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Poly(N-Isopropylacrylamide) based BioMEMS/NEMS for cell manipulation

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Poly(N-Isopropylacrylamide) based BioMEMS/NEMS for Cell Manipulation

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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DEDICATION

To Viviana, Ana Alexandra, Aitor, Leonardo, Maria Dolores and Family
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In recent years, BioMEMS/NEMS have been primary elements associated with the research and development efforts in the bioengineering area. International and federal funding has effected 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.

This research was concerned with the development of a novel BioMEMS device for cell manipulation. The functioning of the device is based on the use of thermally responsive polymer networks, which differs dramatically from existing approaches. This approach is cost effective, requires low power and uses a minimal amount of on-device area, which makes it suitable for personal medical diagnostics and battle field scenarios.

The device integrates the technologies associated with reversibly binding surfaces and dielectrophoresis, (DEP). The DEP field drives a sample into contact with a binding surface. This surface can be controlled to provide different levels of target selectivity. This system provides a separation strategy that does not suffer from fouling issues.
The binding surfaces are fabricated from LCST polymers. The LCST 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 selection of targets. Furthermore, size-exclusion “trenches” were patterned into the binding surfaces. The trenches permit the passage of the small objects in order to provide size-exclusion separations. In order to expand the discrimination size range from the micron to the submicron scale, two techniques for submicron patterning of cross-linked reversibly binding surfaces were investigated. The patterning techniques associated with electron-beam lithography and the combination of softlithography and a focused ion beam patterning were found to generate well-defined patterns that retained their thermo-responsiveness.

The combination of DEP and reversibly binding surfaces for bio-particle manipulation is a significant contribution to microfluidic based separations in BioMEMS/NEMS. The developments associated with this research provide a novel technology platform that facilitates separations, which would be difficult to achieve by any other existing methods.
CHAPTER 1
INTRODUCTION

Bioengineering applications of micro and nano technology, (BioMEMS/NEMS), have become progressively more established and have found widespread use in applications that require their interaction with biological systems. Biotechnology has advanced to the level of more than a one hundred billion dollar research and development effort in the last 50 years, [1]. For example, in 2005 the National Institutes of Health, (NIH), increased the funding for the National Institute of Biomedical Imaging and Bioengineering to $297.6 million, which was targeted toward development of state-of-the-art instrumentation for bioengineering and biomedical technologies. Some of the BioMEMS research areas for diagnostics, sensing and detection applications are:

- Gene diagnosis and therapy,
- Cell sorting, manipulation, and identification,
- Bacteria analysis,
- DNA molecular sizing,
- Environmental microbial sensing,
- Tissue Engineering,
- Drug delivery systems.
The largest and most significant research area in BioMEMS is diagnostics. Many BioMEMS devices, for diagnostic applications, differ notably in their designs, fabrication processes, materials and 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, sorting of biological particles and sorting of individual cells is of paramount importance in microfluidic based diagnostics, [2-6]. 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, [7-9]. 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 for their operation. Recent advances in BioMEMS/NEMS and microfluidics exhibit notable results for sorting and separation mechanisms based on optodynamics, hydrodynamics, electrokinetics and advanced materials, [10-15]. For instance, flow cytometry, (FC), dielectrophoresis, (DEP), optical tweezers and stimuli sensitive polymers are examples of current approaches, [16-21].

Each of the sorting techniques mentioned exhibits various advantages and drawbacks, which depends on the specific application. FC is a technique for examining, counting and sorting microscopic particles suspended in a microfluidic flow. This technique allows for the analysis, usually on the basis of their relative fluorescence, of the physical and/or chemical characteristics of single cells. Complex computerized instruments are used to pass a stream of single cells or particles through the measuring
apparatus, which makes it unsuitable for a non sequential process. Normally, the cells are stained with a light-sensitive dye, placed in a fluid and passed in a stream before a laser or other type of light. This is a method for measuring the number of particular cells in a sample and the percentage of live cells in a sample. In addition, the method can be used to ascertain certain characteristics of cells such as size, shape and the presence of tumour markers on the cell surface. FC can also be utilized for cell sorting and manipulation by using electrical or mechanical means to divert and collect cells, with one or more measured characteristics, which fall within a range of interest. Cells/particles can be selectively charged during a pass and on their exit can be deflected into separate paths of flow.

In the MEMS area, FC has been widely studied and developed for many important applications, [22-24]. Although there has been great progress, within the MEMS field, the majority of the proposed systems fall in the category of cell-holding procedures. Therefore, the MEMS devices are only designed for microfluidics control and retain the cell sample. The actual sample analysis is performed by a complex “out of the chip” optical system. The most common method for sample manipulation and attachment in these MEMS devices is DEP and most of the optical system, sensors and data analysis reside outside the chip, [25-27].

Recently, some research groups have integrated the optical part with the rest of the device, [28]. However, the system’s complexity is augmented and different issues such as power consumption, portability and operational ease are still important obstacles to be overcome. Another important matter is the fact that FC MEMS are mainly intended
for the sample’s optical analysis more than as a sample sorting or a sample
preconcentration mechanism, [29].

Optical methods normally require excessive “outside the chip” infrastructure for
proper implementation. Currently, the optical tweezers approach is one of the most
exploited optical methods. Optical tweezers are widely used to lock in, analyze and
manipulate biological samples, [30-32]. The operational principle for optical tweezers
relies on the optical gradient force, which competes with the radiation pressure.
Therefore, very tight focusing is required in order to produce a stable optical trap. The
trapping area is much smaller than typical cells, which results in the force, exerted on the
cells, being very non-uniform. In addition, optical tweezers require very high optical
power, (1 to 100 mW), to produce a sufficient trapping force, (-1 to 100 pN). The high
optical power density could result in photodamage of cells, [33]. One possibility for
reducing this effect is the use of infrared lasers. Another drawback related to MEMS
optical tweezers is that focusing of the laser for each sample is a time consuming
approach. Therefore, the use of optical tweezers is not a suitable sorting method for
substantial sample size.

DEP is the phenomenon that a particle, which is influenced by an inhomogeneous
electric field, experiences. DEP imposes a force proportional to the gradient of the
electric field. Due to its functional ease, DEP has been a widely used principle for lab-
on-a-chip MEMS devices. Dielectrophoretic based particle sorters are very suitable for
integration with other system components. Different types of particle sorters have been
presented in the literature, [34-37]. The majority of the approaches combine DEP with
different techniques such as optical systems. DEP systems suffer from particle adherence
to the microelectrode and can cause cell damage due to long time exposure to the electric field, which is a concern.

In contrast to the previous two methods, the stimuli sensitive polymers approach presented in this dissertation exploits the fact that “Intelligent” thermally responsive polymers swell and contract with changes in temperature, [38]. In order to reduce the complexity of the separation and sorting tasks, there exists a growing interest in these responsive materials for catch and release type applications, [39-40]. Almost two decades have elapsed since the first demonstration of the existence of "Intelligent" or “Responsive” hydrogels, [41]. Presently, these materials are being increasingly utilized in the BioMEMS/NEMS area. Environmentally sensitive hydrogels offer unique opportunities for a wide range of BioMEMS/NEMS sensor-actuator applications. Environmentally sensitive hydrogels offer excellent bio-stability and bio-compatibility. In addition, fabrication methods for environmentally sensitive hydrogels are relatively inexpensive, [42].

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, [43-44].

The poly(NIPAAm) hydrogel exhibits a Lower Critical Solution Temperature, (LCST). In addition, poly(NIPAAm) exhibits remarkable hydration-dehydration changes, in aqueous solution, in response to relatively small changes in temperature, [45]. Below the LCST, poly(NIPAAm) chains hydrate to form an expanded structure. Above the LCST, poly(NIPAAm) chains dehydrate to form a collapsed structure. The physical
structures and properties of poly(NIPAAm) are readily controlled by simply changing the temperature without changing the chemical structure of the polymer. Therefore, temperature responsive poly(NIPAAm) can be employed in drug delivery systems, cell manipulation, solute separations, concentrations of dilute solutions, immobilization of enzymes, coupling of biomolecules and photosensitive materials, [46-50]. These characteristics made poly(NIPAAm) an ideal candidate for this research. The most common example for this technology is the use of a thin intelligent polymer network film that integrates sensing and actuation at the material level, [51].

The area of intelligent polymer networks has developed rapidly in recent years. However, there remain a large number of possibilities for new procedures and improvements to reduce the complexity of separation and sorting tasks. The possibility of reducing the patterning size is due to the latest nano range lithography tools. The ability to reduce the patterning size provides the opportunity to interact with an array of different biosamples such as bacteria and viruses. One major interest, which drives the use of BioMEMS, is the reduction of the sensor element to the scale of the target species. These sensor elements provide higher sensitivity and the reduction of the time to result, which is due to small volumes and higher effective concentrations.

This research studied the effects of different topographic profiles and patterning procedures on the behavior of poly(NIPAAm). Additionally, this research studied the impact on the interaction of poly(NIPAAm) with bacteria in order to generate microfluidic based separations for sensor/actuator mechanisms. This research provided a novel technology platform that facilitates separation processes, which are difficult to achieve by any other method, [52].
Figure 1.1 presents the sorting and separation mechanisms utilized, during this research, as stimuli for responsive polymer networks.

![Figure 1.1: Separation Mechanism Using Stimuli Responsive Polymer Networks](image)

Starting with a mixed biosample population, (A), microfluidics was exploited to allocate the interaction of the samples with a deposited and patterned thin layer of the polymer network, (B). Application of external stimuli results in physical and chemical property changes to the polymer network that produced an attach or release effect, which was dependent on the external stimuli, (C). The combination of other effects such as DEP and polymer patterning was exploited in order to take full advantage of the polymer network response. Application of these effects maximized the sorting and separation of the biosamples, (D).

