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Fabrication challenges and perspectives on the use of carbon-electrode dielectrophoresis in sample preparation

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Abstract: The focus of this review is to assess the current status of 3D carbon-electrode DEP (carbonDEP) and identify the challenges currently preventing it from its use in high throughput applications such as sample preparation for diagnostics. The use of 3D electrodes over more traditional planar ones is emphasized here as a way to increase the throughput of DEP devices. Glass-like carbon electrodes are derived through the carbonization of photoresist structures made using photolithography. These biocompatible carbon electrodes are not ideal electrical conductors but are more electrochemically stable than noble metals such as gold and platinum. They are also significantly less expensive than common electrode materials, both in terms of material cost and fabrication process. CarbonDEP has been demonstrated for the manipulation of microorganisms and biomolecules. This review is divided in three main sections: 1) carbonDEP fabrication process; 2) applications using 3D carbonDEP; and 3) challenges and perspectives on the use of carbonDEP for high throughput applications.

1. Introduction

Dielectrophoresis (DEP) is a well-known technique for particle manipulation that has enabled a number of applications such as sorting, patterning, and filtering[1–4]. The technique exploits the interaction between an electrical dipole induced on polarizable particles and an electric field gradient. Particles can be made to move towards a maximum (positiveDEP) or minimum (negativeDEP) gradient value depending on the polarizability differences between the targeted particle and the suspending media. Furthermore, a given particle suspended in a given media can display both DEP behaviors as the frequency of the field changes. The use of different materials or combination of them, including electrodes, electrical insulators, conductive liquids and composites, and fabrication approaches have led to the emergence of a number of techniques to implement DEP such as metal-electrode DEP, carbon-electrode DEP, insulator-based DEP and contactless DEP. These and other techniques have been recently reviewed and compared by this author [5] and hence out of the scope of this

paper. As expected, no single technique is universal and each of them present advantages and disadvantages depending on the application sought. Briefly, carbon-electrode DEP compares advantageously to metal electrodes in terms of fabrication and device costs as well as electrode stability. When compared to insulator-based DEP, the use of carbon electrodes allows for the use of only tens of volts, instead of hundreds, to polarize the DEP devices and less probability of inducing Joule heating in the sample during experiments.

The focus of this review is to assess the current status of 3D carbon-electrode DEP (carbonDEP) and identify the challenges to its use in high throughput, practical applications such as sample preparation. Sample preparation is currently expensive, both in terms of cost and time, and there is a critical need for approaches that are capable of processing large sample volumes to rapidly extract and concentrate targeted particles. The use of 3D electrodes over more traditional planar ones is emphasized here as a way to increase the throughput of DEP devices. After enlarging the cross section of a microfluidics channel to increase flow rate, traditional planar electrodes positioned on the surface of these enlarged channels cannot affect the particles flowing in the bulk of the channel. In contrast, 3D electrodes as high as the channel and dispersed through its cross section can. A further consideration is that when increasing the cross section, a taller channel is preferred over a wider one to keep the device footprint as small as possible. This review is divided in three main sections: 1) current fabrication processes; 2) demonstrated applications using 3D carbonDEP; and 3) challenges and perspectives of carbonDEP for high throughput applications.

2. Current Fabrication of carbon-electrode Dielectrophoresis devices

Heat treatment of an organic precursor in an inert atmosphere, a process generally known as pyrolysis, yields a carbonaceous material known as glass-like carbon, or also by its well-known trademark names of glassy carbon and vitreous carbon [6]. This carbon is amorphous, with a structure featuring many interlaced carbon ribbons [7]. It is characterized as glass-like because of the conchoidal nature of its fracture. This material is also electrically conductive, featuring an electrical resistivity of 1×10^{-4} Ω/m when pyrolysis is performed at temperatures around 900 °C in an atmosphere such as nitrogen[8]. Such resistivity is similar to that of indium tin oxide but four orders of magnitude more than Cu. Nonetheless, it still allows for the use of low-voltage sources, <20 V_{pp}, to implement a practical DEP force when the gaps between electrodes are in the order of tens of micrometers. A well-known advantage of glass-like carbon over other electrode materials such as Au or Pt is that it is more electrochemically stable. This allows for a stronger DEP force when using carbon electrodes

since a higher voltage can be applied without electrolyzing the sample. In terms of materials, a polymer precursor for glass-like carbon work is significantly less expensive that noble metals. For example, SU-8 is a negative-tone photoresist commonly used as precursor and its current cost is in the order of USD$1 per ml ($1.2/g accounting for average SU-8 density). A gram of gold or platinum for evaporation are around $60 and $50 respectively [9]. Although these prices can vary based on the supplier, quantity purchased and market, they remain an order of magnitude more expensive than carbon precursors.

