Cell permeabilization using a non-thermal plasma

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Abstract. The atmospheric pressure glow discharge torch (APGD-t) is a miniature pulsed RF (13.56 MHz) plasma source specifically designed to efficiently produce and transport reactive species to and interact with biological samples. When applied to adherent cell cultures, the APGD-t is capable of inducing temporary cell permeabilization. In this work, we evaluate the maximum radius of macromolecules able to enter into HeLa cells following a plasma treatment using a size exclusion approach with dextran molecules. We observe that this maximum radius is below 6.5 nm. We also show that no degradation occurs when a plasmid DNA suspended in culture media is plasma-treated at the operating conditions leading to cell permeabilization. The plasma-assisted DNA transfection of the HeLa cells with the hrGFP-II-1 plasmid using the APGD-t is demonstrated. Local transfection efficiencies obtained are as high as 35%.

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1. Introduction

Low-power, cold (non-thermal) plasmas have been used for several years for modifying various surfaces to improve their biocompatibility. As typical examples of application, one can list various surface modifications promoting cell growth and adherence, wettability enhancement or passivation. Novel plasma sources are now being developed for the direct treatment of living biological entities such as cells, bacteria and spores. On the one hand, the lethal effect of the plasma is used and applications are found in the sterilization of medical devices and the deactivation of yeasts and bacteria [1]. On the other hand, the plasma is used to trigger and/or participate in complex biochemical processes leading to chemical and/or physical modifications of the live cells without causing necrosis. Pioneering work by Stoffels and her colleagues with the plasma needle [2] has demonstrated the ability of plasma to detach mammalian cells without causing necrosis, to deactivate bacteria and to activate surfaces [3]. The floating electrode dielectric barrier discharge (FE-DBD) developed by Fridman and his colleagues has been used to sterilize human skin without inducing damage or causing blood coagulation [4], to initiate apoptosis in melanoma cancer cell lines [5], to hasten blood coagulation [6] and to demonstrate the sterilizing effects of charged particles produced by the non-thermal plasma [7]. Our laboratory has developed a device capable of treating mammalian cells at power levels low enough to avoid necrosis. The atmospheric pressure glow discharge torch (APGD-t) (figure 1) [8] has been used to modify surfaces to promote cell adhesion and proliferation either by activation using an helium plasma [9] or through the deposition of plasma polymer tracks [10] and, in more subtle manners, to detach human endothelial cells (HAAE-1) from Petri dishes without causing necrosis [11]. In the context of the present work, mammalian cells were exposed to the APGD-t plasma stream and were temporarily permeabilized [9] thus opening the door to the transfer of foreign material into the live cells; i.e. a plasma-assisted cell transfection process. To our knowledge, only one other group has demonstrated the ability to transfect cells using a plasma [12, 13]. However, they only reported on the transfection capability of the plasma source and no attempt was made to identify the plasma-assisted cell transfection mechanisms.
To better understand the effects of non-thermal plasma on cell permeabilization, we evaluate the maximum size of dextran molecules that can be introduced into HeLa cells after exposure to the plasma afterglow produced by the APGD-t. Moreover, we investigate the effect of this plasma afterglow on the DNA sugar-phosphate backbone to verify the possible introduction of breaks in the plasmid DNA used in plasma-assisted cell transfection. Finally, mammalian cell transfection is demonstrated with this non-thermal atmospheric pressure plasma source.

