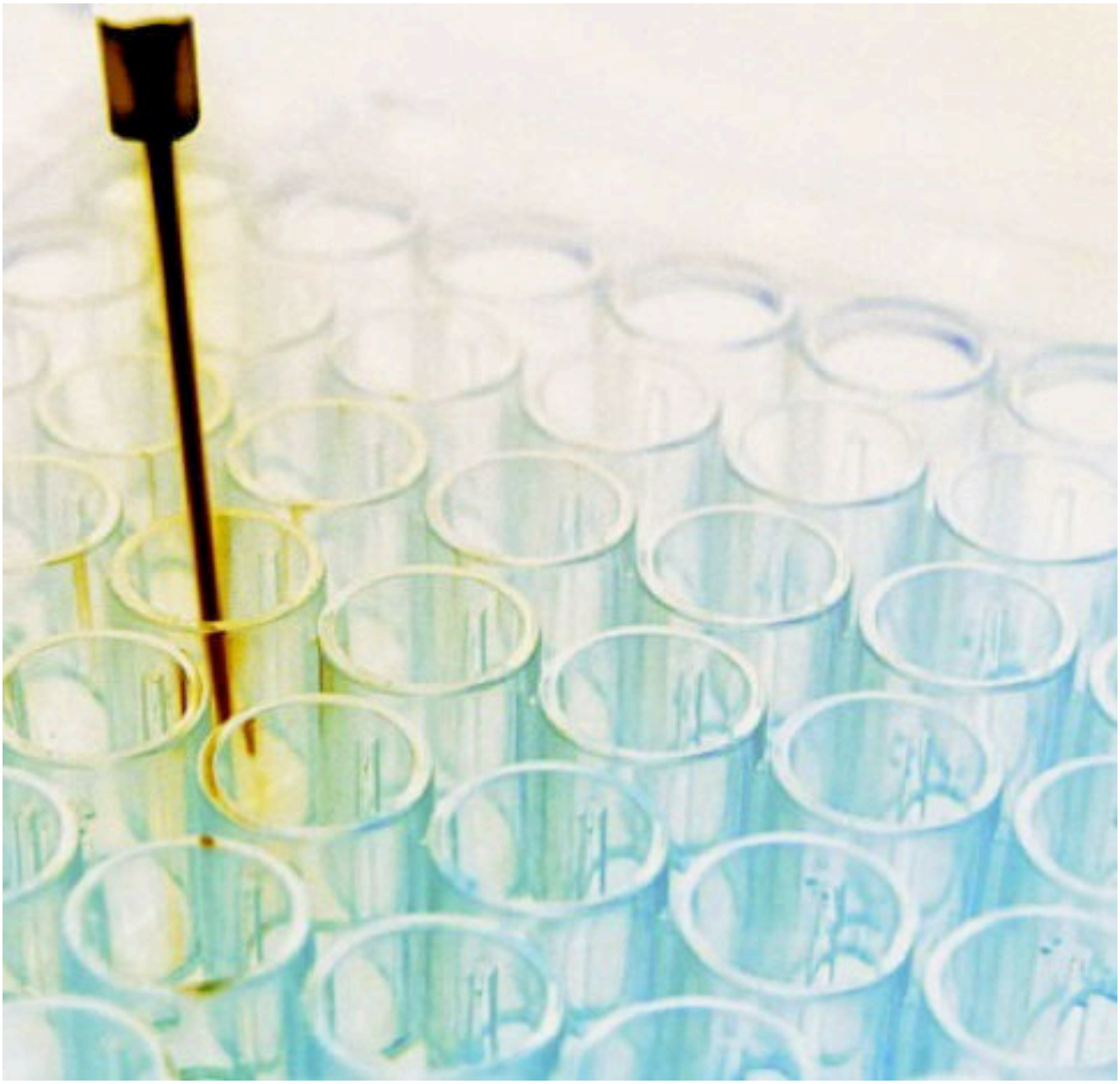

iWPCT

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INDIRECT TREATMENT OF CANCER CELLS BY AIR SPARK AND CORONA DISCHARGE