The complete process to fabricate carbonDEP devices is illustrated in figure 1 and has been detailed elsewhere [10, 11]. The important steps are 1) photolithography of SU-8, 2) carbonization and 3) coupling of the carbon electrodes in the microfluidics networks. SU-8 photolithography is implemented in two steps: a) fabrication of planar interdigitated fingers that will become carbon connection leads to the base of the 3D carbon electrodes and b) fabrication of SU-8 geometries that will become the 3D carbon electrodes. A current limitation of this fabrication process is the choice of substrate. Since SU-8 carbonization is performed at around 900 °C, current substrates are limited to silicon, silicon oxide or fused silica (sapphire could be used as well but it is more expensive). Once a 3D SU-8 topography is fabricated, it is introduced in a tube furnace and heat treated under an inert atmosphere, usually nitrogen. The carbonization process features the following stages: a) a temperature ramp from room temperature to 200-300 °C at 10 °C/min followed by a 30 minute dwell at 200 °C, to allow for any residual oxygen to be evacuated from the chamber and prevent combustion of the polymer as the temperature is raised further; and b) a temperature ramp from 200 to 900 °C at 10 °C/min with a one-hour dwell at 900-1000 °C. The samples are then naturally cooled down to room temperature. Near-isometric shrinkage occurs during pyrolysis and is especially important to DEP because the gaps between carbon electrodes are wider than those fabricated in SU-8. After pyrolysis, the carbon electrodes and the areas in between are de-scummed using oxygen plasma to eliminate any carbon residues between the electrodes that could lead to an electrical short-circuit during experiments. In an optional step, a thin SU-8 layer of around 2 μm can be patterned around the carbon electrodes to electrically insulate the connection leads and to planarize the surface around the base of the electrodes. The fabrication sequence presented often results in dense arrays of carbon electrodes with height ~100 um which effectively increase the cell capacity/square centimeter of carbonDEP devices. Furthermore, the process is highly reproducible given the use of commercial precursors like SU-8 and the use of photolithography for patterning. Shrinkage is currently characterized after carbonization and is also highly reproducible for a given carbonization process. Ongoing work is on further understanding the impact of different pyrolysis parameters on shrinkage and the properties of the carbon

electrodes. The aim is at a design tool that provides guidelines on the SU-8 structure to make, and how to carbonize it, in order to get carbon electrodes of specific dimensions and properties.

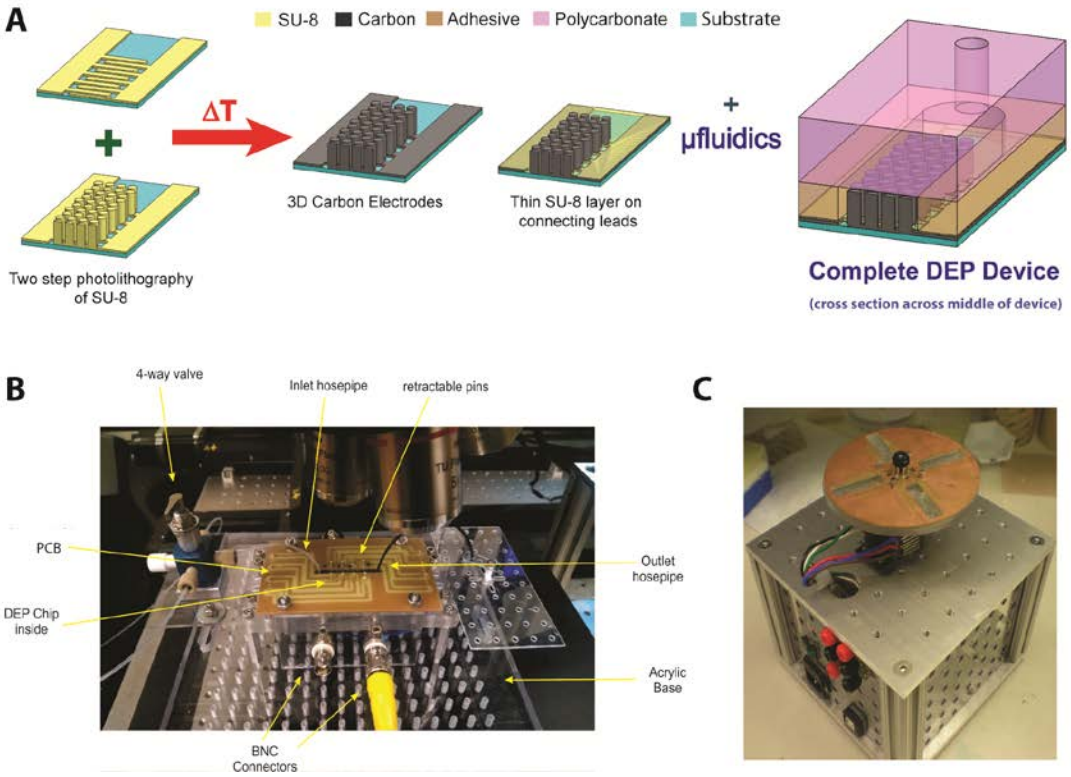

Fig. 1. *Fabrication of carbonDEP devices: a) Fabrication of carbon electrode array and microfluidics network. Two different experimental platforms used in carbonDEP: b) Typical experimental setup when using a syringe pump and c) one based on centrifugal microfluidics. The size of the disc is that of a common music CD. Note the absence of tubing, instead the carbonDEP chips are mounted in one of the four compartments on the disc and the sample is moved thanks to centrifugal forces. The signal from a function generator is routed to the chips through a slip ring, visible in the figure under the disc.*

