Nonthermal Plasma Bio-Active Liquid Micro and Nano-Xerography

Gregory Fridman, Mengyan Li, Peter I. Lelkes, Gary Friedman, Alexander Fridman, and Alexander F. Gutsol

***Abstract*—Method of biochemical patterning which allows for micro- and nano-scale resolution on nonplanar substrates is presented. Utilizing this method, bio-molecules (including DNA, proteins, and enzymes) can be delivered to charged locations on surfaces by charged water buffer droplets. Charging of water droplets is accomplished using dielectric barrier discharge (DBD) plasma. DBD was effectively stabilized in the presence of high concentration of micron-size water droplets. The concepts of the proposed method, as well as first experimental results supporting the idea are discussed in this paper.**

***Index Terms*—Biomedical equipment, plasma applications, plasma materials-processing applications.**

I. Introduction

PATTERNING of different bio-chemical molecules on surfaces has many applications ranging from biosensors, used in genetic discovery and monitoring of dangerous toxins, to tissue engineering constructs where surfaces control tissue assembly or adhesion of cells. Most existing methods of biochemical patterning are suitable only for planar surfaces. In addition, micro- and nano-scale patterning often relies on complex sequences of lithography-based process steps.

Many methods are available for printing today. The most common ones, that led us to our idea, are inkjet printing and its derivative, electro-spray technology, and laser printing technologies. The disadvantages of inkjet printing technology are splashing caused by droplets accelerated to high speeds hitting target surface, low precision caused by diffusion, and, in many of the variations of the technology, like electro-spray, high dependence on chemical composition of the ink, i.e., printer having to be heavily modified to be able to print a slightly different substance. Laser printers address many of the issues brought up by Inkjets but raise many of their own; for example, toner particles undergo high mechanical strains that bio-chemical molecules and DNA are likely not to survive. There also is a multitude of issues associated with heating.

We are developing a method of biochemical substance patterning, or printing, which allows for micro and nano-scale resolution on planar and nonplanar substrates. Our goal is to print droplets of buffer containing bio-molecules (including DNA), peptides, and cells. We accomplish this by creating droplets of the molecules or cells in their respective buffer solution, then charging the droplets in dielectric barrier discharge (DBD) plasma, and finally delivering them onto substrate with charge prewritten onto the substrate via well-known xerography (micron-resolution) or charge stamping (nanometer-resolution) (Fig. 1).

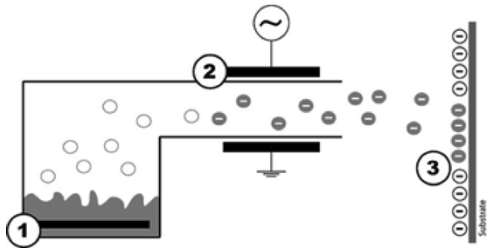

Fig. 1. Plasma "BioPrinter" prototype with ① droplet atomizer, ② DBD plasma reactor, and ③ charged droplet deposition substrate.

DBD was selected as the plasma source because of its lower volumetric power; we are able to charge droplets in a more "gentle" way than competing technologies and more powerful plasma sources [1], [5]–[7]. DBD is also a nonthermal discharge able to function under room temperature and pressure conditions which are required for survival of most bio-chemicals. In addition, DBD design allows us to charge large volumes and throughput more liquid than most available technologies resulting in faster printing. Finally, we have precise control of the droplet flow speed (carrier gas flow speed) and are able to virtually eliminate splashing problems that occur with other methods like electro-spray [2]–[5]. Slower droplet speeds also allow for better control over printing precision and rate.

