Non-Thermal Plasma BioPrinter with Nano-Scale Precision

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Abstract

We have developed a method of biochemical patterning which allows for micro and nano-scale resolution on non-planar substrates. In this method, bio molecules (including DNA) are 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. Discussed in this paper are theoretical estimations as well as experimental data.

1. Introduction

Patterning of different biochemical 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. Two most common ones, that led us to our idea, are Inkjet and Laser printing technologies. Inkjet sprays ink droplets onto paper (Fig. 1) while in a Laser printer toner particles are charged and then deposited onto paper in the pre-charged locations. Disadvantage of Inkjet printing technology is 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 electrospraying – 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. Toner particles undergo high mechanical strains that biochemical molecules and DNA are likely to not survive. There also is a multitude of issues associated with heating.

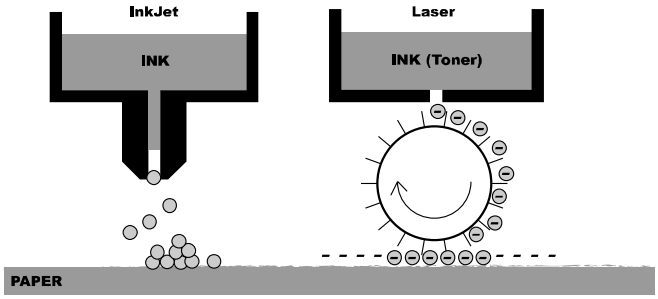

Fig. 1. Inkjet and Laser printing technologies as prototypes of the Plasma BioPrinter

After looking at the existing state-of-the-art technologies, we have developed a method of biochemical substance patterning, or printing, which allows for micro and nano-scale resolution on non-planar substrates. In this method we utilize ideas of both Inkjet and Laser printing technologies as well as a popular derivative of Inkjet printing – electrospraying technology. Our goal is to print biomolecules (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 pre-written charge (Fig. 2). DBD was selected as the plasma source because of its lower power – we are able to charge droplets in a more "gentle" way than competing technologies and more powerful plasma sources [1]. DBD is also a non-thermal discharge which is required for survival of most biochemicals. In addition, DBD design allows us to charge larger 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 electrospraying. Slower droplet speeds also allow for higher precision control in printing.

