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Experimental Study of Atmospheric Pressure Argon Plasma Jet–Induced Strand Breakage in Large DNA Molecules

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ABSTRACT: In recent years, the atmospheric pressure plasma jet (APPJ) has been extensively studied for potential biological and medical application. It has been shown that a bactericidal effect is primarily due to the inactivation of the DNA repair mechanism or a DNA damage response in the microorganism. The damage is attributed to the interaction of biomolecules with reactive species produced by the plasma. In this experiment, the influence of the environmental medium on the efficacy of an argon plasma jet in the breakdown of large DNA (λ DNA) molecules was studied using characterization of the APPJ by electrical and optical methods. The DNA was suspended in four different solutions: physiological saline (PS), water, phosphate buffered saline (PBS), and Tris-EDTA (TE) and exposed to a plasma jet for three different periods of time (60, 90, and 120 s). The molecular responses of λ DNA were analyzed in gel electrophoresis. The largest amount of breakage was found to occur in water, with significant damage found at 120 s. PBS showed a linear degradation pattern. The free-radical scavenging properties of the medium may play a significant part in preventing strand breakage, and this must therefore be taken into account when exposing microorganisms and human cells during experiments. The crucial role of medium properties highlights the need for further studies of organisms in their natural surroundings.

KEY WORDS: atmospheric pressure argon plasma jet, λ DNA, PBS, water

I. INTRODUCTION

In recent years, atmospheric pressure plasma (APP) has been extensively studied for potential biological and medical applications. APP has an important role in the activation and/or inactivation of biomolecules including nucleic acids, lipids, and proteins in aqueous media. APP has been applied for study as a biodecontaminant, in biofilm inactivation, and for dentistry applications, sterilization, tissue treatment, and wound healing.[1–4]

One of the most commonly used APPs is an atmospheric pressure plasma jet (APPJ). The APPJ represents an indirect source because the plasma produced between two electrodes is transported to the treatment materials using a feed gas, which is typically helium, argon, or nitrogen. This technique confers the ability to treat irregular surfaces

and oddly shaped objects.[5,6] Argon as the feed gas has been used in wound healing and inactivation of isolated and vegetative microorganisms *in vitro*.[6–11]

Although the bactericidal effect of plasma is acknowledged, most of the molecular mechanisms of action are still unknown and require investigation. In our previous experiment, we indicated that the argon feed jet was responsible for killing prokaryotic and eukaryotic cells.[12] It is vital to determine the nature of the plasma's influence on biomolecules before its potential application on complex structures such as living tissue can be realized. Studies have shown that the bactericidal effect is primarily due to inactivation of the DNA repair mechanism or DNA damage in the microorganism; the plasma jet has been shown to induce damage to cellular and plasmid DNA.[13] Study of the plasma jet in large DNA (λ DNA) molecules has continued to yield important new insights.[14–16]

The medium used for suspension of biomolecules and microorganisms during plasma treatment can also influence an induced effect.[1,17] Damage has been shown to be due to the interaction of biomolecules with reactive oxygen species of the cellular medium, because plasma modulates vital properties of media, and the toxic nature of plasma can also be altered by the surrounding media.[11,18] Most of the studies on strand-break formations in DNA have been performed in an aqueous DNA solution. The analysis of DNA breakage after suspension of large DNA molecules in water exposed to an argon plasma showed that exposure to treated medium alone can be involved in DNA breakage.[15] Information on the interaction between plasma and the environment is very limited, and research on varying efficiencies using different media is noticeably absent from the literature.[11]

Central to studying DNA damage under various APP conditions is understanding the physical and chemical processes that are crucial for advancing related biomedical applications such as cancer therapy and wound healing.[19] In this study, we investigate the influence of environmental media on the efficiency of plasma in the breakdown of large DNA molecules. The molecular responses of λ were analyzed after exposure to an argon (Ar) plasma jet in four different solutions (environments): (1) physiological saline (PS), (2) ultrapure water, (3) phosphate buffered saline (PBS), and (4) Tris–ethylenediaminetetraacetic acid (EDTA) (TE) medium. These four solutions differ in terms of their properties for buffering capacity (pH control), osmotic stability, and chemical composition.