The microfluidic channel that contains the electrodes is fabricated separate and currently out of pressure-sensitive double-sided adhesive (PSA) and polycarbonate (PC). It is important to note that the following procedure can be used for other DEP approaches, not only for carbonDEP. A microchannel is first cut from an adhesive laminate using a cutter-plotter and is aligned to the channel inlet and outlet, which had been previously drilled in a PC substrate. The carbon electrode array is then manually aligned with the PSA-PC stack such that the electrode array is contained inside the microfluidic channel. Experimental devices are then sealed using a rolling laminator. The advantages and limitations of this process have been recently explored [12]. Although channel widths less than 200 μm are not possible with a general purpose cutter, the use of patterned adhesive has allowed for very rapid prototyping that is inexpensive and independent from a cleanroom. Moreover, based on this author's experience the PSA approach yields devices that are less prone to leaks at high pressure than

microchannels made using soft lithography, and bond by plasma treatment; and also than SU-8 channels bonded to their cover with SU-8 [11]. If needed, the connection between chip and syringe pump is implemented by pressure-fitting 1/16" OD tubing into the channel inlet and outlet and sealing the joint with general purpose 2-part epoxy glue. Commercial connectors, such as NanoPort™ or connectors fabricated in-house, can also be used but these approaches add to the cost of the device and are not absolutely necessary. If centrifugal microfluidics is used in combination with carbonDEP as previously demonstrated [13], no tubing is required.

3. Selected Applications of carbonDEP

This section will provide a succinct overview of selected applications enabled by carbonDEP. Their common denominator is the use of carbonDEP in sample preparation, by concentrating and purifying a targeted population from a sample. All these applications were implemented using an experimental setup similar to that shown in Fig. 1B and an experimental protocol which featured three main stages: 1) extract targeted particles from the flow using positiveDEP to trap them on the electrodes; 2) wash the particles by holding them on the electrodes against a flow of washing solution; and 3) turn the electrodes off to release the targeted particles and collect them at the exit of the channel. Four applications are presented next: a) elimination of natural contaminants to increase sensitivity of PCR-based protocols [14], b) enrichment of bacterial persisters from an antibiotic-treated sample [15]; c) trapping of lambda DNA [16], towards future protocols for enriching biomolecules in diagnostic applications; and d) extraction of a diluted yeast cell population from large sample volumes [17]. These results attest to the potential of carbonDEP in sample preparation once the challenges presented in the next section are overcome. Other works with carbonDEP that are not detailed here include the use of a syringe pump-based platform to purify an *E. coli* population [18] and to implement rapid electrical lysis of yeast and mammalian cells [19]. CarbonDEP was also coupled to centrifugal microfluidics to implement a yeast viability assay [13]. Such experimental setup is shown in fig. 1C. The significance of this last work was a proof of concept of a system that can lead to an automated, self-contained platform that does not require complicated setup, *i.e.* no tubing, connectors, syringe pumps, and will enable a user-friendly sample-to-answer platform.

3.1 Increasing the sensitivity of PCR-based protocols

An important challenge in PCR protocols is to eliminate inhibitors of polymerase that may be present in the biological/environmental sample such as bile salts in faeces, heavy metals and humic substances in soil, collagen in food samples, heme in blood, phenolic compounds, and proteinases in milk [20–22]. Different approaches to overcome PCR failure due to inhibitors present in the sample are constantly being developed but the unfortunately the list of inhibitory substances is rather large. Strategies to overcome PCR inhibition have included sample-washing steps, density gradient centrifugation, gel electrophoresis, column chromatography and even the use of additives such as bovine serum albumin [21–24]. However, these methods tend to be cumbersome, time demanding and often expensive.

3D carbonDEP was demonstrated as a module to prepare samples prior to PCR processing [14]. The objective was to extract a viable yeast population from a flow, trap it on the electrodes and wash off any inhibitors using an inhibitor-free buffer. Comprehensive details of this work can be found elsewhere. A stock of *S. cerevisiae* yeast cells was first grown following standard protocols and then partitioned into two sets of samples. Each of these was spiked with a different concentrations of humic acid, which is the most prevalent PCR inhibitor in soils and natural surface waters [22, 25]. Concentrations ranged from 1 to 100 ug/ml. Control experiments were first done using only PCR, no DEP, to determine the limit of detection. Results are reproduced here in figure 2A. Yeast cells were only detected when the concentration of humic acid was less than 10 ug/ml. The other set of samples spiked with humic acid was then subjected to a DEP protocol before PCR. Once the viable cells are trapped on the electrodes, Sabouraud broth was used as the washing buffer. Upon turning the field off, the washed cells were released, collected from the DEP chip and run through the exact same PCR protocol than before. Results are shown in fig. 2B, starting with the concentration of 10 ug/ml. The importance of this work was the removal of humic acids from a sample, an important step that could enable PCR detection of targeted organisms in an environmental sample. The use of the DEP protocol allowed for an increase on sensitivity from 10 to 75 ug/ml in an assay that took 40 min for a 200 ul sample. Another important advantage of DEP is that discriminating between viable and non viable cells is relatively straightforward. This capability reduces the possibility of false positives in PCR when using a DEP protocol, since only viable cells are fed to the PCR analysis stage.

