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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. © *2011 American Institute of Physics*. [doi:10.1063/1.3521502]

Cold atmospheric pressure plasmas offer a unique environment in plasma medicine, allowing treatment of soft materials, including biomaterials such as living tissues. Single plasma devices can be as small as 25 μm,[1,2] thus approaching the size of a typical cell and allowing very precise treatment reducing damage to the surrounding healthy living cells. Several biomedical applications have already been identified; examples include biocompatible implant coatings, skin diseases, blood coagulation, cancer therapy, tissue removal, cosmetic treatments, sterilization and decontamination, wound healing, dental tooth carries.[3–8] 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.[5]

Little is known of the influence that plasma has on DNA. While qualitative work is a good indicator,[9,10] 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, the formation of double strand breaks is important as these are difficult for cells to repair. The motivation for investigations of DNA damage is twofold. A fundamental understanding of plasma-induced DNA damage for informing reliable risk benefits analysis. Furthermore, DNA serves as a useful indicator with biomolecules 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 biomolecules.

In this study, plasmid DNA in solution was exposed to the effluent of a rf atmospheric pressure plasma jet,[11,12] and simultaneously, the absolute density of ground state atomic oxygen in the jet was measured. The plasma is ignited between two parallel stainless steel electrodes confined between two quartz windows. The discharge gap is 1 mm, 1 mm wide, and the plasma channel is 30 mm long. The plasma is operated in helium [with 1 slm gas flow (slm denotes standard liter per minute) and velocity of 16.75 m s^{-1}] with 0.5% admixture of oxygen. The plasma is driven with an excitation frequency of 13.56 MHz, and the applied power varied between 20 and 50 W. Approximately, 1–2 W power is delivered into the plasma. Under all conditions, the plasma operates in a low-density alpha-mode where a stable, homogeneous glow discharge is sustained between the two electrodes.[11,12] An effluent is emitted from the plasma bulk into ambient air and the investigations presented are performed a distance of 2 mm from the end of the electrodes (see Ref. 16). 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.[12]

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.[13] Neutral species, reactive particles in particular, and UV radiation dominate its characteristics.[11,12] Reactive oxygen species and reactive nitrogen species, e.g., O, O_3, $O_2(a\ ^1\Delta_g)$, OH, N, N_2, and NO, are particularly known for their aggressive influence on biomolecules.[14] Measurements have shown ground state atomic oxygen, and ozone densities penetrate with relatively high densities as far as 100 mm from the nozzle of the plasma channel.[11,12] In fact, synergistic processes involving two or more of the above species are likely mechanisms for DNA damage.

Atomic oxygen can be expected to be one of the more important species for DNA damage, and absolute atomic oxygen densities are measured in the bulk plasma using diagnostic based modeling (DBM) (Ref. 15). DBM uses an active combination of numerical simulations with optical emission spectroscopy. Excellent quantitative agreement has been found between measured atomic oxygen densities with direct laser spectroscopic measurements.[16]

Plasmid DNA pCDNA3.1 (Invitrogen) was purified using Qiagen plasmid maxi kits after propagation in *E. coli*.[17] The DNA concentration was determined spectroscopically. Subsequently, the DNA was diluted to a concentration of

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14 ng/μl in 10 mM phosphate buffered saline (PBS) and 10 mM tris-EDTA. PBS is isotonic, contains sodium chloride and sodium phosphate, and is moderately radical scavenging, while tris-EDTA is known to be a strong radical scavenger, in particular of OH radicals.[18] The effluent of the plasma was allowed to interact with the plasmid DNA solution for varying treatment times and applied rf powers. The samples were 2.8 mm deep. 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. The three forms listed above correspond to DNA with no strand breaks, with only single strand breaks, and with double strand breaks, respectively. The use of the yields of these forms to distinguish between and analyze the rates of single strand break (SSB) and double strand break (DSB) in DNA is well established for both radiation and direct radical activity.[19,20] Because no form is governed solely by the effects of either single SSBs or DSBs, these rates must be obtained by fitting predicted dose-response behaviors to some or all of the plasmid forms to provide quantitative rates for each process. Traditionally, this is achieved by fitting to only the SC and LIN forms for the SSB and DSB rates, respectively, using equations that are designed to be linear in the fitting parameters to allow for simple linear regression to be used.

