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DNA strand scission induced by a non-thermal atmospheric pressure plasma jet

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The DNA molecule is observed to be very susceptible to short-term exposures to an atmospheric pressure plasma jet. The DNA damage induced by plasma-generated species, *i.e.* excited atoms, charged particles, electrons and UV light is determined.

The use of non-thermal plasmas in bio-medical applications has been driven by the development of cold plasma devices capable of being operated in the open atmosphere.[1] The generation of atmospheric pressure plasmas removes the need for costly high vacuum systems and avoids the inherent difficulties of treating living tissue under vacuum conditions. Moreover, since such plasmas are 'cold' they are ideal for treating materials that are easily damaged by high temperatures, *e.g.* soft plastics and biological media, indeed exposure of any cellular material to temperatures only a few degrees higher than ambient may cause irreparable damage.

A recent extended review provides an update on research related to applications of non-thermal plasmas in medicine and a discussion of the possible mechanisms of interactions between plasma and living matter.[2] In this review the authors presented a very broad overview of the use of 'cold' plasmas in the clinic including: tissue sterilization, wound healing, tissue regeneration, treatment of melanoma skin cancer and dental cavities. However, these applications are still in the earliest stage of their development and their introduction into general medical practice may be some years away. The main reason for this is a lack of a deeper understanding of the physical, chemical and biological mechanisms underlying the interaction of a non-thermal atmospheric pressure plasma and living cells, tissues and organs.

In the present work we have investigated the formation of single- and double- strand breaks in DNA molecules induced by an atmospheric pressure plasma jet (APPJ). The complexity of plasma-generated species, *i.e.* excited atoms and molecules, charged particles, electrons and UV light gives a variety of possible pathways by which DNA can be damaged. Therefore, our aim is to understand the interaction of particular components of the plasma with DNA.

A schematic view of the experimental set-up is presented in Fig. 1. Two tubular metal electrodes are separated by a 3 cm gap. A quartz cylindrical tube is inserted between the electrodes and helium is flowed through the tube with the velocity of 10 m s^{-1}.

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The two electrodes are connected to a RF power supply capable of producing sinusoidal shaped voltage pulses with an amplitude of 4 kV and repetition rate of 3.2 kHz. A discharge is ignited in the gap between electrodes; the discharge current typically reaches a peak value of 19 mA under these conditions. The mean power transferred into the discharge is around 20 W. At the open end of the reactor a plasma plume is launched into the surrounding air. The general working principle of the generation of such plasma jets is described by Kedzierski *et al.*[3]

In this experiment we used extra-chromosomal plasmid DNA (pBR322, 4361 base pair) extracted from *E. coli* bacteria and purified to ensure only a relatively small amount of residual proteins. Plasmid DNA was dissolved in autoclaved ultra-pure water and 2 μL of such an aqueous DNA (200 ng μL^{-1}) solution was deposited onto mica slides that were then dried at room temperature. The DNA covered mica slides were then placed perpendicularly to the 'cold' plasma jet formed in helium.

Samples located at the tip of the jet were exposed for different time durations. It should be noted that the temperature of the tip of the plasma jet was measured using a mercury thermometer and a thermocouple and was found to be no more than 10 °C above ambient. Thus, no significant thermal effects on the plasmid DNA are expected. Moreover, control experiments were also performed by leaving a few samples at room temperature, while the other samples were treated. In this control measurement, 85–75% of intact DNA was recovered from the mica slides. Additionally, experiments were also performed, where the dry DNA samples were exposed to a
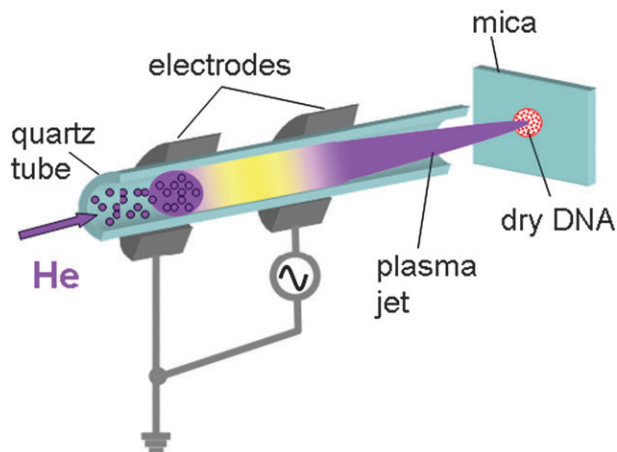

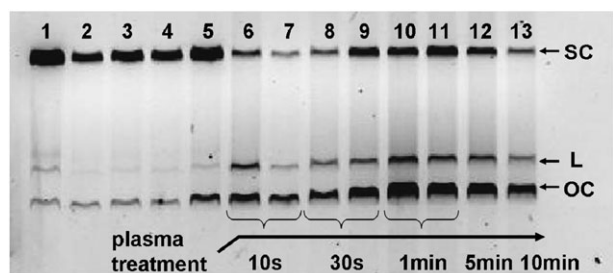**Fig. 1** A schematic view of the experimental set-up.