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Cold atmospheric plasmas are rich sources of reactive oxygen and nitrogen species (RONS) which have potential to selectively target cancer cells without any harmful impact on healthy tissues. The most common cold plasma sources for cell treatments are plasma jets using noble gases (Ar, He) [1]. We use discharges generated in atmospheric air, which reduce the operation costs and produce much larger concentrations of RONS [2]. We present *in vitro* study of the indirect effects of transient spark (TS) and streamer corona (SC) discharges that were shown to be suitable to kill cancer cells with minimum impact on normal cells. Both discharges were generated in non-homogenous electric field with liquid media samples placed under the grounded electrode, so the plasma effect was mostly mediated by neutral RONS (such as H_2O_2, $^{\bullet}OH$, NO_X, O_3). In the first setup, TS discharge was operated between a high voltage hollow needle electrode and the grounded mesh. A gas flow (30% O_2 in N_2, 0.5 L/min) through the needle drove neutral active species toward the samples. The effect of TS was investigated on human cervical cancer HeLa cells and monkey kidney normal Vero cells in 100 µL PBS. The viability of cells was determined after 4 and 24 hours incubation by trypan blue exclusion test. The cytotoxic effect increased with treatment time: after 4 h incubation 25% of both HeLa and Vero cells were dead, but after 24 h incubation Vero cells recovered. The cytotoxic effect was probably induced by activation of apoptotic mechanism: apoptotic HeLa cells turned into dead cells after 24 h while apoptotic Vero cells recovered. In the second setup, positive SC in air was operated between high voltage needle electrode and a grounded wire in a single or multiple corona geometry, with the only gas flow by corona ionic wind. The grounded stainless steel wire was placed above the wells of 96-well plates with 100 µL of medium with cells. The indirect plasma effect was investigated for various treatment times on human malignant melanoma cells A 375 and human embryonic kidney cells HEK 293T and performed with single SC per well or on 8 wells in a row using the multiple SC (Fig. 1). The viability of cells was evaluated by metabolic activity MTT-test. We observed the change in the cell shape after 24, 48 and 72 hours of incubation. The viability of A 375 cells decreased with treatment time to nearly all dead cells after 5 min. The tests of this SC setup on normal cells are still under way. We extrapolate that these cold air plasma sources (TS and SC), similar to commonly used plasma jets operating He or Ar, have a good potential to be applied for *in vivo* cancer treatments.

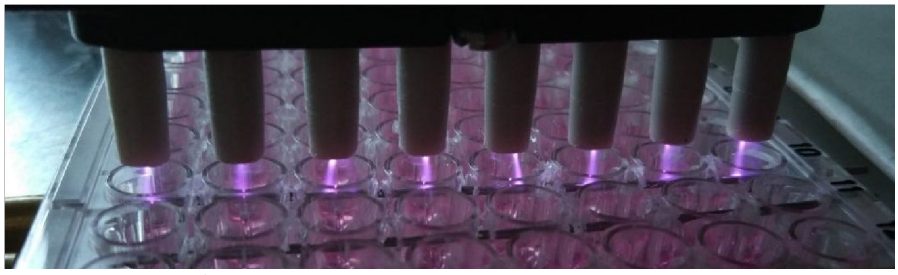

Figure 1 Treatment of cancer cells in 96–well plate in multiple streamer corona setup.

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References

[1] M. Keidar, A. Shashurin, O. Volotskova *et al.*, *Phys. Plasmas*, **20**, 057101 (2013)
[2] K. Hensel, K. Kučerová, B. Tarabová *et al.*, *Biointerphases*, **10**, 029515 (2015)

Indirect treatment of cancer cells by air spark and corona discharge

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INTRODUCTION

Commonly used cold plasma sources for cell treatments are plasma jets using noble gases (Ar, He) [1]. We use DC driven discharges generated in atmospheric air, which reduce the operation costs and produce higher concentrations of RONS [2]. We present *in-vitro* study of the indirect effects of transient spark and streamer corona air discharges. Both discharges were generated in non-homogenous electric field with cells in a liquid media placed under the grounded electrode, so the indirect plasma effects were mostly mediated by RONS (such as H_2O_2, $^{\bullet}OH$, NO_X, O_3).

