

Viable cell handling with high aspect ratio polymer *chopstick* gripper mounted on a nano precision manipulator

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Abstract This paper presents the development of an optimized contact technique for viable cell manipulation utilizing a high aspect ratio polymer *chopstick* gripper. The gripper consists of a 2 μm thick metal heater layer and a 60 μm thick SU-8 layer and is fabricated by a typical UV-LIGA process using SiO_2 as sacrificial layer. The grippers were completely released, de-tethered and assembled as end-effectors on to a nano precision manipulator to perform cell manipulation. Successful pick-and-place of a suspended normal rat kidney cell in phosphate buffered saline solution was demonstrated. The major cell-damage mechanisms associated with contact techniques were identified and alleviated by optimizing the handling force and operating temperature of the polymer gripper. The viability of cells handled with this optimized contact technique was demonstrated by labeling cells with a fluorescent dye. The developed technique will enable incorporation of simple, viable, and repeatable cell handling capabilities into the generic micromanipulators used in the biological laboratories.

1 Introduction

Viable handling of individual cells in aqueous medium is a challenging task due to the fragile nature of the cell

membrane and also due to the stringent environmental conditions required by the cells for their healthy survival. Since a wide range of cell analysis are performed in the biological laboratories for understanding cell behavior, it is highly desirable to have repeatable and convenient cell handling capabilities to be incorporated into the typically used micromanipulator set-ups.

The various techniques used to achieve controlled cell manipulation can be broadly classified into contact and non-contact techniques. The non-contact techniques predominantly employ optical principles (Ashkin and Dziedzic 1987; Buican et al. 1987; Ferrari et al. 2005; Chiou et al. 2005); examples include opto-electronic tweezers and laser tweezers. A major disadvantage of these systems is the potential damage they can cause to biological specimens, resulting in a decrease in the amount of active lifetime of the cell and consequently the time available for studying the sample. Magnetic (Gosse and Croquette 2002; Haber and Witz 2000) and ultrasound (Kim et al. 2004) fields have also been used to demonstrate manipulation of cells in aqueous media. The development of microfluidic technology using micromachining techniques enabled the development of a wide range of lab-on-a-chip devices. These devices were used in cell manipulation techniques by incorporating different principles like dielectrophoresis (Mohanty et al. 2003), magnetic/electric fields (Lee et al. 2004), and optical traps (Munce et al. 2002). Though capable of trapping single and multiple cells, these methods are typically cell specific and require electrode or magnetic arrangements.

The most generally used contact technique employs vacuum suction using micropipettes for holding cells (Lee et al. 1994; Sato et al. 1987; Zhezhi et al. 1998). Due to its low degree of controllability, it is relatively difficult to achieve precise cell manipulation using this technique.

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Another contact technique that is seeing rigorous development these days involves the use of micro-fabricated devices, such as microgrippers and microprobes. These types of MEMS devices provide a wide range of options in terms of design, performance and material compatibility. Although cell handling capability using such MEMS-based grippers (Nguyen et al. 2004; Chronis and Lee 2005; Zhou et al. 2004; Jager et al. 2000) has been demonstrated, none of these works have demonstrated optimized viable cell manipulation.

In this work, we report a highly repeatable, optimized contact technique for viable cell manipulation by optimization of cell handling force, operating temperature and operating voltage of polymer microgrippers.

2 Polymer microgripper

The polymer gripper shown in Fig. 1 consists of a thick ($\sim 60 \mu\text{m}$) polymer layer and a thin ($\sim 2 \mu\text{m}$) metal layer. The polymer was chosen to be SU-8, a widely used negative photoresist for fabricating high aspect ratio microdevices. The SU-8 has an added advantage for electrothermal actuation application as it has high coefficient of thermal expansion ($\sim 52 \text{ ppm/C}$). Gold was used as the heater layer due to ease of deposition by electroplating and its biocompatibility nature. SU-8 was proven to be biocompatible in several independent studies (Kotzar et al. 2002; Voskerjian et al. 2003; Lu et al. 2006). Kotzar et al. (CWRU, NASA, Cleveland Clinic) reported sterilized SU-8 passed all three tests performed to qualify its biocompatibility. The gripper operates by expansion of the polymer due to the heat generated by the electrical current applied through the metal. The gap between the gripper tips can be varied by varying the electrical current applied.