2. Materials and methods

2.1. APGD-t

The APGD-t has been extensively described in a previous paper [8]. An atmospheric pressure plasma jet is formed by the injection of a plasma-forming gas (helium in the cell permeabilization work) inside an annular space defined by a central capillary electrode and a quartz confinement tube, itself coated with a conductive paste. The capillary electrode consists of a small, 0.007 in (0.1778 mm) ID (inner diameter) by 0.014 in (0.3556 mm) OD (outer diameter) stainless steel tube. The quartz tube has an ID of 2 mm and an OD of 4 mm in its straight section, and tapers to 500 $\mu$m ID downstream in the nozzle section. The downstream end of the capillary electrode is recessed by 1 mm inside the quartz nozzle. Helium is injected at a flow rate of 0.5 liter min$^{-1}$. No gas is injected in the capillary electrode. Square-wave amplitude-modulated 13.56 MHz RF power (100 Hz pulsations) is applied to the device at various duty cycles, thus producing power levels varying from approximately 1 to 10 W. Most experiments were conducted at 5% duty cycle, and a corresponding plasma power of 3.5 W. The end of the
The nozzle is positioned 3 mm away from the bottom of the Petri dish or well to be treated. Since the electrical field is confined inside the plasma device, the treated sample is only exposed to the plasma afterglow. A motorized X–Y platform is used to move the sample and treat each plate in a selected pattern. The speed of the motorized platform was set at 5 mm s\(^{-1}\). All operating parameters were controlled using the NI-LabVIEW\textsuperscript{TM} control interface.

### 2.2. Cell culture

HeLa cells (ATCC #CCL-2) and culture products were purchased from ATCC. The cells were cultivated in minimum essential medium eagle (MEME) supplemented with 10% fetal bovine serum, 100 µg ml\(^{-1}\) of streptomycin and 100 µg ml\(^{-1}\) of penicillin. Cells were expanded in 100 mm Petri dishes (Sarstedt #83.1802.003) and incubated at 37°C with 5% CO\(_2\). The day prior to cell treatment, the cells were plated at a density of 10\(^5\) cells per well in 12-well plates (Corning #3512) for green fluorescent protein (GFP) plasmid transfection studies and 3 × 10\(^5\) cells per 35 mm glass bottom culture dish (MatTek #P35G-1.0-14-C) for the dextran studies. The human recombinant GFP plasmid (hrGFP-II-1) was purchased from Stratagene (#240143) and produced in \textit{E. coli} DH-1 (ATCC #33849) competent cells. The plasmid was purified using Quiagen Plasmid Giga kits (#12191).

### 2.3. Dextran permeability

For the dextran molecule (the so-called dextrans) studies, the growth media was removed and 132 µl of fresh complete growth media containing 80 µg µl\(^{-1}\) of 3, 10, 40 and 70 kDa dextrans (Molecular Probes #D3308, #D22910, #D1829 and #D1818) was added to cover the glass section of the Petri dish. Immediately after plasma treatment, 1 ml of fresh media was added to the treated cells. The treated cells were rinsed three times with media and immediately imaged with a Zeiss confocal microscope (LSM 5 Exciter). The excitation wavelengths for the 3 and 70 kDa was 543 and 488 nm for the 10 and 40 kDa dextrans. The detection wavelengths for the 3 and 70 kDa dextrans spread between 560 and 615 nm, and between 505 and 530 nm for the 10 and 40 kDa dextrans.

### 2.4. Plasmid transfection

For the transfection studies, the growth media was removed and replaced by 400 µl of fresh complete growth media containing 20 µg of hrGFP-II-1 plasmid. Immediately after plasma treatment, 1 ml of fresh media was added to the well. Cells were incubated for 24 h before counting under fluorescence microscopy.

### 2.5. Effect of non-thermal plasma on DNA

Twenty five µl of plasmid DNA solution at a concentration of 0.05 µg µl\(^{-1}\) (using water, phosphate buffered saline (PBS) or MEME supplemented with 10% foetal bovine serum, 100 µg µl\(^{-1}\) of streptomycin and 100 µg µl\(^{-1}\) of penicillin as the resuspension medium) was put in a 96-well plate (Corning #3596). With the nozzle end positioned 3 mm above the bottom of the well, the samples were treated for 1–30 s at a power of 3.5 or 10 W. Control samples were
Table 1. Hydrodynamic radii of dextrans. Adapted from [14].

| Molecular weight (kDa) | Hydrodynamic radius (nm) |
|-----------------------|--------------------------|
| 9.5                   | 1.86                     |
| 39.1                  | 4.78                     |
| 73.0                  | 6.49                     |
| 110                   | 7.82                     |
| 250                   | 11.46                    |

also prepared. After treatment, 5 µl of each sample was analyzed by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining.

