NON-CONVENTIONAL BIOMEMS FOR BIOSAMPLES MANIPULATION

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ABSTRACT

This paper presents the fabrication processes for a novel, none Conventional BioMEMS approach for Bio Samples manipulation. The functioning of the device is based on the use of Hydrogels networks, which differs dramatically from existing solutions. This approach is suitable for "Lab On a Chip" scenarios. The device integrates different technologies associated with reversibly binding surfaces and Dielectrophoresis, (DEP). This system provides a novel separation strategy for Bio Samples that does not suffer from fouling issues. The binding surfaces are fabricated from thermally responsive polymer networks,. These polymers experience hydration-dehydration changes in response to temperature fluctuations. Therefore, separation efficiency can be "dialed in" as a function of temperature to prompt the selective of targets. The developments associated with this research provide a technology platform that facilitates separations, which would be difficult to achieve by any other existing methods

1. INTRODUCTION

In recent years, BioMEMS/NEMS have been primary elements associated with the research and development efforts in the bioengineering area. International and federal funding have applied an enormous increase in the development of state-of-the-art bioengineering and biomedical technologies. Most of the BioMEMS/NEMS related applications are associated with diagnostics, sensing and detection. Procedures for separation and manipulation of biological components play a paramount role in the function of these bioengineering mechanisms [1].

The most significant research area in BioMEMS is diagnostics. Many BioMEMS devices for diagnostics applications differ notably in their designs, fabrication processes and materials, as well as, their application areas. Most of these devices are utilized to detect biological samples such as microorganisms, cells, proteins, viruses, and DNA. Related to cell identification, the separation, manipulation, and sorting of biological particles and individual cells is of paramount importance in microfluidic based diagnostics [2]. In particular, there is a growing need for portable devices that detect and quantify biological species, isolate specific cell subpopulations from complex matrices and support living cell arrays [3]. Moreover, the areas of low-cost personalized medicine, battlefield diagnosis, and homeland security require inexpensive, low power, reliable devices. In addition, the devices for these areas must be capable of both raw sample processing and detection, which does not involve high skills training in order to operate. Recent advances in BioMEMS/NEMS and microfluidics exhibit notable results for sorting and separation mechanisms based on optodynamics, hydrodynamics, electrokinetics and advanced materials [4]. Depending on the final application, each of the above sorting techniques exhibits various advantages and drawbacks.

In contrast to the previous methods, the stimuli sensitive polymers approach presented in this paper exploits the fact that "Intelligent" thermally responsive polymers swell and contract with changes in temperature [5]. An example of one thermally responsive hydrogel is the cross-linked Poly(N-Isopropylacrylamide). Poly-NIPAAm is normally used to undergo volume transitions by taking advantage of its thermally sensitive hydrophobic interactions within the polymer network [6].

This paper presents the development of a none conventional and novel cell sorting integrated BioMEMS device based on the use of "Intelligent" lower critical solution temperature, (LCST), polymers. This novel sorting and manipulation strategy hasn't been previously exploited or reported, so it can be considered to be an important contribution to the BioMEMS field.

2. EXPERIMENTAL

2.1. Design

A novel technology platform that facilitates separation processes, which are difficult to achieve by any other method is presented. In order to implement the separation strategy, a BioMEMS device was fabricated. The device integrates different technologies to achieve

particle manipulation and sorting. It is based on four main concepts: "intelligent'" reactive polymer network layers, dielectrophoresis (DEP), Joule effect heating, and microfluidics.

Thermal responsive high aspect ratio poly-NIPAAm trenches are fabricated in order to exploit their swelling-collapse behavior. When the trenches are open, objects smaller than their width can be driven down into the channel by DEP and held there upon closing of the trench. Objects that do not fit into the trench are not retained upon closing the channels, and can be passed over the trench, offering size-selection separation. Figure 1 illustrates the basic concept described in detail.

2.2 Fabrication

The poly-NIPAAm patterning process started applying RCA cleaning to n-type <100> Single Side Polished 2" silicon. Different SU-8 PR from MicroChem Corporation (SU-8 2005, 2025 and 100) was used to spin coat the sample using a ramping spinning at 500 rpm for 5 s and a second speed depending on the SU-8 selected and the desired thickness. For SU-8 100 the second speed was set to 1500 rpm for 25 seconds; this results in a coating thickness of 120 µm. The sample is then given sufficient relaxation time of a one hour to allow reflow to complete and to prevent problems with step coverage on the silicon. A pre-exposure bake is performed using temperature ramping to reduce stress. The sample is baked on a hotplate for 5 min at 65^{o}C, and finally allowed to bake at 95^{o}C for 40 min. Exposure is performed using a Karl Suss Mask Aligner UV light source for 35 s through a mask defining the locations of the SU-8 on the wafer. The SU-8 structures on the cantilevers are the trenches with a 15 µm width and an opening space of the same size. The post exposure bake was carried out at 65oC for 3 min, 95^{o}C for 12 min and then left in room temperature for 20 minutes to avoid thermal stress. The substrates were developed using MicroChem SU-8 developer with gentle agitation for 20 minutes and then hard baked at 150^{o}C for 2 hours. Figure 2, show images of the poly-NIPAAm patterns.