This dissertation presents the fundamentals of stimuli responsive polymer networks. The polymerization method employed for this research is presented in Chapter two. Chapter three explores the processing steps, which were applied to fabricate the integrated BioMEMS device. The microfluidic channels integration, which was used for the interaction of the poly(NIPAAm) with biological samples, is explained. The
collection of methods utilized for poly(NIPAAm) processing is presented. These methods were employed in order to generate 3D structures with different topographic characteristics and a size range down to the sub-micron regime. The integration of all the processing techniques, the study and analysis of the attach and release mechanism between the biomaterial and the biological samples and other related procedures such as DEP are also presented. Chapter four is devoted to the presentation of the complete approach to the experiments and the analysis of the results. Chapter five presents the conclusions achieved through this research and some possible applications.
CHAPTER 2
STIMULI RESPONSIVE POLYMER NETWORKS

There have been broad studies for drug delivery, separations and diagnostics applications using polymer systems that undergo phase transitions in response to external stimuli. These “Intelligent” polymers are known to respond to a variety of stimuli such as pH, temperature and the electric field, [53-56]. One of the main temperature-responsive categories is the alkyl acrylamide polymers. An example of this category is poly(N-isopropylacrylamide) or poly(NIPAAm), which undergoes a sharp phase separation at its lower critical solution temperature, (LCST), when submerged in water. The existence of this reaction in water is the reason why they are called hydrogels. This endothermic process is driven by a gain in entropy, which is associated with the release of hydrophobically bound water molecules, [57]. Poly(NIPAAm) has a LCST, in water, when heated above 32°C, (89.6°F), [58]. A large quantity of information is available for these materials since they have been employed extensively as biomaterials. However, Poly(NIPAAm) gels are only responsive in the presence of aqueous solutions with relatively low ionic strength, which significantly limits the types of fluids that can be manipulated, [59]. The hydrogel’s temperature and pH responses have been completely characterized. The hydrogel exhibits tunable phase transitions, which are sharp and may be exploited in many BioMEMS applications.
The LCST of these thermally sensitive polymers can be tuned to a desired temperature range by copolymerization. Copolymerization with a more hydrophilic comonomer raises the LCST while use of a more hydrophobic comonomer lowers the LCST, [60]. Carboxylic acid monomers such as acrylic acid, (AA), or methacrylic acid, (MAA), which are readily available, have been copolymerized with NIPAAm. These copolymerizations, which utilize traditional free radical polymerization techniques, are employed to form random copolymers with desired temperature and pH-responsive properties, [61]. The degree of the physical reaction of swelling increases dramatically with the incorporation of ionizable comonomers. Moreover, the transition temperature increases as the ionized groups make the polymer more hydrophilic, [62]. The swelling behavior of the hydrogel permits their use in sensor-actuator mechanisms where the volume phase transition rate is known to scale with the square of the gel dimension. Therefore, swelling should increase dramatically with microscaling, [63]. However, the time scale is also a function of gel composition, microstructure and surface properties [64].

There is also a difference in the swelling response to increasing and decreasing temperature. The collapse of the gel network is significantly faster than the swelling process, [65]. The Flory-Rehner theory has been used to study and interpret the anisotropic swelling of hydrogels. The theory qualitatively captures the experimental features observed in poly(NIPAAm) based networks. The Flory-Rehner theory has been widely used to analyze the phase transition of networks, [66].
The osmotic pressure, \( \Pi \), of the network can be described by six variables:

\[
\Pi = \Pi(d, \phi, \phi_0, f, N_c, \chi).
\] (1)

The parameter \( d \) is the dimension in which the network can swell. The volume fraction of the swollen gel is given by the \( \phi \) parameter. The volume fraction of the preparation state is given by the \( \phi_0 \) parameter. The degree of ionization is given by the \( f \) parameter. The number of segments between cross-links is given by the \( N_c \) parameter.

The Flory-Huggins interaction parameter \( \chi \) is a function of the temperature, \( T \), [67]. Figure 2.1 presents a plot of the equilibrium polymer volume fraction of the network as a function of the reduced temperature with \( 1 - 2\chi \).

![Figure 2.1: Phase Behavior for Swelling in Surface Attached Networks](image)

In this phase diagram, the isobars illustrate how the material’s discontinuous volume transition depends on different factors such as the ion content, the state of preparation, the cross-link density and its chemical composition. In the plot, the volume
fraction in the preparation state, \( \phi_0 \), was set to 1.0, the segment between cross links, \( N_c \), was set at 500 and different degrees of ionization, \( f \), were applied. Figure 2.1 reveals the capability to “tune-up” the network’s precise thermal response depending on the specific application’s parameters.

One of the main objectives of research with hydrogels is the evaluation of their responses as building materials for BioMEMS using several criteria. A few of these criteria are the ease of fabrication of the hydrogel structures, the kinetics of the volume phase transition as a function of gel size and composition, their ability for sensor-actuator mechanisms, anisotropic swelling of the hydrogel, modeling of the swelling geometry, and the response to different stimuli.

2.1. Photopolymerization of NIPAAm

In order to create the poly(NIPAAm) structures, a technique known as Soft-lithography is applied. This technique is described in detail in Chapter 3. Soft-lithography generates poly(dimethylsiloxane) or PDMS molds, which are applied to produce the polymer features. To obtain the polymer biomaterial, NIPAAm, (97 % Aldrich), was purchased, [68]. N, N’-Methylenebisacrylamide, (BisAAm, Chemzymes Ultra Pure), was obtained from Polysciences Inc. and used as a cross linker. An initiator, 2,2-dimethoxy-2-phenyl-acetophenone, (DMPA, 99%, Aldrich), was used. Acrylic acid was purchased from Fisher, (99.0%), N, N-Dimethylformamide, (DMF), was purchased from Aldrich. All the chemicals were used without further purification. Poly(NIPAAm) was prepared on a Pyrex glass surface. The preparation of the substrates included, in the given order, the actions:
• Submerged in sulfuric acid for 10 minutes,
• Washed with isopropanol,
• Rinsed in deionized water,
• Dried with dry nitrogen gas,
• Cleaned for 15 minutes in a plasma cleaner,
• Soaked in a photosensitive silane coupling agent for 15 minutes,
• Heated in an oven at 100°C for 10 minutes,
• Rinsed with deionized water,
• Dried with dry nitrogen gas.

The photopolymerization process began with 0.1% (w/w) of initiator, [DMPA], 2.5% (w/w) of cross-linker, [BisAAm], in a test tube with 1ml of Acetone. The test-tube was stirred for 5 minutes and then purged with nitrogen to remove the dissolved oxygen.

The mix was injected into the soft-lithography PDMS molds using capillary forces. UV light was applied, for 8 minutes, to photo-initiate the grafting of NIPAAm while nitrogen was used to purge the polymerization chamber of any oxygen. The thiocarbamate group of the sensitizer was bonded to the glass surface by coupling the silane agent with the hydroxyl groups on the glass surface. Figures 2.2-2.5 present the processing steps for the poly(NIPAAm) structures.
In Figure 2.2, the final thickness of the SU-8 photoresist was 40 μm. The trench structures were attached to the two lateral square features, which were used as the inlet and outlet ports for the capillary injection of the mix.

Figure 2.3 presents the details of the SU-8 trenches where the PDMS was applied to generate the molds.
In Figure 2.4, the PDMS was applied to obtain the master molds and the poly(NIPAAm) was “In-situ” photo-polymerized onto the substrate.

Figure 2.5 displays the excellent pattern fidelity that was achieved using soft-lithography. The final Poly(NIPAAm) thickness is 40 μm.
CHAPTER 3
SYSTEM FABRICATION

The processing methods, which were developed and applied to fabricate the proposed BioMEMS device, are presented in this chapter. The device integrated different technologies to achieve particle manipulation and sorting. The device was based on the main concepts embodied in “Intelligent” reactive polymer network layers, Dielectrophoresis, (DEP), the effect of Joule heating and microfluidics.

Thermal responsive high aspect ratio polymer trenches were fabricated in order to exploit their swelling/collapse behavior in order to separate biological particles with micron and submicron dimensions. The swelling/collapse behavior of the polymer was described in Chapter 2. When the trenches are open, objects smaller than their width can be driven down into the channel through the use of DEP. Once the objects are driven into the trenches they are captured by closing of the trenches. Objects that do not fit into the trench are not retained when the trenches are closed and pass over the trenches. The opening and closing of the trenches provides the mechanism for size-selection separation.

This novel approach represents a key technology platform that facilitates separation processes that are difficult to achieve by any other sorting or manipulation method.
Figure 3.1 presents the basic concept associated with this sorting mechanism. It displays the four main building blocks of the system: DEP, microfluidics, heating element, and the Poly(NIPAAm) structures.

The fluid flow enters a micro chamber thru microfluidic channels. In the micro chamber the particles are driven down by negative dielectrophoresis forces. The open-close effect of the poly(NIPAAm) captures the particles and effects size sorting.
3.1. Poly(NIPAAm) Processing

3.1.1. Soft-Lithography

Soft-lithographic methods use an elastomeric stamp or mold, which is prepared by casting the liquid prepolymer of an elastomer against a master that has a patterned relief structure, in order to create structures such as microfluidic channels, [69]. Photo-lithography is used for fabrication of the masters.

Most of the research associated with soft-lithography has used poly(dimethysiloxane) or PDMS as the elastomer. PDMS has several properties that make it well suited for soft-lithography, [70]. PDMS is biocompatible, permeable to gases and can be used for cell culture(s). PDMS is optically transparent above 300 nm. Since PDMS is elastomeric, it can contact non-planar surfaces conformally, [71].