The DNA molecular weight ladder used in each gel was the TrackIt™ 1 kb DNA Ladder (Invitrogen #10488-072). For the digestion study, the plasmid was incubated with 10 units of restriction enzymes Hind III (Invitrogen #15207-12) and 10 units of EcoRV (Invitrogen #15425-010) in 1× REact® 2 (Invitrogen # Y92500) at 37 °C for 1 h. The resulting digested plasmid was analyzed by electrophoresis in a 1% agarose gel and visualized by ethidium bromide staining.

3. Results and discussion

3.1. Dextran size exclusion

Dextrans are glucan biopolymers consisting of several D-glucose molecules joined into chains of varying length. Dextrans have a high solubility in water and their structure when in solution consists of expandable coils. The average molecular weight of the dextran is a key property as it determines the average radius of the particles in solution. Table 1 presents the equivalent hydrodynamic radii of the dextrans used.

Dextrans are not able to freely diffuse through the cell membrane and therefore, can be used to reveal a plasma-induced diffusion process. A size exclusion study was performed using fluorescently labeled dextrans of different radii. Results from this study are presented in figure 2. It can be observed that the 3 and 10 kDa dextrans readily enter the cells only in the plasma treated area (figures 2(a) and (b)). Using the same excitation and detection intensities than for the 10 kDa dextrans experiment, a lower fluorescence intensity could be observed for the cells treated with the 40 kDa dextran (figure 2(c)). The larger size of the 40 kDa dextran (radius of ∼ 4.8 nm) in comparison with the 10 kDa (radius of ∼ 1.9 nm) seems to limit its diffusion through the pores created by the plasma treatment. To determine the maximum radius of the pores created, the experiment was repeated using a 70 kDa dextran having an equivalent hydrodynamic radius of ∼ 6.5 nm. Using the same excitation and detection intensities as for the 3 kDa dextran (since both dextrans have the same fluorochrome), no significant fluorescence could be detected. As a matter of fact, even with higher excitation fluorescence and longer detection time no significant fluorescence could be seen (data not shown). This suggests that the 70 kDa dextran was not able to diffuse inside the cells through the pores created, hence the maximum radius of the pores created is below ∼ 6.5 nm.
To assess the possible transfection efficiency that could be obtained using this technique, the complete plasma-treated pattern is presented in figure 3. It can be observed that the cells lying just outside of the plasma-treated track were not permeabilized and did not incorporate dextrans. However, most of the cells in the plasma-treated track did incorporate some dextran molecules. This possibility for localized cell permeabilization could lead to the application of this technique to localized cell transfection. These results also suggest that the mechanisms responsible for cell permeabilization are limited to where the plasma comes in close contact with the cells for the conditions we used.

These observations corroborate well with the two proposed mechanisms by which pores are created in the cell membrane: cell charging [15] and lipid peroxidation [9]. In the cell charging theory, the electrons streaming out of the plasma afterglow build up on the cell membrane creating a Coulomb force high enough to deform the cell from its ellipsoidal, attached form, to a partially detached, more spherical conformation. The deformation of the cells during the switching of conformation could create a shear stress high enough to partially disrupt the wall of the cell thus creating transient pores. Heavy charged particles (i.e. ions) of the plasma have been shown to play a major role in bacterial deactivation [7]. However, ions are mostly present in direct application of the plasma as these charged species rapidly recombine in the afterglow [16].

Figure 2. Confocal (a)–(c) and bright field (d)–(f) images of dextrans incorporated in HeLa cells following plasma treatment.
Therefore, ions are less likely to play a significant role in cell permeabilization using a device like the APGD-t, since the plasma forming region is confined inside the nozzle and the treated cells only exposed to the afterglow.

In the second theory, the reactive species of the plasma reaching the cell membrane react with the lipid bilayer and ultimately create transient pores. Lipid peroxidation may be initiated when any plasma-produced species having a reactivity sufficient to abstract a hydrogen atom from a methylene group reaches the cell membrane. The hydroxyl radical (OH•) is the most probable reactive species to initiate lipid peroxidation as it is readily produced by the APGD-t discharging in air [8]. OH• is known to react rapidly with organic molecules and for its ability to initiate lipid peroxidation [17]. The propagation of the lipid peroxidation leads to the cross-linking of the fatty acid side chain which can lead to formation of transient pores. Since OH• reacts rapidly with any molecule in its vicinity (hence, it will hardly diffuse), it could explain why there is no cell permeabilization outside of the plasma-treated track. It is also known that ultrasounds cause homolytic fission of H2O to H• and OH• [17]. Since the use of ultrasound to create transient pores in the cell membrane has been demonstrated [18], this seems to further confirm the theory for OH• participation in the plasma treatment.