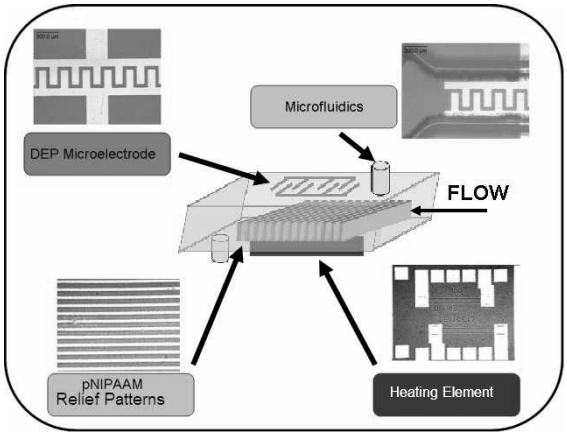

Figure 1 Proposed Integrated BioMEMS Device.

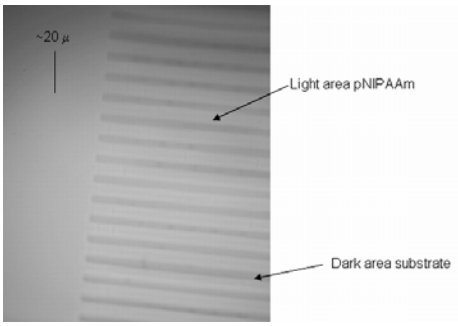

Figure 2 Transferred Poly-NIPAAm Structures on Substrate.

A microelectrode array was fabricated on top of a glass substrate using the conventional photolithography method using Futurrex NR9-1500PY negative photoresist (PR). The glass substrates were cleaned using a sulfuric acid solution for 5 min, then dipped in 50:1 HF for 30 s to improve adherence, the PR was spin coated at 3000 rpm for 30 s; next, the sample was soft baked for 2 minutes at 150^{o}C on the hot plate and followed by UV exposure for 35 s. A post exposure bake is applied for 1 minute at 100^{o}C and finally developed for 20 s with Futurrex RD6 developer. In the next process step, 1800 Å of Au were deposited on the substrate via sputtering and lift-off applied using acetone, isopropanol, DI water rinsed, and nitrogen dried.

In order to modify the thermally sensitive hydrophobic interactions within the polymer network, a nichrome resistor was fabricated. The fabrication process started by cleaning the glass substrates in a sulfuric acid solution for 10 minutes, rinsed with DI water and desiccated at 200^{o}C on the hot plate for 10 minutes. Next, the resistor network was patterned using negative photoresist NR7-3000 PY (Futurrex, Inc., Franklin, NJ) on the wafer. The nichrome resistor was deposited in an electron- beam evaporator for a total thickness of 1800 Å.

The method applied to fabricate the microfluidic channels is based on SU-8 patterning. The microfluidic structures have been fabricated using a novel developed method; in this method, SU-8 PR is used as an intermediate layer between two substrates.

3. RESULTS AND DISCUSSION

3.1 Microfluidic Channel

The SU-8 fabricated microfluidic channels were tested using fluorescent polystyrene beads (PolyScience PA) of 6 µm nominal size. These beads were added to a DI water solution with a final concentration of ~1 x 106 ml^{-}

[1] as visual targets to be observed through a fluorescent microscope FITC filter cube. For a 10 µl min^{-1} flow (1 x 10-8 m3 min^{-1}), the v obtained for the DUT is ~0.013 ms^{-1}. The flow's Reynolds number was calculated in order to determine the relative turbulence of the flow stream. Because of the Reynolds number value (~0.2), a strategy for sample and poly-NIPAAm interaction due to the laminar flow must be implemented

3.2 Dielectrophoresis

The Electric field E was numerically simulated using finite element analysis. Figure 3 shows the interdigitated microelectrode array simulation using ANSOFT HFSS software.