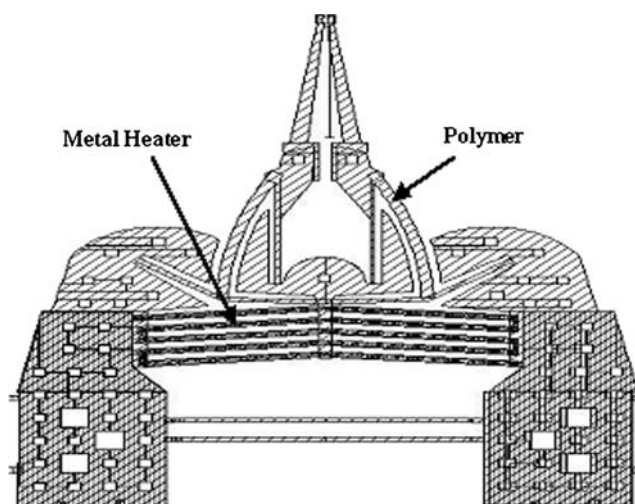


Fig. 1 Design of the polymer *Chopstick* microgripper

2.1 Design

The design of the polymer gripper (Fig. 1) was motivated by the necessity to optimize its performance in aqueous media in which the cell manipulation is performed. Chevron-type actuator was preferred over the conventional hot-arm cold-arm type, since the thermal gradient required to obtain optimum actuation of the latter is compromised in aqueous medium (Sameoto et al. 2004). The long gripper arms were designed in the shape of a *chopstick* in order to ensure sufficient isolation of the cell handling tips from the metallic heater layer, which undergoes the maximum temperature change.

2.2 Fabrication

The high aspect ratio polymer grippers were fabricated by a UV-LIGA process (Fig. 2). $5 \mu\text{m}$ thick, low temperature oxide (LTO) was used as the sacrificial layer. Gold layer was electroplated through $2 \mu\text{m}$ thick S1813 molds and a $60 \mu\text{m}$ thick SU-8 was patterned over that. The fabrication process was optimized so that there is minimal residual stress in the polymer. A high residual stress in the SU-8 results in the gripper tips to be spread apart after their complete release and also affects its actuation. Hence, the post exposure bake of SU-8 was performed at a low temperature of only 65°C for a longer duration ($\sim 1 \text{ h}$) so that there is minimum shrinkage in the polymer during cooling, which results in a lower residual stress. After developing, the grippers were hard baked at 140°C for 1 h, which was crucial to improve the structural rigidity of the polymer. The grippers were then released in 7:1 buffered oxide etch (BOE) using a timed etch process, so that the released grippers remain tethered to huge anchor pads on the substrate. A special de-tethering technique using focused-ion-beam (Colinjivadi et al. 2006) was developed in order to effectively release these grippers without any damage to the polymer/metal interface. These released grippers were mounted on to custom-made ceramic pads made of aluminum nitride using a conductive epoxy.

2.3 Finite element modeling and characterization

Finite element modeling using Ansys was performed to optimize the gripper design and also to understand its behavior in air and aqueous media. The material properties used for the simulations are listed in Table 1 (Incropera and Dewitt 1996; Nguyen et al. 2004; Roch et al. 2003). Resistivity of the electroplated gold was measured using a four-point probe set-up and it was found to be $4.5 \mu\Omega \text{ cm}$. The specific heat capacity of SU-8 was assumed to be