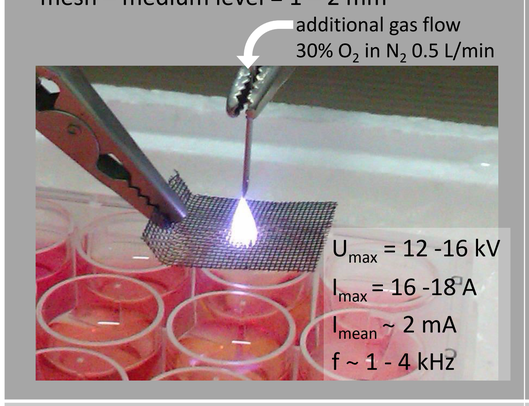
MATERIAL AND METHODS

Two types of discharges – transient spark (TS) and multi-point streamer corona (SC) were used to study the effect of cold plasma generated at atmospheric air on eukaryotic cells. Both discharges were driven by positive polarity DC high voltage and were generated in ambient air. **TS** discharge had an additional gas flow through the HV needle to drive neutral active species toward the sample. In **SC** discharge, this gas flow effect was maintained by the electric wind.

COLD PLASMA DISCHARGES

TRANSIENT SPARK DISCHARGE

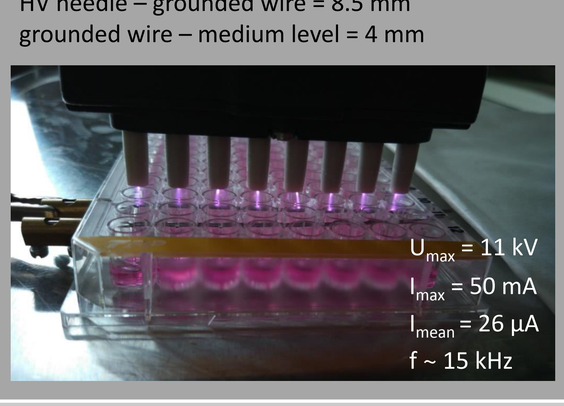
distance:
HV needle – grounded mesh = 10 mm
mesh – medium level = 1 – 2 mm



MULTI-POINT STREAMER CORONA DISCHARGE

(designed for 96-well plate treatment)

distance:
HV needle – grounded wire = 8.5 mm
grounded wire – medium level = 4 mm



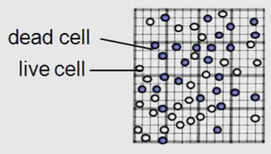
EUKARYOTIC CELLS

Medium: 100 µL **PBS**
HeLa human cervical cancer cells
Vero monkey kidney normal cells

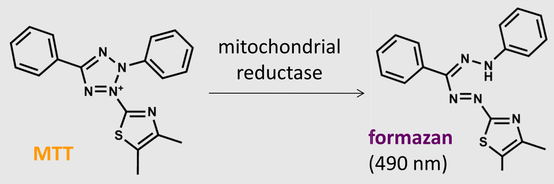
Medium: 100 µL **DMEM**
A 375 human malignant melanoma cells
HEK 293T human embryonic kidney cells