II. Experimental Setup

Experimental setup and schematic illustrations of the Plasma Bio-Printer are presented in Fig. 2(a)–(c). Experimental process is as follows. ① Liquid is supplied by a variable flow mini-pump at 130 ml/h. ② Piezoelectric Ultrasonic Nebulizer (atomizer) then produces 1–4-μm droplets. ③ Aerosol flow into the system is controlled by the ball valve and ④ by supplying carrier gas into the Nebulizer. ④ Carrier gas is supplied in two locations to allow control of the flow rate as well as droplet concentration via gas dilution. ⑥ Air, Argon, or Helium can be used as carrier gases ⑤ which are cooled by liquid Nitrogen to ensure minimal water vaporization of droplets in the discharge. Our gas flow through plasma ranges from 1 to 2 L/min (4–8 cm per sflow speed through plasma gap), thus staying laminar with

Manuscript received August 30, 2004; revised February 13, 2005. This work was supported in part by the Ben Franklin Nanotechnology Institute and in part by the Defense Advanced Research Projects Agency (DARPA).

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Digital Object Identifier 10.1109/TPS.2005.848602

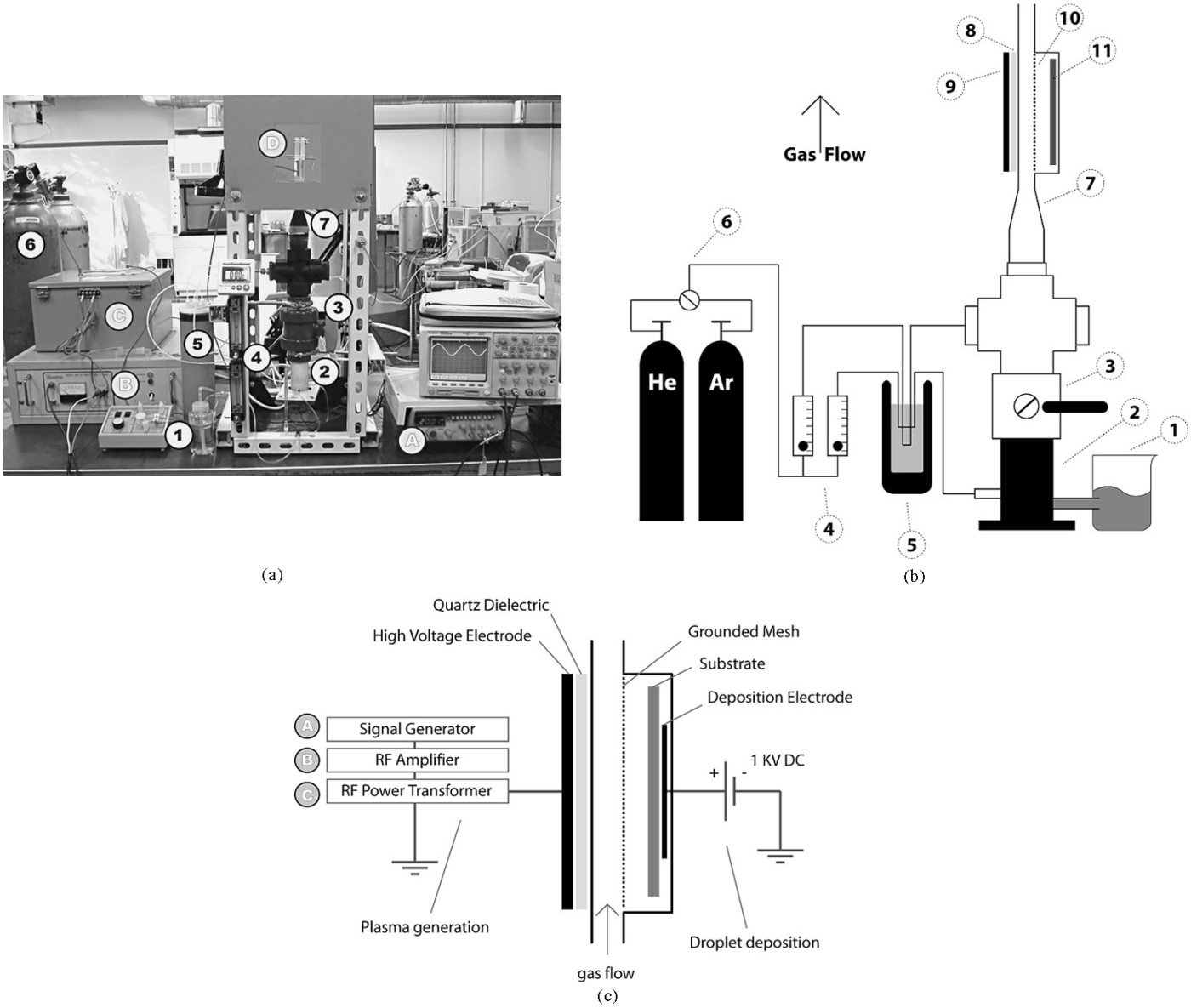

Fig. 2. (a) Plasma BioPrinter experimental setup. (b) Plasma BioPrinter setup schematic. ① Liquid supply (bottle and variable flow pump). ② Ultrasonic nebulizer. ③ Droplet flow control valve. ④ Gas flow control. ⑤ Liquid nitrogen cooler. ⑥ Carrier gas supply. ⑦ Flow conversion nozzle. ⑧ Quartz dielectric barrier. ⑨ High-voltage electrode. ⑩ Grounded mesh electrode. ⑪ Target substrate. (c) Printer head setup schematic with DBD plasma generator. Ⓐ signal generator. Ⓑ RF current amplifier. Ⓒ RF power transformer

