Intracellular or Extracellular: a Way to Plasma Protection

Ruonan Ma[1], Hongqing Feng[2], Fangting Li[1, 3], Yongdong Liang[2], Qian Zhang[1], Weidong Zhu[4], Jue Zhang[1, 2], Kurt H. Becker[5] and Jing Fang[1, 2].

[1] *Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, 100871, China*
[2] *College of Engineering, Peking University, Beijing, 100871, China*
[3] *College of Physics, Peking University, Beijing, 100871, China*
[4] *Dept. of Applied Science and Technology, Saint Peter's College, New Jersey, 07306, USA*
[5] *Dept. of Applied Physics, Polytechnic Institute of New York University, New York, 11201, USA*
E-mail: zhangjue@pku.edu.cn

With the development of plasma medicine, atmospheric pressure cold plasmas have shown promising results in various biomedical applications. However, safety concerns need to be addressed when a plasma is applied directly to living cells or tissue. The reactive oxygen species (ROS) produced by plasmas are considered to be the key constituents that induce biological effects, but they can also induce oxidative stress and consequently cell death, if the dosage is not properly controlled. As a result, anti-oxidative defenses must be taken to protect the nearby vulnerable tissue in plasma treatments.

In this study, both intracellular (genetic engineering) and extracellular (scavengers) measures were tested in an effort to evaluate anti-oxidative protection for cells against atmospheric pressure cold plasma treatment. As we know, oxidative stress pathway plays a very important role in the resistance to plasma processing for the eukaryotic cells. Hereby, for intracellular protection, we constructed two overexpression mutant strains through recombinant plasmid (pACT2-SOD1 and pACT2-SOD2) transforming the wild *Saccharomyces cerevisiae* strain. Superoxide dismutase (SOD) catalyzes dismutation of superoxide anion (O2 -·) to less harmful hydrogen peroxide (H2O2), which is then decomposed by Catalase into H2O and O2. SOD in concert with Catalase form the first and most important line of antioxidant defense. A series of scavengers: SOD, L-Histidine and D-Mannitol with different concentration gradient were employed as extracellular protection.

The intracellular protection, wild-type and extracellular protection strains are respectively exposed to a direct current, atmospheric pressure, cold air plasma mircojet. The intracellular ROS is measured with dichlorodihydrofluorescein diacetate (DCDHF diacetate) through fluorescence microscopy. Relative survival rate of plasma treated strains was performed using XTT assays. To evaluate the protection effects in the long term, full growth curves of each strain were obtained by tracking the cell growth after plasma treatment up to 28 hours. Reactive oxygen species (ROS), such as hydroxyl (·OH), singlet oxygen (1O2) and O2 -· were detected by end-on optical emission spectroscopy (OES). A promising precautionary measure in future clinical applications of plasmas will be provided in this work.