CELL DETACHMENT USING ELECTRIC DISCHARGE PLASMAS

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ABSTRACT

In this work we investigate the effect of a small non-thermal plasma on cultured cells. Plasma is an ionized gas, containing electrons, positive/negative ions, radicals and various excited species. The final goal of the research is to be able to manipulate single cells in a tissue. This will find many applications in fine surgery. In this work we use a novel non-thermal atmospheric plasma source, the “plasma needle”.
We have chosen Chinese hamster ovarian cells (CHO-K1) as a model system to study plasma-cell interactions. When these fibroblasts are exposed to the plasma we observe instantaneous detachment of cells from the surface and loss of cell-cell interaction. Cell viability is tested using propidium iodide (PI) and cell tracker green (CTG) fluorescent staining in combination with a confocal laser scanning microscope (LSM). Cells that are detached during the treatment remain alive and can reattach within several hours. The influence of the plasma is restricted to sub-millimeter areas, the surrounding cells are not affected. Due to this high precision and refinement, plasma treatment may become a novel surgical technique.

INTRODUCTION

Plasma treatment

Plasma can be generated by an electric discharge in gas. A non-thermal plasma contains highly energetic electrons while the ions and neutral species remain at (almost) room temperature. It is a reactive medium that is capable of superficial and refined treatment of various materials. Our "plasma needle" is a discharge generated using rapidly oscillating (radio-frequency) voltage. It appears as a radiating glow at the end of a metal pin [1].
One of the important advantages of non-thermal plasma treatment is that it can be applied to heat-sensitive surfaces. In the biomedical technology plasmas are for example used for coating of artificial implants [2] and sterilization of medical equipment [3]. For direct application of plasma on the human body there are some more requirements. The plasma source needs to be electrically and chemically safe and it may not cause any thermal damage.
Before introducing plasma treatment as a medical technique we must carry out a fundamental study to identify all possible plasma-cell interactions. In this work we use CHO-K1 cells and observe their responses to the treatment. The versatility of fibroblasts makes them a good model system for our study.

Potential plasma effects

Potential plasma effects on living cells include UV emission, reactive radicals, electrical properties and thermal effects. UV irradiation can be harmful to cells [4], however the UV radiation from our plasma is limited to a low dose in the UV-A region. The reactive radicals are capable of unique chemical reactions with cell constituents. Their reactivity is needed to trigger specific cell reactions. Therefore in moderate concentrations radicals can be beneficial.
Threshold values for influence of electric currents and electric fields to living cells has been extensively studied [5]. In our range of use this will not pose any problem.
Temperature measurements of our plasma have demonstrated that no thermal effects will occur during plasma treatment. The temperature during plasma treatment does not rise above 32^0C or two degrees above the ambient.

EXPERIMENTAL

Plasma operation

The discharge type is an atmospheric radio-frequency (RF) glow, shown in figure 1. The frequency used is about 10 MHz. Plasma appears as a faint flow with less than one millimeter diameter at the tip of the powered electrode. The buffer gas that is used is helium, which is inert and non-toxic. The power input into the plasma is 0.1-0.2 W.

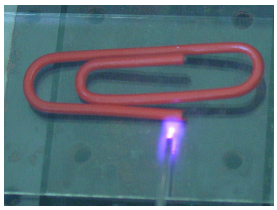

Figure 1. Plasma needle

Cell treatment and visualization

To prepare samples for treatment cells are transferred onto sterilized object glasses (26x10x1 mm) and placed in multi-well dishes. Cells are incubated two or three days prior to treatment. Before treatment cells are covered with a layer of Phosphate Buffered Saline (PBS) to prevent drying out. Typical treatment time of the individual cells is 5 to 10 seconds.

Cells are studied with a light microscope and with a confocal laser scanning microscope (LSM 510 Zeiss). Typically we use dual staining of propidium iodide (PI) and cell tracker green (CTG) to distinguish between living and dead cells.

RESULTS AND DISCUSSION

Treatment with the plasma needle induces cell responses in a restricted area. A typical plasma treated spot is merely 0.2 mm wide, however it is also possible to make marks of 50 μm size.

If plasma powers are used in the range of 0.1 to 0.15 W we observe the appearance of voids where cells are completely detached, and areas with rounded cells as can be seen in figure 2.

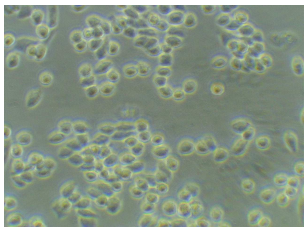

Figure 2. Rounded and partly detached living cells, 15 min. after plasma treatment.

Viability testing (CTG) of the rounded cells proves that these cells are alive. The rounded cells are relatively easy to remove, and can be transferred to another Petri dish. If we observe the rounded cells for several hours after the treatment we see reattachment of these cells to the glass surface and to each other (figure 3).

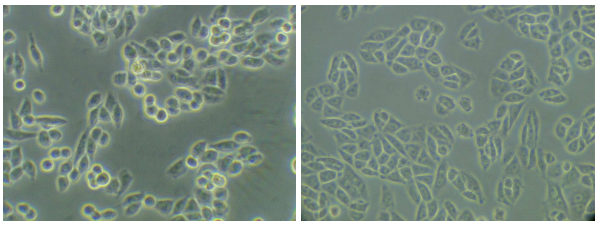

Figure 3. Rounded cells start reattaching after one hour (left) and are reattached after four hours (right).

The mechanism of this interesting process is not completely understood yet, however we expect it to be a mainly (plasma)chemical effect. It is most likely that radicals emitted from the plasma destroy the adhesion molecules of the cells. Under these conditions, the action of the plasma is restricted to the cell exterior, so that the cells themselves remain undamaged.

To achieve cell detachment without necrosis it is important to keep plasma power low and exposure time short. If not applied carefully, plasma treatment may lead to membrane blebbing and necrosis.

CONCLUSIONS

If plasma treatment is applied carefully, cell detachment can be achieved with a high precision in an area as small as 0.1 mm diameter. Detached cells remain viable, they restore contacts with other cells and reattach to the bottom within a few hours. Based on this observation we conclude that plasma action is limited to the exterior of the cell and only adhesion molecules are affected.

The final aim of this research is achieving controlled manipulation of cells in tissue with high precision. The plasma needle may evolve into a novel surgical tool for refined removal or rearrangement of cells. Since cell membranes are not perforated during treatment, no inflammatory reaction in a tissue environment is expected.

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