low Reynolds number; ⑦ connection from Nebulizer to plasma is made in such a way as to ensure laminar flow. ⑧ Plasma region consists of a 40 × 80 mm gap with 1-mm-thick quartz dielectric. ⑨ Gold was thermally evaporated onto quartz to act as one electrode. ⑩ Second electrode is an open stainless steel mesh, ⑪ which provides us with the ability to extract charged droplets from plasma onto target substrate using an external electric field.

In plasma, droplets are charged to their "floating potential." A potential at which flow of electrons onto a droplet is balanced by the flow of positive ions. Bohm's sheath model [3], [8] allows us to estimate the floating potential as φ_{fl}

$$\varphi_{\text{fl}} = -\frac{T_e}{2}\ln\left(\frac{M_i}{2\pi m_e}\right) = -4.7\ \text{V} \tag{1}$$

where T_e is electron temperature, M_i is ion mass, and m_e is electron mass. For droplet of radius $r = 500$ nm, we can estimate its charge to be

$$q_{\text{drop}} = \varphi_{\text{fl}} c = \varphi_{\text{fl}} 4\pi\varepsilon_0 r \approx 1000 \text{ electrons} \tag{2}$$

where c is droplet capacitance and ε_0 is permittivity of free space. In DBD discharge in Argon at room temperature and pressure, number of electrons $n_e \approx 10^{12}$ cm^{-3} and their velocity $v_e \approx 10^8$ cm/s we can calculate electron flux [5], [8]:

$$\Phi_e = 4\pi r^2 \frac{n_e v_e}{4} \approx 10^5 \ \frac{\text{electrons}}{\text{s}}. \tag{3}$$

Thus, a 500-nm-radius droplet will gain 1000 electrons in $5 \cdot 10^{-3}$ s; and at the flow speed of 4 cm/s through plasma we can estimate that the droplet will be charged after $2 \cdot 10^{-2}$ cm

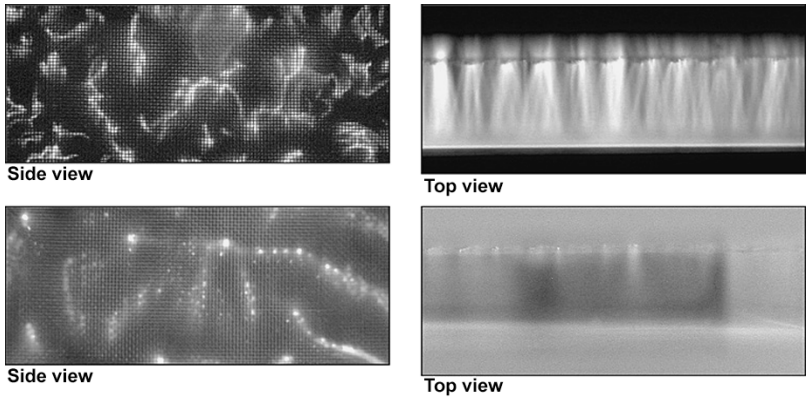

Fig. 3. DBD plasma with water aerosol at room conditions. Top: chilled Argon; bottom: chilled Helium.