The influence of these four solutions on plasma properties was also examined as it relates to strand breakage. Our results suggest that a broad spectrum of efficiency of λ DNA is possible by modulating the properties of solutions with plasma, and the level of reactive species is affected by the interactions between plasma and the solutions.

II. EXPERIMENT

The experimental setup for APPJ electrical and optical measurements is shown in Fig. 1. For this study, the plasma jet was generated in a glass capillary tube with an inner diameter of 3.0 mm and an outer diameter of 5 mm. Argon was used as the working gas, and flow rate was controlled by a volume flow meter. The flow rate of argon gas was

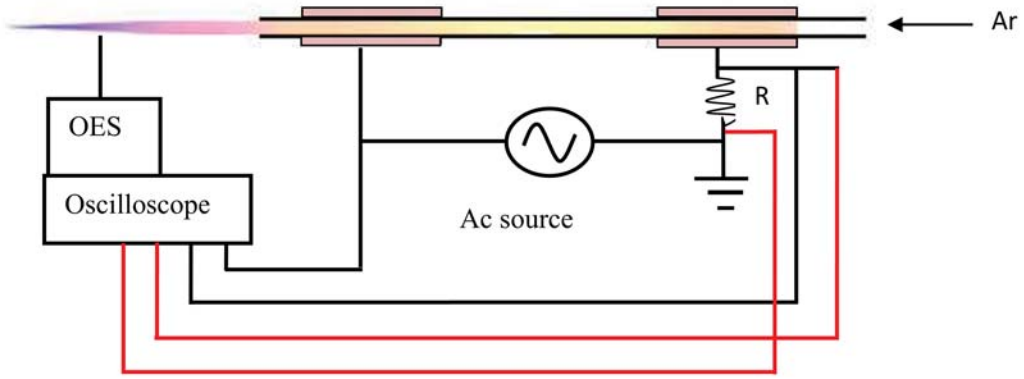

FIG. 1: Schematic diagram of the generation and characterization of the APPJ

restricted to below 5 L/m so that flow velocity would not exceed the limit for a laminar argon flow. A sinusoidal voltage of 3.5 kHz was applied for excitation and sustaining of discharges. The 1-cm-wide electrodes were made of aluminum foil that wrapped the capillary tube. The distance between the inner edges of the two electrodes was 15 cm. The ground electrode was on the upstream side. The active electrode was on the downstream side and was 0.5 cm apart from the tube orifice.

The voltage applied to the discharge was measured using a high-voltage probe, and discharge current was recorded using a current probe. In addition, a digital oscilloscope (Tektronix TDS2000) was used to obtain the voltage and current waveforms (Fig. 2). Optical emission spectra (OES) were collected perpendicular to the jet using an optical spectrometer (Ocean Optics) with a spectral range of 180–1100 nm and a resolution of

