Atmospheric Oxygen Plasma Trigger to Activation of Macrophage-like Cells

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Abstract: Plasma is used to mimic external factors to macrophage-like cells for making sure whether cell proliferation or activation. In this experiment, trace ROS is introduced to culture media and observe the variation of different treatment time. The results indicate cell got damage in the long irradiated period and lag the rate of cell cycle; in addition, cells survival is still confirmed.

Keywords: Atmospheric plasma, Macrophage-like cell, ROS, Activation.

1. Introduction

Macrophages are one kind of long-lived innate immune cells that engulf foreign substances such as bacteria and invasive pathogens, which is called phagocytosis process. Its main function is as scavenger in the immune system[1]. It also can make cell signals to emit cytokine, Interleukin-1 (IL-1), arising spontaneous inflammation-promoting reaction for recruiting other immune cells, i.e., pass the antigen to other defensive cells to protect our body[2].

In the immunotherapy, macrophage in vitro activation is difficult to clarify due to its mechanism complexity. In the previous studies, reactive oxygen species play a distinct role in biological processes. Using adequate particles to macrophage cells, ROS can enhance intensity of engulfment and cell proliferation; otherwise, it could make macrophage to ignore harmful signals[3]. For example, cancer cells increasing in large scale produce massive ROS to mislead macrophage not to eat them. As is known to all, monocyte is activated by IFN-γ and LBP. In this study, atmospheric plasma is introduced as reactive oxygen species source to treat macrophage cells for stimulating cellular polarization to M1 phenotype[4] and furtherly understand the impact of different kinds of voltage and irradiated time on cell growth curve. In addition, ROS is going to replace biology factors to activate monocyte.

2. Experimental setup

Dielectric barrier discharge as plasma source is used to generate reactivate species. AC power supply with frequency of 10 kHz is used for plasma generation. Applied voltage to the discharge electrode is based on 4.2 kV. Material gas is oxygen with the fixed flow rate at 1 slm. For the plasma device, steel mesh is used as powered electrode, copper foil as grounded outer electrode wounded on aluminium oxide tube that is used as dielectric layer.

The J774.1 macrophage-like cell line was cultured in RPMI-1640 medium containing 10% FBS at 37 °C under constant CO2 (5%) in the incubator. Cell population was adjusted to 0.5 ×10¬5 cells per ml, and 100 µl was placed in each well of 96-well plate. After 24 hours, cells are irradiated with the plasma indirectly through the liquid of culture media. Irradiation distance between the torch edge and cells is fixed at 12 mm, as shown in Fig. 1. Generated ozone concentration is around 90 ppm. The gas temperature of plasma irradiation should be below 37 °C. The survival rate of the macrophage-like cells before and after the plasma irradiation is estimated by cell counting kit-8, and measured in 24, 32, 48 hr. One of indexes of macrophage cell activity is phagocytosis. Therefore, irradiated cells were placed in the microplate overnight and then the latex beads coated with IgG FITC is added to the cells to measure amount of engulfment.

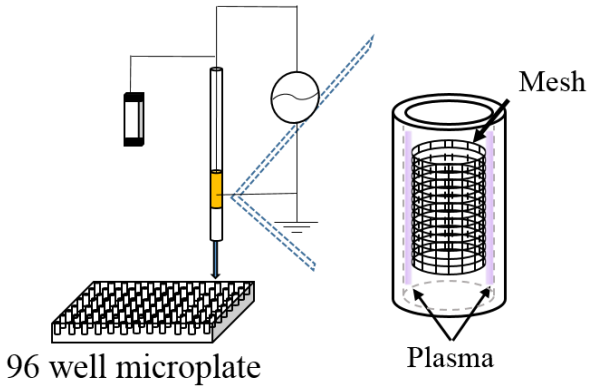


Fig. 1. Schematics diagram of plasma treats on cells.

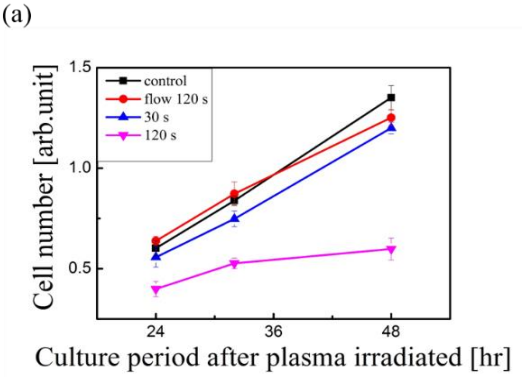
3. Discussion and Results

The number of the macrophage cells decreases and the cell cycle speed becomes slow with the plasma irradiation period, as shown in Fig. 2(a). The growth rates tend to decrease in the case of longer irradiation period from 30 to 120s, as shown in Fig. 2(b). The cells would suffer damages from active species in the plasma. These results suggest that the number of cells can be reduced by the plasma irradiation keeping each macrophage cell activity constant.