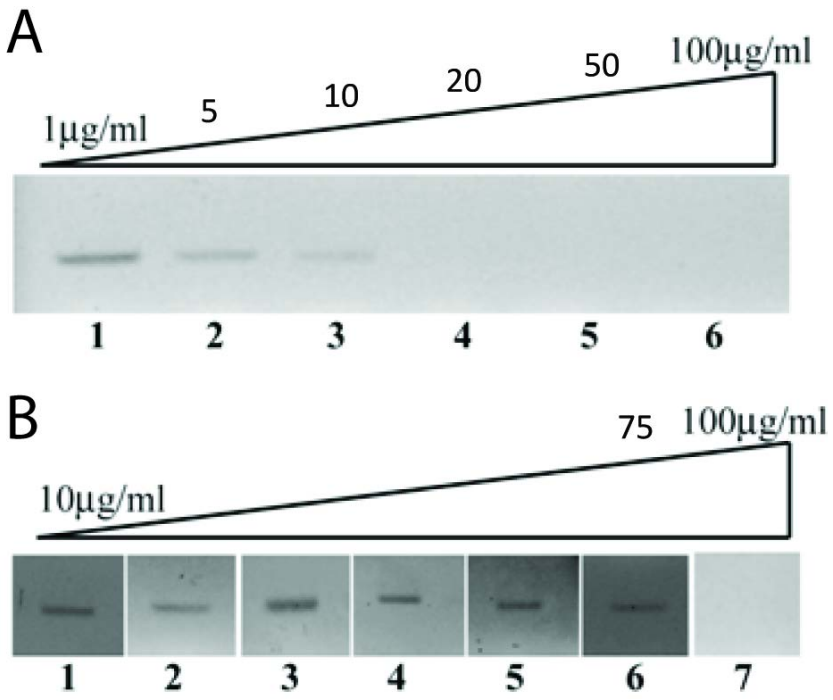

Fig. 2*. PCR results demonstrating A) the inability of only PCR analysis to clearly detect yeast cells when the concentration of humic acids in the sample is above 10 ug/ml and B) significant improvement obtained when using a carbonDEP-based sample preparation module before PCR analysis: humic acids concentrations up to 75 ug/ml are effectively removed from the sample to enable yeast detection. From (23).*

3.2 Enriching bacterial persisters after antibiotic treatment

Bacterial persistence is thought to be responsible for treatment failures, post-therapy relapses, and lengthy treatment regimens in diseases such as leprosy and tuberculosis. Besides making it difficult to treat infections, the persistence phenomenon may also increase the probability of emergence of genetic resistance, thus contributing to the short lifespan of antibiotics after they reach the market. Therefore, there is a pressing need for new experimental tools to address the phenomenon of bacterial persistence. Purification would facilitate the characterization of these subpopulations using conventional ‘omics-based approaches [26]. While fluorescence-activated cell sorting (FACS) is the most common enrichment technique and provides high-throughput fractionation of cell populations, this technique requires cells to be differentially labeled, which could potentially change the phenotype of the organism and hinder downstream analysis. 3D carbonDEP was used for the isolation and purification of bacterial cells remaining viable after a 24-hour treatment with isoniazid (INH), a frontline anti-tuberculosis drug. *M. smegmatis* was used in this work since it is a model organism to investigate

mechanisms of dormancy or drug-cell interactions in mycobacterial infections, such as tuberculosis. The objective was a tool that allows for purification of a large-enough population of targeted cells to enable its analysis using proteomics and transcriptomics[15].

The results obtained demonstrated DEP-based enrichment of intact *M. smegmatis* cells from a mixed input population of INH-treated cells comprising about 90% intact cells and 10% damaged cells. After following an experimental protocol similar to the one presented above, the intact cell population was enriched from 90 to up to 99% purity and recovered from the carbonDEP chip. Importantly, up to $3X10^4$ intact targeted cells were recovered per assay. Taking into account that most of the 'omics-based approaches for downstream analysis of a purified population require a sample containing at least 10^5-10^6 targeted cells, serial assays could provide the user with enough material.

3.3 DNA concentration

The DEP behavior of DNA under the influence of an electric field gradient within a carbon electrode array has also been studied[16]. Similar carbon electrode geometries than those used in the previous applications were used to trap YOYO-labeled λ-DNA immersed in a phosphate buffer with electrical conductivity of 187 uS/cm. The goal of this work was to demonstrate the capability of carbonDEP to trap biomolecules. This last consideration is important towards the development of a carbonDEP-based sample preparation module capable of 1) purifying a targeted bioparticle population; 2) conduct electrical lysis on such population [19]; and 3) concentrate the DNA and other internal organelles extracted during lysis.