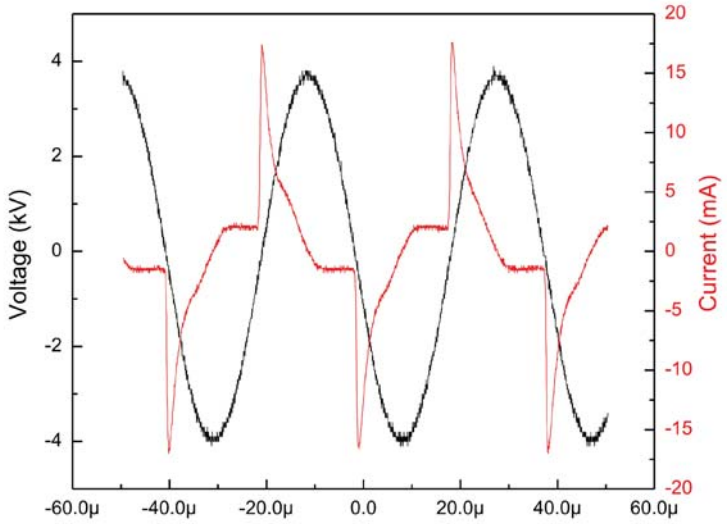

FIG. 2: Current and voltage waveforms of the APPJ

0.2-nm full width at half-maximum (Fig. 3). OES data were compiled using a personal computer equipped with relevant Lynex software for both driving and acquisition. We were thus able to obtain the relative irradiance of the active species in the plasma. During OES measurement, exposure time was 100 ms. Emission intensities of the active species were collected at a position of the plasma jet (2 cm from the end of the nozzle) through an optical fiber with a diameter of 100 μm.

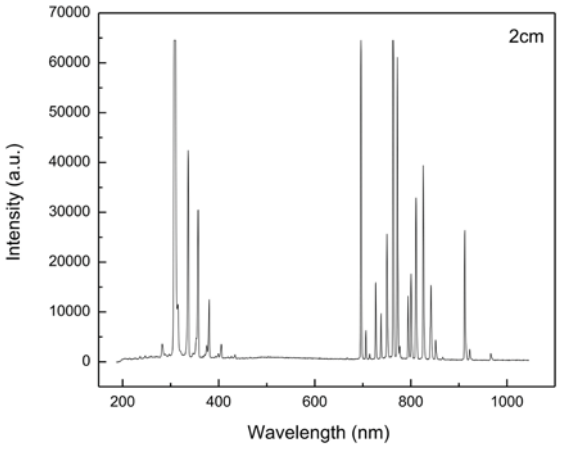

FIG. 3: OES of the APPJ

A. Electrical and Optical Characterization of Plasma Jet

Before applying the plasma jet for any purpose, it is necessary to determine the basic discharge parameters of electron temperature (T_e) and electron density (n_e). Different methods are available to measure both parameters, with the most commonly used being the Langmuir probe, the microwave interferometer, laser Thomson scattering, and optical spectroscopy (OES).

In the case of APPJ, the OES-based technique, suitable for measurement of both electron temperature and electron density, is based on the measurement of relative intensities of two or four spectral lines of the same atomic species. The intensity of spectral lines depends on KT_e and is proportional to the population density of excited states. Hence, KT_e can be determined using the well-known Boltzmann plot[20]:

$$KT_e = \frac{E_2 - E_1}{\log\left[\frac{I_1}{I_2}\frac{\lambda_1}{\lambda_2}\frac{g_2}{g_1}\frac{A_2}{A_1}\right]}, \quad (1)$$

where indices 1 and 2 refer to the first and second spectral lines, I is the measured intensity of selected spectral lines, K the Boltzman constant, E the excited states energy, g the statistical weight, and A the transition probability.

The Boltzman plot method is valid if the discharge plasma under study is in complete local thermodynamic equilibrium (LTE).[21] But in our experiment, it is unlikely that LTE will be maintained due to the low plasma density. Although this method may not be used for the exact determination of KT_e and n_e, it can still provide us with approximate values of plasma parameters under varying working conditions of APPJ discharge. In the spectrum, argon lines are observed to be in the range of 180–900 m, and KT_e is determined by selecting two argon spectral lines. The intensity of these spectral lines is obtained from the observed spectrum, shown in Fig. 4. Values for E, g, and A for the selected lines are taken from the National Institute of Standards and Technology atomic spectra data sheet. Using all of these values in Eq. (2), KT_e can be easily determined. Electron density n_e can be determined using the relative intensity of atomic and ionic spectral lines in the Boltzman-Saha equation[22]:

$$n_e = 2\left(\frac{I_1}{I_2}\right)\left(\frac{\lambda_1}{\lambda_2}\right)\left(\frac{A_2}{A_1}\right)\left(\frac{g_2}{g_1}\right)\left[\frac{2\pi m_e kT_e}{h^2}\right]^{\frac{3}{2}} \exp\left[-\frac{E_1 - E_2 + E_i}{kT_e}\right], \quad (2)$$

where I_1 is the intensity of the Ar–I line; I_2 the intensity of the Ar–II line; λ_1, λ_2 the wavelength; A_1, A_2 the transition probabilities; and g_1, g_2 the statistical weights of level (neutral and ionized, respectively).