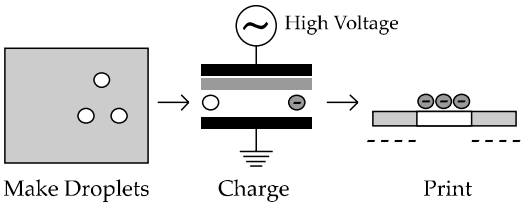

Fig. 2. Plasma "BioPrinter" prototype

2. Experimental setup

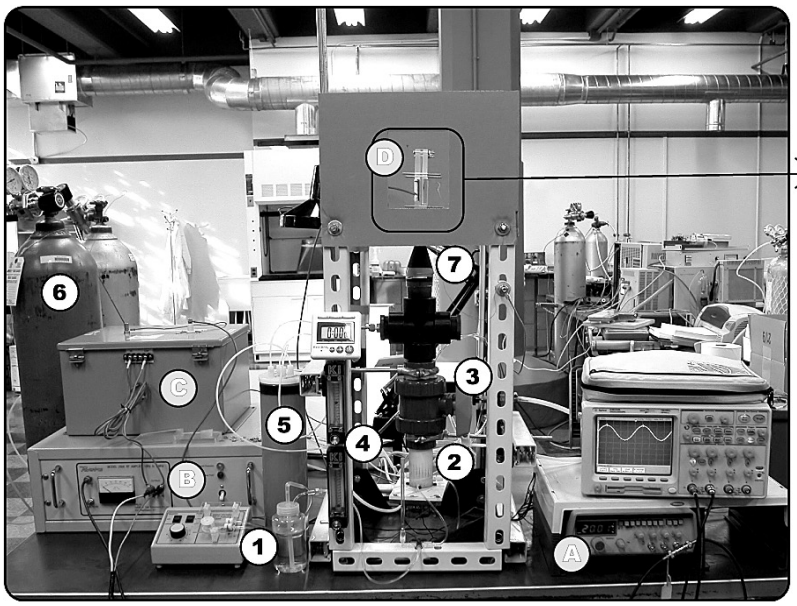

Fig. 3.1. Plasma BioPrinter Experimental Setup

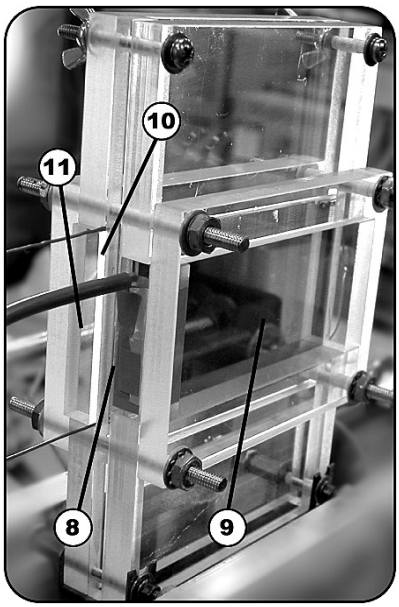

Fig. 3.2. Plasma Discharge & Printer Head

Plasma Generation

10KV 20KHz AC is generated by:

A. Signal generator (sine, triangle, square waveform)
B. High frequency current amplifier
C. Power transformer

Voltage is then applied to:

D. Printer head

Experimental Setup

1. Liquid supply (bottle and variable flow pump)
2. Piezoelectric "Ultrasonic Nebulizer"
3. Droplet flow control valve
4. Carrier gas flow control
5. Liquid Nitrogen cooler
6. Carrier gas supply
7. Flow conversion nozzle

Printer Head

8. Quartz dielectric
9. High voltage electrode
10. Grounded mesh electrode
11. Target substrate

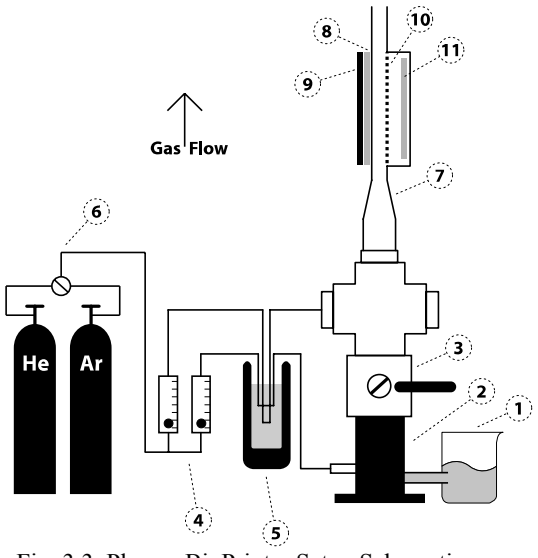

Fig. 3.3. Plasma BioPrinter Setup Schematic

Schematic illustration of the Plasma BioPrinter is presented in Fig. 3.3 (all number are same on Fig. 3.1, 3.2, and 3.3). Experimental process is as follows. Liquid is supplied by a variable flow mini-pump at 130ml per hour (#1). Piezoelectric "Ultrasonic Nebulizer" (atomizer) then produces 1 up to 4 micrometer droplets (depending on the surface tension of the supplied liquid) (#2). Droplet flow into the system is controlled by the ball valve (#3) and by supplying carrier gas into the Nebulizer (#4). Carrier gas is supplied in two locations to allow for control of the flow speed as well as droplet concentration (#4). We have ability to test the system with air, Argon, or Helium as carrier gases (#6) which are cooled by liquid Nitrogen to ensure minimal water vaporization (#5). Our gas flow through plasma ranges from 1 up to 2 litters per minute (4 up to 8 cm per second in plasma), thus staying laminar with low Reynolds number; connection from Nebulizer to plasma is made in such a way as to ensure laminar flow (#7). Our plasma region consists of a 40x80x1 mm quartz dielectric barrier (#8). Gold was thermally evaporated onto quartz to act as an electrode (#9). Second electrode in an

open stainless steel mesh (#10) which provides us with the ability to extract charged droplets from plasma onto target substrate (#11) using an external electric field.