Fig. 2 An image of an agarose gel containing pBR322 irradiated to several APPJ exposure levels. The different DNA conformers are labelled as SC-supercoild, L-linear and OC-open circular.

Fig. 3 The effect of helium stream and plasma exposure time upon the change in abundance of each of the plasmid forms. The different DNA conformers are labelled as SC-supercoild, L-linear and OC-open circular.

helium stream with the same flow rate, but without applying the electrical excitation necessary to create the plasma jet. No detectable damage of DNA due to its exposure to a stream of flowing helium was observed. After exposure, the DNA samples were recovered with water and analysed by 0.8% agarose gel electrophoresis, pre-stained with SYBR green I dye. One of the images obtained from the electrophoresis of post-treatment DNA is presented in Fig. 2. The following samples were analysed: (a) solution control (sample no. 1); (b) non-irradiated samples removed from the mica slides (samples no. 2–3); (c) samples treated with the stream of the He gas but no plasma (samples no. 4–5) and (d) plasma irradiated samples (sample no. 6–13).

Plasmid DNA is a good indicator molecule to use for recognition of single- and double-strand breaks in helix DNA owing to its different topological states: supercoiled (SC) represents the undamaged molecule; open circular (OC) and linear (L) conformers are formed due to the disruption of a phosphodiester bond on one and both strands, respectively. The mobility in the gel electrophoresis of these three forms is different; thus, three particular forms can be separated. The intensity of the bands indicates the relative concentration of different types of DNA conformers. The percentage amount corresponding to each form is plotted as a function of the plasma exposure time in Fig. 3. Results from at least three different samples with the same irradiation conditions were summed. A mean value was calculated for each form and an error bar calculated as one standard deviation from the mean. Rapid degradation of supercoiled DNA is observed, up to 60% within the first ten seconds of He plasma treatment, followed by a period, when there was a slower accumulation of damage. Upon exposure, the amount of linear DNA was initially observed to increase by 10%, and after ten seconds remained constant. Most remarkable is the production of open circle forms due to single strand breaks in DNA, yielding a 70% rise after ten minutes of He plasma treatment.

The low-temperature plasma treatment of dry plasmid DNA by cold plasma has previously been shown to lead to a very strong degradation of DNA with many double-stranded fragmentations observed under exposure time up to 1 min but by using different devices for plasma generation.[4–6] In contrast, our results show only a weak (below 10%) multifragmentation of DNA, which is observed as smeared bands in the gel image.

Optical emission spectra (OES) were also recorded to monitor the plasma plume composition using a TRIAX320
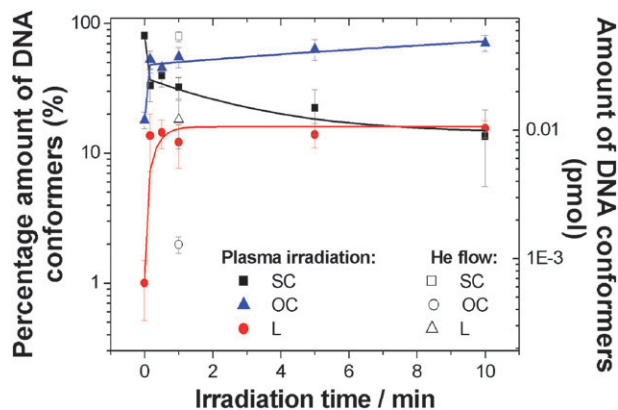
spectrophotometer. The optical emission spectra spanned the range from 280–800 nm (Fig. 4). The shown spectrum is very rich in emission lines that can be attributed to He, N_2, N_2^+ and also to radicals $OH^\bullet$ and $O^\bullet$. These species are mainly products of the helium plasma jet's interaction with the ambient air. The strongest emission lines are assigned as excited and ionic nitrogen molecules, *i.e.* N_2^* and N_2^+. The near infrared region exhibits a contribution of reactive oxygen compounds with strong emission atomic lines (OI) at 777, 715 and 627 nm, as well as O_2^+ and O_2^* lines at 631 and 760 nm, respectively. The emission of an OH line is observed at 309 nm. It should be noted that it is well known that hydroxyl and oxygen radicals can contribute significantly to the damage of DNA owing to their chemical reactivity.[7] The existence of intensive UV radiation in the plasma jet is expected to occur below 300 nm. In order to investigate the contribution of such UV light to DNA damage, a LiF filter with transmission of UV light in the range of 120–750 nm was placed between the jet and the DNA sample which was then exposed for one minute. Single strand breaks were still observed (10% damage) but no double strand breaks were observed.

