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빛의 노출에 대한 MDAMB231 와 MCF-7 세포의 세포 수명 연구

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Cell life study of MDAMB231 and MCF-7 cells under the exposure of illuminator

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ABSTRACT

The basic structural and functional unit of life is the cell. A variety of species are there that extends from unicellular e.g. bacteria to multi-cellular creatures e.g. human beings. If the cells are grown on cell culture dish for the sake of experiments then they need to be stored in CO_2 incubator at 37°C and 5% CO_2 supply to keep them alive for long time. In this paper, a study regarding the life of MDAMB231 and MCF-7 human breast cancer cells is described without keeping it in CO_2 incubator. We kept the cells at normal room environment without CO_2 supply at 18°C under the exposure of illuminator at 18°C room temperature. This study is important before doing transfection experiment on cells so that the cells are not dead due to excessive exposure of illuminator which is exposed to monitor the situation of cells during transfection experiment in optical tweezers setup. We have done experiment with open cover of cell culture dish in which cells are directly exposed to room environment. After experiment, result is that both types of cells are alive for 3 hours while keeping them at above mentioned conditions while opening the cover of cell culture dish.

Key Words: cell viability, microscopy, optical tweezers, gene transfection

1. Introduction

Cell is the basic building block of life in all living things. It only takes one biological cell to create an organism. In fact, there are countless species of single celled organisms, and indeed multi-cellular organisms like us. A single cell is able to keep itself functional by owning a series of 'miniature machines' known as organelles. Major organelles of any type of cells are mitochondrion, cytoplasm, nucleus, endoplasmic reticulum and cell membrane which is also known as plasma membrane. MDAMB231 and MCF-7 are important breast cancer cell lines that were isolated from the cancer patients. Now days a lot of research is being done on MDAM231 and MCF-7 because of large number of breast cancer patients in the world. Researchers are trying to find the most efficient and safe solution to wide spread diseases. Usually it is treated with surgery and then possibly with radiation or chemotherapy, or both. Beside these curing methods, researchers are also trying to cure these cancerous cells by another technique called transfection. Transfection is the process of deliberately introducing nucleic acids into the cell. It includes any means of artificial introduction of foreign DNA into cultured eukaryotic cells. There are many transfection methods like chemical transfection[1], gene gun[2], microinjection[3], atomic force microscopy(AFM) based transfection[4] and laser-assisted transfection. Currently, laser-assisted transfection method is one of the most attractive transfection techniques. The use of lasers provides the distinct benefit such as high transfection efficiency, good post transfection cell viability, simplicity of operation. In almost all laser setups, sample is illuminated by lamp to monitor the condition of the cells

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during experiment.

2. Motivation

It is worth noting that the cells under observation can be damaged by the exposure of laser or even illumination light. MDAMB321 and MCF-7 cells are grown by cell-culturing techniques. These cells are grown in single layer form and are adhered to the bottom of cell culture dish where cover should always be closed to avoid the cells from external contamination. Furthermore, the cells are stored in CO_2 incubator at 37°C and 5% CO_2 supply to keep them breathing. This is the most favorable environment for the cells. However, most laser setups do not have CO_2 supply. Also the temperature of most of the laser setups is kept below 20°C for better operation of laser equipments. Furthermore, another serious consideration while doing experiment of cells is the size of cell culture dish on which cells are grown and adhered at the bottom of the cell culture dish. Usually while doing some experiment on the cells, the cover of cell culture dish is closed to prevent the external contaminations from interacting with the cells. But sometimes, due to laser setup and experimental limitations and requirements, it is required to do experiment without cover of cell culture dish. Like in our laser setup, the cell culture dish is placed in between the objective lens and condenser lens and while doing transfection experiment, cell culture dish is required to move in between objective lens and condenser lens along their axis. Due to this, we need enough space for movement of cell culture dish and height of the cell culture dish is a big hurdle in proper focusing of laser by objective lens.