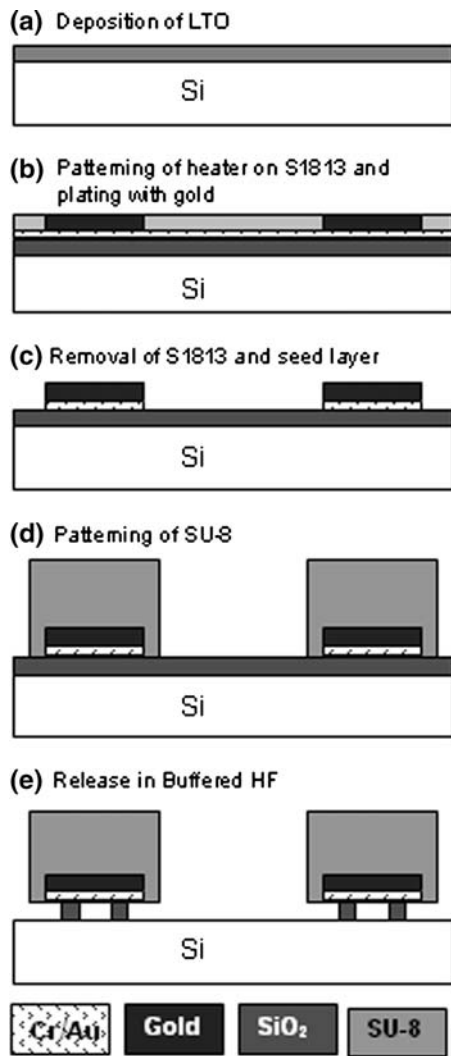


Fig. 2 Fabrication sequence for the high aspect ratio polymer microgripper

1,500 J/kg K, consistent to that of typical epoxies. In order to optimize the system resources, sequential simulation was performed by first running electro-thermal simulation and subsequently feeding its result as input to a structural simulation. Since the gripper is finally mounted on ceramic pads leaving its body to be overhanging, the losses to substrate were minimal and were neglected in the model.

Since the polymer gripper operates with surface temperature significantly lower than 800°C, the radiation losses from the device are negligible (Geisberger et al. 2003). Hence, the significant heat loss mechanism occurring in the polymer gripper is the heat loss by convection to the surrounding media. Since the beams of the metallic bent beam heater layer are closely spaced, conduction through the media between the beams should not be neglected in the model. Hence, the respective media (air or fluid) was added in the gaps between the beams and also surrounding them. A

Table 1 Material properties used for finite element modeling of the polymer grippers

Material property	Gold	SU-8	Air	Water
Electrical resistivity ($\Omega\text{-m}$)	4.5e-8	1e10		
Thermal conductivity (W/m-K)	315	0.2	0.0252	0.6
Young’s modulus (GPa)	78	4.05		
Coefficient of thermal expansion (ppm/°C)	14.2	52		
Temperature coefficient of resistance (°C)	0.0037			
Specific heat capacity (J/kg-K)	128.74	1,500	1,032	4,182
Convection coefficient (W/m ² -K)			200	4,000

convection coefficient depending on the media of actuation was applied to the exposed surfaces of the gripper. It is known that the convection coefficient for a device in a particular media is dependant on various parameters like the density, viscosity and conductivity of the media, the material of the device and geometry of its surface (Kreith and Bohn 1993). In order to perform the electro-thermal modeling of the polymer grippers, they were simulated by applying different convection coefficients on their surfaces and the value which gives the best fit with the measured values was taken as the convection coefficient for the respective media. By this approach, the convection coefficient applied to our device in air and aqueous liquid environment were 200 and 4,000 W/m² K, respectively.

Before fabricating the devices, it was necessary to determine the optimum thickness ratio between the metal and polymer layers. From the simulation results shown in Fig. 3, it could be observed that a higher polymer to metal thickness ratio was desirable to minimize the out-of plane actuation of the gripper due to bi-morph effect. Hence the thickness of the polymer and metal layers were set at 60 and 2 μm , respectively.

Figure 4a, and b shows the steady state temperature distribution along the gripper in aqueous media. It can be seen that the gripper tips are maintained almost at room temperature due to low thermal conductivity of the

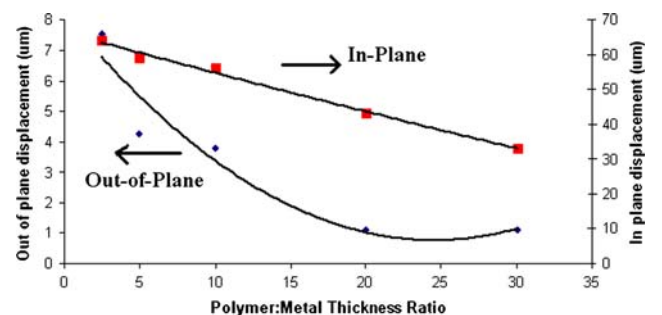


Fig. 3 Ansys results showing the effect of polymer to metal thickness ratio on out-of-plane displacement of the polymer gripper

