Dual Helix Dielectrophoretic Cell Separator Fabricated Using the Direct-Write Spindle Deposition Technique

N. Guérin, M. Lévesque, D. Therriault

Laboratory for Multiscale Mechanics, Mechanical Engineering Department, École Polytechnique de Montréal, Montréal, QC, Canada

ABSTRACT

This paper reports the fabrication of a 3D cell separator fabricated using the direct-write spindle deposition technique. This device is designed to use insulator-based dielectrophoresis as a driving force for the separation of particles. The curvature of its helix-shaped channel produces the inhomogeneous electric field that is the basis of dielectrophoresis. A 3D helix-shaped separator presents advantages compared to reported 2D dielectrophoretic separator. The constant curvature of its channels gives it a better efficiency than a 2D planar spiral-shaped separator for a given trajectory path length. This device is fabricated by extruding sacrificial filaments on rotating spindles which are assembled and joined to inlet and outlet reservoirs. Once encapsulated in epoxy, the filaments are melted and removed in order to create channels. This novel approach allows the fabrication of complex 3D shapes and shows potential for the miniaturization of medical testing equipment.

Keywords: cell separator, dielectrophoresis, direct-write, helix, 3D

1 INTRODUCTION

Cell separation is a key step in many biomedical procedures and is usually accomplished by large and expensive equipment[1] which limits the possibility of testing samples in the field. The miniaturization of diagnostic equipment would facilitate medical testing at the point of care.

Dielectrophoresis (DEP) is a promising technique for cell separation and many cell separators based on it have been reported[2-4]. When polarizable particles are subjected to an inhomogeneous electric field, a nonzero force is applied on them. If different types of particles have different electrical properties, thus different polarizabilities, DEP can separate them. In many DEP separators, the inhomogeneous electric field is created by the shape of the electrodes applying the voltage. This method has limitations such as the heating of the fluid through the Joule effect and the possible apparition of bubbles due to electrolysis of the medium. Both can influence the flow of the medium and render the separation inefficient.

Those problems are overcome by the use of insulator-based DEP, or iDEP in which the electric field's inhomogeneities are created by the geometry of the separation zone in the device. With this method, the electrodes can be placed in the reservoirs containing the particles and medium, away from the zone where the separation takes place. This distance prevents the Joule heating and electrolysis from disrupting the separation process.

A few promising devices that rely on iDEP have been proposed[2-4]. These devices use different geometries in order to create an inhomogeneous electric field, which is

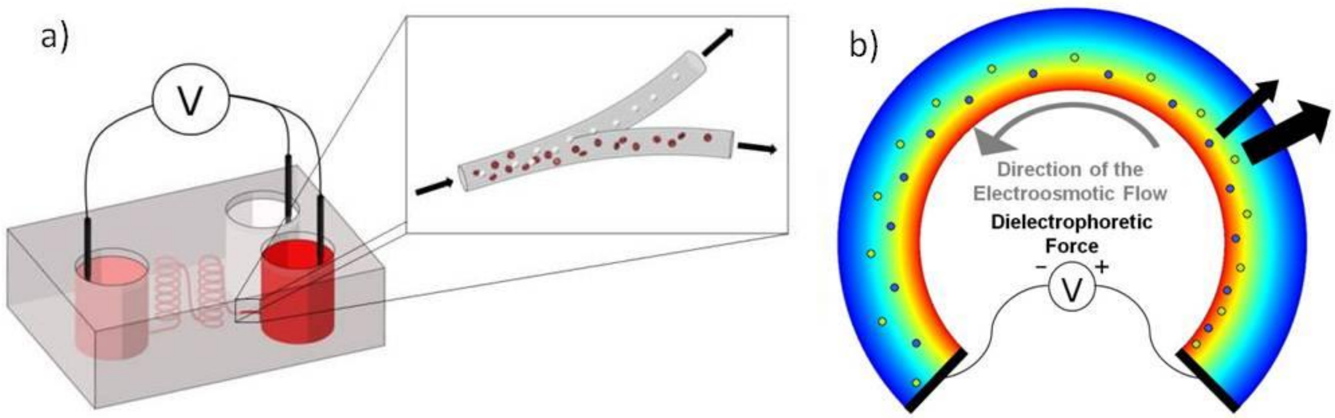

Figure 1 – a) Illustration of the proposed 3D cell separator. The inlet (left) contains white and red blood cells that are separated by the application of the voltage (V). The two outlets (right) contain each type of cells separated. b) Schematic of the principle of operation of the separator. The black arrows represent the dielectrophoretic forces that separate the particles. The gray arrow represents the direction of the electroosmotic flow, which makes the particles circulate in the channel.

necessary to separate particles. Such geometries include an insulating hurdle[2], a serpentine[3], and a spiral shape[4].