3. System parameters and estimations

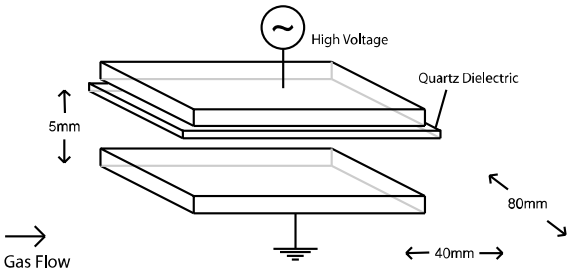

Fig. 4. Dielectric Barrier Discharge (DBD) plasma setup

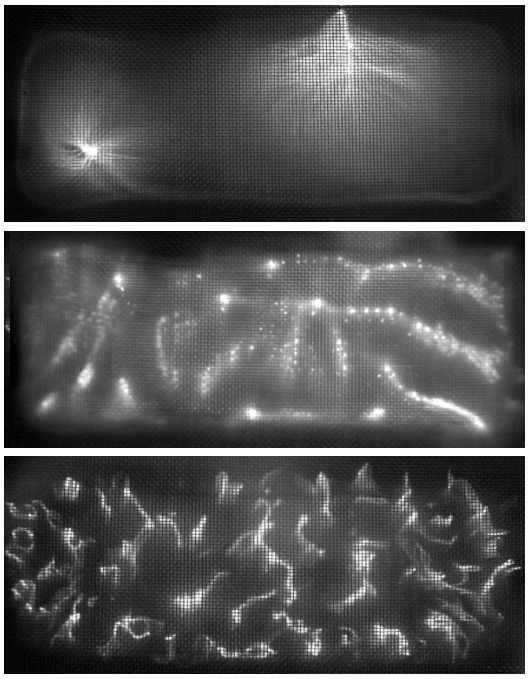

Fig. 5. DBD in (top to bottom) dry Argon, Helium with water aerosol and Argon with water aerosol

Dielectric Barrier Discharge plasma is non-thermal, atmospheric pressure discharge. Water is an electro-nagitive liquid – water molecules arrange themselves in such a way that negative ends of the droplets are pointing outward and positive ends – inward, thus forming a charge layer close to surface, creating a potential drop between the layer itself and the outside [1]. DBD plasma is electron + positive ion gas so we have an abundance of charges to shield the potential drop thus charging the droplet negatively. Thus, water, in the ionized gas, will charge to its "chemical" potential that has been measured to be $\Delta\varphi = 0.26V$ [1]. In plasma, ion collisions are inelastic and ions easily transfer their kinetic energy to the surrounding gas. Electron collisions are very elastic due to electron's small size, and thus electrons give up their energy slower. Ion temperatures are close to those of the surrounding gas while electrons are far more energetic. When droplet enters plasma region it gathers electrons for its potential is initialy different from that of the plasma immediately surrounding it; thus the droplet attracts electrons, gaining negative charge. Positive ions start flowing to the negatively charged droplet, attracted by the newly created electric field, and balance the flow of energetic electrons, shielding the droplet from plasma. Some electrons, however, are energetic enough to overcome the barrier and stay on the droplet. As a result, droplet gets charged to a certain potential, refered to as "floating potential" – a potential at which flow of electrons is balanced out by the flow of positive ions. Bohm's sheath model, describing behavior of a Langmuir probe, allows us to estimate the floating potential as φ_{fl} (4.7 V at room temperature and pressure in electron + positive ion gas):

