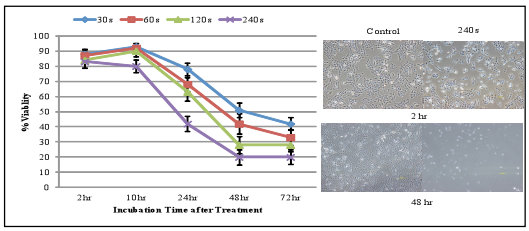


Fig. 2. Growth kinetics of T98G cells at 2 and 48 hours after treatment by DBD plasma.

Figure 2 shows the growth kinetics of T98G cells. Cells proliferation kinetics have been studied at 24, 48, 72 hours after plasma treatment, following trypsinization and counting total cells per plate by using a trypan blue dye and hemocytometer. This growth kinetics assay showed that our plasma have inhibitory effect on the growth of T98G cells in exposure and incubation time dependent manners. Maximum effect is shown by 240 seconds plasma exposure; it inhibits the growth of cells up to 80% at 48 and 72 hours after treatment and its viability range is 19-20%.

We have also measured the electron energy band structure for each biological material in valence band by γ-FIB. We can measure work-function (φ(w)), center energy of the valence band (ε0) and energy band structure function fe(x) in each samples by taking the inverse-Fast Fourier Transform (IFFT) to Auger transform function T(Ek), which is identical to the secondary electron's distribution function. For the *E.coli*, it is found that the work-function (φ(w)) is is (7.79 eV, 7.49 eV) for the (before, after) the plasma treatment, respectively.

Since the yeast, *E. Coli,* and cancer cells are immersed in water during plasma treatment in this experiment, it is likely that plasma has altered the pH properties of water media and this eventually affects the cell surface morphology, which results in the electron energy distribution in the valence band of these surface structures[4].

4. Conclusion

The electron energy band structure for the microbial and living cells has been changed by plasma treatment, in which both the work function and central energy of the valence band are accordingly decreased toward the their surface.

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