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Reactive oxygen species influence on plasma-treated HL-60 cells

Jaroslav Kristof[1], Mahedi Hasan[2], Md Jahangir Alam[1], Abubakar Hamza Sadiq[2], Farhana Begum[1], Kazuo Shimizu[1,2]

[1] Graduate School of Medical Photonics, Shizuoka University, Hamamatsu, Japan,

[2] Graduate School of Science and Technology, Shizuoka University, Hamamatsu, Japan.

e-mail: Kristof.jaroslav@shizuoka.ac.jp

Certain diseases, such as cancer, continue to pose significant risks to the population. Treatments can often be harmful, and physicians must carefully weigh the benefits and risks. Chemotherapeutic drugs, while effective against cancer cells, also damage healthy, fast-dividing cells in the body. One promising solution is targeted drug delivery, which aims to deliver chemotherapy drugs specifically to cancer cells, minimizing harm to healthy tissue.

Our method involves encapsulating chemotherapeutic drugs within immune cells—specifically, macrophages or neutrophils—which then transport the drugs directly to cancer cells. This approach can significantly reduce the required drug dosage and associated side effects.

In this study, HL-60 cells were used as a model for immune cells. The HL-60 cell line (RCB0041), derived from human promyelocytic leukemia, has the ability to differentiate into granulocytes and macrophages. The cells were seeded in 25 cm² flasks with 10 mL of culture medium and incubated at 37°C in a 5% CO_2 environment using an AS ONE CO_2 incubator (model E-22).

A film-type dielectric barrier discharge (DBD) electrode operating in atmospheric-pressure room air was used for treatment. Figure 1 shows the experimental chamber setup, and Figure 2 illustrates plasma generation at the electrode surface. The electrode comprises a dielectric layer sandwiched between two conductive layers and operates at approximately 4.3 kV (peak voltage). A positive sawtooth waveform with a frequency of 5 kHz was used.

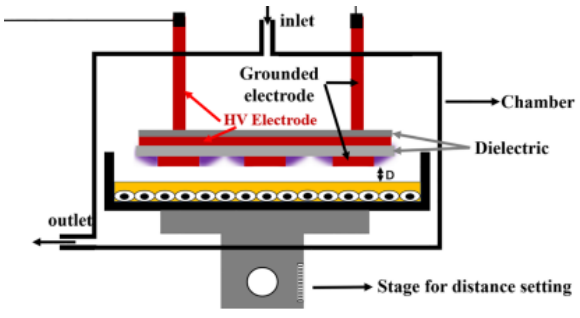

Figure 1: Schematic of cell treatment using the microplasma dielectric barrier discharge (DBD) electrode within the experimental chamber.

As a model drug, we employed the fluorescent dye DiYO-1, which has an excitation peak at 491 nm and emission at 509 nm. Cell viability was assessed using Calcein 450 AM (excitation at 405 nm, emission at 450 nm). The effects of treatment were evaluated using flow cytometry.

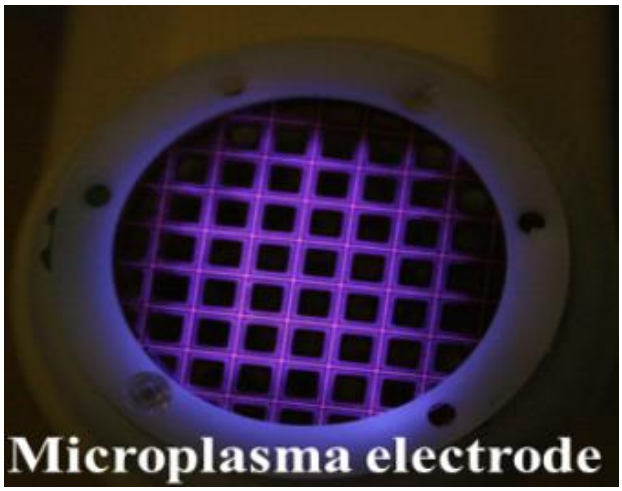

Figure 2: Plasma generation on the surface of the microplasma dielectric barrier discharge (DBD) electrode during operation.

Our results demonstrate successful intracellular delivery of dyes with molecular weights of up to 1270 Da (DiYO-1) and 150 kDa (Fluorescein isothiocyanate–dextran) [1,2], using plasma treatment. Further, we investigated the role of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$) in facilitating this process. The involvement of these species was assessed using specific scavengers applied prior to plasma treatment.

Sodium pyruvate, used to scavenge H_2O_2, resulted in increased cell viability. Dimethyl sulfoxide (DMSO), a scavenger of hydroxyl radicals, led to enhanced uptake of DiYO-1. Conversely, sodium azide, a singlet oxygen scavenger, reduced DiYO-1 uptake. These findings suggest that optimizing the levels and types of reactive oxygen species is critical for improving drug delivery efficiency.

References

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