MAGNETIC MICROPALLETS FOR SINGLE ADHERENT CELL RECOVERY AND ANALYSIS

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ABSTRACT

This paper presents a novel microtechnology, magnetic micropallets, for the recovery of single adherent cells from large mixed populations. This work significantly advances the base technology, the micropallet array, which itself is effective for the analysis of adherent cell populations and isolation and selection of individual cells. We greatly improved the method of recovery of individual micropallets by incorporating ferromagnetic iron oxide nanoparticles into their structure such that single micropallets can be manipulated and collected magnetically.

KEYWORDS: Micropallets, Magnetic, Cell Sorting, Single Cell

INTRODUCTION

The increased appreciation in recent years of the cellular heterogeneity of both diseased and healthy tissues has provided motivation for study of tissues at the level of the single cell. Likewise, the recognition of intrapopulation differences between individual cells in culture, even from the same source or cell line, is driving demand for new technologies to effectively isolate single, unperturbed cells from large populations for individual study. We have previously reported a bioMEMS-based microtechnology for the separation, isolation, and recovery of single adherent cells from culture with minimal perturbation. This technology, micropallet arrays, enables the selective recovery of a single adherent cell from culture amongst populations of tens of thousands of cells [1, 2].

THEORY

Micropallet arrays consist of hundreds of thousands of microscale polymer pedestals on which single adherent cells are held. Micropallets are fabricated from a transparent and biocompatible photopolymer using standard photolithographic techniques. Any micropallet can be released from the glass substrate on demand using a pulsed laser, carrying the adhered cell with it and coming to rest on the array's surface. Collection of released micropallets was previously accomplished using a simple inversion process that caused any released micropallets to fall into an array of collection wells [2]. This method has critical drawbacks including the requirement to search the collection wells to locate the recovered micropallet, the large volume of collection fluid in which the cell is recovered (which is ill-suited for single cell analysis methods), very low throughput capacity, and increased rates of contaminating loosely adherent cells that detach from non-targeted micropallets of the array. A micropallet that is responsive to magnetic fields eliminates these drawbacks by enabling its collection and manipulation via magnetic means subsequent to its release. We proposed that a magnetic micropallet could be fabricated using a composite material that is both ferromagnetic and photopatternable, that the final micropallet would itself be magnetically responsive, and that single, viable cells could be collected and precisely delivered using the magnetic micropallet platform.

EXPERIMENTAL

Magnetic micropallets were fabricated using standard photolithographic techniques from a photopatternable polymer that was made in-house by dissolving EPON 1002F resin in solvent and adding a photoinitiator [3]. The resultant 1002F photopolymer is similar to SU-8 photoresist, but exhibits much lower autofluorescence [3]. The photopolymer was made magnetic by the addition of ferromagnetic iron (II, III) oxide nanoparticles. Microstructures made from this material exhibited ferromagnetism. The iron oxide nanoparticles were mixed into the 1002F photopolymer by mechanical stirring and centrifugation was used to remove aggregates of nanoparticles and thereby improve overall homogeneity of the composite material. The photopatterning of the ferromagnetic photopolymer was accomplished using a collimated light source and SU-8 developer following the same recipe as prescribed for standard 1002F photopolymer [3].

NIH/3T3 cells were maintained in culture and seeded onto micropallet arrays that had been coated with human fibronectin. Cells were incubated for 3 h at 37 °C/10% CO_2 to allow them to adhere to the micropallets. Individual micropallets holding single cells were released using a pulsed laser focused at the micropallet and glass substrate interface, as previously described [2]. Released micropallets were collected using a collection probe based on a 1 mm diameter permanent magnet. For clonal expansion experiments to demonstrate viability of recovered cells, micropallets holding single cells were transferred into individual wells of a 96-well tissue culture plate containing cell culture media and cultured for a period of 1 week. For RT-qPCR analyses, single 3T3 cells stably transfected with rat *neu* were collected, lysed and analyzed for mRNA expression of mouse *ß-actin* and rat *neu* using a TaqMan PreAmp Cells-to-Ct Kit.

RESULTS AND DISCUSSION

We showed that incorporation of iron oxide nanoparticles had minimal impact on the photopatternability of the polymer, Figure 1, and no effect on the viability of cells cultured on its surface, Figure 2. Microscopic image clarity of cells on magnetic micropallets was good with minimal signal degradation as compared to standard micropallets, Figure

3, and micropallets were easily collected after laser release using a collection probe based on a small (1 mm) permanent magnet, Figure 4. The collection probe enables precise delivery of micropallets to downstream analysis vessels.

