Workshop: Plasmas in Medicine

COLD ATMOSPHERIC PRESSURE PLASMA JET INTERACTIONS WITH PLASMID DNA: FIRST EVIDENCE FOR SINGLE-EVENT DOUBLE STRAND BREAK INDUCTION

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Cold atmospheric pressure plasmas offer a unique environment in plasma medicine, allowing treatment of soft materials, including bio-materials such as living tissues. Single plasma devices can be as small as micro-meters allowing very precise treatments reducing damage to surrounding healthy living cells. Several bio-medical applications have already been identified, examples include bio-compatible implant coatings, skin diseases e.g. psoriasis, blood-coagulation, cancer treatments, tissue removal, and cosmetic treatments. It is essential to correlate direct plasma parameters with effects on bio-materials.

Little is known of the influence plasma has on DNA. While qualitative work is a good indicator [1,2], it is vital to quantitatively determine the nature of this influence before any potential application on living tissue can be realized. The motivation for investigations of DNA damage is two-fold. For applications such as skin treatments and wound healing it is vital that DNA damage is avoided. However, for cancer therapy controlled DNA damage may be desired. In particular formation of double-strand breaks is important as these are difficult for cells to repair. Furthermore, DNA serves as a useful indicator of interactions with bio-molecules in general and the damage caused to it can be readily quantified.

The effect of a cold (< 40^{o}C) radio-frequency driven atmospheric pressure plasma jet on plasmid DNA has been investigated. Plasmid DNA, in different solutions, is exposed to the effluent of an RF atmospheric pressure plasma jet, operated in a helium oxygen gas mixture. The discharge operates in a homogeneous alpha mode, and the electric field direction is perpendicular to the gas flow, confining charged particles inside the plasma core. The different plasmid DNA buffer solutions offer varying degrees of radical scavenging. The effects on DNA are correlated with absolute measurements of ground state atomic oxygen (measured using diagnostic based modelling [3]) and helium metastables (measured using laser diode absorption spectroscopy) in the jet. Gel electrophoresis was used to analyse DNA forms (linear, open circular and supercoiled) post-treatment. The experimental data is fitted to a rate equation model that allows for quantitative determination of the rates of single and double strand break formation. A rate equation model was used to fit the experimental data and determine quantitative rates for formation of single strand breaks (SSBs) and double strand breaks (DSBs). An important feature of this model is the capability to distinguish between genuine DSBs caused by a single event and pairs of SSBs lying close enough to each other to mimic DSBs. Figure 1(a) shows the relative ratio of three different types of DNA in 10 mM PBS, after various exposure times to the plasma jet. The solid lines show the fit to the rate equation model, the goodness of fit shows that the rate equation model is applicable.

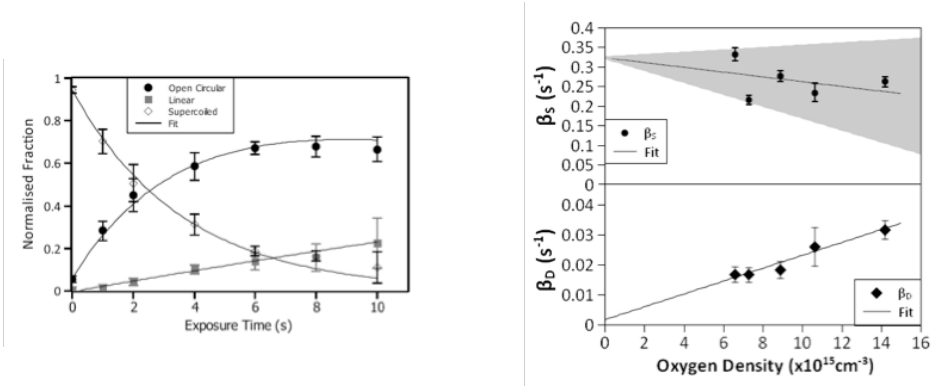

Fig. 1: (a) Fraction of supercoiled, open-circular and linear DNA after plasma exposure to plasmid DNA in 10 mM PBS for varying exposure times. Error bars are the SE of three measurements and account for statistical effects alone. Solid lines show the best fit to the rate equation model. (b) Rate of single and double strand breaks in the plasmid DNA in 10mM PBS as a function of absolute atomic oxygen density. The error bars are the uncertainties in the parameters as derived from the fitting process. The lines are best-fit lines derived from a weighted linear least squares fit to each of the data sets.

While single strand breaks are easily repairable, since the damaged strand can replicate the undamaged, double strand DNA breaks can result in significant changes at the cellular level, including carcinogenesis or apoptosis. Formation of double strand breaks correlates well with the atomic oxygen density. We have been able to exclude UV and in combination with other measurements we have strong indications that neutral components in the jet are effective in inducing single-event double strand breaks.

As is shown in figure 1(b), the best-fit straight line describing β_D has an intercept of 0.0016 ± 0.0034 s^{-1} and a slope of $(2.2 \pm 0.4)10^{-18}$cm^{3}s^{-1}, showing a clear correlation with neutral oxygen density and an intercept which could statistically be zero. The slope of this line then hints to there being a component or components in the plasma which are very effective at producing DSBs and that these components are well correlated with the atomic oxygen density. It is however not fully conclusive to assume that it is the atomic oxygen itself which is responsible for DSB production. The density of other species within the plasma may also correlate with atomic oxygen. In contrast, the rate of SSBs shows no evidence of dependence on atomic oxygen density. The best-fit straight line describing β_S has an intercept of 0.32 ± 0.09 s^{-1} and a slope of $(-6.1 \pm 9.1)10^{-18}$cm^{3}s^{-1}. Since the range of values for the slope span zero (one standard deviation), there is no evidence for SSB formation being correlated with atomic oxygen density. It is very unusual to find a means to form DSBs that does not also form SSBs. Since β_S is typically two orders of magnitude greater than β_D it is not certain this is the case here in spite of the lack of correlation between β_S and atomic oxygen density, since the effect of this species on β_S may be masked by other components which give rise to SSBs.

Reference

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