Two different mold or stamp fabrication methods were applied during this research. One method was based on the use of positive, standard microelectronic, photoresist. Use of this method allowed fabrication of poly(NIPAAm) structures up to 1.5 μm in height. Another method, which involved the use of SU-8 photoresist, allowed fabrication of poly(NIPAAm) structures up to 120 μm in height. The two methods are described in detail in the following sections.
3.1.1.1. Standard Photoresist Patterning

In this approach the masters were made of positive patterned photoresist and used as molds for PDMS embossing, (Shipley 1813). Silicon wafers were used as the substrate. The substrates were RCA cleaned, DI water rinsed and dehydration baked on a hot plate at 200°C for 10 minutes, [72]. Preparation of the substrates continued with HMDS and photoresist spin-coating prior to UV exposure at the mask aligner. Once the desired photoresist structures were developed, the photoresist was hard baked at 155°C for 2 hours. A mix of PDMS, (Dow Corning), and curing agent was prepared in a 10/1 ratio and allowed to outgas for 1 hour inside a laboratory hood. The PDMS mix was placed on top of a 2 inch <100> n-type silicon wafer and placed on a hot plate at 90°C for 90 minutes. Afterwards, the PDMS mix was allowed to cool down for 2 hours in order to obtain the final, desired, patterns. Openings at the edge of the PDMS patterns were constructed in order to apply capillary force effects to the poly(NIPAAm). After the poly(NIPAAm) accepted the shape of the PDMS patterns, it was photopolymerized, “in-situ”, as described in Chapter 2. Figure 3.2 presents the final photoresist structures, which were obtained with this soft-lithography method.

![Figure 3.2: Bright Field Images of the Photoresist Structures](image)
3.1.1.2. SU-8 Photoresist Patterning

An alternate method to generate high aspect ratio structures incorporated the use of SU-8 photoresist, (PR). The SU-8 photoresist is a thick, negative, high aspect ratio and epoxy-photoplastic resist. SU-8 photoresist is utilized primarily as a substitute, in several MEMS applications for the LIGA, (lithographie, galvanoformung und abformung), process. The LIGA process, which the use of the SU-8 photoresist replaces include:

- Fabrication of plastic micro-molds or metal micro-molds by electroplating, [73],
- Microfluidics for SU-8 microchannels [74],
- Fabrication of photoplastic structures such as micro-gears, [75],
- Micro-coil fabrication, [76],
- Rapid prototyping using laser machining
- Bonding material for optical MEMS.

Very high aspect-ratio micro-structures with vertical sidewalls and high resolution have been reported using the LIGA technique, [77]. However, the need for a synchrotron radiation source limits the application of the LIGA technique. In addition, the process is inherently time consuming and expensive. The SU-8 photoresist can be spin coated in a standard spinner with thicknesses ranging from 1 $\mu$m to 300 $\mu$m and thicknesses up to 2 mm have been reported with multilayer coatings. Combined with standard lithographic processes, the use of SU-8 photoresist offers excellent opportunities in MEMS applications and packaging, which makes its use very popular in the MEMS community.
The processing of SU-8 photoresist has been described by various groups, [78-81]. SU-8 photoresist has very suitable properties of thickness and chemical stability. Additionally, SU-8 photoresist possesses good mechanical and optical properties. The disadvantages associated with the use of SU-8 photoresist are concerned with stress, adhesion selectivity and resist stripping. The adhesion SU-8 photoresist is good with materials such as silicon and gold, [82]. However, on materials such as glass, nitrides, oxides and other metals the adhesion is poor. The SU-8 photoresist can easily be removed from the surfaces of such materials during the development process. On many surfaces such as silicon or glass, which are suitable for spinning the SU-8 photoresist, the thermal expansion coefficient mismatch is large. This mismatch causes large amounts of stress at the material interface, which is due to shrinkage of the SU-8 photoresist while cross-linking during curing. The stress effect is pronounced in large SU-8 photoresist structures and, if poor adhesion is obtained during processing, the photoresist delaminates easily.

The challenges for fabricating an ultra-thick micro-structure with SU-8 photoresist include coating, baking, the developing efficiency and the tremendous residual stress formed after curing. Different groups have reported that a micro-structure with a height of 1.2 mm can be formed by double coating the SU-8 photoresist layers, [83], [84]. However, multicoating is a time consuming process. The surface flatness is also a critical issue. The solvent content in subsequent coating layers may be different, which results in a much more complicated lithography process. Single coating of an SU-8 photoresist film, thicker than 1mm, is not practical using a conventional spin coater due to the high viscosity of the photoresist. As a photoplastic material, the SU-8 photoresist
is chemically stable and resistant to most acids and other solvents. Consequently, after cross-linking it is difficult to remove and suitable methods of stripping, which are compatible with other materials in the structure, are often not effective or their use is not desirable.

The process was initiated, by applying RCA cleaning to an n-type <100> Single-Side-Polished 2” silicon wafer. Different MicroChem Corporation SU-8 photoresists, (SU-8 2005, 2025 and 100), were used to spin coat the sample, [85]. Initially, ramping spinning at 500 rpm for 5 s was employed. Depending on the selected SU-8 photoresist and the desired thickness, a second ramping speed was employed. For example, the second speed was set to 1500 rpm for 25 seconds, which resulted in a coating thickness of 120 $\mu$m, when SU-8 100 photoresist was used. After spin coating, the sample was allowed to relax for one hour in order to allow reflow to complete and to prevent problems with step coverage on the silicon. A pre-exposure bake was performed using temperature ramping to reduce stress. The sample was baked on a hotplate at 65 °C for 5 minutes. Immediately afterward the temperature was ramped and baking continued at 95 °C for 40 minutes. The pre-exposure bake was deliberately extended to allow for maximum solvent evaporation. A Karl Suss Mask Aligner UV light source was used for exposure. A mask, which defined the locations of the SU-8 photoresist on the wafer, was utilized and exposure persisted for 35 seconds. The SU-8 photoresist structures on the cantilevers formed the trenches. The trenches possessed a width of 15 $\mu$m and an opening space of the same dimension. The post exposure bake was carried out at 65°C for 3 minutes and 95°C for 12 minutes. Afterwards, the samples were left at room temperature for 20 minutes in order to avoid thermal stress. The substrates were
developed using a MicroChem SU-8 photoresist developer. The development process consisted of gentle agitation for 20 minutes followed by a hard bake at 150\(^\circ\)C for 2 hours.

The poly(NIPAAm) patterning process is presented in Figure 3.3

---

**Figure 3.3: SU-8 Photoresist Processing for Poly(NIPAAm) Patterning**

Figure 3.3 A-E presents the poly(NIPAAm) patterning steps. A silicon or glass substrate is cleaned for the SU-8 patterning, Figure 3.3A. Figure 3.3 B displays the patterned SU-8 structures. The SU-8 photoresist structures were used as molds for the PDMS, Figure 3.3C. The PDMS mix was placed on top of the patterned SU-8 photoresist and placed on a hot plate at 90\(^\circ\)C for 90 minutes. Afterwards, the sample was allowed to cool down for 2 hours in order to obtain the desired final patterns. Figure 3.3D, represents the process where the PDMS membrane was peeled-off and placed on top of a new substrate. The new substrate could be either silicon or glass, which has been preprocessed with the silane binding agent. Openings at the edge of the PDMS patterns
were constructed in order to apply capillary force effects on the poly(NIPAAm). Figure 3.3E, the poly(NIPAAm) took the shape of the PDMS patterns and was photopolymerized “In-situ” as described in Chapter 2.

A SEM was used to characterize the height SU-8 structures, which were found to be 120 μm. Figure 3.4 presents a SEM image of the SU-8 photoresist structures.

![Figure 3.4: SEM Image of the SU-8 Photoresist Structures](image)

Responsive, high aspect ratio, poly(NIPAAm) trenches were fabricated in order to exploit their, temperature dependent, swelling/contraction behavior to separate submicron biological particles from real samples. When the trenches are open, objects smaller than the width can be pulled into the channel and held there upon closing the trench. Objects that do not fit into the trench are not retained upon closing the channels, and are passed over the trench. This mechanism offers size-selection separation. Figure 3.5 presents a DAPI, (4', 6-diamidino-2-phenylindole), image of the final poly(NIPAAm) structure, which was synthesized by this method.
3.1.2. E-Beam Lithography

Recently, most of the hydrogel related research has focused on the fabrication process of structures using micro-fabrication techniques, [86]. One of the most common fabrication approaches is the use of uv-light photolithography in order to pattern structures, [87]. Different techniques for fabricating sub-micron length scales that span a wide range are available. This section presents a fabrication process using an e-Beam Lithography, (EBL), system within a Scanning Electron Microscope, (SEM), which was enhanced with a Nanometer Pattern Generation System, (NPGS), [88]. General purpose scanning electron microscopes compare well, in terms of controllability and stability, with dedicated e-Beam Direct Writers and dedicated CD SEM’s. These tools provide medium throughput, high quality and lithography capability for nanotechnology devices.

Extensive development of commercial EBL systems has provided various modes of use for positive and negative resists. However, work still continues to be performed with the common polymer PMMA resist on converted SEMs, [89]. PMMA is one of the most common positive e-beam resists employed today since it possesses the highest resolution, [90]. There are many other negative resists available such as COP, SAL and
P, (SI-CMS), which work by cross-linking the polymer chains together. Cross-linking of the polymer chains renders them less soluble in the developer, [91].