However, as this only makes use of some of the available data, a more robust model has been used in this work, which also makes use of the complete nonlinear behaviors that are derived from the rate equations governing damage in the plasmid system. This allows for all of the data to be used in a single fit, reducing the errors associated with the fitting procedure. The conclusions drawn from the data presented in this paper are in no way dependent on the use of this more robust model rather than the traditional one; the more robust model simply leads to smaller error bars since all of the available data are used in the analysis.

Although the details of this model will be described elsewhere,[21] we give a brief description here for completeness; it is entirely analogous to the radiation damage kinetics of DNA but with time of exposure to the plasma replacing the absorbed dose as the independent variable. The model has three key parameters: β_S, the rate constant for SSB formation; β_D, the rate constant for single-event DSB formation; and ρ, related to the ratio of the persistence length of the DNA duplex to the total plasmid length. The transformation from SC$\rightarrow$OC occurs as a first order reaction with the rate constant β_S, the transformation SC$\rightarrow$LIN occurs also as a first order reaction with the rate constant β_D, while the rate for the transformation OC$\rightarrow$LIN involves both rate constants and ρ since this parameter acts to describe the stochastic probability of two SSBs from independent events lying close enough to mimic a DSB in producing the LIN form. The equations governing this concentration dynamics can be solved analytically to give

$$S = S_0 e^{-(\beta_S+\beta_D)D},$$

$$C = e^{-\beta_D D}[C_0 e^{-(1/2)\beta_S^2\rho D^2} + S_0(e^{-(1/2)\beta_S^2\rho D^2} - e^{-\beta_S D})],$$

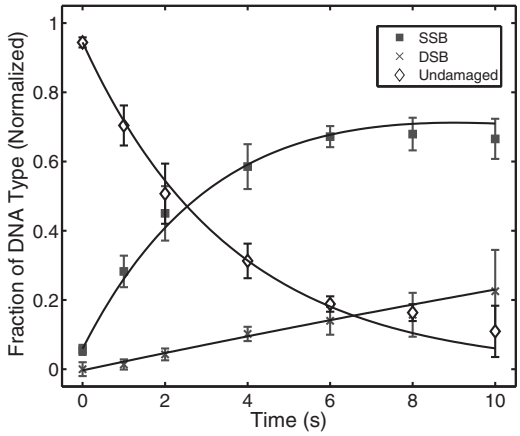

FIG. 1. Fraction of supercoiled, open-circular, and linear DNA after plasma exposure to plasmid DNA in 10 mM PBS for varying exposure times. Error bars are the SE of three measurements and account for statistical effects alone. Solid lines show the best fit to the rate equation model.

$$L = 1 - (C_0 + S_0)e^{-(\beta_D+(1/2)\beta_S^2\rho D^2)}, \quad (1)$$

where D is the time of exposure to the plasma jet. A weighted nonlinear least-squares fit was performed, fitting the dependence on D of the observed ratio of the three forms simultaneously. The fit parameters were β_S, β_D, S_0, and C_0. The fit results depend only very weakly on the value of ρ, which was set to a value corresponding to ten base pairs throughout the analysis, although including this as a fitted parameter results in no change to the conclusions arising from the fitting process. The effect of coincident SSBs is explicitly accounted for in this model through the term $e^{-(1/2)\beta_S^2\rho D^2}$ and is distinguishable from the effect of true DSB, which occurs through all terms containing β_D. Furthermore, computational experiments have demonstrated that the fitting procedure we used is robust at distinguishing between these two effects.[21]

The influence of UV emitted from the plasma was investigated by placing a MgFl slide between the effluent and plasmid DNA solution; UV from the plasma can penetrate the $MgFl_2$ slide, while radicals are blocked. There was no evidence for SSBs or 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. Furthermore, the pH of the sample was measured before and after exposure to the plasma jet, and no change was observed.