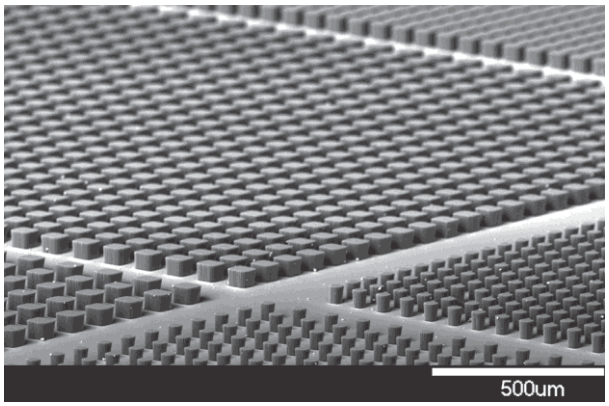

Figure 1: An array of test structures fabricated with the ferromagnetic photopolymer. 10 μm features with 5:1 aspect ratios are possible.

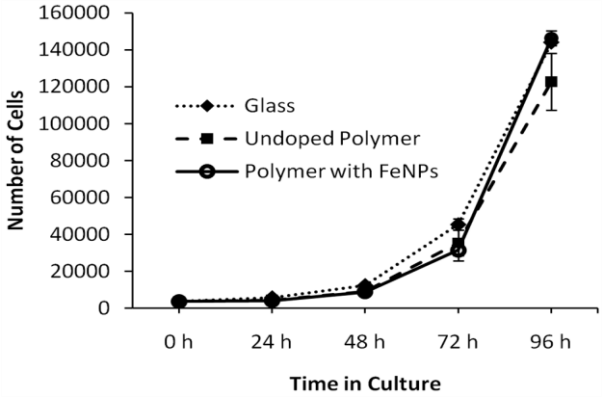

Figure 2: Growth rate of 3T3 cells on human fibronectin coated substrates of glass, standard photopolymer, and photopolymer with iron oxide nanoparticles.

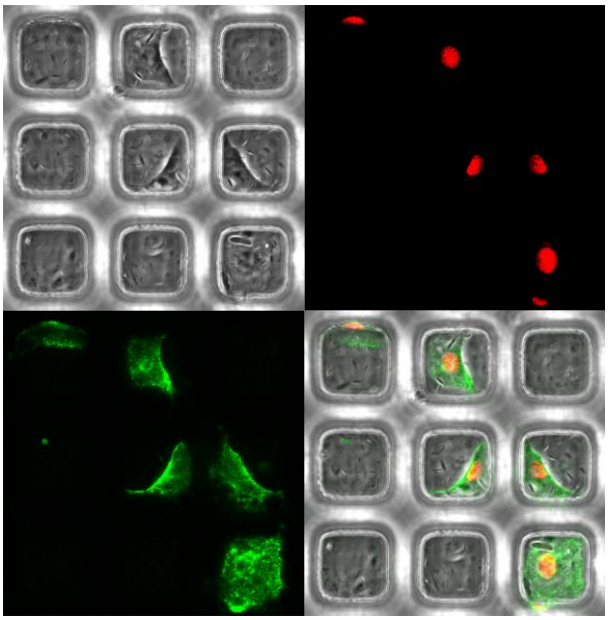

Figure 3: Multichannel immunofluorescent image of 3T3 cells stably transfected with rat neu on magnetic micropallets. Channels are (left to right, top to bottom): Phase contrast, nuclear stain, HER2/neu cell surface marker, combined channels.

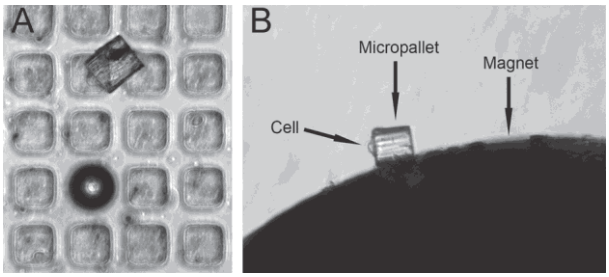

Figure 4: A) Micropallet with adhered 3T3 cell resting on surface of array after laser release. Circular object is the remnants of a bubble created as a by-product during laser release. B) A micropallet with adhered cell attracted to a magnet after recovery.