The design of the APGD-t lends itself to another potential action on the cells. The relatively high gas flow used by the APGD-t to transport the reactive species could cause high local pressures and shear stresses onto the cells. These mechanical forces could also have an impact on cell permeabilization.

3.2. Non-thermal plasma effects on DNA

Reactive oxygen species, which are readily produced by the discharge of the APGD-t in air [8], are known to oxidize DNA bases in cells. DNA mutations can be generated when the oxidized DNA is not properly repaired by the cell. DNA sugar-phosphate backbone breaks are another...
Figure 4. Direct plasma treatment of the naked plasmid hrGFP-II-1 resuspended in water (a), media (b) and PBS (c). Lane L: molecular weight ladder. Lanes 0–30: plasma treatment at 3.5 W for 0–30 s. Lane 30*: plasma treatment at 10 W for 30 s.

The highly reactive plasma produced by the APGD-t on the foreign naked DNA to be inserted into the cells during transfection must be investigated. The use of an agarose gel is a standard and simple way to separate DNA by molecular weight. The hrGFP-II-1 plasmid used in this study is a small circular DNA construction comprising the minimum properties that can allow an autonomous GFP gene transcription in mammalian cells. Figure 4 shows the plasmid DNA integrity in water, culture media, and PBS as visualized after plasma treatment at various powers. In figure 4(a), 1.25 µg of hrGFP-II-1 plasmid in pure water was treated for different periods of time from 0 to 30 s using the same operating conditions applied in the dextran experiments (3.5 W). In lane 30*, a 30 s treatment at 10 W was used to overexpose DNA to the plasma. Under these conditions, the plasmid was almost totally degraded.

Without plasma treatment (lane 0), the hrGFP-II-1 plasmid is mostly in one conformation (the lower band). A less condensed circular form is also present (higher bands). After only 1 s of plasma treatment in water (figure 4(a), lane 1), a third lower band appears. This could be the result of DNA condensation. A smear under the principal bands is produced proportionally to the plasma exposure duration. This white shadow is most probably the result of a DNA degradation that produces fragments of DNA of varying lengths. This degradation shows that the water does not protect the plasmid from the deleterious effects of the plasma. In figure 4(b), the same experiment was repeated but using cell culture media instead of pure water as the resuspension medium. It can be observed that, in media, even after a plasma exposition of 30 s at 10 W, the DNA remains unaffected. This suggests that some components in the media are able to protect the plasmid DNA from plasma degradation. The media is mainly composed of a carbonate buffer with salts, amino acids, a phenol indicator and vitamins required for cell growth. The hrGFP-II-1 plasmid was treated using the same conditions but with the plasmid resuspended in PBS (figure 4(c)). The PBS reduces the plasma degradation on plasmid DNA when compared to water but some degradation is still present (figure 4(c), PBS, lanes 30 and 30*). This suggests that
the buffer is, at least in part, responsible for plasmid DNA protection. The effect of the composition of the resuspension medium on plasma degradation is not fully understood. The protective effect of the media could be a result of its different buffering capability, or the presence of an additional component. The presence of radical scavengers, such as vitamins (present in the media, but not in PBS) could help to protect the plasmid DNA from plasma degradation. The mechanism by which plasmid DNA suspended in water is degraded by the APGD-t is also not fully understood. The radicals, ionized molecules and/or free electrons produced in water by the plasma could attack the DNA sugar-phosphate backbone and lead to DNA breaks. This mechanism seems to be limited in PBS and not powerful enough to break DNA in culture media. Importantly, dextran incorporation and GFP transfection in the present work were done in culture media, thus decreasing the chances of DNA break during the process.

