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Effect of the atmospheric pressure nonequilibrium plasmas on the conformational changes of plasmid DNA

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The cold atmospheric pressure plasma, which has been widely used for biomedical applications, may potentially affect the conformation of DNA. In this letter, an atmospheric pressure plasma plume is used to investigate its effects on the conformational changes of DNA of plasmid pAHC25. It is found that the plasma plume could cause plasmid DNA topology alteration, resulting in the percentage of the supercoiled plasmid DNA form decreased while that of the open circular and linearized form of plasmid DNA increased as detected by agrose gel electrophoresis. On the other hand, further investigation by using polymerase chain reaction method shows that the atmospheric pressure plasma jet treatments under proper conditions does not affect the genes of the plasmid DNA, which may have potential application in increasing the transformation frequency by genetic engineering. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3212739]

The atmospheric pressure nonequilibrium plasmas (APNPs) have been widely used in many applications such as materials processing,[1,2] water treatment,[3] and synthesis of nanomaterial[4,5] and biomedical applications.[6–26] Although the effective electron temperature T_e can reach several electron volts, the heavy particles, which undergo frequent collision with energized electrons, can still remain at low temperature due to orders difference of their mass. The collisions can result in the generation of high concentrations of ions and radicals. Because the gas temperature of the APNPs can be controlled at room temperature and there is no harmful radiation, researchers now pay more and more attention to the biomedical applications of APNPs, such as treatment of living tissues and tumor. Recently, Stoffels *et al.*[17] and Fridman *et al.*[20] found that APNPs can induce cell apoptosis process. Since the DNA damage can induce cell apoptosis process, the plasma may affect DNA and the genes, which may be potentially used in the plasma-mediated plant genetic transformation technique.[27] Therefore, it is necessary to investigate the impacts of plasma on characteristic of plasmid DNA.

In this paper, the effects of APNP on plasmid DNA are investigated. The plasmid used in this study is pAHC25, which contains scorable and selectable marker genes (*gus* and *bar*), both under the control of the maize ubiquitin promoter.[28] It is widely used as a vector in the plant transgenic technique. The possible factors that may affect the characteristic of the plasmid DNA are discussed in this paper. The action mechanisms are also investigated by using agarose gel electrophoresis and polymerase chain reaction (PCR).

The schematic of the experimental setup is shown in Fig. 1. A detailed description of the experimental setup can be found in Ref. 13. The gas temperature of the plasma plume is at room temperature. The power consumed by the device is about 2 W for the pulse frequency of 8 kHz and applied voltage of 8 kV. All the experimental results reported in this letter, the pulse frequency f of 8 kHz, pulse width t_{pw} of 1.0 μs, and applied voltage V of 8 kV are fixed. The Eppendorfs containing the plasmid pAHC25 are placed right under the nozzle. The distance between the nozzle and the surface of the plasmid is fixed to 45 mm. The working gas $He/O_2(1\%)$ with a flow rate of 2 l/min is used.

The concentration of the plasmid used is measured by the Ultraviolet Spectrophotometer (U0080D, HITACHI). The plasmid is diluted with distilled water to 0.1 $\mu g/\mu l$ in a 2 ml

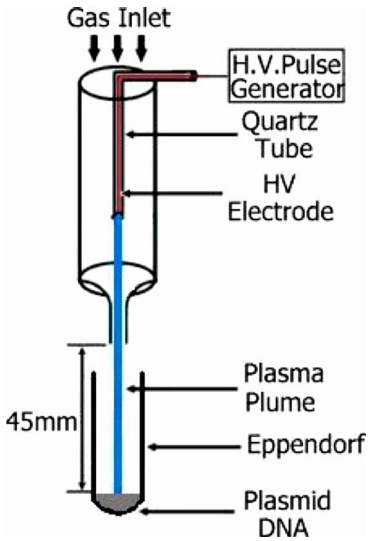

FIG. 1. (Color online) Schematic of the experimental setup.

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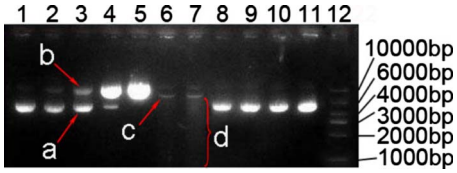

FIG. 2. (Color online) Lane 1–7: plasma treatment for 10 s, 30 s, 1 min, 2 min, 4 min, 8 min, and 16 min, respectively. Lane 8–10: control group treated with the working gas at the same flow rate with plasma off for 10 s, 4 min, and 16 min, respectively. Lane 11: untreated control. Lane 12: DNA Marker. a: supercoiled conformation, b: open circular conformation, c: linearized conformation, and d: fragmentized conformation.