The separator presented in this paper uses the principle of iDEP and a 3D helix-shaped channel in order to overcomes the shortcomings of 2D planar configurations such as the loss of efficiency for longer channels. This article presents the operating principle, fabrication and testing of a novel 3D helix-shaped iDEP cell separator which is expected to have to a better separation efficiency for a given path length than a 2D spiral separator, due to its constant channel curvature.

2 EXPERIMENTAL METHODS

2.1 Operating principle

The 2D spiral-shaped separator proposed by Zhu and his team has the advantage of being very versatile and costumizable[4]. The voltage applied can be tailored for optimal separation. The width and length of the channel can also be modified to suit different types of particles. However, the effective length of the channels is limited. Since the curvature of the channel is responsible for the inhomogeneity of the field, when the curvature decreases, the separation efficiency also decreases. It follows that, as the channel becomes longer in a 2D spiral, the curvature becomes lower. Hence, the separation efficiency is greatly reduced.

The separator presented in this paper overcomes this shortcoming by the use of the third dimension. The equivalent of the 2D spiral in 3D is the helix, which is the shape of common mechanical coil springs. This shape allows the curvature of the channel to be constant which would lead to a better separation efficiency for a given path length.

Figure 1-a shows the helical-shaped cell separator featuring an innovative 3D shape. Figure 1-b shows the forces acting on the particles. When a voltage (V) is applied between the inlet and outlet reservoirs. The curvature of the channel creates an inhomogeneous electric field which allows the dielectrophoretic forces to act on particles according to their electrical properties.

2.2 Fabrication

The 2D devices are usually fabricated using conventional microfabrication techniques such as soft lithography. These techniques do not allow the fabrication of complex 3D shapes. Some 3D devices have been reported [5, 6] but in most cases, their shape are either very simple or consist of an assembly of 2D shapes. The 3D device presented here must then be fabricated using a novel technique that allows the fabrication of complex 3D shapes.

The fabrication of our 3D separator device involves the direct write fabrication method[7]. In this method, a fugitive ink is extruded by a pneumatic dispensing system. The fugitive ink used is made of 30 wt% of microcristalline wax (Strahl and Pitsch inc.) and 70 wt% of petroleum jelly(Unilever). This ink is deposited on a rotating 1.2 mm diameter epoxy spindle using a 100 µm steel nozzle at a pressure of 700 kPa. The extruded filament is 110 µm in diameter. The rotation of the spindle is provided by a precisely adjusted electric motor (Mycos Technologies). The spatial positionning and displacement of the nozzle is controled by a computer-controlled robot (Fisnar).

Figure 2-a shows the deposition of a helix on a spindle. As the spindle rotates, the deposition nozzle is moved along its length which creates a helix. The separator is made of two helices with opposite rotating directions. The rotating direction of the helix is controlled by the direction of the nozzle movement.

The microchannels connecting the helices to the reservoirs are deposited on a glass substrate using the direct-write technique and the same 100 µm nozzle. A gap is left between the inlet and outlet reservoirs to install the helices.The walls of the reservoirs are created by direct-write and are filled with fugitive ink to prevent epoxy seepage during the encapsulation phase.

Figure 2-b shows the assembled fugitive ink part. The epoxy spindles with their helices are glued upright on the substrate and connected to each other and to the deposited filaments using fugitive ink.The ink assembly is encapsulated in epoxy (Epon 862/Epikure 3274). After full polymerization, the fugitive ink is removed by application of heat and vacuum. Figure 2-c shows the relsulting microchannels in the bulk epoxy. The total length of the channel is approximately 5 cm.