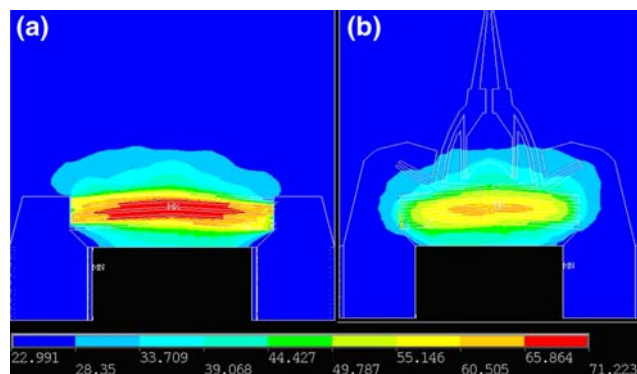


Fig. 4 Ansys results showing temperature distribution on the (a) metal and (b) polymer surface of the polymer microgripper for $V_{in} = 0.25$ V in aqueous media ($h = 4,000$ $\text{Wm}^{-2}\text{K}^{-1}$)

polymer and also due to convection losses to the surrounding media.

Due to the substantial difference in the thermal conductivity and heat capacity of the metal and the polymer, it was necessary to study the transient response of the gripper in the aqueous media. Figure 5 shows the thermal transient response of the gripper in aqueous media. It could be observed that the maximum actuation frequency of the gripper is dependant on the SU-8 layer as it takes a longer time than the metal to reach a steady state value during heating and cooling cycles.

Characterization of the polymer gripper was performed using a micromanipulator set-up controlled by a Labview program. The Labview program controls the Keithley Source Measuring Unit (model 2430) to source voltage and records the measured values of current and resistance using a four-point probe set-up. These resistance values were used to calculate the change in temperature across the gripper using the formula,

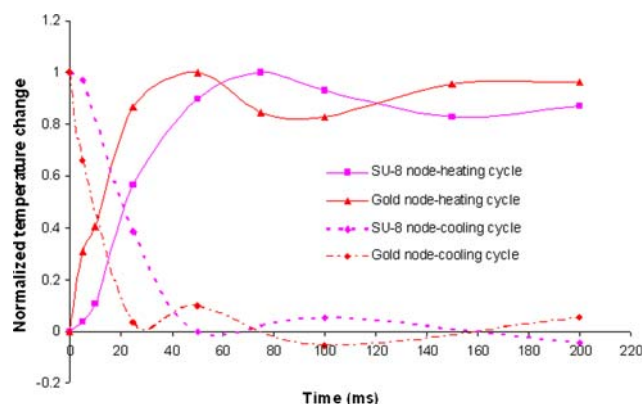


Fig. 5 Ansys results showing the thermal transient response of the polymer gripper. The delay in heating and cooling of SU-8 compared to gold is observable

$$\Delta T = \frac{R_1 - R_0}{(R_0 \times \alpha)}, \quad (1)$$

where R_0 is initial resistance, R_1 is measured resistance value at voltage V_1 and α is temperature coefficient of resistance of the metallic layer (gold).

National Instruments Vision development module was incorporated into our Labview program for measuring displacement by image analysis. Figure 6a, and b shows the displacement, input power and average temperature change of the polymer gripper in air and phosphate buffered saline (PBS) solution. It can be seen that the gripper requires higher voltage and power for actuation in the liquid medium compared to the air medium due to substantial loss of heat to the liquid. A 10 μm displacement in air requires 0.17 V, 2.8 mW and only 7°C increase in temperature, whereas a similar displacement in PBS solution requires 0.7 V, 50 mW and 18°C temperature change. It should be noted that while the Ansys simulations give the temperature profile along the gripper body, the measured values indicate only the average change in temperature occurring across the gripper.