In the case of the flow 30s, the growth rate of the macrophage cells decreases with increase of the culture period till 48 h, even though the initial cell population is maximum among cell with different parameters. When the macrophage cells are irradiated with the oxygen plasma for 30 s, the growth rate of cells increases with the culture period. This result suggested that the macrophage cells irradiated with the plasma for 30 s activated by the plasma irradiation.

In order to evaluate the ability of macrophage cells, the phagocytosis characteristic of the cell is observed. Phagocytosis capacity is one of measured of the activity of macrophage. Figure 3 shows changing the plasma

(a)



(b)

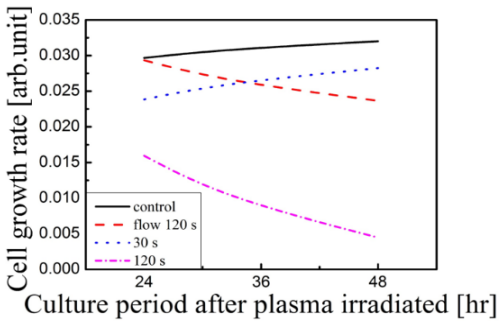


Fig. 2. Survival cell number is affected by plasma irradiation time. (a) treatment time is from 30 to 120 s (b) the cell growth rate of (a).

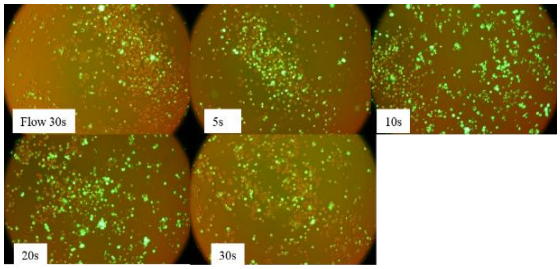


Fig. 3. Fluorescence microscopic image of macrophage fed with fine beads.

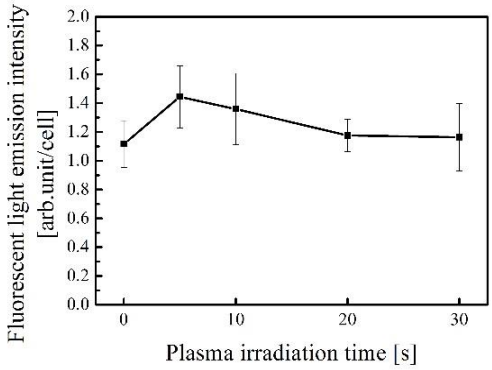


Fig. 4. Phagocytosis of macrophage cell while feeding latex beads.

irradiation period, the light spot on the figures are fluorescent beads engulfed by macrophage. The phagocytosis for one cell becomes maximum at 5 and 10 sec of the plasma irradiated period. In the case of 30s, the plasma irradiation induces the cell damage, and phagocytosis capacity would be decreased. Figure 4 indicates the fluorescent light emission intensities from the engulfed beads per one cell. This figure suggests that the activity of macrophage cell becomes maximum when the cells are irradiated by oxygen plasma for 5 sec. The increase in number of macrophage cells suffering the plasma irradiation is not significant comparing with the gas flow case. Cells are damaged depending on irradiation period, in the shorter area, it seems macrophage stimulated by ROS in the plasma to enhance the engulfment. One of the mechanism for the enhancement is that the combination of the introduced ROS by the plasma and the mitochondrial ROS is proper to the cell activity[5]. On the contrary, the homeostatic balance is destroyed. Despite the applied voltage of plasma is low, accumulating amount of compounds that free radicals are combined with the culture media to form can't be ignored such as hydrogen peroxide and nitric oxide.

4. Conclusion

After plasma irradiated, cells continuous to grow up as a slight extent in 30 s, i.e., cell activity is last; besides, the longer period causes the cell growth rate turns to downward-sloping due to active species. Intriguingly, phagocytosis of macrophage is activated with kind of ROS in the shorter irradiated time.

Due to those free radicals, new chemical substances are created immediately Despite those harmful molecule, the results of cell growth are still could be confirmed that plasma not leads cell death.

5. References

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