DNA concentration by positiveDEP was observed at frequencies between 10 and 50 kHz. However, it was concluded that a frequency of 10 kHz provides the most rapid and strongest concentration of DNA around the carbon electrodes (Fig. 3A). NegativeDEP was observed at 75 kHz (Fig. 3B) while no effect was observed at frequencies beyond 200 kHz. An experimental protocol similar to that described above was then implemented for the concentration and retrieval of λ-DNA. The electrode array was polarized using a sinusoidal signal with a frequency of 10 kHz and magnitude of 16 V_{pp}. The flow rate in the channel was 2 µl/min. Results are shown in Fig. 3C. As in the case of the previous two applications, the elution profiles are divided into three phases: 1) 'sample', where the DNA is extracted from the solution and excess volume collected at the outlet; 2) 'washes', when pure buffer is flushed through the channel; and 3) 'eluates', indicating the potential polarizing the electrodes has been turned off causing the release of the targeted particle. As evidenced by the peak of higher

DNA concentration after the field is turned off, DNA was retained on the carbon electrodes against the flow as long as the electrodes were polarized.

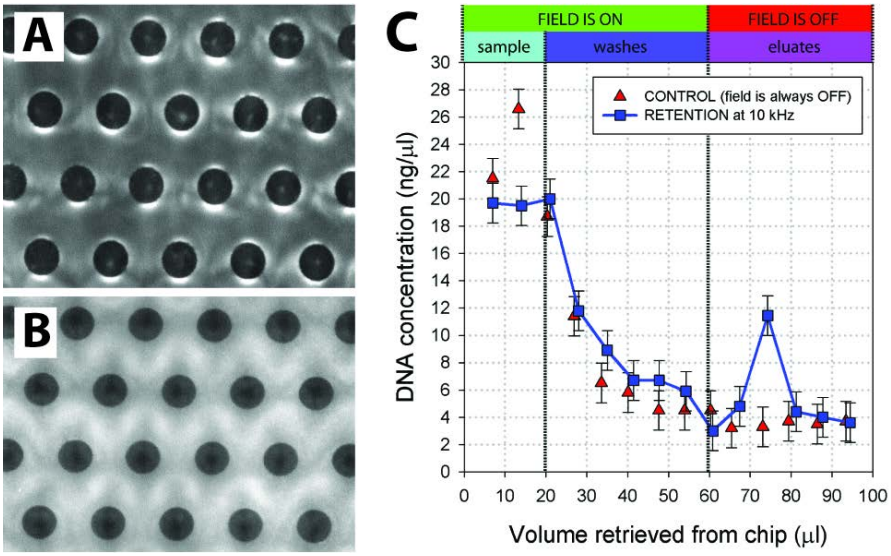

Fig. 3. *A) λ-DNA tagged with YOYO-1 trapped around the carbon electrodes (dark circles) using positiveDEP at 10 kHz. B) λ-DNA tagged with YOYO-1 positioned in the field gradient minima using negativeDEP at 75 kHz. C) Experimental results showing the trapping (diminished concentration of DNA with respect to control in the first fractions retrieved from the chip) and release of DNA for collection at the end of channel (blue peak with respect to control immediately after the field is turned off).*

3.4 Purification of a diluted cell population from large sample volumes

In recent work, carbonDEP was used to process up to 4 ml of a sample featuring a diluted yeast cell population, down to 10^2 cells/ml. As before, the aim was at trapping, washing and recovering an enriched, purified cell fraction that would facilitate downstream analysis. Islam et al demonstrated a method to trap the few cells present in the original 4 ml volume, wash them and re-suspended in much smaller volumes, ~20 ul. In such a way the cell concentration of a sample was increased from 10^2 to 10^4 cells/ml[17]. These results are significant in the context of timely diagnostics of the cause of clinical sepsis, food and environmental contamination. For example, prompt diagnosis of the pathogen behind a suspected infection can enable the administration of the correct treatment to eradicate it before it results in life-threatening conditions such as sepsis. Although current detection techniques such as mass spectroscopy, PCR and a number of biosensors are mature to detect a specific pathogen with high specificity[27–29], they require an idealized sample. This is, a sample volume of few microliters with a pathogen concentration of at least $10^{3\text{-}4}$ copies/ml suspended in media that is friendly to the analytical technique to be used. Sample preparation is currently expensive, both in terms

of cost and time, and there is a critical need for approaches that are capable of processing large sample volumes to rapidly extract and concentrate those few particles that resemble the targeted particle. For example, when looking for *E. coli*, extracting all particles with diameter 0.5-2 um from few milliliters of sample. Using an experimental protocol similar to that detailed in the sub-sections above, a maximum enrichment of 154.2 ± 23.7 was implemented using carbonDEP. In this case, a diluted yeast cell population was extracted from up to 4 ml of sample when the flow rate in the channel was 10 ul/min, leading to assay times of 6 to 7 hours. This time can be considered long but is significantly shorter than the 24-48 hours currently required to detect a pathogen in a diluted sample. Although the experimental conditions used in this work were idealized and still far from practical application, the objective was an initial proof of concept and using such experimental data to validate a methodology that will eventually enable *a priori* design of future devices. For example, to optimize electrode geometry, gaps between electrodes, and positioning of the electrodes within the microfluidics channel.

