Atmospheric pressure plasma jet interactions with plasmid DNA

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Résumé
The effect of a cold < 40°C radio frequency-driven atmospheric pressure plasma jet on plasmid DNA has been investigated. Gel electrophoresis was used to analyze the DNA forms post-treatment. The experimental data are fitted to a rate equation model that allows for quantitative determination of the rates of single and double strand break formation. The formation of double strand breaks correlates well with the atomic oxygen density. Taken with other measurements, this indicates that neutral components in the jet are effective in inducing double strand breaks.

Introduction
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 25 micrometers, thus approaching the size of a typical cell and allowing very precise treatment 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. In particular, the ability of non-thermal plasmas to inactivate micro-organisms has shown great promise for sterilization and in general decontamination on for example living tissues - wound healing, dental tooth caries, and on foods. Plasma interactions with living tissue should keep cell damage to a minimum. In general cell death should only be induced when necessary in a manner that the body can renew and repair itself i.e. apoptosis.

Little is known of the influence plasma has on DNA. While qualitative work is a good indicator, it is vital to quantitatively determine the nature of this influence before any potential application on living tissue can be realized. 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. The motivation for investigations of DNA damage is two-fold. A fundamental understanding of plasma induced DNA damage for informing reliable risk benefit analysis [1]. Furthermore, DNA serves as a useful indicator with bio-molecules in general and the damage caused to it can be readily quantified. As an initial step it is essential to correlate direct plasma parameters with effects on bio-molecules.

In this study plasmid DNA in solution was exposed to the effluent of an rf atmospheric pressure plasma jet and simultaneously the absolute density of ground state atomic oxygen in the jet is measured. An effluent is emitted from the plasma bulk into ambient air and the investigations presented are performed a distance of 2 mm from the jet nozzle. The maximum gas temperature measured inside the plasma core using rotational bands of nitrogen is 75 C, while in the effluent applied to the DNA it is 40 C. The effluent of the plasma is allowed to interact with the plasmid DNA solution for varying treatment times and applied rf power. After exposure to the plasma jet gel electrophoresis was used to separate different DNA forms: supercoiled (SC), open circular (OC), and linear (LIN). For each set of conditions, the full study was repeated in triplicate with the standard error (SE) of each set of three measurements being used in subsequent fitting analysis. A system of rate equations governing damage in the plasmid was used to determine rates of each of the three forms.

The influence of UV emitted from the plasma was investigated by placing a MgF₂ slide between the effluent and plasmid-DNA solution; UV from the plasma can penetrate the MgF₂ slide while radicals are blocked. There was no evidence for single strand breaks (SSBs) or double strand breaks (DSBs) detected, even up to exposure times of 1 min. Exposure of the DNA to applied rf radiation only, without a plasma, and also gas flow only without a plasma were also investigated and again no influence was found.
The interactions at the plasma liquid interface are complex with a large variety of relevant species and a broad range of densities and particle fluxes. The discharge configuration is such that the electric field direction is perpendicular to the gas flow and plasma channel exit nozzle. This confines the charged species within the electrode gap inside the plasma bulk region. With no direct power input and very short mean free paths for charged species the effluent, upon interaction with the surrounding ambient air, is devoid of charge carriers. Neutral species, in particular reactive particles, and UV radiation dominate the effluent characteristics. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), e.g. O, O₃, O₂(a1Δg), OH, N, N₂, NO, are particularly known for their aggressive influence on biomolecules.

In order to investigate the role of radical damage to the plasmid-DNA, 10 mM Tris-EDTA – a substance known to be a much stronger radical scavenger than PBS – was used as a buffer solution. 100 mM Tris-EDTA mimics the radical scavenging environment found in cells in protecting DNA from damage. However the damage incurred to the DNA in 10 mM Tris-EDTA is greatly reduced compared to that in water or PBS. From this we can conclude that radicals play an important role in DNA damage.

Figure 1 shows the rates for SSB and DSB formation as a function of absolute atomic oxygen density measured in the core of the plasma bulk. It should be emphasized that there may be other plasma species or a combination of two or more species either in the plasma or induced downstream products causing DNA damage. However, as a starting point atomic oxygen has been identified as a relevant species, either directly or indirectly, correlated to DNA damage.

Fig. 1: Rate of single and double strand breaks of the plasmid DNA in 10 mM PBS as a function of absolute atomic oxygen density. The error bars are the uncertainties in the parameters $\beta_S$ and $\beta_D$ 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.

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. It is very unusual to find a means to form DSBs that does not also form SSBs. Since $\beta_S$ is typically two orders of magnitude greater than $\beta_D$, it is not certain this is the case here in spite of the lack of correlation between $\beta_S$ and atomic oxygen density, since the effect of this species on $\beta_S$ may be masked by other components which give rise to SSBs. Ozone and singlet delta oxygen densities were also measured and do not show a direct correlation to either single or double strand break rates.

References