Apoptosis induced by Nanosecond Dielectric Barrier Discharge Plasma

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Application of non-thermal plasmas on cultured cells [1] and in vivo [2] was shown to be a promissing strategy in cancer treatment. Recently, many groups reported on apoptotic effect of different plasma sources based on direct exposure to Dielectric Barrier Discharge Plasmas (DBD) operated in ambient air and multiple plasma jet devices allowing to deliver plasma in a flow of noble gases. DBD plasma sources have large and scalable active area and together with high Reactive Oxygen Species (ROS) production rate and low operating cost seem to be the most convenient tool for skin and subcutaneous cancer treatment. Nanosecond repetitively pulsed DBDs demonstrate better homogeneity compared to AC driven DBDs [3] and, hence, result in more uniform effect.

A dielectric barrier discharge with a cylindrical electrode covered by glass (very similar to the DBD devices described in [1,2]) is used for treatment of immortalized HMEC cell lines. HMEC cells were incubated following standard procedure during 4 days at 37 C with 5% CO_2. The confluent cell layers were obtained at the bottom of a plastic well (Falcon 24-well). The gap between dielectric and cells was 2mm. The high voltage pulses of 40 ns and 10 kV in amplitude were applied with a frequency of 500 Hz. The treatment time varied between 10s and 120s and resulted in 25-300 J of total dose. The micrographs performed 24 hours after treatment show a number of detached cells, cells in the process of detachment, elongated and bloated cells. The media with detached cells and the cell layer were then collected after trypsinisation. Flow cytometry analysis was performed to distinguish normal, apoptotic and necrotic cells using Annexin V and Propidium iodide labeling. A significant dose effect was demonstrated on the number of apoptotic cells (Figure 1) with almost 100% of apoptotic cells corresponding to 120s of treatment time. The number of necrotic cells was of the order of 1% and was found to be independent of the dose. The experiments with Jurkat cell lines are now in progress.

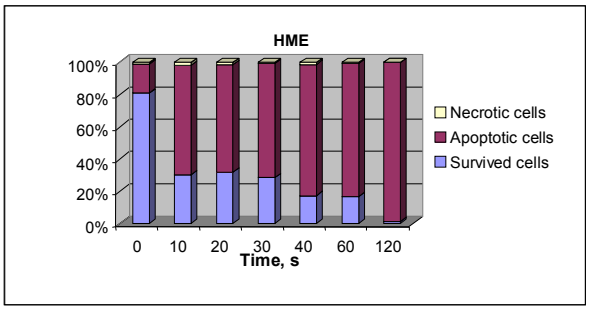

Figure 1: *Apoptotic, necrotic and survived cells 24H after plasma treatment*

References

[1] Sensenig R. et al., Ann Biomed Eng (2010), 39(2), 674-687
[2] Vandamme M. et al., Plasma Process Polym (2010), 7, 264–273
[3] Jiang H., IEEE Trans on Plasma Science (2011), 39(11), 2076-2077