3.3. Plasma transfection

The dextran experiments suggest that there is a size limit to the entrance of macromolecules in plasma-treated cells. The hrGFP-II-1 plasmid used to study the effect of the plasma on DNA has 4856 base pairs (bp). The radius of this plasmid is estimated to be under 6 nm in its uncondensed form [19]. Because the plasma afterglow produced by the APGD-t does not seem to have degraded the plasmid DNA suspended in the culture media, a transfection experiment was performed on HeLa cells. The hrGFP-II-1 plasmid was resuspended in culture media to avoid the degradation by plasma. Twenty four hours after plasma treatment, the fluorescent cells were observed under a fluorescent microscope and precise locations were counted. Even though transfection was uneven around the track, some regions showed a transfection efficiency up to 35% (figure 5).

Plasmids from two different purifications were used for cell transfection. As can be observed with the result of the electrophoresis (figure 6(a)), one plasmid has a more compact conformation (A, lower band around 3000 bp) than the other (B, no lower band). To ensure that this band does not originate from a contamination, both plasmids were digested with restriction enzymes (EcoR V and Hind III). Since the hrGFP-II-1 plasmid possesses only one possible cleaving site for each restriction enzyme, two bands (780 and 4076 bp) appeared in figure 6(b) for each purification, thus demonstrating that both plasmids are identical. Therefore, the lower
Figure 6. Gel electrophoresis results for two different hrGFP-II-1 plasmid purifications. (a) Non-digested. (b) Digested with restriction enzymes EcoR V and Hind III. Lane L: molecular weight ladder. Lane A–B: plasmid purifications.

band in purification A can only result from a condensed conformation of its circular form. The diffusion of the more condensed and smaller form of the plasmid A inside the cell should be easier than that of the less condensed form B. The transfection experiment confirmed this hypothesis by demonstrating a two-fold increase in the transfection efficiency in the case of plasmid A when compared to plasmid B (data not shown).

Different techniques can be used to condense DNA like the modification of the ionic condition [19] or the use of poly-L-lysine [20]. These are already used to increase the DNA transfection efficiency in other techniques. The more condensed form of the plasmid DNA could act on the transfection efficiency in two ways. Obviously, a smaller plasmid means that a larger quantity of DNA will enter inside the cell when the pores are formed. Consequently, with more plasmid copies inside the cells, the probability of transfection increases accordingly. Unfortunately, the cell membrane is only the first barrier to overcome in cell transfection. A large percentage of the plasmid DNA never reaches the cell nucleus where it can be transcribed due to poor efficiency of intracellular trafficking and to the DNA degradation that takes place inside the cell [21]. Therefore, the more condensed form of the plasmid could also help increase the transfection efficiency by protecting the plasmid and facilitating the intracellular trafficking toward the nucleus. These arduous intracellular steps leading to transfection and ultimately, to the production of the green fluorescent protein, could also explain why the transfection obtained around the track was more uneven than in the case of dextran.

4. Conclusion

We reported that the APGD-t, a miniature atmospheric non-thermal plasma source producing an afterglow jet in helium, is capable of creating pores between 4.8 and 6.5 nm in radius in the cell membrane. Transfection of the hrGFP-II-1 plasmid in HeLa cells is possible with this device and local efficiencies up to 35% were observed. Moreover, the size of the DNA was shown to have an effect on plasmid-assisted transfection efficiency. We also confirmed that the non-thermal plasma can damage DNA. However, under very mild operating conditions (3.5 W with the present device), no degradation is observed. The further development of the APGD-t could lead to exciting new tools in biomedical research and clinical therapies.
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References