A novel technique for e-Beam lithography has been developed using poly(NIPAAm) as a resist to pattern a silicon substrate. The topographic profiles of the structures achieved were characterized and analyzed using an Atomic Force Microscope, (AFM). The results showed that the poly(NIPAAm) behaves as a negative resist for e-Beam lithography. Therefore, it can be exploited in a direct patterning procedure for a thermally responsive hydrogel in order to achieve nano scale structures.

3.1.2.1. Resist Preparation

The hydrogel utilized during this research as a resist for e-Beam lithography was poly(NIPAAm). This polymer was dissolved using cyclohexanone. Afterwards, the solution consisting of the polymer and the solvent was mixed and stirred for 24 hours at room temperature. The poly(NIPAAm) concentration was set at 5%. It is important to note that the solvent normally used for PMMA, which is Anisol, did not dissolve the poly(NIPAAm) at this concentration level. The cyclohexanone and the poly(NIPAAm) were acquired from Aldrich, [68].

The resist was spin-coated at different speeds onto Silicon substrates in order to analyze the speed effect on the resist’s final thickness. The speed values were selected in accordance with the PMMA vendor suggestions and employed for 45 seconds each, [92]. Following the spin coating the resist was baked on a hot plate at 140°C for 75 seconds.
3.1.2.2. E-Beam Exposure

E-Beam lithography was performed, for each machined pattern structure, using a JOEL SEM Model 840, which was enhanced with the Nabity NPGS system. The exposure parameters, which were used for resolution optimization, were 30 kV for the acceleration voltage, 24 pA for the beam current and an e-beam dose range between 300 μC/cm² and 720 μC/cm².

3.1.2.3. Resist Developer

Once the e-Beam lithography process was performed, the resist was submerged into a MIBK/IPA developer. A MIBK/IPA developer ratio of 1:3, which is used for PMMA, was applied for 70 seconds. Application of the developer was followed by 20 seconds in IPA and a rinse for 20 seconds in DI water. In order to complete the development process the samples were rinsed and dried with no post or hard baking step performed on the samples.

The topographic profile of the fabricated structures was characterized and analyzed using an Atomic Force Microscope, (AFM). Figure 3.6 presents a comparison of the effect of different spin speeds and the final film thickness. The thicknesses are in angstroms, (Å), for poly(NIPAAm) and PMMA. The data was obtained using a profilometer, (Tencor Alpha Step 200).

The results exhibit a similar behavior, among the two resist polymers, for the spin speed effect on the final film thickness.
Figure 3.6: PMMA and Poly(NIPAAm) Film Thickness vs. Spin Speed

The two dimensional image of the patterns acquired using an Atomic Force Microscope, (AFM), is presented in Figure 3.7.

Figure 3.7: Two Dimensional AFM Image of the Poly(NIPAAm) Patterns

The scan size was 40 μm at a scan rate of 0.5 Hz. In Figure 3.7, the smallest feature sizes obtained are in the nanometer scale range as illustrated.
Figure 3.8 displays the three dimensional image of the poly(NIPAAm) patterns. The different section analysis test points appear as inverse triangles.

![Three Dimensional AFM Image and Section Analysis](image)

Figure 3.8: Three Dimensional AFM Image and Section Analysis

In addition, Figure 3.8 presents the section analysis of the patterns. The average final thickness value for the patterns was 140 nm. The achieved side walls profile is also displayed in the image.
3.1.3. FIB Patterning

A possible alternative for poly(NIPAAm) patterning is the use of focused ion beam, (FIB), systems combined with soft-lithography. FIB systems have proven to be very useful in micro and nano processing. The utility of FIB systems is primarily due to their ability to selectively remove and deposit material without the use of a patterned resist mask, [93]. Applications for FIB in the fabrication of MEMS/NEMS have been described with emphasis on the processing. FIB techniques can also be very useful during prototyping of microstructures, [94]. This patterning technique demonstrated an interesting approach to the achievement of nano scale structures of poly(NIPAAm).

3.1.3.1. Sample Preparation

Silicon samples were prepared and supplied with poly(NIPAAm) using soft-lithography. These samples were rinsed with DI water and allowed to dry. The samples were taken to the FIB system, which was located at USF’s NNRC, (Quanta 3D USF), where they were patterned. The SEM/FIB was configured and optimized for PMMA patterning.
Figure 3.9 presents the structures obtained by combining soft-lithography and FIB patterning. The large features were obtained with soft-lithography and the trenches were obtained by FIB patterning.

![SEM Image of FIB Patterned Cross-Linked Poly(NIPAAm)](image)

Figure 3.9: SEM Image of FIB Patterned Cross-Linked Poly(NIPAAm)

### 3.2 Dielectrophoresis Effect

Current approaches to cell sorting, most frequently, exploit differences in cell density, specific immunological or receptor-ligand interactions to isolate target cells. These techniques are often inadequate. Therefore, sorting devices capable of identifying and selectively manipulating cells through novel physical properties are desirable. Electrical-field-induced forces on polarizable particles have been the subject of extensive studies in recent years because of their potential applications for particle characterization.
and manipulation. Particles, including biological cells, become electrically polarized under the influence of applied fields. The induced polarizations can, in turn, interact with applied fields, which results in net electrical forces on the particles. Dielectrophoresis, (DEP), is an important phenomenon for the manipulation and distinction of various dielectric particles, [95]. DEP possesses the ability to influence cells or particles strictly as a function of their intrinsic dielectric differences without any modification to them. Therefore, DEP has become one of the most attractive manipulation techniques. In recent studies, many research groups have revealed the dielectric properties of various biological and separated polystyrene micro-beads such as human peripheral blood mononuclear cells, yeast, leukocytes, erythrocytes, neuronal cells and malaria, [96].

During the past few decades, the application of dielectrophoresis for collecting, positioning and separating particles suspended in liquids has advanced tremendously due to improvements in micro-fabrication techniques.

Two approaches, which depend on migration and retention, have been demonstrated for DEP separation. DEP migration exploits the opposing polarities of DEP forces exerted on different particle types. One type is attracted toward high-field regions by positive dielectrophoresis while the other type(s) are repelled by negative dielectrophoresis. Therefore, different particle types are focused at different regions of a microelectrode structure and spatial separation is achieved, [97]. DEP retention exploits the competition between DEP and fluid-flow forces. Particles experiencing a weaker DEP force, either negative or a small positive, are eluted by fluid flow. However, particles experiencing strong positive DEP forces are trapped at electrode edges against the drag of the fluid flow.
There has been great interest in a family of techniques collectively termed field-flow fractionation, (FFF). FFF techniques exploit the velocity gradient of a hydrodynamic flow profile to achieve particle fractionation, [98]. In these techniques, a laminar fluid profile is established in a thin chamber such that fluid moves faster with increasing distance from the chamber walls. A force field is applied perpendicular to the flow direction and across the flow profile. The force field causes particles to be forced toward a so-called accumulation wall of the chamber, which is typically the bottom wall. In normal-mode FFF, particles are sufficiently small that the diffusion and accumulation forces counteract and an equilibrium concentration profile results along the force-field direction and hence across the fluid velocity profile. Thereby particles are carried by the fluid at velocities that depend on their equilibrium profile. Dissimilar particle types exhibit different characteristic concentration profiles. The different concentration profiles are carried at different velocities, which causes the different particle types take different times to traverse the separation chamber. Therefore, they can be separated by collecting them at appropriate times at the chamber exit. This principle was exploited during this research in order to deflect the particles down to the poly(NIPAAm) trenches where the catch and release mechanism was executed to manipulate the samples.

The movement of dielectric particles inside a microfluidic channel is dominated by the dielectrophoretic velocity induced by a non-uniform electric field and the hydrodynamic velocity imparted by the fluid flow. The total velocity is given by:

\[
V_{\text{Total}} = V_{\text{DEP}} + V_{\text{Hyd}} \ .
\]  
(3.1)
The dielectrophoretic velocity results from the equilibrium between the dielectrophoretic force and the viscous drag force, which is represented by:

\[
V_{\text{DEP}} = \frac{F_{\text{DEP}}}{f_{\text{Drag}}} = \frac{F_{\text{DEP}}}{6\pi \eta r}
\] (3.2)

The DEP force acts on neutral bodies in nonhomogeneous electrical fields. It differs with respect to the aspect of neutrally from the electrical force, which acts only on charged bodies in an electrical field, \(E\). In the presence of the field, \(E\), the body becomes polarized. The polarization of the body makes it equivalent to an electric dipole. In an electric dipole there exists an excess of positive charges area and an excess of negative charges in another area and the two areas are separated by some distance. This dipole establishment for an initially neutral dielectric particle, which is subjected to a nonhomogeneous field is illustrated in Figure 3.10).

![Figure 3.10: Dielectric Particle in a Nonuniform Electric Field](image)

Since the field is nonhomogeneous, the forces acting on the two ends are not equal and the total net force is nonzero. The force, \(F\), is given by:
\[ F = \nabla \cdot (Q_{+}E(r + d) - Q_{-}E(r)), \quad (3.3) \]

where \( Q_{+} \) and \( Q_{-} \) represent the excesses of positive and negative charges on the body. If the distance associated with the induced dipole, \( d \), is small compared to the distances with respect to which the field varies appreciably, then

\[ Q_{+} \approx Q_{-} \quad (3.4) \]

and

\[ E(r + d) \approx E(r) + (d \cdot \nabla)E. \quad (3.5) \]

Substitution of the approximations given by equations 3.4 and 3.5 yields:

\[ F = (p \cdot \nabla)E \quad (3.6) \]

where

\[ p = Qd \quad (3.7) \]

is the induced dipole moment of the body.
The time-averaged dielectrophoretic force that represents the interaction force between the electric field and the induced dipole moment is given by:

\[ F_{DEP} = 2\pi r^3 \varepsilon_m \text{Re}(f_{CM}) \nabla E^2, \quad (3.8) \]

and

\[ f_{CM}(w) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2 \varepsilon_m^*}, \quad (3.9) \]

where \( r \) is the radius of the particles, \( \varepsilon_m^* \) and \( \varepsilon_p^* \) are the complex permittivities of the suspending medium and particle respectively, \( E \) is the local electric field and \( \text{Re}(f_{CM}) \) is the real part of the polarization factor, (Clausius–Mossotti factor), which is dependent on the applied field frequency and the conductivity medium. \( \text{Re}(f_{CM}) \) can have values between +1 and -0.5.