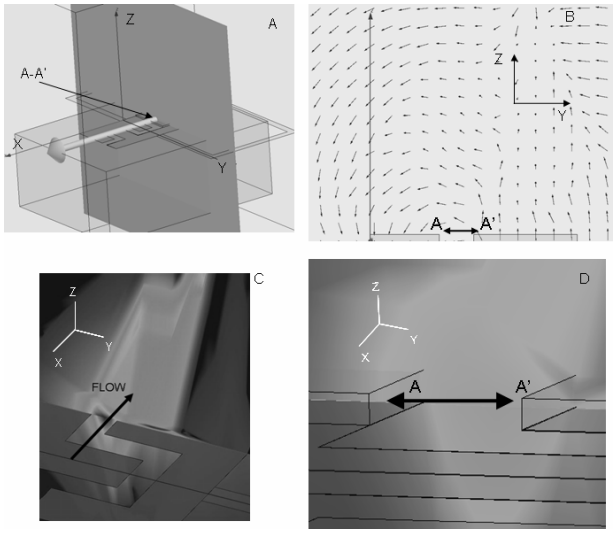

Figure 3 Simulation of the Electric Field E on the Microelectrode Array.

In figure 3.A, a plane is defined at the center of one finger section of the microelectrode array; the gap A-A' distance is 50 µm. The plane is perpendicular to the flow inside the microfluidic channel. In 3B an AC signal is applied to the microelectrode array producing an electric field shown at the perpendicular plane. The generated vectors indicate the direction of the field which according to equation 3 will produce a DEP force perpendicular to the flow inside the microfluidic channel. Figure 3C shows the field intensity, which as expected is larger near the array reducing in the z axis direction. Finally, figure 3D shows a detailed view of E near to the A-A' cross section between to electrodes of the array. In the final device DEP force is necessary to deflect the particles inside the microfluidic channel which presents a laminar flow behavior as explained before. In order to bind the samples and the poly-NIPAAm, this DEP effect must be applied inside the microfluidic channel.

3.3 Poly-NIPAAm relief patterns

The high aspect ratio trenches made of the UV polymerized "In situ" poly-NIPAAm using softlithography were tested to confirm their temperature responsiveness, as shown in figure 4.

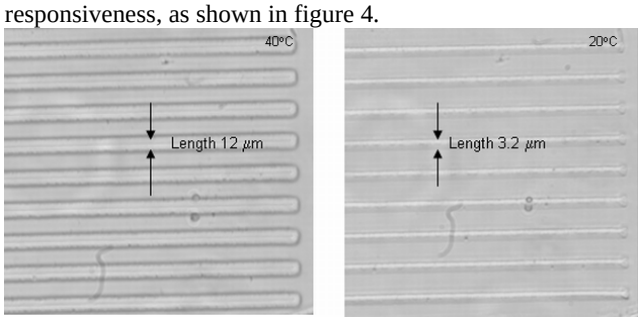

Figure 4 Bright Field Images of the Poly-NIPAAm Swelling Behavior at 20°C and 40°C

The BioMEMS device exploits the opening and closing effect of the fabricated trenches to allocate the particles inside them in order to generate a sorting mechanism. Figure 8 shows the microbeads mix; while the 6 µm beads move inside the trenches, the 20 µm beads stay on top of the structures (figure 5).

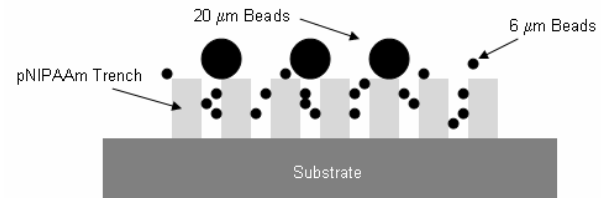

Figure 5 The 20 µm Beads Remain on Top of the Poly-NIPAAm Structures.

The experiment started programming a syringe pump to generate an input flow of 10 µl min^{-1}. Fluorescent polystyrene beads of 6 µm and 20 µm nominal size were added to the DI water solution with a final concentration of ~1 x 106 ml^{-1} at 60°C. At this temperature and flow values, the trenches are opened but the particle density attached to the trenches is almost zero as expected due to the laminar flow regimen inside the microfluidic channel. Negative DEP force is applied perpendicularly to the flow in order to deflect the particles down to the trenches.

Figure 6 shows the images sequence of the particles' path. In A and B, the flow contains 6 µm and 20 µm particles at 60°C. The attached particle density is zero. Next, DEP is applied in C and the particles start to be deflected to the trenches increasing their density. Figure 6 frames D, E and F show the capture process. It can be observed that the particle density increased and just one of the 20 µm bead remains attached on top of

the trenches as shown on frame F. As designed, the sorting mechanism has been confirmed by the 6μm beads shown inside the trenches.