CELL VIABILITY ANALYSIS

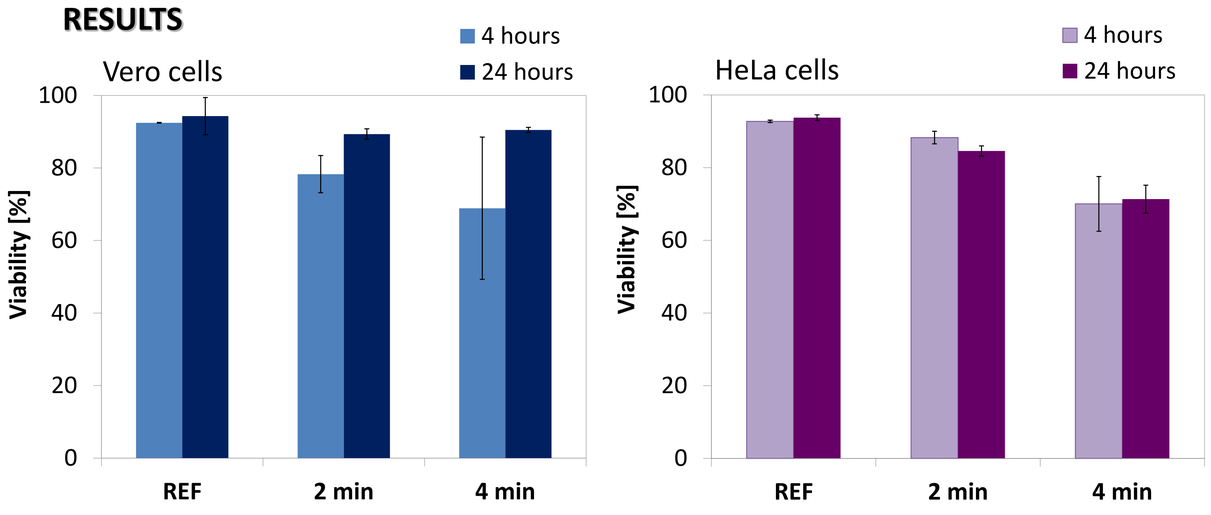
Trypan blue exlusion test



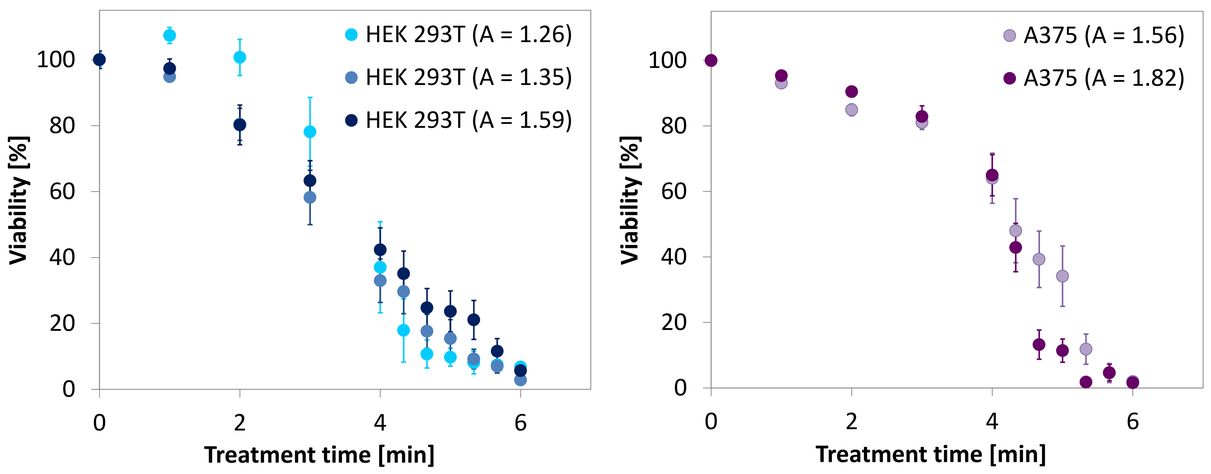
Metabolic activity MTT-test



RESULTS



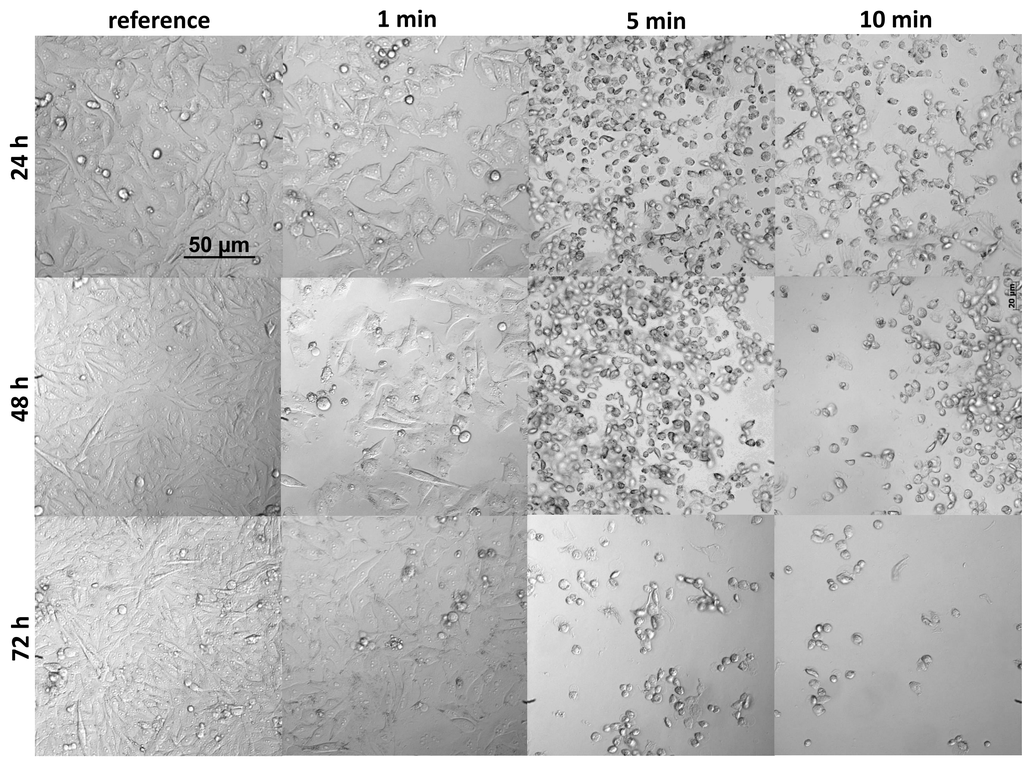
Viability of normal **Vero** and cancer **HeLa** cells after indirect **TS** discharge treatment (2 or 4 min treatment time) after 4 and 24 hours incubation.



Viability of normal **HEK 293T** and cancer **A375** cells in dependence on **SC** discharge treatment time after 24 hours incubation. The "A" value refers to the absorbance of control sample which is proportional to initial metabolic activity of cells.

RESULTS

- Cell viability decreased with increasing treatment time with all tested cell lines.
- The temperature of treated medium after 10 minutes **SC** treatment increased from initial 24˚C to 31˚C, therefore we consider the **SC** effect as non-thermal.
- Cytotoxic effect of **TS** discharge in **HeLa** and **Vero** cells was ~ 25% after 4 min of treatment and 4 h incubation. After 24 h incubation the cytotoxic effect on **HeLa** cells remained, while **Vero** cells recovered.
- Cytotoxic effect of **SC** discharge in **A375** and **HEK 293T** cells increased with treatment time and nearly all cell were destroyed after 6 min treatment (24 h incubation). However, **HEK 293T** cells were slightly stimulated by plasma treatment under 2 min.
- Various cell lines can respond to various plasma sources differently under different conditions. In different cultivation media plasma induces different reactive species.
- Longer incubation time experiments with **A375** and **HEK 293T** cells are currently under way.