Substitution of equation (3.8) in equation (3.2) and simplifying shows that the dielectrophoretic velocity is proportional to the cube of the particle radius and the real component of the polarization factor with a fixed medium conductivity and a fixed electric field. Consequently, the DEP force can be used to separate, position or deflect particles with different sizes and dielectrophoretic affinities under the appropriate electric field distribution. With the electric field gradient of the microelectrode along the perpendicular direction to the fluid flow, particles with different dielectrophoretic velocities can be selectively fractionated in a microfluidic channel.
The Electric field, $E$, can be numerically simulated through the use of finite element analysis. Figure 3.11 presents a simulation for the array of inter-digitated microelectrodes. The simulation was performed with the ANSOFT HFSS software, [99].

Figure 3.11: Simulation of the Effect of the Electric Field, $E$, on the Microelectrode Array

In Figure 3.11(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 Figure 3.11(B) an AC signal is applied to the microelectrode array. The applied signal produces an electric field in a plane perpendicular to the surface plane of the microelectrodes. The vectors indicate the direction of the field, which will produce a DEP force perpendicular to the flow inside the
microfluidic channel. The strength of the field is given by equation 3.8. Figure 3.11(C) displays the field intensity, which, as expected, is larger near the array and reduces in the z axis direction. Figure 3.11(D) presents a detailed view of the electric field, $E$, close to the A-A’ cross section between to electrodes of the array. In the final device, the DEP force is necessary to deflect the particles into the microfluidic channels, which exhibit laminar flow behavior as explained in Section 3.4. The induced DEP force could be, either negative or positive. If the DEP force is negative the particles would move away from the microelectrode array. If the DEP force is positive the particles would move toward the microelectrode array. The direction of the DEP force depends on the final value of the Clausius–Mossotti factor, which is indicated in equation 3.8. In order to bind the samples and the poly(NIPAAm), the DEP effect must be applied inside the microfluidic channel.

3.2.1. Fabrication

A microelectrode array was fabricated on top of a glass substrate with, Futurrex NR9-1500PY, negative photoresist, (PR), using conventional photolithography. The initial processing of the glass involved several steps, which consisted of the substrate being:

- Cleaned with a sulfuric acid solution for 5 minutes,
- Dipped in 50:1 HF for 30 s to improve adherence,
- PR was spin coated at 3000 rpm for 30 s,
- Soft baked, on a hot plate, for 2 minutes at 150°C,
- Exposed to UV light for 35 s,
• Baked, post exposure, for 1 minute at 100°C,

• Developed for 20 s with Futurrex RD6 developer.

After the initial processing, 1800 Å of Au was deposited on the substrate via sputtering. Lift-off was performed using acetone and isopropanol. The device was DI water rinsed and nitrogen dried. The cross section and bright field image of the final inter-digitated microelectrode array are presented in Figure 3.12.

![Figure 3.12: Au Microelectrode Inter-Digitated Array](image)

### 3.3 Thermal Actuation

In order to modify the thermally sensitive hydrophobic interactions, within the polymer network, micro-machined thermal actuators were installed in the final device.

A micro-heater to thermally affect the hydrogel was fabricated using conventional techniques, [100]. A thermal insulation layer of SiO₂ was thermally grown up to 3000 Å. The SiO₂ was patterned by employing standard photolithography and diffusion openings...
were defined. Boron diffusion was applied on the substrate in order to obtain the desired doping profile. Finally, aluminum contacts were patterned to connect the diffused resistor. The final temperature obtained was, according to the Joule effect, proportional to the geometric properties and the current and voltage applied to the resistor, [101]. Figure 3.13 presents two diffused resistor prototypes, which were fabricated as part of the research.

Another proposed method to generate the heating element in the system was to use a nichrome resistor heater. Using the Joule heating effect, which is determined by:

\[ Q_R = I^2 R \text{ Watts} \quad (3.10) \]

where \( I \) is the current applied to the resistor in amperes and \( R \) is the resistor’s final resistance value in ohms.
Thin film heaters can be made of different materials on a variety of substrates. The thin film circuit substrate was selected to ensure the performance and reliability of the circuit. The main requirement for the substrate, for this research, was the electrical insulation properties. A number of thin film resistor materials such as: tantalum nitride, polysilicon, tungsten, and platinum can be used for different applications. However, the most popular material is the nickel-chromium alloy called nichrome, which is 80% nickel and 20% chrome. Nichrome was selected as the material for fabrication of the resistor film for this research. Thin film resistors, (thickness less than 24,000 Å or 0.1 mil), offer the advantage of a low temperature coefficient of resistance, (TCR), and finer line definition capabilities over their thick film equivalents. Nichrome was selected since it can be easily e-beam deposited on glass substrates and allows standard processing for patterning. Nichrome resistors are corrosion-resistant and, with proper annealing, offer a very low TCR, which results in precise and thermally stable resistors.

The resistor fabrication process started by cleaning the glass substrates in a sulfuric acid solution for 10 minutes, rinsing with DI water and desiccating, on the hot plate, at 200°C for 10 minutes. Next, the resistor network was patterned using, Futurrex NR7-3000 PY, negative PR on the wafer, (Futurrex, Inc., Franklin, NJ). The nichrome resistor was deposited in an e-beam evaporator for a total thickness of 1800 Å. Typical sheet resistance values for nichrome range from 100 to 200 Ohms-per-Square, with a tolerance of 10% of the nominal value and a TCR of $0 \pm 50$ ppm per °C. Figure 3.14 presents the cross section and the bright field image of the final structure fabricated, which yielded a final resistance value of 1.9 KΩ.
3.3.1 Heat Transfer

In order to design the heating element, it was necessary to estimate the heat transfer applied to the microfluidic channel. The total amount of heat necessary to effect a change in temperature, from room temperature and above the LCST, is given by:

\[ Q_w = fc\Delta T \]  \hspace{1cm} (3.11)

where \( f \) is the fluid flow, \( c \) is the specific heat and \( \Delta T \) is the change in temperature. To obtain a change in temperature of 20°C with a fluid flow of 10 μl per min the total heat, \( Q_w \), required was 6 x 10\(^{-3}\) Kcal per hour.
The heat loss, due to transfer by convection and conduction through the channel’s side walls and top part, can be calculated using Fourier’s law of heat conduction, which is given by:

$$Q_f = \mu A \Delta T$$

(3.11)

where $\mu$ is the thermal conductivity, $A$ is the area and $\Delta T$ is the change in temperature. The total amount of heat, $Q_f$, applied to the channel was 0.012 Kcal per hour, which is equivalent to 0.014 W per hour, in electric units. This value was used to calculate the total current required for the nichrome heating device, which was in accordance with the final measured resistance value.

### 3.4 Microfluidics Integration

In the final device the poly(NIPAAm) structures were placed inside microfluidic channels to interact with biological samples. Microfluidic systems constitute a steadily growing sector of the MEMS field. Flow channels for fluids are fundamental building blocks of microfluidic systems. Flow channels in microfluidic systems are analogous to wires or thin film electrical interconnects in conventional integrated circuits, [102]. There is a clear trend to reinvent microfluidic components, as disposables, by replacing classic MEMS/NEMS materials such as silicon or pyrex with inexpensive polymer materials such as PDMS, parylene or SU-8 photoresist, [103]. Two different microfluidic channel integration methods were developed during this research. One was based on the use of PDMS which was bonded to the substrate. The other method utilized SU-8
photoresist as an intermediate layer between two surfaces in order to create microfluidic channels. PDMS has had a large impact on research and development. The PMDS impact is due to its ease of processing, in standard laboratory conditions, and its biocompatibility predestines, which make it suitable for rapid prototyping in microfluidics, [104]. This elastomer is widely used in microfluidic applications to form components such as channels, valves, and diaphragms, [105]. PDMS is a low cost material that offers many advantages. PMDS can be processed by easily molding. PMDS is elastic and can form fluid seals effectively. PDMS is commonly used as a bulk material, [106]. The predominant fabrication process associated with PDMS is bulk molding. The principle of the PDMS patterning process used during this research is discussed in the following section.

**3.4.1 PDMS Microfluidic Channel Fabrication Process**

The initial phase for the Silicon-PDMS molding, used during this research, was bulk silicon processing using an induction coupled plasma, (ICP), a deep reactive ion etcher, (DRIE), which employed the advanced silicon etch, (ASE), process, [107]. The ASE process was patented by Bosch GmhH. The basic idea behind the anisotropic reactive ion etching efforts, in use today, is to find a balance between trench side wall passivation and trench bottom etching. Trench bottom etching is activated by the bombardment of ions from the plasma discharge. The method known as the “Bosch Process” is a room temperature process based on continuous cycling of subsequent passivation and etching steps. The Bosch Process achieves high aspect ratio microstructures. Aspect ratios over 20 and etching depths up to 500μm can be achieved.
A typical etching rate is 2μm per min, the selectivity to resist is 75:1 and the selectivity to silicon dioxide is 250:1, [108].