4. Challenges and Perspectives of carbonDEP in high throughput applications

DEP has been shown to discriminate many types of particles from a background but it has been mostly done at throughputs that are too low to enable practical application in sample preparation. The first step to increase the throughput of a microfluidics device is to expand its cross section A such that the flow rate Q can be increased without increasing flow velocity V (recall the mass continuity equation, $Q=VA$). An increase of A instead of V is desired because the DEP force competes directly with the hydrodynamic drag force acting on the particle, which is dependent on V when implementing a Stokes flow. Furthermore, an increase on channel height rather than width is desired to maximize the number of devices per square area of the fabrication substrate. Once the cross section is increased to improve throughput the next step is to maintain efficiency. DEP is a short range phenomena and addressing all particles flowing in an expanded cross section becomes a challenge. It is here where 3D electrodes have a significant advantage over traditional planar ones. In comparison to planar electrodes that only address particles flowing close to the channel surface, 3D electrodes span the complete channel cross section and ensure that all particles experience a DEP force. The use of 3D electrodes over more traditional planar ones has proven to be a viable way to increase throughput, as long as the electrodes span the whole channel cross section. Hence, the challenge is to push the limits of current fabrication techniques, or innovative ones, to make the electrodes as high as possible while maintaining narrow gaps between them. Besides carbon electrodes, few other approaches have been implemented to fabricate 3D micro electrodes

including electroplated metals[30, 31], metallized polymer or silicon structures [32–34] and casting of conductive resins[35]. Although not made using microfabrication, other 3D electrodes have been implemented for DEP. Besides the wire electrodes used in DEP experiments before the 1990s, the works by Fatoyinbo et al [36], Park et al [37] and Abidin et al [39] are good examples of elegant metallic 3D devices. In particular, the approach of Fatoyinbo and colleagues of drilling chambers through intercalating layers of metal and insulator so to create electrodes throughout the periphery of the chamber has led to commercial devices for rapid cell characterization (3DEP 3D Dielectrophoresis Cell Analysis System commercialized by Labtech.com). When compared to other microfabrication techniques, the fabrication of carbon electrodes features less steps and/or is less expensive than all of them. For example, carbon electrodes are obtained from carbonized photoresist following a photolithography process that is a mere step in other fabrication sequences. The patterning of metal or other conductive materials is usually done in three ways: etching or lift-off for thin films and electroplating for thicker layers. Regardless of the choice, a sacrificial polymer mask or mold must be implemented; while these structures can already be used as carbon precursors. The carbonization process implemented in the fabrication of carbonDEP devices compares advantageously to metallization in that it does not require high vacuum, expensive metals or expensive infrastructure such as an evaporator or sputterer. On the other hand, carbonization is a longer process (only when compared to thin metal films though), requires high temperatures, and the shrinkage of the polymer precursor presents a challenge to fabricate very small gaps between electrodes. Casting of conductive resins is in principle a more straightforward solution than carbonDEP, but the fabrication of a master mold that features extensive arrays of small electrodes, like those employed in carbonDEP with narrow gaps between them, and is robust enough to enable several molding cycles is a significant challenge. Silicon has been a material of choice to make micromolds, and in this case the mold is fabricated using non-trivial approaches that can quickly make it very expensive. Furthermore, the casting operation may not yield highly reproducible results given the complexity of casting a relatively thick resin in a mold with very small cavities. These problems would not be present when casting macro electrodes with a surface exposed to the channel containing the targeted particles, but these are no different than surface electrodes and their disadvantages.

Although highly reproducible for a specific precursor material and pyrolysis protocol, shrinkage of the precursor structure during carbonization is an important challenge in carbonDEP because it reduces the mechanical stability of the electrodes and also widens the gap between them. Two competing parameters can be taken into account: electrode aspect ratio (EAR) and gap aspect ratio (GAR). Electrode aspect ratio is given

by the height of the electrode over its diameter. High aspect ratio electrodes, tall and slender, are less stiff than short, fat ones when made with the same material and when both feature the same cross section. In DEP, one requires the electrodes to not touch each other and generally aims at having a uniform gap throughout the complete height of the electrodes. Thus, a certain degree of stiffness is desired. The cross section of the electrode, circular, triangular, etc. can also be tailored to increase the moment of inertia of the structure and prevent bending[11]. Current DEP devices feature an electrode height of around 100 um with an aspect ratio between 2 and 4. The shrinkage during carbonization is near isometric and depends on the ratio between surface area and volume of the polymer precursor[38]. A structure featuring a high ratio shrinks more than one with small ratios since there is more area available for material evaporation during carbonization. The vast majority of electrodes used so far in carbonDEP featured 200 um-high, 50 um-diameter SU-8 posts which featured a 50% shrinkage.

