solution for an hour. Slides were then transferred to an electrophoresis cell, covered with the electrophoresis solution (0.3M NaHO, 0.1 mM Na2EDTA) for 20 minutes to allow DNA unwinding and then a current was applied (20 V, 300 mA, 20 minutes). Slides were stained with 60 μ l of a 20 μg/ml ethidium bromide solution and observed under an epifluorescence microscope. Comets were scored by means of the Comet Assay IV Analyzer (Perceptive Instruments Inc.). The results show that the exposure to plasma produced cytotoxicity and genotoxicity on B16 cells in a dose-dependent manner. It was observed that the effect of non-thermal plasma was higher in cells of melanoma of mouse comparing to the effect observed in the lymphocytes.

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Effect Of Plasma Pen Treatment And Plasma Activated Medium (PAM) On Cancer And Normal Cells

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Non-thermal atmospheric pressure plasma has recently found an ever growing use in medicine; including development of new cancer treatments. The most significant factor, produced by plasma that influence cancer cells are reactive oxygen and nitrogen species (RONS). RONS react with the surrounding air, cellular aqueous media and with cells themselves; however, the exact mechanism of their interaction with the cells is not yet fully understood. Some of the studies suggest that plasma is able to induce apoptosis in cancer cells and has a potential to selectively kill cancer cells without causing a major destruction of normal cells [1]. Plasma can be applied both directly on cell or tissues or indirectly – by plasma-activated medium (PAM). It is a cellular medium, which was treated by plasma and then applied onto the cells, so the cells interact only with RONS produced in PAM [2].

The aim of this study was to test *in vitro* the effect of plasma on cancer cells A375 (human melanoma epithelial cells) and normal cells HEK293T (human embryonic kidney cells). As a medium we used DMEM with 10% FBS. The first part focuses on direct treatment of cells by our design of air corona plasma pen [3,4]. In the second part, we evaluated the effect of PAM on the cells. We used discharges generated in atmospheric air, unlike the majority of plasma jets used for biomedical application that use rare gases (He, Ar). Cell viability was measured using the MTT test.

In the first setup, cells were treated with the corona plasma multipen. The cells were placed in 96-well plate with 100μl of medium and the corona discharge operated in between the 8 pen needles and steel wire above the medium surface. Medium temperature did not exceed 34°C. The cell viability was measured after 24-hour incubation and it was evaluated in dependence on time of plasma treatment. Viability of both types of cells decreased with the time of plasma treatment with no selectivity on cancer cells. After 5 minutes of plasma treatment almost all cells were dead ($>$ 95 %).

In the second setup, the effect of PAM on cells was tested in various experimental setups aiming to find the most effective way of PAM production. We used transient spark or streamer corona discharges with different parameters in combination with electro-spraying of the used medium. The discharges were operated in between the high voltage needle and a grounded mesh. The cells viability was evaluated after 24- and 48-hour incubation. We investigated the cell viability dependence on time of plasma treatment, on the used discharge regime, treatment before and after FBS was added, and on the amount of PAM added to the cells.

To conclude, cold plasma has the potential to be used in cancer treatment, because it can be used on live cells and tissues. The use of direct plasma is more technically demanding and still possible mainly in surface *in vivo* procedures, because endoscopic plasma application into the organism is more difficult to implement. Therefore the use of PAM seems to be perspective, since PAM can be prepared before and its *in vivo* application by injections is relatively easy.

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References

[1] J. Zirnheld, S. Zucker, T. DiSanto et al., *IEEE Transactions on plasma science*, **38**, 948-52 (2010).
[2] H. Tanaka, M. Mizuno, K. Ishikawa et al., *IEEE Transactions on plasma science*, **42**, 3760-3764 (2014).
[3] A. Polakovič, L. Strížencová, M. Kotrbancová et al.: *Cold air plasma source for biomedical applications based on DC corona discharge*, 6th ICPM, p. 300, Bratislava, Slovakia, 4-9 September 2016.
[4] K. Kučerová, A. Polakovič, H. Gbelcová et al.: *Indirect treatment of cancer cells by air spark and corona discharge*, 4th IWPCT, p. 57, Paris, 27-28 March 2017.

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