[1] Laroussi M 2005 Low temperature plasma-based sterilization: overview and state-of-the-art Plasma Process. Polym. 2 391–400
[2] Stoffels E, Flikweert A J, Stoffels W W and Kroesen G M W 2002 Plasma needle: a non-destructive atmospheric plasma source for fine surface treatment of (bio)materials. Plasma Sources Sci. Technol. 11 383–8
[3] Stoffels E, Kieft I E, Sladek R E J, van den Bedem L J M, van der Laan E P and Steinbuch M 2006 Plasma needle for in vivo medical treatment: recent developments and perspectives Plasma Sources Sci. Technol. 15 S169–80
[4] Fridman G, Peddinghaus M, Fridman A, Balasubramanian M, Gutsol A and Freidman G 2005 Use of non-thermal atmospheric pressure plasma discharge for coagulation and sterilization of surface wounds Proc. 17th Int. Symp. on Plasma Chemistry pp 1066–7
[5] Fridman G, Shereshevsky A, Jost M M, Brooks A D, Fridman A, Gutsol A, Vasilets V and Friedman G 2007 Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines Plasma Chem. Plasma Process 27 163–76
[6] Fridman G, Peddinghaus M, Ayan H, Fridman A, Balasubramanian M, Gutsol A, Brooks A and Friedman G 2006 Blood coagulation and living tissue sterilization by floating-electrode dielectric barrier discharge in air Plasma Chem. Plasma Process 26 425–42
[7] Fridman G, Brooks A D, Balasubramanian M, Fridman A, Gutsol A, Vasilets V N, Ayan H and Friedman G 2007 Comparison of direct and indirect effects of non-thermal atmospheric-pressure plasma on bacteria Plasma Process. Polym. 4 370–5
[8] Léveillé V and Coulombe S 2005 Design and preliminary characterization of a miniature pulsed RF APGD torch with downstream injection of the source of reactive species Plasma Sources Sci. Technol. 14 467–76
[9] Yonson S, Coulombe S, Léveillé V and Leask R L 2006 Cell treatment and surface functionalization using a miniature atmospheric pressure glow discharge plasma torch Proc. 17th Int. Symp. on Plasma Chemistry pp 1066–7
[10] Leduc M, Coulombe S and Leask R L 2009 Atmospheric pressure plasma jet deposition of patterned polymer films for cell culture applications IEEE Trans. Plasma Sci. 37 927–33
[11] Coulombe S, Léveillé V, Yonson S and Leask R L 2005 A miniature atmospheric pressure glow discharge torch (APGD-t) for local biomedical applications Pure Appl. Chem. 78 1147–56
[12] Sakai Y, Khajoee V, Ogawa Y, Kusuhara K, Katayama Y and Hara T 2006 A novel transfection method for mammalian cells using gas plasma J. Biotechnol. 121 299–308
[13] Ogawa Y, Morikawa N, Ohkubo-Suzuki A, Miyoshi S, Arakawa H, Kita Y and Nishimura S 2005 An epoch-making application of discharge plasma phenomenon to gene-transfer Biotechnol. Bioeng. 92 865–70
[14] Armstrong J K, Wenby R B, Meiselman H J and Fisher T C 2004 The hydrodynamic radii of macromolecules and their effect on red blood cell aggregation Biophys. J. 87 4259–70
[15] Stoffels E, Sakai Yama and Graves D B 2008 Cold atmospheric plasma: charged species and their interactions with cells and tissues IEEE Trans. Plasma Sci. 36 1441–57
[16] Laroussi M, Mendis D A and Rosenberg M 2003 Plasma interaction with microbes New J. Phys. 5 41

New Journal of Physics 11 (2009) 115021 (http://www.njp.org/)
[17] Halliwell B and Gutteridge J M C 2007 Free Radicals in Biology and Medicine (New York: Oxford University Press)

[18] Schlicher R K, Radhakrishna H, Tolentino T P, Apkarian R P, Zarnitsyn V and Prausnitz M R 2006 Mechanism of intracellular delivery by acoustic cavitation Ultrasound Med. Biol. 32 915–24

[19] Rybenkov V V, Vologodskii A V and Cozzarelli N R 1997 The effect of ionic conditions on dna helical repeat, effective diameter and free energy of supercoiling Nucleic Acids Res. 25 1412–8

[20] Mann A, Richa R and Ganguli M 2008 DNA condensation by poly-L-lysine at the single molecule level: Role of DNA concentration and polymer length J. Control. Release 125 252–62

[21] Vaughan E E, DeGiulio J V and Dean D A 2006 Intracellular trafficking of plasmids for gene therapy: Mechanisms of cytoplasmic movement and nuclear import Curr. Gene Ther. 6 671–81