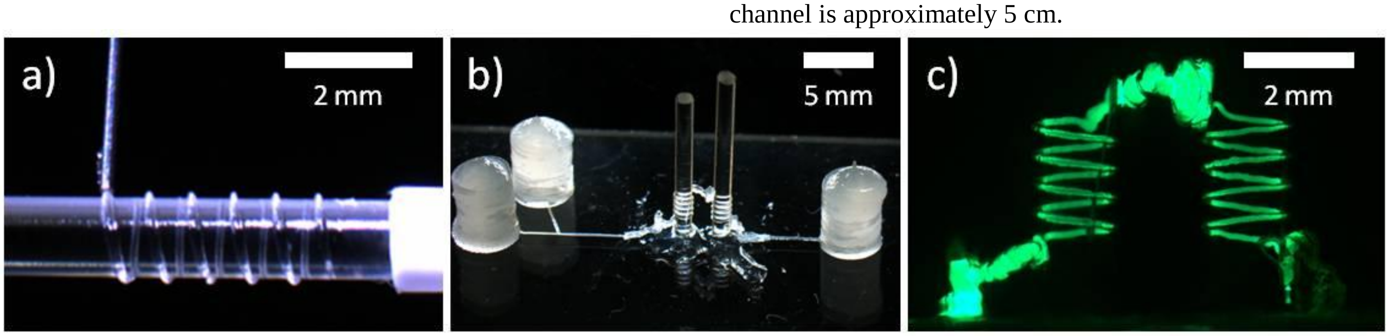

Figure 2 - a) The direct write spindle deposition technique allows the fabrication of helices. b) The deposition of the filaments and reservoirs is complete and the device is assembled. c) Fluorescent microscope side view image of the infiltrated device using fluorescein dye (Sigma-Aldrich)after encapsulation and ink removal.

2.3 Separation experiments

In order to separate particles, a medium with controlled conductivity made of deionized water and sodium chloride fills the channel and the reservoirs. Figure 3-a shows the 4 and 10 µm diameter polystyrene beads that are added to the inlet reservoir.

A voltage is applied between the inlet reservoir and the outlet reservoirs. This voltage creates an electric field that is stronger near the inside wall of the helix channel and weaker near the outside wall as illustrated in Figure 1-b. This gradient, due to the difference in the path length of the electric current, is responsible for the magnitude of the DEP force. The force is directed from high field values to low field values. The particles are then pushed outward. The electric field also creates an electrokinetic force which is directed along the length of the channel. This makes the particles circulate through the separator.

The two types of particles used have different electrical properties due to their different sizes, which results in DEP forces of different magnitude. This difference creates a physical separation between the particle types allowing to separate them.

When the mixed particles enter the channel from the inlet reservoir, the particles have a random position in the channel. The first helix will push the particles toward its outside wall. The particles will be acted upon by forces of different magnitude but, given a long enough exposition to the electric field, they will all reach the outside wall.

At the end of the first helix, the curvature changes direction which means that the oustside wall of the first helix becomes the inside wall of the second one. The particle are then pushed toward the "new" outside wall. Since they are all aligned on the inside wall, the particles that are subjected to the greater force will be pushed outward faster. At the end of the second helix, the particles will be physically separated. In order to make sure that the particles do not all reach the oustside wall in the second helix, it must be either shorter or wider than the first.

When they reach the Y junction, the flow will be split and the each type of particles will travel in one of the branches of the junction. When they reach the reservoirs, the particles can be collected.

3 RESULTS

3.1 Manufactured prototype

The current prototype shown on Figure 2-c has a continuous channel that connects the inlet and outlet reservoirs. The straight and helix-shaped parts of the channel have a sufficiently smooth surface. The shape of the helices is also very regular. Because of the spindle deposition technique, the channel curvature is constant which will lead to a constant DEP force and a better efficiency.

During the fabrication process the substrate on which the device was assembled detached itself from the encapsulating epoxy. The result is that the channels that links the reservoirs to the helices have a free surface. This does not cause any problem since air is a good insulator. However, the connections between the different parts are crude. This may compromise the separation process since the shape of these connections amy induce unpredictable electric field gradients that can push particles in uncontrolled directions.

Another flaw of the prototype is the diameter of the helix parts of the channel. In order to separate efficiently, the second helix must have a wider channel to prevent the particles from all being aligned close to the outside wall. During the fabrication, a slightly higher deposition pressure was used in order to create a wider filament but it is not significantly wider. This will probably result in an inefficient separation.

The next prototypes need to have smoother and more regular connections. The length of the second channel or its diameter must be better controlled in order to have a shorter or wider channel.