Based on above discussion, it is now obvious that we have to do experiment on MDAMB231 and MCF-7 breast cancer cells under the exposure of illumination light , without CO_2 supply, at 18~20°C of experimental room temperature and without the cover of cell culture dish. So there is maximum chance of direct interaction of external contaminations with the cells. After transfection experiment, many of the cells are dead and it can be thought the cells are dead due to unfavorable environment for the cells as mentioned above. To rule out this confusion, we have done experiment while keeping it at previously described conditions.

3. Experimentation

3.1 Preparation of MDAMB231 and MCF-7 Cells

For transfection experiment, single layer MDAMB231 and MCF-7 cells were grown on cell culture dish using cell culture techniques. These cells are adhered at the bottom of the cell culture dish. An important point in cell culturing is to shake the cell culture dish very well so that cells are spread uniformly at the bottom of cell culture dish. If cell culture dish is not shaken well, then aggregated cells are produced that are not good for the transfection experiment. Furthermore, after cell culturing, the experiment should be done after 24 hours so that the cells are adhered at the bottom properly.

3.2 Experimental Setup

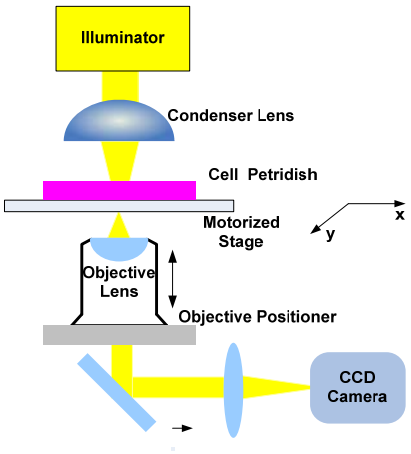


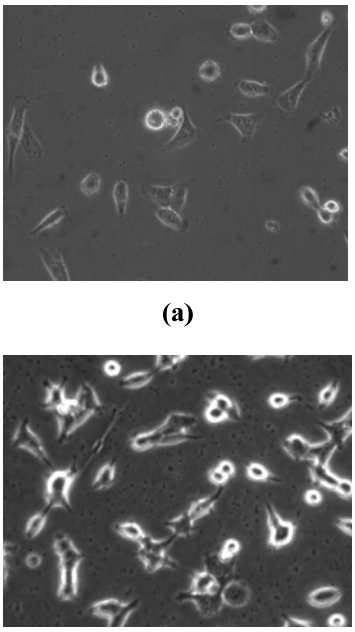
Fig. 1. Experimental setup for both MDAMB231 and MCF-7 cells

Experimental setup for this experiment is shown in Fig. 1. Setup contains a motorized stage that is capable of moving in lateral direction. Objective lens can move in axial direction by objective positioner. CCD camera is there to monitor the situation of the cells during the experiment. After culturing the cells; the cell culture dish is placed at motorized stage under the exposure of illumination light. The illuminator is basically a halogen lamp having 100W max rated output power. The intensity of light should be optimum to get the maximum cell visibility. Initially, the intensity of the illumination is set at a level at which cells can be observed easily. For experiment, many cell culture dishes are prepared and cells are kept under the illumination light from 15~180 minutes step by step. This process is same for both MDAMB231 and MCF-7 cells.

4. Results

After the experiment and checking the cells under microscope it is found that both types of cells are alive and are not affected by the external environmental conditions as it can be seen from Fig. 2. If the cells are dead then they start floating in the cell culture media. In addition, living cells show some brightness under the microscope. If the cells are dead then they lose their brightness, become ugly and we can easily distinguish between living and dead cells by their appearance under the microscope. Overall, MDAMB231 and MCF-7 cells can survive at 18°C without CO_2 supply, while directly exposed to air by removing the cell culture dish cover, and

under the exposure of illumination light.



(a)

(b)

Fig. 2. Microscopic image of (a) MDAMB231 and (b) MCF-7 cells after experiment

5. Conclusion

On the basis of above discussion, it can be concluded that both type of cells can survive in above unfavorable conditions and these conditions will not affect the transfection experiment that will be done as a next step to this experiment.

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