Equation (1) is used to calculate T_e for different sets of spectral lines, with the average value of T_e calculated to be 0.41 eV. Equation (2) is used to calculate n_e for different sets of lines, with the average values of $n_e = 1.48 \times 10^{16}$ cm^{-3}.

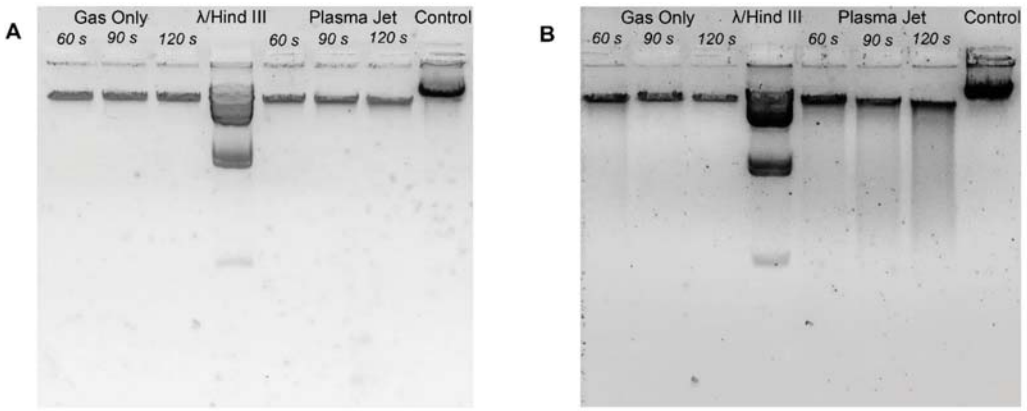

FIG. 4: Typical photographs taken during UV (ultraviolet) observation of the gel electrophoresis of DNA bands. The duration of exposure and exposure agent with a control and λ/HindIII is shown in each image. (A) λ DNA suspended in TE buffer. Visible movement of the bands exposed in plasma is seen, compared to gas only and control. There is little shearing. (B) λ DNA suspended in PBS buffer. There is visible movement of the DNA band as well as increased shearing with respect to time in plasma jet exposure.

B. Preparation of λ DNA

For this study, 100 μL of λ DNA (Fermentas; SD0011; 48502 base pairs) at a concentration of 0.3 μg/μL was suspended in 900 μL of one of the four mediums (PBS, PS, water, or TE). The suspension was centrifuged at 10,000*g* for 20 s to dilute the λ DNA concentration to 0.03 μg/μL. From this solution, 100 μL was supplied to seven different wells of a 48-well microtiter plate.

1. Exposure of λ DNA to Ar Plasma Jet

The Ar plasma jet was applied to each well for 60, 90, and 120 s. Similarly, Ar gas without plasma was applied to three other wells for 60, 90, and 120 s. The distance between the tip of the jet and the surface of the microtiter plate was 2.0 cm. Fluctuations in DNA damage were observed at distances >3 cm.[5] Thus, the distance between the tip of the jet and the surface of the microtiter plate was kept at 2.0 cm. To obtain a comparative result, distance, gas flow, and voltage constant were kept constant, and only treatment medium and time of exposure were varied. A well that was left untreated in either plasma jet or gas was designated as the control.

C. Agarose Gel Electrophoresis

A gel with 1.0% agarose concentration in 0.5× 45 mM Tris-borate, 1 mM EDTA (TBE) was prepared. The solution was heated in a microwave oven until the agarose dissolved. Ethidium bromide (0.5 μg/mL in H_2O) was incorporated into the warm solution. A comb that formed eight slots (0.8 × 0.1 × 0.7 cm) in the gel was positioned above the plate. Warm agarose solution was poured into the mold. After the gel was completely set, it was transferred to an electrophoresis tank, and electrophoresis buffer (0.5× TBE) was poured to cover the gel to a depth of ~1 mm.