Dry etching and molding is a fast and flexible production process for polymer microstructure products based on a sequence of process steps, one, of which, is a DRIE step, [109]. The high aspect ratios, directional freedom, low roughness, high etching rates and high selectivity with respect to the mask material of DRIE allow a versatile fabrication process of micro-moulds for subsequent molding and embossing. The feasibility of this process has been demonstrated for feature sizes of several micrometers up to tens of micrometers. The layouts of the mould inserts are etched by DRIE into silicon. The etched structures are used for PDMS embossing.

Silicon wafers <100> 4 inch, n-type were DRIE processes using a Unaxis, PlasmaTherm 770 ICP system to produce microfluidic channel molds on Silicon for the final application during this research. The DRIE conditions used in order to obtain the structures on the Silicon wafers are presented in Table 3.1.

<table>
<thead>
<tr>
<th>Passivation Step Parameters:</th>
<th>Etch Step Parameters:</th>
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</thead>
<tbody>
<tr>
<td>Pressure: 20mTorr</td>
<td>Pressure: 20mTorr</td>
</tr>
<tr>
<td>Time: Morphed from 5 sec. to 5.5 sec.</td>
<td>Time: Morphed from 5.2 sec. to 4 sec.</td>
</tr>
<tr>
<td>O₄F₈ flow: 70 sccm</td>
<td>SF₆: 100 sccm</td>
</tr>
<tr>
<td>Ar: 40 sccm</td>
<td>Ar: 40 sccm</td>
</tr>
<tr>
<td>Electrode Power: 1W</td>
<td>Electrode Power: 16W</td>
</tr>
<tr>
<td>Coil power: 850W</td>
<td>Coil Power: 850W</td>
</tr>
</tbody>
</table>
The structures obtained by DRIE, during this research, are presented in Figure 3.15. The final depth was 8μm.

Figure 3.15: SEM Image of the DRIE Patterned Micro-Channels

Figure 3.16 displays the channel’s topographic profile.

Figure 3.16: SEM Image of the DRIE Anisotropic Etching
A typical Bosch Process side wall effect is outlined in Figure 3.17. The passivation and etching steps produced this profile.

![Figure 3.17: SEM Image of the DRIE Bosch Process Side Wall Profile](image)

### 3.4.1.1. Silicon-PDMS Molding

PDMS was poured into the Silicon molds and cured for 1 hour at 65 °C using a 10:1 ratio of elastomer and curing agent. After curing the PMDS was peeled from the mold revealing the microfluidic channels. The channels are presented in Figure 3.18.

![Figure 3.18: PDMS Microfluidic Channels](image)
3.4.2. SU-8 Photoresist Microfluidic Channel Fabrication Process

Another method applied to fabricate microfluidic channels, during this research, was based on SU-8 photoresist patterning. Polymerized SU-8 photoresist possesses excellent chemical and mechanical properties. The properties of the polymerized SU-8 photoresist have made possible many novel microfluidic devices whose capabilities have been demonstrated, [110]. However, the fabrication of high-aspect ratio microfluidic channels is still under extensive investigation. During this research, a novel method was developed for the fabrication of microfluidic structures. SU-8 photoresist was used as an intermediate layer between two substrates. One of the substrates contained the DEP microelectrode array and the other contained the poly(NIPAAm) trenches. Polished, 1.1mm thick, microscopic glass substrates were cut to fit the design of the device. Prior to the lithography process, the glass substrates were cleaned in a boiling Piranha solution, (H₂SO₄:H₂O₂= 3:1), for 10 min.

The photolithography masks were generated using layout design software, (AutoCAD). The SU-8 photoresist process was the same as described in Section 3.1.1.2 in order to obtain the high aspect ratio structures. The bonding method between substrates utilized a highly transparent UV-sensitive adhesive and a UV light source. PDMS with a 1:1 ratio was used for sealing the microfluidic devices and the capillary tubing. The process for fabricating the SU-8 photoresist microfluidic devices is presented in Figures 3.19 and 3.20.
Figure 3.19 presents the microfluidic channel cross-section where the upper substrate, (Black), contains the DEP microelectrode array and the lower substrate, (Gray), contains the polymer trenches. The SU-8 photoresist was patterned to form the microfluidic channels. Finally, both substrates were bonded and the channels’ inlet and outlet ports, (I/O), were attached and sealed using PDMS.
Figure 3.20: Microfluidics Integration

Figure 3.20 presents the complete microfluidics integration process. In step A, the SU-8 photoresist is patterned according to the channel design. In step B the channel is aligned to the DEP microelectrode array and the poly(NIPAAm) trenches. In step C, the substrates are bonded and the channel formed using the SU-8 photoresist as an intermediate layer. Figure 3.20D presents a bright field image of the final channel structure. Figure 3.20E displays the DEP Microelectrode inside the microfluidic channel.
CHAPTER 4
EXPERIMENTS AND RESULTS

4.1. Poly(NIPAAm) Patterning

The topographic profile of the Soft-lithography-FIB patterned structures, which were fabricated, was characterized and analyzed using an AFM. AFM imaging was performed in the FIB exposed area using an Asylum Research's MFP-3D system and uMasch silicon cantilevers with nominal spring constants of 1nN under ambient air and deionized water. The sample was submerged in 50°C water and maintained at that temperature by recycling the water droplet for 15 minutes. The effects of temperature, on the gel, were obtained by systematically scanning a small area of 10 scan lines as the fluid equilibrated with the surrounding air temperature. Sectional data was taken on the same position of the infrastructure for comparison. The AFM results illustrated the swelling behavior of the poly(NIPAAm) due to a change in water temperature from 45°C to 25°C. The swelling resulted in an 80% volume transition in the vertical plane. The FIB patterning demonstrated nano-scale range patterning capabilities.
An SEM image of the patterns generated is presented in Figure 4.1. The lighter colored areas are the Poly(NIPAAm) structures.

Figure 4.1: SEM Images of Soft-Lithography and FIB Patternning

Figure 4.1 presents a structure with dimensions less than 500 nm. Figure 4.2 presents the AFM section analysis for the FIB patterned structures test.

Figure 4.2: AFM Measured Section Analysis

Figure 4.3 presents the AFM scans for temperatures of 45°C and 25.6°C. The swelling properties of the Hydrogel with changes in temperature are clearly illustrated.
4.2. High Aspect Ratio Trenches Thermal Responsiveness

The high aspect ratio trenches, which were constructed of the UV polymerized “In-situ” poly(NIPAAm) using soft-lithography, were tested to confirm their temperature responsiveness. The collapse/swelling behavior expected was obtained by applying a 20°C temperature change, (ΔT). DI water was poured on the samples at different temperatures above and below the LCST of the poly(NIPAAm).
It was observed that at 40°C, (104°F), the trenches were open. However, using DI water at room temperature causes the poly(NIPAAm) to swell and induces a closing effect on the trenches. This effect is a key technology platform that facilitates separation processes that are difficult to achieve by any other method. Figure 4.4 presents the poly(NIPAAm) swelling behavior due to temperature stimuli.

![Figure 4.4: Bright Field Images of the Poly(NIPAAm) Swelling Behavior](image)

**4.3. Particle Entrapment**

A series of experiments were performed in order to analyze the particles catch and release effect inside the high aspect ratio trenches. Fluorescent polystyrene microbeads, (PolyScience PA), were added to a mix consisting of DI water, 20µm diameter beads and 6µm diameter beads. This mix was applied to the top of the trenches at different temperatures and different beads concentration values in order to examine their behavior. Figure 4.5 presents the trench structures, submerged in water, with a temperature above the LCST of the poly(NIPAAm). There is no microbeads mix present in Figure 4.5.
The BioMEMS device exploits the opening and closing effect of the fabricated trenches to entrain particles inside them in order to generate a sorting mechanism. Figure 4.6 presents an image with the microbeads mix present. The 6 µm beads move inside the trenches, while the 20 µm beads stay on top of the structures.

Figure 4.6: Particles Moving Inside the Trenches
Figure 4.7 illustrates the behavior of the beads mix diagrammatically. The size sorting mechanism is applied to 20 μ and 6 μm diameter microbeads.

After the surface was scoured with cold water, the thermal responsive polymer swelled “closing” the trenches and capturing the particles inside. This closing and capture behavior is displayed in the sequence presented in Figure 4.8.

The areas inside the circled point display the captured particles and the closed trenches. In Figure 4.8A, cold water was applied and the particles moved inside the trench. In figure 4.8 B, the trenches start to close. In Figure 4.8C, the particles are captured.

The experiment was repeated using different microbeads concentrations. One set of experiments contained 6 μm and 20 μm microbeads. Other sets of experiments
contained only the 6 µm microbeads. In the experiments with a mix containing the two
different diameter size microbeads, the majority of the 20 µm beads were removed.
However, some of them remained on top of the trenches and a significant amount of
particles were not trapped in the trenches.

It appears that there is some interaction that produces a “sticking” effect between
these beads and the polymeric surface, which produces a “failure to release” of the
particles. After the trenches were scoured again with hot water, very few, if any, of the
particles that were trapped were able to be removed. This situation is presented in Figure
4.9.

![Figure 4.9: Bright Field Image of the Failure to Release Effect](image)

Although some applications such as particle entrapment for analysis don’t require
the release of the particles from the trenches, the scope of the application is reduced by
the lack of a release mechanism. The final BioMEMS device must be capable to
integrate both the capture and release mechanisms.
4.4. Cell Attachment

An experiment was developed to analyze the interaction behavior of the poly(NIPAAm) and *Escherichia coli* bacteria. Two silicon substrate samples were prepared and supplied with Poly(N-isopropylacrylamide) on each. These samples were rinsed with RO/DI water and allowed to dry. In preparation for the experiment, a hybridization oven and a water bath were both set to 38°C. One of the samples was placed in the oven while the other was left out at room temperature, (22°C). A 50 ml conical tube of rinse water, (RO/DI), was placed in the water bath and another was left at room temperature. Two cultures of *E. coli* w/green fluorescent protein, (GFP), were prepared and allowed to equilibrate at the two operating temperatures. The cultures were prepared as follows:

- A single colony of *E. coli* w/GFP was added to 1 ml of minimal media and resuspended by vortexing,
- The culture, (0.5 ml), was added to two tubes containing 1.5 ml of minimal Media,
- Each of the two cultures were mixed by vortex and allowed to equilibrate at the two defined temperatures.