The ability to magnetically collect individual micropallets with adhered cells is a substantial and far-reaching improvement over the previous method of collection, i.e., inversion of the entire array over collection wells. The inversion method has a previously-reported success rate of 63% [2], but also presents confounding issues such as possible contamination by non-target cells from the array that lose adherence to their micropallet substrates and very slow throughput capabilities. Magnetic collection methods greatly reduce these problems and also enable the delivery of the collected micropallet/cell to a very precise location and/or small volume vessel. This is critically important for single cell analysis techniques such as PCR analysis, which require the analyte to be present in very small volumes of fluid.

Single cells held on magnetic micropallets were collected and analyzed directly using single cell real time quantitative PCR (RT-qPCR) analysis for mRNA expression of mouse *ß-actin* and rat *neu*. Both transcripts were detected for each single cell analyzed, with average threshold cycle (C_t) values of 26.80 ± 0.26 and 22.99 ± 2.20, respectively, Figure 5.

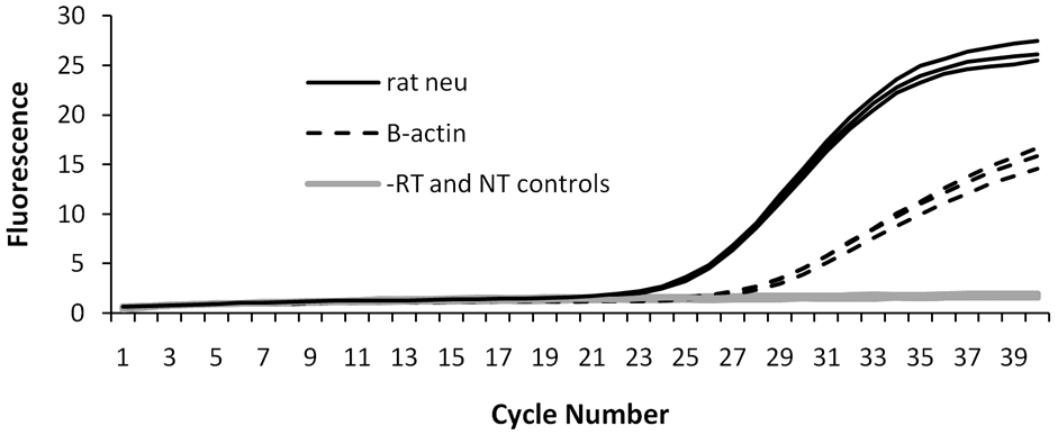

Figure 5: RT-qPCR traces from analysis of a single 3T3 cell stably transfected with rat neu collected on a magnetic micropallet. Black solid lines show the amplification of rat neu and black dashed lines show the amplification of mouse β-actin, both of which were analyzed in triplicate. No template (NT) and no RT enzyme (–RT) controls are represented by gray lines.

In addition to the proof-of-principle experiments demonstrating feasibility of single cell molecular analyses, we also demonstrated that cells are able to survive the magnetic collection and transfer process. Single 3T3 cells that were magnetically recovered using magnetic micropallets were expanded into clonal populations of thousands of cells after recovery and culture, Figure 6.

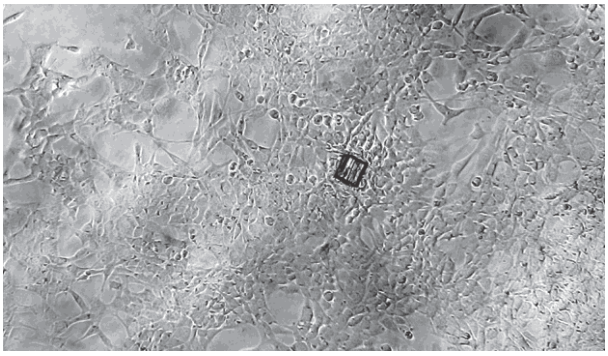

Figure 6: Clonal population of cells expanded from a single collected 3T3 cell. The design of the magnetic probe allows the micropallet to be released completely from the collecting magnet. Cells remain viable throughout the collection process.

CONCLUSION

We significantly advanced the base micropallet arrays technology by creating a magnetically responsive micropallet, greatly improving the recovery process of released micropallets. Throughput of micropallet recovery has been immensely increased and we demonstrated that cells survive the collection process. The precise control afforded by this advancement enables single cell PCR analysis of recovered single cells, which was previously not possible.

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