After exposure to the APPJ, the treated samples, control, and λ/Hind III were separately mixed with 2 μL of gel-loading buffer type I (0.25% bromophenol blue, 0.25% xylene cyanol superfine (FF), and 40% [w/v] sucrose in H_2O) in a ratio of 5:1 using a micropipette. Each mixture was applied on the slot using a different micropipette. The arrangement of the mixture is shown in Fig. 4. The lid of the gel tank was closed and attached to electrical leads so that the DNA migrated toward the positive anode. A voltage of 50 V was applied, and run time was 100 min, fixed.

D. Observation of DNA

At the end of the run time, the gel was examined by transmitted UV light in a Gel Doc system, and the gel was photographed.

E. Data Analysis

Three independent sets of experiments were conducted using all four media. Data were obtained for each medium. The optical density of the bands was processed with Image Studio Lite. The length of visible smear was measured to record the shearing length of each band.

III. RESULTS AND DISCUSSION

Figure 4 shows typical results of agarose gel electrophoresis of λ DNA molecules exposed to the plasma jet and to gas only with control and λ/HindIII. Figure 4(A) shows the movement of λ DNA bands in TE medium. To contrast, Fig. 1(B) shows the movement of λ DNA bands in PBS medium. The increased visible movement of band and shearing effect is seen with respect to the time in plasma jet exposure compared to gas only and control. We measured band concentration for each experimental point and length of shearing effect. We found that a linear DNA fragment of a given size migrates at different rates, and the distance migrated is related inversely to number of nucleotide pairs. Table 1 shows that the length increased with respect to time. The shearing length of gas only was not calculated, because there was insignificant change in length compared to that of the control. As shown in Fig. 4, shearing length was directly proportional to exposure time. Water is shown to have significant breakage with respect to time ($p < 0.006$).

TABLE 1: Length (mean ± standard deviation) of λ DNA shearing at different plasma exposure times

	Shearing length (cm) with exposure time			
Medium	**60 s**	**90 s**	**120 s**	**p Value**
Water	0.38 ± 0.13	0.71 ± 0.14	1.04 ± 0.19	0.006*
PS	0.50 ± 0.21	0.88 ± 0.33	1.21 ± 0.40	0.096
PBS	0.83 ± 0.60	1.3 ± 1.02	1.71 ± 1.02	0.526
TE	0.06 ± 0.06	0.17 ± 0.19	0.27 ± 0.32	0.541

Analysis of variance: Significant difference is marked with an asterisk (*).
PBS, Phosphate buffered saline; PS, physiological saline; TE, Tris-EDTA.

We examined the protective effects of four media against strand breaks induced by plasma jet in a quantitative manner. TE and PBS are well known as free-radical scavengers, and TE is especially used in a molecular biology laboratory setup. PBS is also used for stabilization of DNA for short durations of time. In cases of TE and PBS, as shown in Fig. 5A and B, respectively, relative degradation increased with respect to time. Relative to control, degradation does not differ significantly for different time durations and exposure conditions. However, there is an increase in degradation with respect to time.

In the human body, cells are usually suspended in extracellular fluid that is isotonic with PS. Distilled water is deficient of minerals. As shown in Fig. 5C and D (PS and