3 Cell manipulation

Cell manipulation was performed using the polymer *chopstick* grippers by assembling them as end-effectors on to a biological micromanipulator system, which has nano-precision control in the XYZ axes (Fig. 7). Normal rat kidney (NRK) cells suspended in phosphate buffered

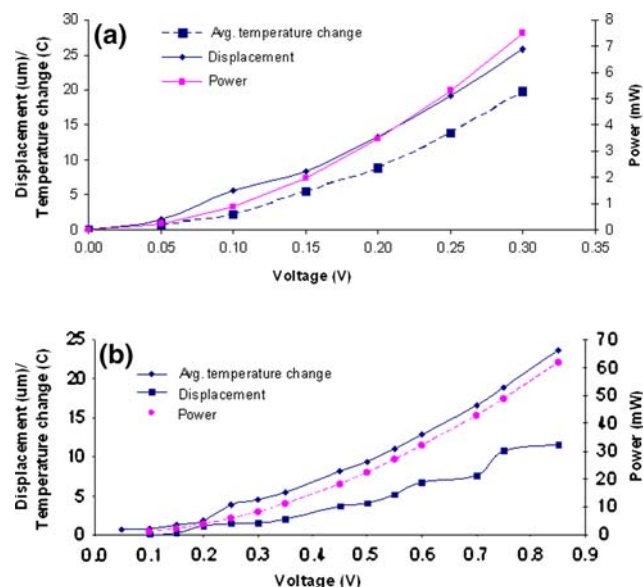


Fig. 6 Measured displacement, average temperature change and actuation power of a polymer gripper actuated in (a) air and (b) PBS solution

solution (PBS) were taken in a petri-dish and placed on the manipulator stage. Figure 8a–f shows a sequence of cell manipulation performed with the polymer microgripper in which a suspended cell was successfully picked, moved and placed in the solution. The tips of the gripper were positioned over the desired cell (Fig. 8a) and actuated to open by applying a small voltage (1 V). Then, the gripper was slowly brought down to the same plane as the cell (Fig. 8b) and closed (0.8 V) so that the cell was safely gripped by the holder. The cell was then slowly lifted in the solution (Fig. 8c), moved to another location (Fig. 8d) and again lowered back to the original plane (Fig. 8e). The cell was finally released (Fig. 8f) by increasing the voltage (1 V). This sequence demonstrates that individual suspended cells could be reliably picked and maneuvered along all three axes using this manipulation set-up.

Due to the fragile nature of the cell membrane, its manipulation requires utmost caution in order to ensure its healthiness during and after manipulation. There are many exogenous factors that could cause non-viability of the cells. The major ones among them are high temperature that increases the permeability of the cell membrane, high mechanical force that could cause traumatic injury and chemical toxicity that could genetically harm the cells. In the following sections, optimization techniques to alleviate these cell damage mechanisms during cell manipulation with the polymer grippers are discussed in detail. In order to perform cell-viability test, adherent NRK cells were labeled with a 2 µg/ml Calcein AM dye (Invitrogen Assay cell viability and live-cell function. <http://www.probes.invitrogen.com/media/publications/442.pdf>) in PBS solution at 37° for 30 min. The labeled cells were washed three times with PBS solution, then trypsinized, washed again and re-suspended in PBS solution just prior to manipulation.

(i) *Operating temperature:* Living cells require an optimum temperature range, not exceeding 10–15°C over

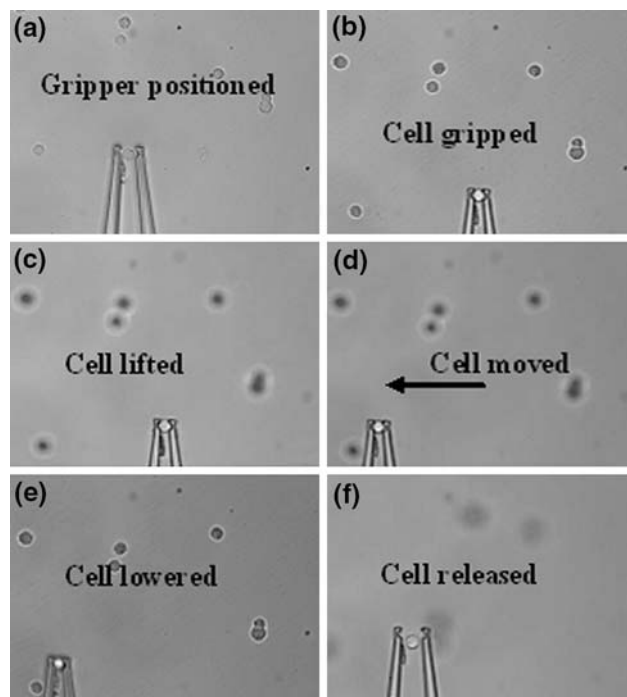


Fig. 8 Sequence showing the successful pick and place of a NRK cell suspended in PBS solution