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

For a r=500nm droplet we can estimate its charge to be:

$$q_{drop} = \varphi_{fl} c = \varphi_{fl} 4\pi\varepsilon_0 r \approx 10^{-16} C \approx 1000 electrons \tag{2}$$

In DBD discharge at room temperature and pressure in Argon number of electrons $n_e \approx 10^{10} 1/cm^3$ and their speed $v_e \approx 10^{10} cm/\sec$ we can calculate electron flux [5]:

$$\Phi_e = 4\pi r^2 \frac{n_e v_e}{4} \approx 10^5 \ 1/\sec \tag{3}$$

Thus r=500nm droplet will gain 1000 electrons in approximately $5*10^{-3}$ seconds; and at our flow speed (4cm/sec through plasma) we can estimate that the droplet will charge after 0.02cm in plasma.

Some of the parameters specific to our plasma setup: plasma frequency:

$$\omega_e = \frac{1}{2\pi}\sqrt{\frac{n_e e^2}{\varepsilon_0 m_e}} \approx 10^9\, Hz \tag{4}$$

Debye radius:

$$\lambda_{de} = 7434\sqrt{\frac{T_e}{n_e}} = 9.1*10^{-5}\, m \tag{5}$$

With average droplet diameter of approximately d=1.7*10^{-6}m we can estimate distance between droplets D=9.2*10^{-6}m. Plasma non-ideality parameter Γ_d, designed to measure strength of interaction between dust particles in plasma (or droplets), shows us whether plasma will be closer to ideal state – complete chaos, or if droplets will "feel" each other forming structures. Estimating non-ideality parameter by a model with a shielded Debye potential, also known as Yukawa model, we obtain [1,4]:

$$\Gamma_d = \frac{Z_d^2 e^2 n_d^{1/3}}{4\pi\varepsilon_0 T_d} e^{\left(-1/n_d^{1/3}\lambda_{de}\right)} = 3.63*10^4 \tag{6}$$

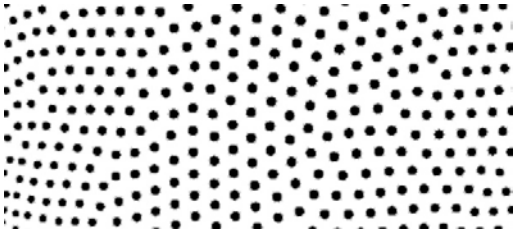
Fig. 6. Coulomb Crystals

In our estimations we came over an interesting fact – many parameters signify to us that plasma will be in highly non-ideal state; i.e. Coulomb Crystals will form. Fig. 6 shows modelled droplet Coulomb Crystal formation. To verify this let us look at the Debye radius λ_{de} (5) and at the distance between droplets. Debye radius signifies distance at which droplets "feel" each other and we can see that it is an order of magnitude higher than our average distance between droplets. Plasma non-ideality parameter (6) also indicates a similar trend: when $1 \le \Gamma_d << 171$ plasma is considered to be in or close to ideal state but as Γ_d approaches 171 and higher plasma is said to be non-ideal and the order should be observed. Γ_d for our system is a few magnitudes higher than for ideal plasma [3]. Fig. 7 shows non-ideal behaviour of streamers in plasma: in both Argon and Helium with and without water and at varying power levels streamers "feel" each other especially in dry cold Argon where they are completely immobile and do not react to even significant changes in gas flow speed.

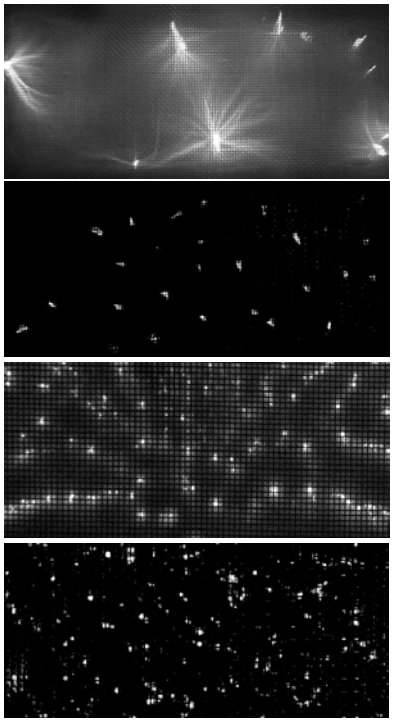
Fig. 7. Streamer patterns in (top to bottom) dry Argon, Argon in water aerosol, dry Helium, Helium in water aerosol.