Photographs of **A375** cells treated indirectly by **SC** discharge for 1, 5 and 10 minutes after 24, 48 and 72 h incubation (light microscope Axio Vert.A1, phase contrast, 20x magnification).

Confluence (proportion of surface covered by adherent cells) of cell culture decreased from initial 90% with increasing plasma treatment time: cells became rounder and started to detach from surface.

CONCLUSION

Indirect effects of cold atmospheric air plasmas generated by transient spark discharge and multi-point streamer corona discharge on cancerous and normal cells were examined.

Both plasma sources affected the viability of cells. However, different vulnerabilities of studied cell lines were observed. Some selectivity of the plasma treatment on cancer **HeLa** cells was found with respect to normal **Vero** cells.

The tests with **TS** suggests the cold plasma can selectively kill cancerous cells, while normal cells can effectively recover after exposure. The tests with **SC** and analysis of cell metabolic activity showed that the cytotoxic effect depends on cell cycle and in certain conditions plasma may stimulate cell viability.

These results demonstrate that generalized statements about the effects of cold plasma on eukaryotic cells have to be done carefully, depending on various plasma sources used on different cell lines in specific conditions.

Supported by Slovak Research and Development Agency APVV 0134-12.

REFERENCES

[1] M. Keidar, A. Shashurin, O. Volotskova, et al., *Phys. Plasmas* **20**, 057101 (2013)
[2] K. Hensel, K. Kučerová, B. Tarabová et. al, *Biointerphases*, **10**, 029515 (2015)