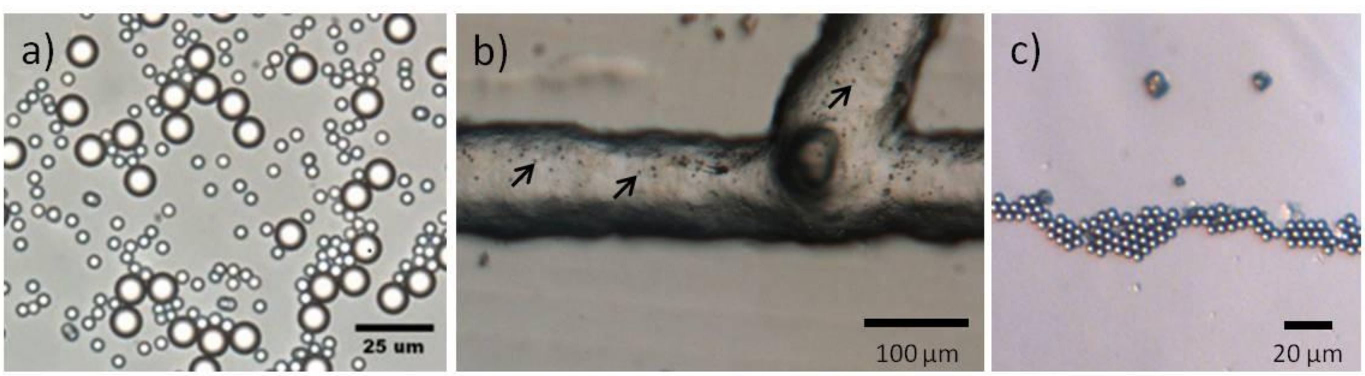

Figure 3 – a) 4 and 10 µm polystyrene particles used for the separation experiments. Those particles are present in the inlet reservoir during the experiments. b) Y-shaped junction during a separation experiment. The arrows point some of the particles point toward particles travelling in the channel during the experiment. Most particles flow to the upper outlet reservoir. c) 4 µm particles present in the upper outlet reservoir after a separation experiment.

3.2 Separation experiment results

Separation experiments have been performed by applying a voltage of 400 VDC between the reservoirs. The particles travelled through the channel but the separation did not take place with the current prototype. Due to the large size of the connection with respect to the channels, the medium flowed at a much lesser speed in the connections than in the channels. This gave time to the 10 µm particles to fall to the bottom of the connections. They were then trapped in the boundary layer and never travelled through the second helix. Figure 3-b shows that only 4µm particles reached the Y-shaped junction. The position of these particles in the channel, some of which are pointed by the black arrows, insured that the vast majority travelled through the branching channel gathered in the upper outlet reservoir. This reservoir lies on the side of the outside wall, which means that the particles that reached the junction were closer to the outside wall. This emphasizes the need to make the second helix shorter or wider to prevent the alignment of the particles on the outside wall. Figure 3-c shows the particles collected in the upper outlet reservoir after a 16 hour sedimentation time. All of the particles collected are 4 µm which confirms that the larger 10 µm particles did not reach the junction. The other outlet reservoir was empty.

4 CONCLUSION AND FUTURE WORK

The fabrication of a dual helix cell separator is a step forward in the development of portable, easy-to-use medical testing devices. It also serves as an example of the flexibility of the direct-write technique.

Other prototypes will have to be fabricated in order to implement modifications to the design of the existing separator. The main improvement will be to the length of the second helix, which must be shortened, in order to effectively separate particles instead of aligning them. In addition, the current junctions between the helices and the channel are crude which can influence the electric field and, thus, the separation efficiency. The next prototypes will have smoother and smaller junctions that will minimize the effects on the electric fields and the slowing of the flow. Finally, a similar separation experiment will be reproduced in a 2D spiral-shaped separator under similar testing conditions in order to evaluate the efficiency gain using the proposed 3D configuration.

REFERENCES

[1] N. Lewpiriyawong, *et al.*, *Analytical Chemistry*, vol. 83, pp. 9579-85, 2011.

[2] Y. Kang, *et al.*, *Biomedical Microdevices*, vol. 10, pp. 243-9, 2008.

[3] C. Church, *et al.*, *Electrophoresis*, vol. 32, pp. 527-31, 2011.

[4] J. Zhu and X. Xuan, *Biomicrofluidics*, vol. 5, pp. 024111-13, 2011.

[5] P. A. Zellner, *et al.*, "Selective E. coli trapping with 3D insulator-based dielectrophoresis using DC-biased, AC electric fields," in *34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS 2012, August 28, 2012 - September 1, 2012*, San Diego, CA, United states, 2012, pp. 6285-8.

[6] M. Yamamoto, *et al.*, *Electrochimica Acta*, vol. 82, pp. 35-42, 2012.

[7] D. Therriault, *et al.*, *Advanced Materials*, vol. 17, pp. 395-9, 2005.