room temperature, for their survival. Hence, it is highly critical that the electrothermally operated polymer gripper does not harm the cell by an elevated temperature at the gripping tips or in the solution. As discussed in Sect. 2.3 using Ansys simulations, there is negligible rise in temperature near the tips of the polymer gripper when actuated in aqueous medium. This conclusion was further strengthened from cell viability tests performed with a wide range of applied voltages (till about 2 V when electrolysis occurs).

(ii) *Operating force:* The operating force of the gripper is one of the major causes for concern in contact based manipulation techniques. The force should be low enough

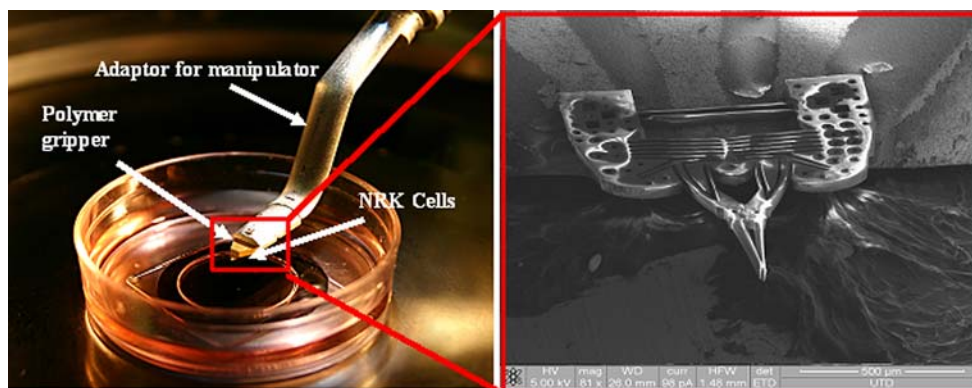


Fig. 7 Set-up for cell manipulation. *Inset* shows SEM picture of a polymer chopstick gripper glued to a ceramic pad with gold conducting lines for applying actuation

to minimize trauma to the cell membrane and also to avoid disturbance in the solution, which might render precise cell handling difficult. In the case of our gripper, the functional material being a polymer has a relatively low value of Young's modulus (~ 4 GPa) and consequently low stiffness. Hence, in order to maintain a lower operating force for our polymeric gripper, the net displacement has to be kept as small as possible. For example, when the gripper is suddenly de-actuated to close, by changing the applied voltage from 0.9 to 0.2 V, the displacement of the tips is 10 μm . But, if the gripper is de-actuated slowly, by varying the voltage in small steps, say from 0.85 to 0.8 V, the displacement of the tips is only 1 μm . Thus, reducing the applied voltage in very small steps, results in lower force at the gripper tips.

Using our cell manipulation set-up, it was observed that the cells were not viable when the voltage was reduced by a drastic value (from 0.9 to 0.2 V) thereby causing a large displacement and consequently large force on the gripper tips when it closes to grip the cell (Fig. 9a, b). This resulted in damage to the cell membrane, causing the Calcein AM dye to leak out of the cell proving that the cell was not viable anymore (Fig. 9c, d). The turbulence caused in the liquid due to this force is also visible in Fig. 9b–d.

Figure 10 shows an alternate, optimized cell handling technique, in which the voltage was reduced in very small steps. During the gripping cycle of the manipulation (Fig. 10a, b), the applied voltage was reduced in very small steps (~ 0.05 V) till the gripper tips gently held the cell. Fluorescent images of the cell (Fig. 10c, d) show that the manipulated cell was healthy both during gripping and after release by the gripper. The experiment was repeated numerous times, and all cells manipulated by this technique were found to be viable.