Once the components were equilibrated at the defined temperatures, the cultures were transferred by pipette to the poly(NIPAAm) membrane spots on each silicon substrate. One spot was left dry on each chip for comparison purposes. Each sample was hybridized for five minutes and then rinsed in their respective rinse water tubes. Each sample was submerged into the tube for ten seconds and then removed for ten seconds. The water velocity, relative to the sample, was 0.56 cm/s, (Re = 1,288, which is laminar
Following the rinse, the samples were given a quick shake to remove the excess surface moisture.

Images presented in Figure 4.10 were collected at a magnification of 250x using an epifluorescence microscope with a FITC filter cube.

Figure 4.10: *E. coli* w/GFP on the Membrane Area of a Silicon Substrate

In Figure 4.10A, the background of the silicon substrate was hybridized for 5 minutes and rinsed at 22°C. In Figure 4.10B, the poly(NIPAAm) membrane area was hybridized for 5 minutes and rinsed at 22°C. In Figure 4.10C, the background of the silicon substrate was hybridized for 5 minutes and rinsed at 38°C. In Figure 4.10D, the poly(NIPAAm) membrane area was hybridized for 5 minutes and rinsed. The results demonstrate that the *E. coli* was attached to the poly(NIPAAm) membrane at 38°C.
4.5. Flow Velocity

The Flow Velocity experiment was carried out in order to determine if water velocity has an impact on whether or not the *E. coli* cells, (w/GFP), are washed off the membrane. The temperature was held constant. All samples were kept at a temperature of 38°C, placed in a hybridization oven and allowed to equilibrate for 10 minutes. Approximately 200 µl of resuspended *E. coli*, (w/GFP), was placed on each spot on the samples. The samples were allowed to hybridize for another 5 minutes. Once hybridization was complete, the samples were individually lowered into RO water, (at 38°C), for the times presented in Table 4.1. After the soak time the samples were pulled up in the water for the same amount of time. These, “down then up”, cycles were repeated fives times for each slide at 38°C.

<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Dip time (seconds)</th>
<th>Total time in water (sec)</th>
<th>Estimated Velocity (cm/s)</th>
<th>Reynolds Number</th>
<th>Estimated cells remaining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>100</td>
<td>0.56 cm/s</td>
<td>1,288</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>50</td>
<td>1.12 cm/s</td>
<td>2,576</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>20</td>
<td>2.8 cm/s</td>
<td>4,293</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>10</td>
<td>3.73 cm/s</td>
<td>8,587</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

After each sample was dipped, they were allowed to dry in the hybridization oven for 5 minutes and subjected to image analysis. The images are presented in Figure 4.11.
Based upon the images displayed in Figure 4.11, it is apparent that the water velocity produced an effect on the ability of the membrane to hold the cells. As the values of the Reynolds number increased the percentage adhesion of the bacteria decreased dramatically. Figure 4.12 presents a plot of the velocity vs. cells remaining.
It is obvious that there is a rapid sloughing effect as the flow velocity increases from 0.56 cm/s. This velocity represents a Reynolds Number, (Re), of 1,288, which is well into the laminar range. Based upon this preliminary data, it appears that the cells are able to stay attached to the membrane for laminar flow conditions. However, the situation reverses for turbulent flow conditions. The next stage of the experiment should be performed with flow conditions corresponding to Reynolds numbers of 1000 and 2000. Results from this experiment should reveal whether or not the velocity of the flow or the laminar/turbulent conditions determines cell adhesion.

4.6. Microfluidic Channel Testing

The PDMS microfluidic channels were attached to a microscope cover slip and observed through the cover slip. The bacteria, *E. coli* w/ green fluorescent protein, (GFP), was introduced at the edge of the microfluidic channel and allowed to move through the microfluidic channels by capillary forces. Figure 4.13 displays the microfluidic channels present on the other side of the cover slip.

![Figure 4.13: FITC Image of the *E. coli* Flowing Inside the PDMS Channels](image)
By adjusting the focal plane, the *E. coli* w/ green fluorescent protein, (GFP), was visible when observed through the FITC filter cube. The *E. coli* bacteria are clearly in the channels.

The SU-8 photoresist fabricated microfluidic channels were also tested using the experimental setup presented in Figure 4.14.

![Experimental Setup: SU-8 Photoresist Microfluidic Channel Testing](image)

The syringe pump, (Harvard Apparatus), was programmed to generate an input flow from 10 µl/min to 600 µl/min. Fluorescent polystyrene beads, (PolyScience PA), of 6 µm nominal size were added to the DI water solution with a final concentration of \(~1\times 10^6\) per ml as visual targets to be observed through the microscope FITC filter cube.

The volume flow rate relationship for a given geometry is given by:

$$F = Av \text{ in m}^3 \text{ per sec}$$  \hspace{2cm} (4.1)

where $F$ is the volume flow rate, $A$ is the cross-sectional area of the flow channel in m$^2$ and $v$ is the average velocity of the fluid in m/s. A volume flow rate of 10 µl/min,
(1x10^-8 m^3/min), produced an average velocity, \( v \), for the DUT of 0.013 m/s. To calculate the Reynolds number of the flow, which is required to determine the relative turbulence of the flow stream, equation 4.2 was applied:

\[
R_	ext{e} = \frac{\rho v L}{\mu}
\]  

(4.2)

where \( \rho \) is the fluid’s density, \( v \) is the fluid’s velocity, \( L \) is the channel length and \( \mu \) is the fluid’s viscosity. As expected, the Reynolds number in the microfluidic channels was very low, which is indicative of a laminar flow behavior. The low Reynolds number value, (0.2), essentially eliminates the presence of any turbulence. In the absence of turbulence certain effects such as mixing are very hard to achieve. These results demonstrate the necessity of implementing a strategy for sample and poly(NIPAAm) interaction in the presence of laminar flow. Figure 4.15 presents an image of the flow at a rate of 10 \( \mu l/min \).

![Figure 4.15: SU-8 Photoresist Microfluidic Channel](image)

Faced with these results it was concluded that the use of the SU-8 photoresist channels was appropriate for the final device.
4.7. Device Testing

The BioMEMS device was fully tested to verify its functionality. The experimental setup is presented in Figure 4.16.

An inverted fluorescent microscope, (LEICA DMI400B), with a CHROMA 31001 cube was used to capture video and still images. The flow, inside the microfluidic channel, was controlled by a Harvard Apparatus PHD 2000 syringe pump, operating in infusion mode. The microscope and syringe pump are pictured in Figure 4.17.
The Device-Under-Test, (DUT), was installed inside a petri dish, which was bonded and leveled using an epoxy layer. The DUT is pictured in Figure 4.18.

![DUT](image)

**Figure 4.18: DUT**

The capillary tubing was attached to the different syringes using a plastic connector which was attached to the capillary tubing. The DEP signal was connected to the gold pads in the device. The DUT contains:

- the microfluidic channel,
- the DEP microelectrode array located at the top of the channel,
- the poly(NIPAAm) trenches located on the bottom substrate,
- the inlet and outlet ports,
- the capillary tubing, which was attached to the inlet port,
- the syringe pump.

The video and still images were processed with IMAGE Pro Plus software, which was installed in an INTEL P4, 1G-RAM, PC. The AC signal for the DEP microelectrode array was generated using a SONY/Tektronix AFG320 Function Generator.
Figure 4.19 presents an image of the top section of the microfluidic channel. The microfluidic channel inlet port is on the left side of the image. The DEP microelectrode array is inside the SU-8 microfluidic channel.

![Figure 4.19: Top Section of the Microfluidic Channel](image)

Figure 4.20 presents an image of the bottom part of the microfluidic channel. The microfluidic channel outlet port is on the right side of the image.

![Figure 4.20: Bottom Section of the Microfluidic Channel](image)

The inlet and outlet ports were drilled in the glass using a diamond coated drill. After drilling, the DEP microelectrode and the poly(NIPAAm) trenches were fabricated next to each port to avoid damage caused by the drilling process.
An inverted fluorescent microscope’s image, of the DUT, is presented in Figure 4.21. The DEP array is on top. The trenches can be seen located at the bottom part of the channel.

![Image](image_url)

Figure 4.21: BioMEMS Device Integration – Device Top View

The experiment was initiated by programming the syringe pump to generate an input flow of 10 μl/min. Fluorescent polystyrene beads, (Polyscience PA), of 6 μm and 20 μm nominal size were added to the DI water solution. The final concentration was set at $1 \times 10^6$ per ml at 60°C. At this temperature and flow values, the trenches were open. However, the particle density attached to the trenches was almost zero. This was expected due to the laminar flow region inside the microfluidic channel. A negative DEP force was applied perpendicular to the flow in order to deflect the particles down to the trenches. Figure 4.22 presents a sequence of images of the particles’ path.
In Figure 4.22A and Figure 4.22B, the flow contains 6 \( \mu \text{m} \) and 20 \( \mu \text{m} \) particles at 60\(^{\circ}\)C. The attached particle density is zero. In Figure 4.22C, DEP force is applied and the particles start to be deflected to the trenches, which increases their density. In Figure 4.22D, Figure 4.22E and Figure 4.22F the capture process is presented. During the capture process the 6 \( \mu \text{m} \) particle density in the trenches is increased and the 20 \( \mu \text{m} \) particles are swept away. In Figure 4.22F just one of the 20 \( \mu \text{m} \) bead remains attached to the top of the trenches. As designed, the sorting mechanism was confirmed by the presence of the 6\( \mu \text{m} \) beads, which are displayed inside the trenches.

