RAPID FABRICATION METHOD FOR PLASTIC MICROFLUIDIC DEVICES WITH EMBEDDED MICROELECTRODES AND ITS APPLICATION TO ELECTROPORATION AND CELL LYSIS ON CHIP

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ABSTRACT

Methods of fabricating microfluidic devices that are scalable, simple, and inexpensive are necessary to ease the transition of microfluidics from an academic to an industrial discipline. Here we present a method for rapid prototyping of microfluidic devices with embedded electrodes in plastic based on electroplating and hot embossing techniques. We then discuss its applicability to electroporation and lysis, two of many potential applications that could benefit from this manufacturing method.

KEYWORDS: hot embossing, thermoplastic, microfluidics, electrodes, electroporation, lysis

INTRODUCTION

Though microfluidic total analysis systems and other types of microfluidic devices enjoy academic popularity, with thousands of papers published on the subject, only in a few limited areas have they achieved commercial success [1]. This can be attributed largely to the unavailability of a robust, standardizable manufacturing and packaging process. Hot embossing of thermoplastics using an electroplated mold has recently been demonstrated as a low-cost method of prototyping microfluidic systems [2]. This process can be adapted to manufacturing applications, allowing researchers to design devices that can be easily scaled up. A further advantage is the so far undemonstrated capability to easily integrate electronics into the plastic chip. In this work we demonstrate that electrodes can be embedded in plastic by a modification of the same process. The deposited metal patterns are mechanically strong when compared to thin metal films but still thin enough to be deformable, allowing us to create 3-dimensional conformal electrode patterns simply by embossing channels on top of the embedded metal.

Applications that involve the electromanipulation of cells have the potential to benefit greatly from the capability to rapidly prototype microfluidic devices with embedded electrodes. In particular, conformal electrode geometries achievable with hot embossing result in a comparatively uniform field over the cross-section of the channel and a high field strength even at relatively low voltages, qualities that are desirable in a point-of-care cell lysis or electroporation device (Figure 1).

THEORY

Electric field-mediated membrane rupture, whether for irreversible lysis or reversible electroporation, relies on the establishment of a transmembrane voltage of around 1 V for eukaryotic cells [3]. By solving the Laplace equation for a spherical cell with a non-conducting membrane, we can find an equation for the transmembrane voltage:

$$V_M = 1.5 \cdot r_{cell} \cdot E_0 \cos \theta$$

Figure 1: (A) CAD Image of an on-chip electroporation and cell lysis device with 2D (B) or 3D (C) embedded microelectrodes. Detailed images show microelectrodes embedded in the plastic microchannel.
where $E_0$ is the external electric field and $\theta$ is the angle between the direction of the E-field and the point on the cell where the potential is measured [4]. In order to reach a membrane potential of 1V, the critical field strength for a 5 micron radius cell is roughly 1.3 kV/cm.

Due to the favorable scaling of electric fields at small lengths, microfluidic devices allow fields of this magnitude to be achieved at low voltages. Additionally, the ability to pattern electrodes inside the channel allows us to substitute a spatially varying field along the direction of flow for the short-duration, high-voltage pulses typically used for electroporation. Simulation results show that narrow pulses of greater than 1kV/cm field strength can be generated with microchannel and electrode geometries achievable by our manufacturing scheme using low DC voltages (see Figure 4). This depends critically on the ability to create conformal 3D electrodes in microchannels, which provides stronger and more spatially homogeneous electrical fields.

**EXPERIMENTAL**

Masks for electroplating channel molds were fabricated using Riston dry-film photoresist laminated onto stainless steel wafers. In order to promote adhesion between the nickel features and the stainless steel substrate, wafers were then treated in a Wood’s nickel strike solution (12.5% HCl, 12% w/v NiCl). Wafers were then immediately moved to a nickel sulfamate electroplating solution and electroplated. The remaining photoresist was removed, leaving raised nickel features 75 microns thick that could be repeatedly embossed into plastic.

Embedded electrodes were fabricated using a similar method (see Figure 2 for a diagram of the fabrication workflow). In this case, the Wood’s strike step was omitted, as adhesion between the nickel features and steel substrate is not desired. The features were intentionally electroplated to a thickness greater than that of the photoresist, which results in the formation of mushroom-like overhangs that anchor the electrodes in the plastic when embossed. Upon demolding, the electrodes would lift off the steel wafer and remain embedded securely in the plastic. For 2D planar electrode structures, the channel mold was embossed into a second piece of plastic and the two pieces were bonded using a solvent-assisted bonding method, creating a closed channel with planar electrodes on one wall. 3D conformal electrode structures could also be fabricated by embossing the channel directly onto the embedded electrodes (see Figure 2E-F).

**RESULTS AND DISCUSSION**

Using the method described above, we have been able to fabricate 75 by 75 micrometer cross section channels in polycarbonate with embedded nickel electrodes (see Figure 3). Simulations indicate that we can achieve sufficient E-field strength in these channels to electroporate mammalian cells at 10 DC volts. Lysis of mammalian cells, or lysis and electroporation of bacterial cells, can be achieved by modestly increasing the applied voltage. By tuning the applied voltage to particular cell types, the selective lysis of particular cell types is also conceivable.

The 3D electrode configuration (figure 4.C) has a much higher field strength at the center of the channel than the 2D configuration (figure 4.B), and less variation in electric field strength across the width of the channel. Under our simulation conditions, the cross section of a channel halfway between two 3D electrodes is completely covered by electric fields of at least 1 kV/cm, compared to only 25% coverage.
with 2D electrodes, ensuring that cells will experience threshold electroporation voltage regardless of their lateral position.

Figure 3: (A) Top view photo of a similar structure of microelectrodes in 2D and (B) in 3D configuration. (C) SEM of an embedded microelectrode fabricated on polycarbonate (note that the plastic filled the gap underneath the mushroom), and (D) microphotograph cross section of an embedded IDE.

Figure 4: Simulations of electric field distribution along 75 by 75 micron microchannels for 2D and 3D configurations, with an operating voltage of 10V. (A) Diagram of the channel showing 3D electrode geometry and view planes. (B,C) Cross-sections of the electric field strength at its maximum, with scale bar to the right. (D, E) Heatmap of the electric field lengthwise along the channel, for 2D (D) and 3D (E) electrode configurations.

CONCLUSION
Hot embossing is a versatile and scalable method of prototyping microfluidic systems. The method of embedding electrodes in plastic that we have presented here further increases the space of applications for this promising manufacturing method. In particular, the ability to create conformal 3D electrodes easily and without expensive vapor deposition steps, shown here to be important for applications that require strong and homogeneous electric fields within microchannels, is a unique and novel application of hot embossing.

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