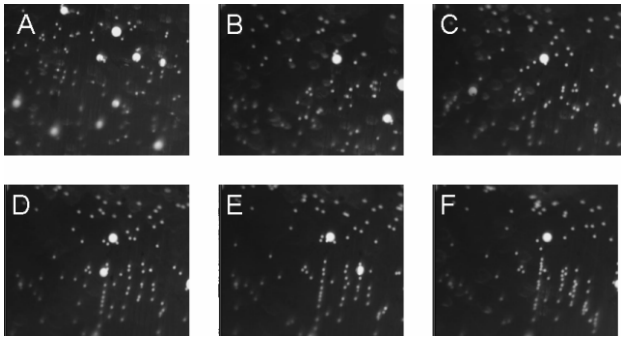

Figure 6 Images Sequence. Capture Mechanism.

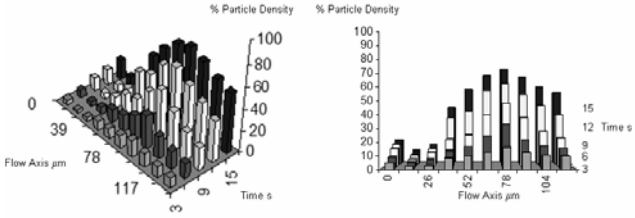

Figure 7 DEP at: 50 KHz, 8 Vpp, 10 μl min-1

DEP was applied changing three main parameters:

- Flow rate
- Frequency
- AC Amplitude

Flow rate was varied from 5 μl min^{-1} to 20 μl min^{-1} while the frequency and the amplitude were varied from 1 to 50 KHz and 1 to 10 Vpp respectively. The image sequences were analyzed using a particle counting tool from MATLAB in order to calculate the particle density inside the trenches. Figure 7 shows the data taken every 3s along the x axis. The plots clearly show the density increase due to the use of DEP

4. CONCLUSIONS

A cell sorting integrated BioMEMS device based on the use of none conventional "Intelligent" thermally responsive polymers has been described, developed and fabricated. This novel sorting and manipulation strategy hasn't been previously exploited or reported, so it can be considered to be an important contribution to the BioMEMS field. Compared to the alternate existing methods, the proposed approach accomplishes the desired characteristics of portability, low complexity, cost effective, power efficient and ease of operation. As illustrated in this paper, the complete integration of different technologies to achieve the results desired has shown promising results related to this technology platform.

Current BioMEMS-based assays suffer from low detection resolution when the target is either in low concentration or is embedded in a complex or dirty matrix. A potential application for the strategy developed is a preconcentration process that enhances sample purity in microfluidic platforms with low power requirements. Another possible application is a multi zone filtering device when the separation and manipulation of mixed multiple bio species is required for diagnostics, sensing and detection.

The integration of different micro and nano fabrication processes supports the development of the strategy presented in this paper representing a major contribution to the field. Further research work is proposed to optimize the microfluidics subsystem, the poly-NIPAAM properties tuning, the different poly-NIPAAm patterning processes and their effects on the BioMEMS/NEMS device, as well as the fabrication and integration of the DEP microelectrode array and the thermal actuation.

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REFERENCES

[1] Bashir, R. (2004). "BioMEMS: state-of-the-art in detection, opportunities and prospects." Advanced Drug Delivery Reviews 56(11): 1565-1586.

[2] Mastrangelo, C. H., M. A. Burns, et al. (1998). "Microfabricated devices for genetic diagnostics." Proceedings of the Ieee 86(8): 1769-1787.

[3] Gambari, R., M. Borgatti, et al. (2003). "Applications to cancer research of "lab-on-a-chip" devices based on dielectrophoresis (DEP)." Technology in Cancer Research & Treatment 2(1): 31-39

[4] Lin, C. H., G. B. Lee, et al. (2004). "Vertical focusing device utilizing dielectrophoretic force and its application on microflow cytometer." Journal of Microelectromechanical Systems 13(6): 923-932New

[5] Wu, X. S., A. S. Hoffman, et al. (1992). "Synthesis and Characterization of Thermally Reversible Macroporous Poly(N-Isopropylacrylamide) Hydrogels." Journal of Polymer Science Part a-Polymer Chemistry 30(10): 2121-2129.

[6] Yoshida, R., K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai, and T. Okano, Comb-Type Grafted Hydrogels with Rapid De-Swelling Response to Temperature-Changes. Nature, 1995. 374(6519): p. 240-242.