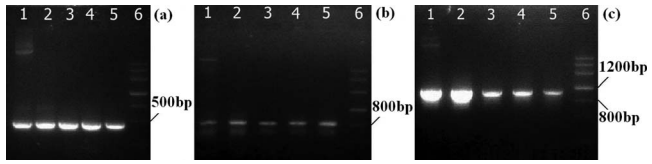

FIG. 4. PCR confirmation for presence of *bar*(a), *ubi-promoter,* (b) and *gus*(c) gene. 1: untreated plasmid pAHC25 as template, 2: treated by plasma for 5 min but not purified pAHC25 as template, 3: Purified open circular conformation as template, 4: Purified linearized conformation as template, 5: Purified open circular conformation and linearized conformation as template, and 6: DNA marker.

Eppendorf and the final volume is 15 μl. After the plasmid is treated with plasma, it was stained by ethidium bromide and analyzed on 0.7% agarose gel electrophoresis in 1×tris-acetate-EDTA (TAE) buffer at 100 V 30 mA for 60 min. For the control experiments, the plasmid is treated by the working gas at the same flow rate with plasma off. Both the plasma treated and the control experimental results of agarose gel electrophoresis are imaged by Gel Imager System (Bio-Rad Gel Doc 2000).

Agarose gel electrophoresis can separate DNA fragments of different length and conformation. Marker represents the DNA fragments with standard base pair (bp) (Fig. 2), which is used as standard to detect unknown size DNA fragments. There are three conformations in a plasmid in the agarose gel electrophoresis image. The slowest, middle, and the fastest bands represent the supercoiled, linearized, and open circular plasmid DNA respectively.[29] In the untreated control group (lane 11) and the air treatment control groups (lane 8–10), the plasmid DNA are with the majority being in the supercoiled form. With the increase of the treatment time (from lane 1 to 5), the proportion of the supercoiled plasmid DNA decreases, while that of the linearized and open circular plasmid DNA increase (lane 1–5). When the plasmid is treated for 4 min, the supercoiled form disappears completely (lane 5). For the treatment time of 1, 2, and 4 min, about 10%, 60,% and 100% of the supercoiled form DNA becomes linearized and open circular forms. Further increase the treatment time to 8 min, the brightness of the band became indistinct and a smeared zone can be seen below, indicating that the plasmid DNA is broken into small fragments because of the treatment of the plasma (lane 6 and 7). Therefore, to have more detail information about the conformational change of the plasmid DNA, in the next, the plasmid is treated for 5, 6, and 7 min, respectively.

Figure 3 shows the experimental results. As can be seen from Fig. 3, when the plasmid DNA is treated for 5 min (lane 2), the linearized and open circular plasmid DNA become much clear (indicated by the arrow c and b, respectively). With the increase of the treatment time (lane 3 and 4), the brightness became weaker and the band became smeared, which indicates that the plasmid DNA was gradually degraded. To further investigate whether the plasma affect the genes of the plasmid, the PCR technology is used.

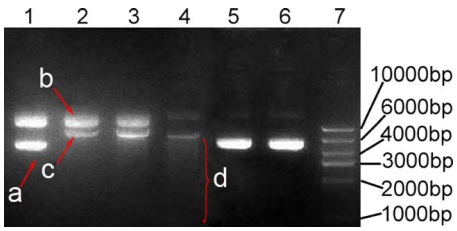

FIG. 3. (Color online) Lane 1–4: treatment by the plasma for 1, 5, 6, and 7 min, respectively. Lane 5: gas treatment with the working gas at the same flow rate with plasma off for 7 min. Lane 6: untreated control. Lane 7: DNA Marker. a: supercoiled conformation, b: open circular conformation, c: linearized conformation, and d: fragmentized conformation.

The PCR is a powerful technique used for the amplification of specific segments of DNA or mRNA.[30] The PCR relies on knowing at least partial sequences of the target DNA a prior and using them to design oligonucleotide primers that hybridize specifically to the target sequences. The target DNA is copied by a thermostable DNA polymerase enzyme, in the presence of nucleotides and primers. Through multiple cycles of heating and cooling in a thermocycle to produce rounds of target DNA denaturation, primer hybridization, and primer extension, the target DNA is amplified exponentially.[31]

The linearized and open circular conformation, obtained after 5 min treatment (Fig. 3), are separated and purified (TiaNgel Midi purification kit), and used as the template for PCR amplification of the *gus*, *bar,* and *ubiquitin-promoter* gene.