(iii) *Operating voltage*: The maximum voltage that can be applied to the polymer gripper is an important criterion for performing repeatable, viable cell manipulation. Since

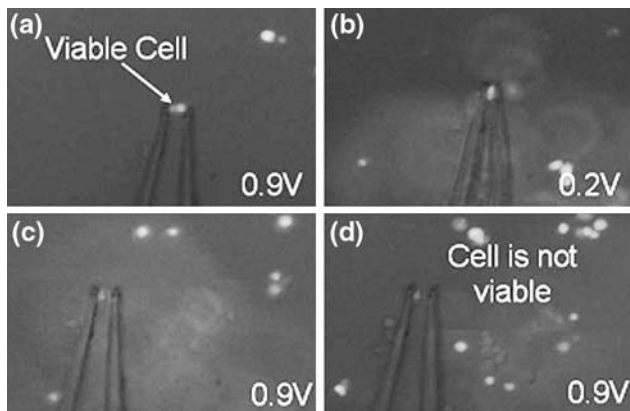


Fig. 9 Non-viable cell manipulation due to high handling force

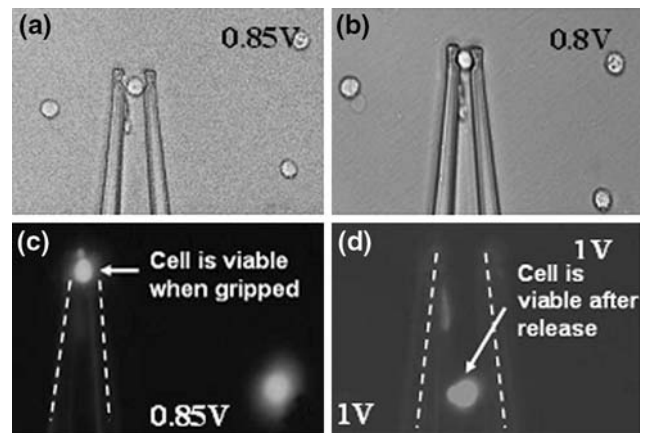


Fig. 10 An optimized cell handling technique showing that the cells are viable during gripping and after release

the diameter of cells in a given population might vary, it is important to ensure that a given gripper design is able to grip cells of different diameters without damaging them. The maximum diameter of the cells that can be held by the polymer gripper depends on the maximum actuation voltage that can be applied to it without causing degradation of the polymer or electrolysis of the solution. From our cell manipulation experiments, we observed that electrolysis starts to occur in the solution after a certain applied voltage (~ 2 V), which causes turbulence, thereby hindering precise cell manipulation (Fig. 11). On increasing the voltage further beyond this value, the polymer over the metallic heater layer gets melted due to excessive heat generated. It was observed that by maintaining the applied voltage to far less than this threshold limit, the designed polymer gripper could manipulate cells over a wide range of dimensions (15–50 μm diameters).

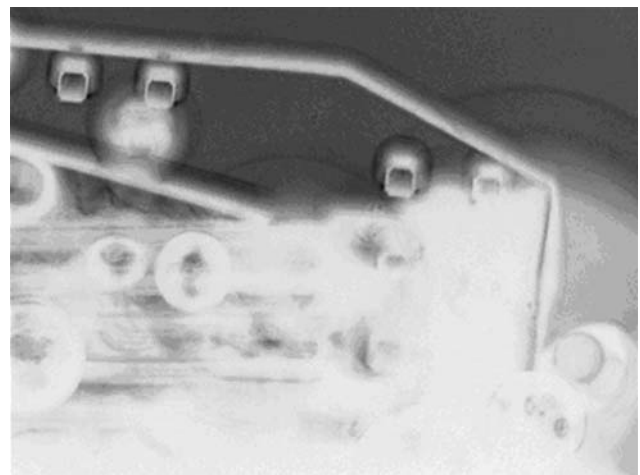


Fig. 11 Electrolysis occurring in the PBS solution due to excessive applied voltage on the polymer gripper

4 Conclusion

An optimized viable cell manipulation technique has been developed using a high aspect ratio polymer *chopstick* gripper. The polymer grippers were fabricated by typical UV LIGA technique and were completely released and assembled as end-effectors on to a generic biological nanomanipulator. Using this set-up, pick-and-place of suspended cells in aqueous medium was successfully demonstrated. The operating force, voltage and temperature of the polymer gripper were optimized and a technique for viable manipulation of cells was developed. Cells of a wide range of diameters were viably manipulated with a given gripper design. This optimized viable cell manipulation technique can be conveniently incorporated on to the generic micromanipulators used in biological laboratories for performing single-cell analysis.

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