Let us get back to droplets. Piezoelectric crystal in Ultrasonic Nebulizer vibrates at 2.4MHz generating droplets of mostly 1.7 micrometer diameter [2]. Depending on the liquid surface tension average droplet diameter at room temperature and pressure is:

$$d = 0.73\left(\frac{T_{liquid}}{rf^2}\right)^{1/3} \tag{7}$$

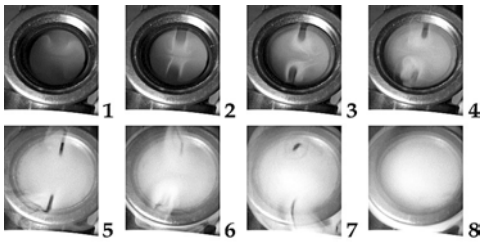

Fig. 8. Droplets exiting Nebulizer

Sonozap corp., makers of the crystal, measured droplet diameters to be in the 1 to 4 micrometer range (via light scattering). Assuming pure water we can estimate droplet concentration to 7.8*10^8 droplets per cm^3. Droplet plus carrier gas mixture is very dense and evaporates very slowly. Fig. 8 shows droplets exiting the Nebulizer at the flow rate of 1 litter of gas per minute (time interval between photos is 1/3 second).

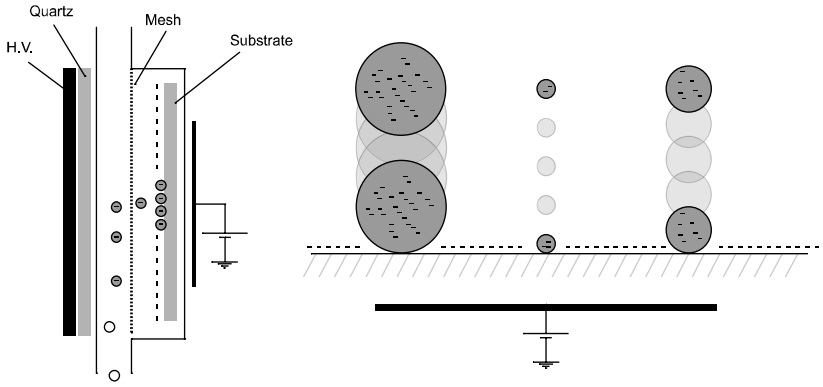

Fig. 9. Charged Droplet Deposition

Our droplets are too small to feel the effect of gravity in the time intervals that concern us [2,3]. Neglecting the effect of gravity we notice that the only two forces acting on the droplet are force exerted by the electric field pulling the droplet onto the substrate and drag force exerted by the gas on the droplet. To find droplet's terminal velocity let's equate Eq (force exerted by electric field E on charge q) and $F_{drag} = 6\pi\eta r v$ (for low Reynolds number or Stoke's flow) [2]. Since we know that droplet charge $q = \varphi c = \varphi 4\pi\varepsilon_0 r$ we can solve for speed:

$$Eq = 6\pi\eta r v \Rightarrow E\varphi 4\pi\varepsilon_0 r = 6\pi\eta r v \Rightarrow v = \frac{2}{3}\frac{\varphi\varepsilon_0}{\eta}E \tag{8}$$

Thus we observe that charged droplet speed under the influence of electric field will not depend on the size of the droplet. This allows us to control droplets much more precisely as well as virtually eliminate splashing problems.

4. Acknowledgements

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