Previous investigations on dry plasmid DNA exposed to UV synchrotron radiation showed the evidence for both single- and double-strand break formations in a wide range of wavelength (below 180 nm).[8] The reported yield of single
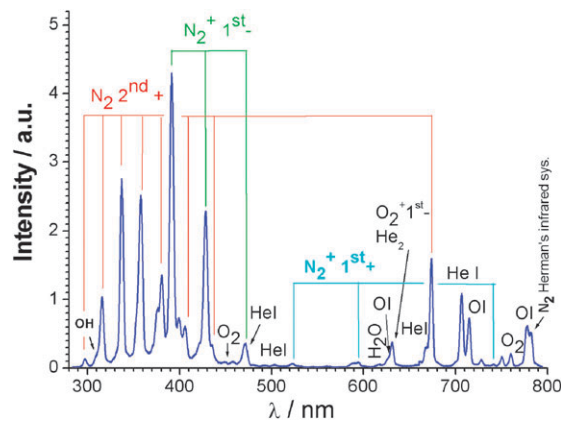

Fig. 4 The optical emission spectrum of the APPJ.

strand breaks is about 20–30 fold higher than one for double strand breaks in dry DNA. Thus, in our experiment, the expected amount of the latter type of damage is below 1%, which is within experimental uncertainties.

The presence of low energy electrons within the plasma jet may be another major source of damage to the plasmid DNA since it has been shown that such low energy electrons can induce breaks in dry plasmid DNA.[9,10] In order to estimate the effect of electrons (and other negative species) on plasmid DNA, a high transmission metallic mesh was placed in front of the sample. A negative potential up to −30 V with respect to ground was applied to the mesh to suppress the electron flux reaching the sample from plasma jet. On decreasing the potential the number of DNA molecules remaining in a supercoiled form was found to be smaller; e.g. 20% of undamaged DNA was observed at the grid voltage of −30 V, but only 8% was observed at −20 V. In view of the collisional nature of the transport these voltages do not simply map onto electron energies, but the effect indicates that the presence of electrons has some bearing on the overall interactions of the plasma jet with DNA.

Similarly by applying a positive potential of 30 V to the mesh one might expect to change the nature of the positive ion flux reaching the sample, though the mesh size was such that one could not be assured of cutting off the ion flux altogether. Applying different positive potentials did not have a significant influence on the extent and nature of the observed damage results. The damage to DNA due to low energy ions is estimated to be no more than 8% of the total modification. The mean energy of ions in the jet is not expected to exceed the ambient gas temperature. There is some record of the fragmentation of DNA components in the condensed phase by the impact of hyperthermal (1–100 eV) ions, i.e. He^+, N^+ and N_2^+ [11–13] all of which are components of our plasma jet. Those studies have shown an increase in intensity of fragmentation products with higher kinetic energy of ions with estimated threshold of 20–30 eV, but it is unlikely that such energies are achieved in our experiment.

To summarize the effect of particular components of a plasma jet on DNA samples the percentage contributions are presented in Table 1. According to our estimation most (more than 60%) of the damage to DNA during plasma irradiation is related to the interaction of excited or/and reactive species. Although in this work we have not made measurements directly of the concentrations of reactive species and the reaction pathways, the role of water and oxygen entrained at the edge of the plume is worthy of further investigation. In fact, DNA damage may turn out to be a useful probe for low concentration of reactive species. Plasma-generated radicals have different lifetimes depending on their reactivity, for example, the lifetime of an OH radical (with several eV of internal energy) in an atmospheric pressure discharge is around one millisecond,[14] though outside the discharge in the free atmosphere it is about 1 s;[15] the lifetime of the excited oxygen molecule in the singlet delta state (about 1 eV) is many tens of seconds. In contrast, based on reported rate constants for electron-positive ion recombination, the lifetime of electrons in the vicinity of the jet is likely to be on the order of microseconds; the lifetime of oxygen atoms will be even less.[16]

In conclusion, in order to understand the effect of particular species of plasma on DNA, we have performed exposure of a DNA sample located in the tip of the jet of an atmospheric pressure helium plasma. Owing to the different life-times of the various plasma-generated species and the interactions between plasma species and the surrounding ambient air, the composition of the plasma jet changes in space. Therefore, in the future we plan to measure the damage induced to DNA samples positioned at different distances.

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Table 1 The percentage damage of the DNA molecule via charged, excited species and UV light during 1 min of the plasma exposure

	UV light	Positive ions	Electrons (and negative ions)	Excited and reactive species
Total damage	10%	8%	20%	62%