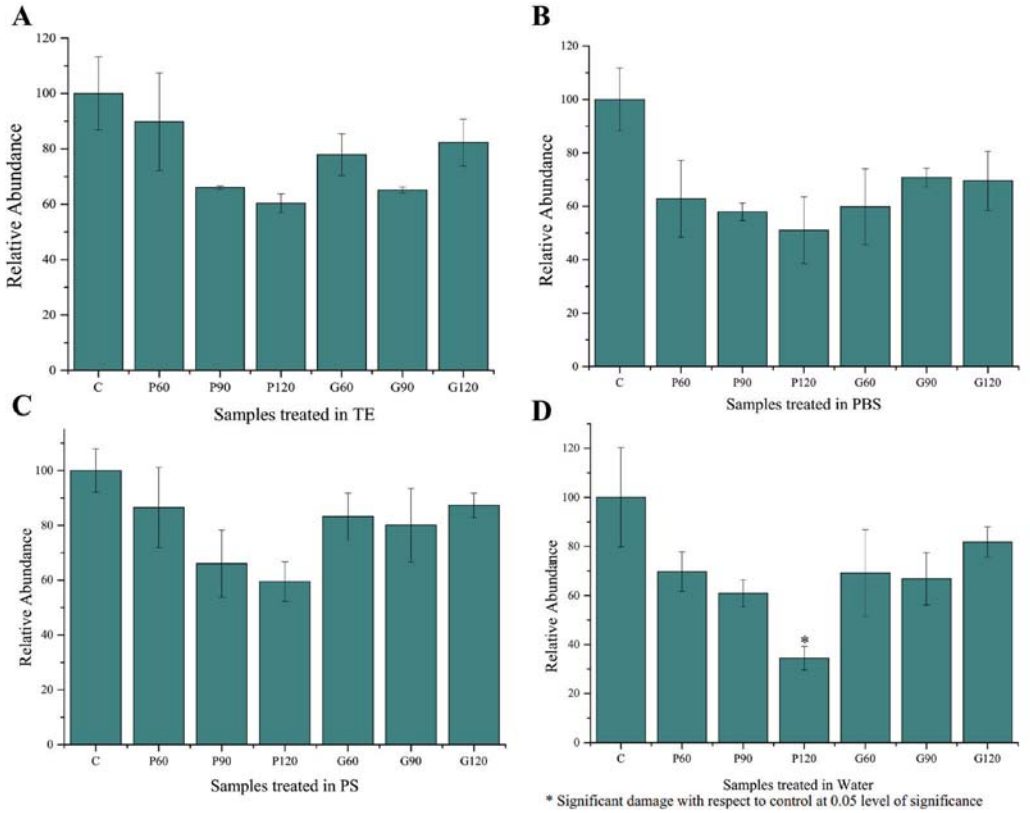

FIG. 5: Relative absorbance of λ DNA in control, plasma jet, and gas in four media. (A) TE; (B) phosphate buffered saline; (C) physiological saline; (D) water

water, respectively), the intensity of the band indicating degradation is quite significant in plasma jet exposure, especially at 120 s of plasma jet treatment in water. Gas showed much less of a change with increased exposure time for both the cases. This clearly shows that the free-radical generation property of PS and water has a significant role in the degradation of λ DNA.

Figure 6 shows the decrease in relative abundance of DNA compared to increases in time (maximum of 120 s) for all media. Water is seen to be the most effective medium for inducing breakage with the most significant damage. PBS shows a linear degradation pattern. TE and PS reveal dramatic changes between 60 and 90 s. The breakage pattern in the plasma jet reacting at 90 and 120 s shows considerable breakage, suggesting a use for low exposure time regarding breakage.[23]

IV. DISCUSSION

By using agarose gel electrophoresis to estimate the effect of APPs on isolated DNA, topological conformations and different lengths of strand-break formation can be ob-