In contrast to EAR, the GAR is given by the ratio between the electrode height and the gap between them. Since the gap between electrodes is a direct determinant of the DEP force, increasing the GAR is more important than EAR to achieve high throughput DEP. Unfortunately, the fact that SU-8 shrinks during carbonization makes increasing the GAR a significant fabrication challenge. For example, the 100 um-high, 50 um-diameter carbon electrodes with a gap between them of 50 um are obtained through the carbonization of 200 um-high, 100 um-diameter SU-8 posts with 15 um gap. The GAR in the precursor SU-8 structures is around 13 but decreases to 2 after carbonization because of the significant shrinkage of the precursor.

When increasing the height of the electrodes is the main objective, GAR and EAR must be optimized simultaneously. Addressing each of them individually is a mistake. For example, increasing the height of the electrodes can be relatively straightforward if their cross section is increased accordingly to maintain an aspect ratio and mechanical stability. Unfortunately, increasing the cross section will also increase the gap between electrodes after carbonization. Hence, the challenge is to fabricate very tall and slender structures that have a very narrow gap between each other. The main determinant is the desired gap between carbon electrodes. This gap will change depending on the application so to use as low polarizing voltage as possible but without creating an opportunity for physical clogging to occur. It is also important to note that the electrodes cannot be too slender or an electrical resistance will develop along their height that will impact the uniformity of the electric field gradient throughout the height of the channel.

Ongoing work is on optimizing the fabrication of different cross sections to increase the moment of inertia of the precursor structures and improve their mechanical stability during the fabrication process. The goal is to

fabricate arrays of SU-8 posts featuring a diameter of 50 um, height of 1 mm and a gap of 10 um between them (EAR=20, GAR=100). Such EAR has been demonstrated before in SU-8 and the main challenge will be the GAR. Special care must be taken on the thickness uniformity of the SU-8 layer during exposure, as air pockets between mask and SU-8 layer introduce undesired light diffraction that leads to broadening of the SU-8 structures. A shrinkage of 50% is expected when carbonizing the targeted SU-8 structures which will yield electrodes of 500 um height and gaps of 60 um. Based on previous results, these gaps will allow for microorganism manipulation as well as mammalian cells.

A challenge to reducing the cost of carbonDEP devices to a level where they can be disposable is the cost of the substrate. Both the precursor SU-8 and infrastructure used for carbonDEP fabrication are not particularly expensive and the main cost is currently on the high temperature substrate that is required for carbonization. Few materials survive the temperatures required during the carbonization of SU-8 ($\geq$ 900 °C) and these tend to be relatively expensive. Choices include quartz, sapphire and aluminum oxynitride if a transparent substrate is required. Silicon coated with silicon oxide as electrical insulator can be used if transparency is not a must. The preferred materials so far have been silicon/silicon oxide and fused silica, given their price and availability. Ongoing work is on using alumina as a substrate to enable disposable devices given its very low cost, and thermal and insulating properties.

Besides these fabrication challenges specific to improving the throughput and cost of carbonDEP devices, a significant challenge to all DEP techniques is the need for low electrically-conductive media to suspend the particles. The dielectrophoretic force is directly dependent on the polarizability difference between the targeted particle and the suspending media. Hence, the use of low polar media is a requisite if a positive DEP behavior from the particle is desired. In general, a positiveDEP force is significantly stronger than negativeDEP and a more active manipulation scheme can be implemented with it. Unfortunately, most biological samples and the media currently used for cell culturing are highly polar due to the increased concentration of salts. This makes the processing of “real-world” samples challenging when using DEP. Re-suspension of such samples in a media that is optimized for DEP is possible and widely implemented using a sedimentation, washing and re-suspension protocol using centrifugation. The DEP buffer features a low electrical conductivity and a tailored pH and osmolality. Although dilution of the original sample with DEP buffer is more straightforward than re-suspension, the former leads to an undesired, significant increase of the sample volume. The sample preparation protocols used in the applications presented above benefit from centrifugation and re-suspension protocol to disperse the cells in a buffer optimized for DEP. In the case of bacteria and fungi, re-suspension is

not expected to lyse or cause significant changes on the organisms; which allows for many potential applications such as diagnosis of infection, food and environmental contamination. In these cases, lysis of all other cells can even be implemented to simplify the problem to the discrimination of bacteria and fungi from cell debris. Furthermore, given the diagnostic nature of these applications, any consequences on the reproduction and progeny of the cells can be omitted. However, the story changes when considering less robust cells such as mammalian cells. In those cases, the choice of media can have a tremendous impact on the immediate survival of the cells. A well-validated DEP buffer used for mammalian cells is a sugar solution using diluted physiological media, which features a conductivity of 500 uS/cm[1, 2, 40]. This buffer has enabled the processing of blood cells and stem cells using positiveDEP. Lu *et al* have shown such solution to be safe for stem cells as long as the cells are exposed to a DEP force for less than 1 minute. No impact on the genetic content and the progeny of the cells were observed in this case. Cell exposure to DEP forces for more than 1 minute but less than 5 yielded no changes in the genetic content but an affected progeny[40]. Hence, care must be taken when designing DEP devices, regardless of the technique, to tailor the exposure of cells to the DEP force: for diagnosis, an experimental window that does not cause immediate changes in the population may be adequate, but this window must be reduced to under a minute if those cells, or most likely their progeny, will be later used for therapy applications. Under this view, the use of DEP focusing instead of trapping will be a more beneficial solution for therapeutics. Ongoing work is on using 3D carbon electrodes to implement streamingDEP, which is the fact of eluting the targeted population in characteristic streams to facilitate their recovery at specific locations (see fig. 4 for an example when using latex particles).