Previous work was performed related to DEP. The *E. coli* bacteria were tested using another microelectrode array to verify the DEP effect on them. Figure 4.23 displays the *E. coli* aligned to the microelectrode array when the DEP was on and scattered six seconds after the DEP was turned off.
DEP was applied while changing three main parameters:

- Flow rate,
- Frequency,
- AC Amplitude.

Flow rate was varied from 5 μl/min to 20 μl/min. The frequency was varied from 1 to 50 KHz. The amplitude was varied from 1 to 10 V<sub>pp</sub>. The image sequences were analyzed using a particle counting routine, from MATLAB, in order to calculate the particle density inside the trenches. Figure 4.24 presents the effect of the DEP on the trench density with a flow rate of 10 μl per min, a frequency of 50 KHz and AC amplitude of 8 V<sub>pp</sub>. The data was taken every 3s along the x axis.
Figure 4.24: DEP with 10 μl per min, 50 KHz, 8 V<sub>pp</sub>

Figure 4.25 presents the effect of the DEP on the trench density with a flow rate of 10 μl per min, a frequency of 50 KHz and AC amplitude of 2 V<sub>pp</sub>. The data was taken every 3s along the x axis.

Figure 4.25: DEP with 10 μl per min, 50 KHz, 2 V<sub>pp</sub>

The plots presented in Figures 4.25 and 4.25 clearly illustrate the increase in density due to the use of DEP.

The experiment demonstrated that the DEP effect on the particles was as proposed. The experiment also demonstrated that the DEP and the flow value are correlated with the particle density. Although not demonstrated in the Figures of 4.24 and 4.25, the highest particle concentration areas coincided with the highest $E$ Field...
produced by the microelectrode array. This result corresponded to the simulation performed, which was presented in Chapter 3.

The next stage of the experiment consisted of injecting cold water into the microfluidic channel. Figure 4.26 presents the image sequence of the particles inside the trenches.

![Figure 4.26: Particles after Cold Water Injection](image)

In Figure 4.26A, the particles clearly occupy a larger area inside the trench. In Figure 4.26B, the flow temperature starts to close the trenches and align the particles while reducing the space. In Figure 4.26C, the trenches are closed and the particles are maintained inside. The sequence of images, presented in Figure 4.26, clearly demonstrates the capture mechanism.

The final experimental stage was focused on the release mechanism. Hot water, at 40°C, was injected into the channel, which caused the trenches to open. The particles demonstrated the same “sticking” effect described in Section 4.3, which did not allow the particles to be released from the trenches. As proposed, DEP was applied and the frequency range was changed from 10-60 KHz to 10-20 MHz, which shifted the Clausius–Mossotti factor. Application of a positive DEP combined with an increase in
the flow value to 20 μl/min generated particle displacement inside the trenches and started the release mechanism. Figure 4.27 presents the image sequence of the release mechanism.

![Figure 4.27: Particles after Hot Water Injection](image)

In Figure 4.27A the particles are inside and attached to the trenches. In Figure 4.27B, Figure 4.27C and Figure 4.27D, the positive DEP force effect on the particles is demonstrated. The particles start to be released, Figure 4.27C, and the particle density inside the trenches is decreased, Figure 4.27D.
Figure 4.28 illustrates the decreasing particle density due to the positive DEP force. As in the negative DEP case, the experiment demonstrated that the DEP and the flow value are correlated with the particle density.

The experiment proved the device’s functionality was in accordance with the proposed mechanisms. The release mechanism is more dependant on the flow value and the voltage peak-to-peak amplitude than the attach mechanism. The release mechanism takes less time.
CHAPTER 5
CONCLUSIONS

A cell sorting integrated BioMEMS device based on the use of “Intelligent” thermally responsive polymers has been described, developed and fabricated. This novel sorting and manipulation strategy hasn’t been previously exploited or reported. Therefore, 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 effectiveness, power efficiency and ease of operation. As illustrated in this dissertation, the complete integration of different technologies to achieve the desired results has displayed promising results related to this technology platform.

This dissertation has described different procedures for the prototyping and fabrication of responsive poly(NIPAAm) hydrogel topographies using soft-lithography, e-beam lithography and soft-lithography combined with focused ion beam patterning. Soft-lithography, together with the use of innovative process materials such as SU-8 photoresist and PDMS, presented the capability of obtaining high aspect ratio polymer structures. Soft-lithography has also proven to provide an excellent approach since its “In-situ” polymerization capability provides significant flexibility to the hydrogel’s property tuning. Although e-beam lithography doesn’t offer such flexibility, it can also
be considered as an alternate patterning solution in order to achieve submicron scale size ranges. The behavior of the poly(NIPAAm) polymer as a negative e-beam resist unveils the possibility of using this technique as a direct patterning procedure for hydrogel based structures. Future experiments will account for relevant factors such as film thickness, e-beam dose, process step temperatures and other issues that need to be optimized for high resolution nano scale range pattern generation. The latter approach, through the combined use of the soft-lithography and FIB patterning also demonstrated a nanometer structure size range, which exploits the advantages of soft-lithography. The AFM structural and functional analysis and results have demonstrated that the hydrogel’s thermal response is maintained after the FIB exposure. In addition, the AFM results demonstrated that the thermal response was maintained while reducing the patterning size and increasing the BioMEMS device interaction range. The increase in the interaction range extends the usefulness of the device to different Biosamples such as Bacteria and viruses, provides a higher sensitivity and a reduction of the device response time.

In the micron size range, the thermally responsive polymer trenches exhibited the desired open and close behaviour due to temperature changes. Fluorescent microscopy results demonstrated the polymer’s thermal response consistency for temperature changes ranging from above to below its LCST. The opportunity to create “smart” trenches to discriminate and capture targets for simple and quick sample processing could be a key technology platform. The existence of such a platform facilitates separation processes that would be difficult to achieve by the other methods mentioned in Chapter 1.
A major challenge related to the proposed approach was the particle-polymer layer release mechanism. Even though the capture mechanism was easily assured, the experiments conducted with the *E. coli* bacteria and the microbeads displayed some attractive sticking to the polymer structures in laminar flow regimes. This activity was probably due to Van der Waals force effect. While some specific applications won’t be affected, the use of a release mechanism based on the reverse dielectrophoresis effect was proposed to overcome these results. The results obtained proved that the proposed approach was correct and the release mechanism was achieved.

With respect to the thermal actuation mechanism, the proposed nichrome resistor approach proved to be the best suited for the integration of the final device. The fabrication process, for the nichrome resistor, illustrated that it was highly compatible with the rest of the device fabrication processes. Nichrome resistors have been widely studied and reported in the literature. Future work related to the actuator’s effectiveness and its processing optimization must be carried out with the objective of reducing the device power consumption and thermal efficiency.

The microfluidics design and fabrication was studied and analyzed to define the proper processing integration procedure. The SU-8 photoresist patterned layer between the two device substrates demonstrated an optimal performance that included a flow range of hundreds of microliters per minute, which make it appropriate for the final applications. Results confirmed the existence of a laminar flow region, which was expected for these types of microfluidics channels. The existence of a laminar flow region simplifies the selection process for a sample deflecting mechanism in order to obtain particle-polymer layer interaction.
The experiments performed to test the *E. coli* attachment-release mechanism to the polymer structures, due to the flow velocity, unveiled the possibility of developing a “shear controlled” sorting strategy as a function of particle adherence. Future work in this area is proposed to define new polymer layer topographies to exploit this principle.

The dielectrophoresis force effect in the device was successfully tested and proved to be adequate, as expected. The particle deflection increased the particles attachment density within the polymer trenches, which improved the sorting capacity of the device. The combination of DEP and a thermally responsive polymer in microfluidics for sorting or particle manipulation has not been reported in the literature. The polymer’s biocompatibility, when applied for cell manipulation, overcomes the DEP major drawback of cell damage due to exposure to a large electric field. Future work is proposed for the design and analysis of new DEP microelectrode array geometries in order to enhance the deflection of the particle to the polymer structures.

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 dissertation, which represents a major contribution to the field. Further research work is proposed to optimize the microfluidics subsystem, the tuning of poly(NIPAAM) properties and the different poly(NIPAAm)
patterning processes, their effects on the BioMEMS/NEMS device. In addition, the fabrication and integration of the DEP microelectrode array and the thermal actuation should be studied with respect to optimization.
REFERENCES


ABOUT THE AUTHOR

Alexandro Castellanos Mier received a Bachelor’s Degree in Mechanic-Electric Engineering from Universidad Veracruzana, Mexico in 1991. He received a M.S. in Control Engineering from Instituto Tecnologico y de Estudios Superiores de Monterrey Campus Monterrey in 1997. Alexandro started teaching electrical engineering before he entered the Master’s program and continued on as a full time professor. Alexandro rose to the position of Chairman for the Electronics and Telecommunications program at Universidad Veracruzana Facultad de Ingenieria where he presided until he entered the Ph.D. program at the University of South Florida in 2003.

While in the Ph.D. program at the University of South Florida, Alexandro functioned for three years as the integrated circuit processing laboratory teaching assistant in the electrical engineering department. Alexandro coauthored publications related to this dissertation. In addition, he submitted papers and participated at international conferences of the Ibero-American science and technology consortium, (ISTEC), and the IEEE. Alexandro also participated as an invited lecturer at different Universities in Mexico.