Figure 1 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. The results for exposure to plasmid DNA in water solution (not shown) reveal quantitatively very similar results to 10 mM PBS; this is in contrast to x-ray irradiation.[17] 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. Figure 2 shows plasma exposure for the same plasma conditions, as were used for Fig. 1, except the DNA was suspended in 10 mM tris-EDTA.

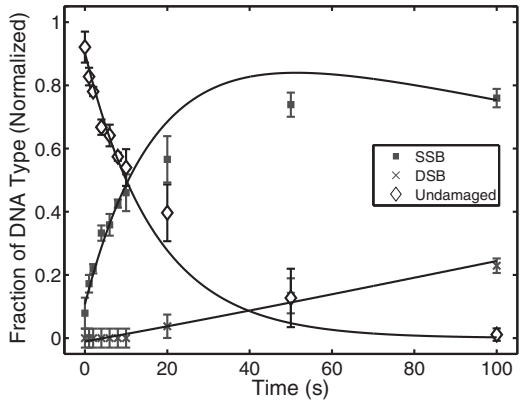

FIG. 2. Fraction of supercoiled, open-circular, and linear DNA after plasma exposure to plasmid DNA in 10 mM tris-EDTA for varying exposure times. Error bars are the SE of three measurements and account for statistical effects alone. Solid lines show the best fit to the rate equation model.

Comparing Figs. 1 and 2, noting that the time scales on the abscissa are an order of magnitude different, the trends are very comparable. 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. Over a timescale of 0–10 s in water and 10 mM PBS solution, there is clear evidence for DNA damage, whereas there is little evidence of DNA breakage in 10 mM tris-EDTA solution.

Figure 3 shows the rates for SSB and DSB formation as a function of absolute atomic oxygen density measured in the core of the plasma bulk. These rates are determined from the fits like that shown in Fig. 1, considering the data for different plasma parameters into one plot. 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 with DNA damage.[14]

As shown in Fig. 3, the best-fit straight line describing β_D has an intercept of $0.0010 s^{-1}$ and a slope of 2.14×10^{-18} $cm^3\ s^{-1}$, showing a clear correlation with neutral oxygen density and an intercept that 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 0.25 s^{-1} and a slope of 1.75×10^{-19} $cm^3\ s^{-1}$. Since the range of values for the slope spans zero (1 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 that give rise to SSBs. Further correlations with other important energy carrying candidates for DNA damage are required to complete our understanding of interactions of cold plasmas with biomolecules.

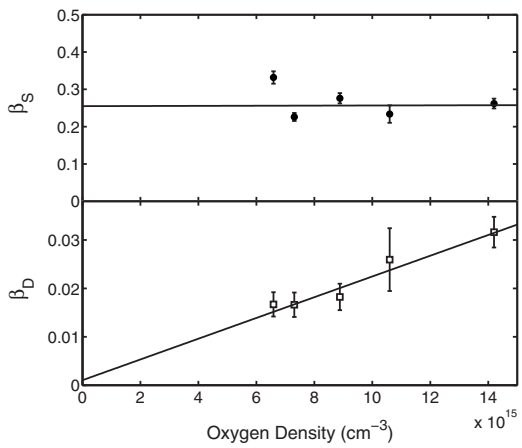

FIG. 3. Rate of single and double strand breaks of the plasmid DNA in 10 mM PBS as a function of absolute atomic oxygen density. The error bars are the uncertainties in the parameters β_S and β_D as derived from the fitting process. The lines are best-fit lines derived from a weighted linear least-squares fit to each of the data sets.

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