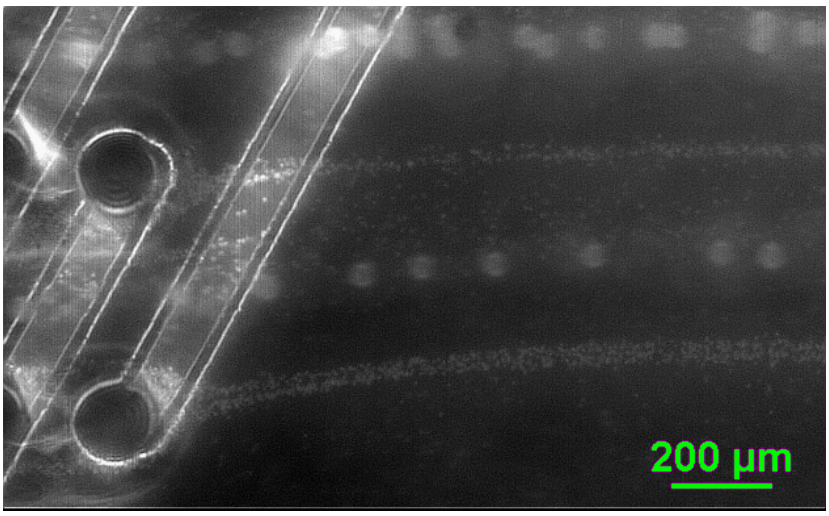

Fig. 4. *Example of streamingDEP when separating 1 um-diameter latex particles from 10 um-diameter ones. StreamingDEP works when the hydrodynamic drag force in the channel overcomes the trapping DEP force. Hence, particles are only focused into specific streams which remain linear throughout the channel thanks to the laminar flow present. In this case, positiveDEP is exerted on the small particles which attracts them to the electrode and hence are eluted in lines collinear with the carbon posts (dark circles). The 10 um-diameter particles experience negativeDEP and are focused and eluted between the carbon electrodes. Experiment performed using a polarizing frequency of 100 kHz, flow rate of 10 ul/min and using DI water as suspending media.*

5. Conclusion

CarbonDEP has been demonstrated for the concentration and purification of microorganisms and DNA. The current experimental protocol shows promise as a sample preparation module in diagnostic applications such as finding the cause of sepsis and food and environmental contamination. CarbonDEP offers advantages over other DEP techniques since 1) the cost of materials is relatively low; 2) glass-like carbon is a biocompatible material that is more electrochemically stable material than noble metals often used in DEP; 3) the fabrication process is relatively straightforward and highly reproducible; and 4) carbonDEP does not require high voltage power sources for operation. Disadvantages include 1) the higher electrical resistivity of carbon when compared to metals; 2) the cost of the substrate and 3) the challenge of making small gaps due to shrinkage of the precursor during carbonization. Further work is needed to increase the throughput of carbonDEP devices and reduce their overall cost in order to become a viable practical solution in sample preparation for diagnostic applications. 3D carbon electrodes have also been demonstrated for cell lysis[19] and optimization of the fabrication process has been shown to yield suspended carbon wires for potential use in biosensors[41]. These facts are encouraging towards a carbon-based lab-on-a-chip (LOC) where integrated preparation of few hundred microliters of sample can be performed. We envision a series of arrays optimized in their shape, dimensions and electrical polarization to achieve different functions. For example, one to use DEP to enrich a targeted population, the next to lyse them and extract their intracellular components, one more DEP stage to enrich a targeted molecule or organelle and a last one to detect to specify the target using carbon elements functionalized with aptamers or bacteriophages. It is important to note that it is highly unlikely that carbonDEP, or any other DEP technique from the point of view of this author, is able to process tens of milliliters of the so-called “real world” samples, such as whole blood and urine.. The likely solution is an integration of DEP with other techniques more amenable to process very large sample volumes such as centrifugation, acoustophoresis [42, 43] or inertial

microfluidics [44, 45]. A major disadvantage of DEP is its dependence on the electrical polarizability of the media. Biological samples tend to have high electrical polarizability thus hindering cell separation since all cells are likely to experience a similar negativeDEP force. By using centrifugation or acoustophoresis before DEP, all cells can be rapidly re-suspended in a media optimized for DEP. The niche for DEP is fine separation using the signature given to the cells by their radius and membrane topography, not only the radius or density as with other techniques. The niche for 3D carbonDEP can be at performing such separation at high throughput and using robust and inexpensive devices. The goal is a step-by-step process to enrich and purify the targeted cells from several milliliters of "real-world" sample into just few microliters in the most rapid way possible to enable timely diagnostics.

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