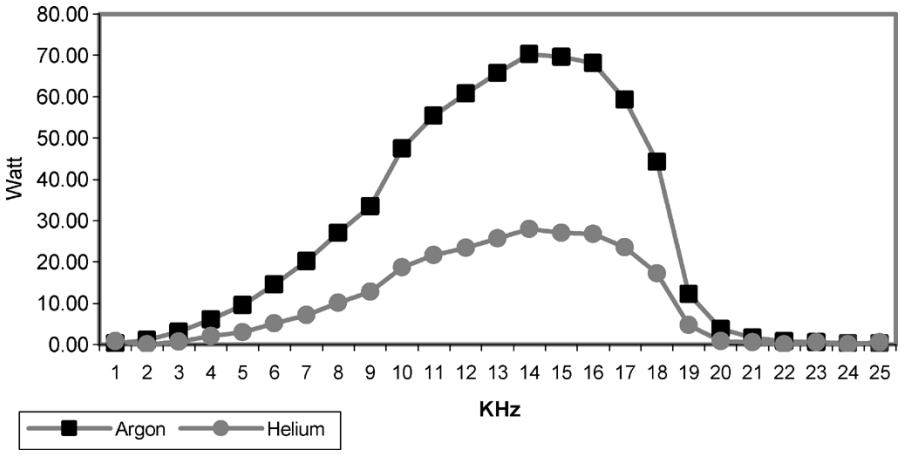

Fig. 4. Power of Argon and Helium discharges with water aerosol present.

in plasma (more details on DBD plasma modeling and particle charging dynamics can be found in literature [4], [5], [8]–[12]).

Piezoelectric crystal in Ultrasonic Nebulizer vibrates at 2.4-MHz generating droplets of 1.7-μm diameter on average at room temperature and pressure [2]. Sonozap Corporation, makers of the crystal, measured droplet diameters to be in the 1–4 μm range (via light scattering). We plan to repeat those experiments at our lab in the near future. Nebulizer liquid throughput of 130 ml/h and flow rate of 1 l/min yields droplet concentration of $7.8 \cdot 10^8$ droplets/cm^3, droplet-carrier gas mixture is very dense and evaporates very slowly at the time scales relevant to our system [2], [3], [7].

Our droplets are too small to feel the effect of gravity during their residence in the system [3], [7]. Neglecting the effect of gravity we notice that the only two forces are acting on the droplet: force exerted by the electric field pulling the droplet onto the substrate $F_{\rm el} = Eq$ (E is applied electric field, q is charge) and drag force exerted by the gas on the droplet $F_{\rm drag} = 6\pi\eta r v$ for low Reynolds number (η is dynamic viscosity, r is droplet radius, v is relative velocity) [2]. To find droplet's drift velocity let us equate the two forces

$$Eq = 6\pi\eta r v \Rightarrow E\varphi_{fl} 4\pi\varepsilon_0 r = 6\pi\eta r v \Rightarrow$$
$$v = \frac{2}{3}\frac{\varphi_{fl}\varepsilon_0}{\eta} E \ \frac{\rm cm}{\rm s}. \tag{4}$$

Thus, we observe that charged droplet drift velocity under the influence of electric field will not depend on the size of the droplet. This allows us to control droplets motion more precisely, as well as virtually eliminate splashing problems [2].