Primers used to the *gus* gene are 5′-*AGTGTACGTATCACCGTTTGTGTGAAC*-3′ and 5′-*ATCGCCGCTTTGGACATACCATCCGTA*-3′ at an annealing temperature of 62□, the sizes of the expected amplification products are 1.05 kb.[32] The *bar* gene was amplified with 5′ -*GTCTGCACCATCGTCAACC*-3′ and 5′ -*GAAGTCCAGCTGCCAGAAAC*-3′ primers at 57□ annealing temperature, obtained an expected amplified production of 443 bp.[33] Primers used to the *ubi-promoter* gene are 5′ -*TGTCACACTTGTTTGAAGTGCAG*-3′ and 5′ -*ACACGAGGTTGGGGAAAGAG*-3′ at an annealing temperature of 61 °C, the sizes of the amplification products are 826 bp.

PCR thermocycling conditions are 30 cycles of denaturation at 94 °C for 45 s, annealing for 1 min and extension at 72 °C for 1 min, then 72 °C for 10 min (Biometra).

The PCR amplified products are analyzed on 1.0% agarose gel electrophoresis in 1×TAE buffer at 100 V, 30 mA for 40 min. The results of agarose gel electrophoresis are analyzed by Gel Imager System.

PCR can detect the existence of a gene, which relies on the specific primers for the gene. It indicates the existence of the gene if a band appears near the expected band in the agarose gel electrophoresis photo. As we can see from Fig. 4, the three types of gene [bar(a), ubi-promoter (b) and gus(c) gene] are also presented in all the plasma treated samples. Therefore, proper plasma treatment does not affect the genes of the plasmid, which may be used in the plasma-mediated plant genetic transformation technique.

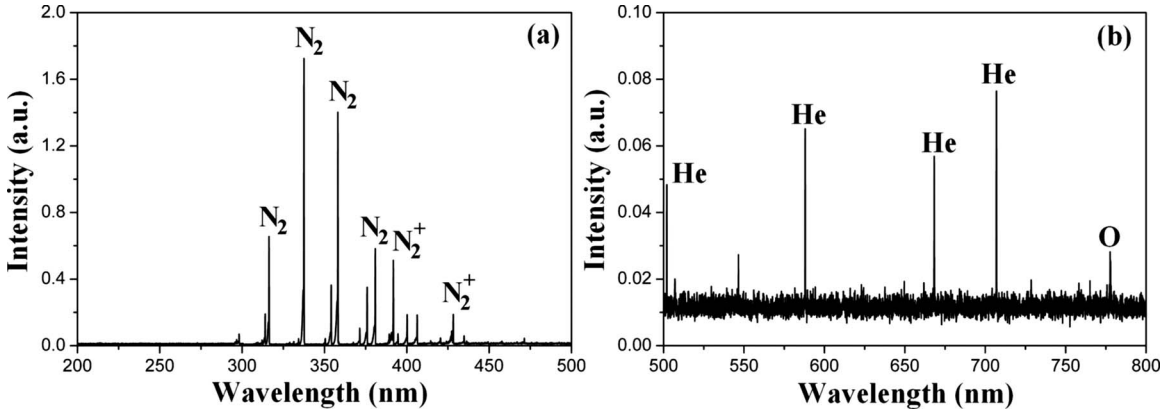

FIG. 5. Typical emission spectra of the plasma plume for $He/O_2(1\%)$ (total flow rate of 2 l/min).

Next, a half meter spectroscopy (Princeton Instruments Acton SpectraHub 2500i) is used to measure the optical emission of the plasma plume. Figure 5 shows the typical emission spectra of the plasma plume for working gas of $He/O_2(1\%)$ at a flow rate of 2 l/min. It can be seen that the emission spectra are dominated by the excited N_2^*, N_2^{+*}, O^*, and He^*. The intensity of the UV radiation with wavelengths in the 200–280 nm range is undetectable, so the UV radiation does not have significant effects to the plasmid DNA. This result is consistent with the conclusion reported by G. Li.[34] Therefore, the most possible factors that cause plasmid transformation of the conformation are the chemically active species. A single scission event can be made on either strand of the double-stranded plasmid, which will result in the release of torsional energy stored in the supercoiled form. The relaxation of the supercoiled plasmid results in the conversion of the relatively compact supercoiled plasmid into an open circle form. If another cleavage event occurs at or near the same location on the opposite strand, the open circle form will be converted to a linear polynucleotide.[35] It is known that, the restriction enzymes which can cleave the DNA at multiple or single special position, can transform the covalently closed circle plasmid DNA into the linear conformation. But the cleavage made by the plasma is random. The chemical structure changes of the plasmid lead to the DNA topology alteration.[36] With the treat time increase, the chemically active species may generate more scissions in the double helix, and the plasmid began to degrade.[37]

In conclusion, proper treatments of plasmid DNA by the plasma plume can change the DNA from supercoiled form to linearized or open circular form. However, according to experimental results by using the PCR technique, plasma treatments of the DNA do not affect the genes of plasmid.

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