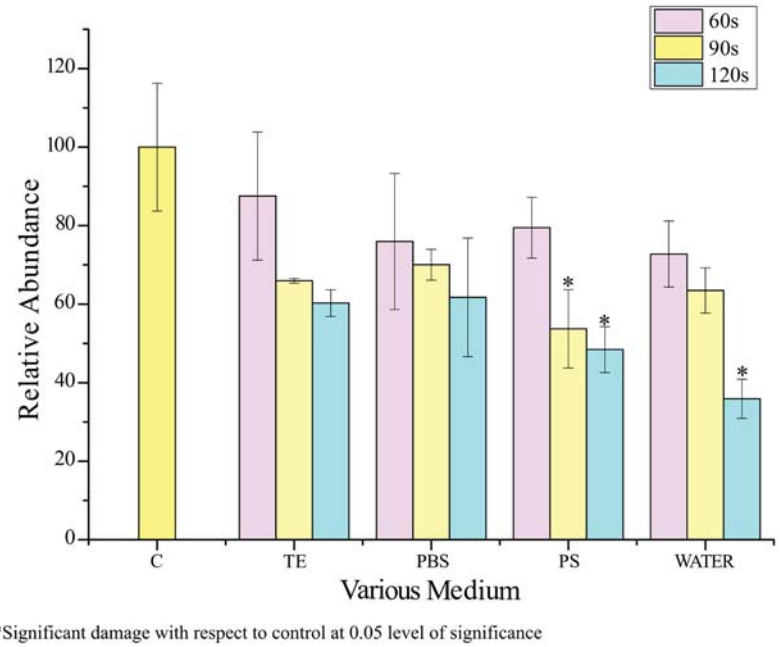

FIG. 6: Comparison of the relative concentration after plasma jet treatment in different media in three different times, with respect to control

served in separate DNA fragments. Although a distance of 2.0 cm was maintained between the source of plasma and λ DNA, we have shown that level of damage remains constant along the entire length of the visible zone of the jet.[5]

Regarding physical operative factors, a group of researchers concluded that no thermal factor contributes to DNA damage, because the APP jet used in their experiments was low in temperature.[24] Moreover, no intense electric field was detected and was therefore excluded as a contributor to strand-break formation.[19] UV light contributed only to a very limited portion of single-strand breaks (SSBs) of DNA and no double-strand breaks (DSBs).[25] The contribution of charged particles was also relatively low, and the concentration of charged particles in the APP zone was very small.[24] The reactive particle component led to negligible changes in nucleobases, but induced both SSB and DBD formation.[26,27] In our previous study, there was no significant change in pH in PBS by argon jet, similar to that of a helium jet.[12,28] However, there was a drop in pH in saline solution by He that contained of oxygen.[28] Quite a number of studies show the production of reactive oxygen and nitrogen species in plasma, and many studies have recommended quantifying the free radical generated from plasma treatment. One study found the generation of an enormous amount of OH radicals using Ar–cold atmospheric plasma.[29]

Plasma irradiation can also affect DNA through an indirect pathway via interactions with solvated electrons and free radicals that are produced by the primary ionizing radiation in a medium containing DNA. This indirect action is considered to be a major con-

tributor in the presence of water.[5] When water molecules are electronically excited, they become dissociated into H and OH radicals.[30] Similarly, OH radicals are also formed by a Fenton reaction from hydrogen peroxide. In turn, these radicals are formed as a result of a superoxide dismutation reaction occurring from highly reactive superoxide radicals.[31] DNA itself is a scavenger and would be expected to compete with other OH radical scavengers, particularly when present in a high concentration.[23]

PBS is isotonic, contains sodium chloride and sodium phosphate, and is moderately radical scavenging, whereas TE is known to be strong radical scavenger, in particular, of OH radicals.[13,23] Similar results in PBS and water were reported by Leduc et al.,[32] although the effect in TE was not observed.

PS, which consists of dilute sodium chloride solution (0.85%), showed DNA fragments that were comparable but insignificant to that of water. Hydrogen peroxide was reported to form in distilled water when it was exposed to all types of plasma.[33] The measured H_2O_2 concentrations were time dependent: the longer the exposure, the higher the concentration.[33] Due to the linear modulation of λ DNA, there is higher accessibility of radicals to sugar moieties, allowing for greater DNA damage.[34,35] This explains the increased breakage seen to occur in water.

The synergistic effect of many plasma components may have the most significant role in the mechanism of DNA strand breaks.[19,27] However, this experiment shows that free radicals play a major part in the generation of DNA fragments due to DNA breakage. The crucial role of medium properties highlights the need for further studies on the suspension of organisms and biomolecules in their natural surroundings.

V. CONCLUSIONS

In summary, the efficiency of plasma to break λ DNA is affected by the interaction between plasma and surrounding environments. The free-radical scavenging properties of the medium play a significant part in causing strand breakage and must therefore be taken into account when exposing microorganisms and human cells to media during experiments.

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