III. Results

We have demonstrated our ability to ignite and sustain DBD discharge in Argon and Helium in the presence of water aerosol (Fig. 3). The discharge operates at room temperature and pressure with power of 50–70 W in Argon and 15–25 W in Helium (Fig. 4).

We have successfully demonstrated our ability to negatively charge water aerosol by depositing it on a positively biased wire, as well as repelling it from a negatively biased wire [Figs. 5(a), 5(b)]. The results did not differ between Argon to Helium.

Additionally, we were able to demonstrate bio-active compound survival by printing Bovine serum albumin (BSA) onto a glass slide followed by fluorescein dye stain. BSA maintains its activity after passing through plasma (Fig. 6.)

IV. Conclusion

Ignition of atmospheric pressure discharge in dense Argon and Helium atmospheres with dense liquid aerosol at atmospheric pressure and temperature was demonstrated. Droplet charging was demonstrated by droplet deposition controlled

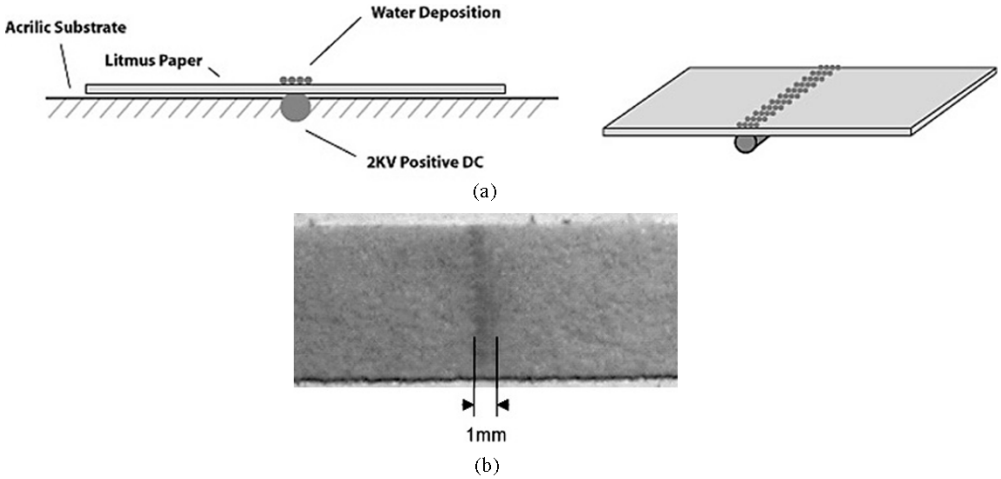

Fig. 5. (a) Schematic of the substrate for deposition on positive electrode. (b) Droplet deposition onto 1 mm positively biased electrode.

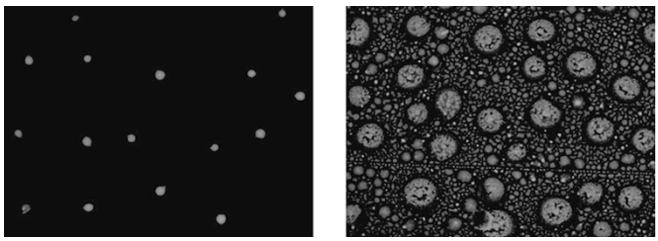

Fig. 6. Bovine Serum Albumin (BSA) fluorescein dye stain after deposition. (Left) 15 s treatment. (Right) 2 min treatment.

by electric field. Additionally, it was demonstrated that DBD plasma in noble gases (under the discussed conditions) has little, if any, influence on bio-active compounds such as BSA (small protein). Detailed investigation of DBD plasma inactivation of various protein and plasma influence on enzymatic activity of various bio-chemicals is being currently performed. Nano-precision is theoretically possible but was not yet demonstrated. Our plans for the near future include fabrication of charge-stamping setup that will provide us with nano-scale electrodes for charged droplet deposition.

ACKNOWLEDGMENT

The authors would like to thank K. Sieber of the Eastman Kodak Company for his assistance with power supply construction.

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