GLASS MICROPALLETS FOR ADHERENT CELL ANALYSIS AND RECOVERY

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ABSTRACT

This paper presents a novel microtechnology, glass micropallets, for the recovery of single adherent cells from mixed cellular populations. This work significantly advances the base technology, the micropallet array, which has been demonstrated for the analysis of adherent cell populations and isolation and selection of individual cells. We report a complete departure from the photolithographic fabrication methods used previously and present a micropallet array made of glass instead of polymeric materials, which improves the technology in several aspects. We demonstrate the glass micropallet array's improved optical properties and its ability to selectively recover adherent cells from large populations.

KEYWORDS: Micropallets, Glass, Adherent Cells, Cell Sorting

INTRODUCTION

It is increasingly evident that cellular heterogeneity within tissues, including recently recognized populations of exceedingly rare cells, e.g., certain progenitor cells, contributes significantly to specific biological behaviors and disease states. Such recognition has motivated attempts to improve upon current analysis methods to be able to characterize tissues down to the single cell level. It has become clear that, for many investigations, methods that utilize population averaging techniques provide inadequate information about the true heterogenic nature of a cellular population, even for populations comprising only cells of the same type or lineage. Thus a technology that can effectively enable characterization of single rare cells amongst a background population, and subsequently their selection and recovery, would likely be found immediately valuable across a wide range of biological investigations.

We have previously reported a microtechnology for the separation, analysis, and recovery of single viable adherent cells from large mixed populations. This technology, the micropallet array, is proven effective for the immunofluorescent analysis and identification of rare cell populations as sparse as 1 in 10,000 cells amongst a background population and its use is demonstrated for the recovery of such cells with maintenance of their viability [1]. Herein we report the advancement of the micropallet array and take advantage of new manufacturing methods to improve the technology in several aspects.

THEORY

Micropallet arrays consist of thousands of microscale pedestals on which single or several adherent cells are held. During fabrication, the micropallet array is chemically treated such that its surface is superhydrophobic. Due to this hydrophobicity, the array exhibits Cassie-Baxter wetting, wherein only the top surfaces of the individual micropallets are wetted upon application of a fluid to the array. This selective wetting acts as the mechanism for cell sequestration to the individual surfaces of the micropallets, as these are the only locales on which the cell can settle and subsequently adhere. Extracellular matrix components are coated onto the top surface of the micropallet array prior to the application of cells, which supports their strong adhesion to the individual micropallets. After cells have been applied to an array and allowed time to adhere, any micropallet can be released from the glass substrate on demand using a pulsed laser focused at the micropallet-glass substrate interface from the underside of the array. The ablative process induced by the pulsed laser generates a small quantity of rapidly expanding gas that dislodges the targeted micropallet from its location, carrying the adhered cell(s) with it. The released micropallet with adhered cells can then be collected and transferred to a downstream vessel for direct analysis of the cell(s) or expansion of the cell(s) into clonal populations.

The original micropallet array technology was developed using one of two high-aspect-ratio photoresists: SU-8 or 1002F [2]. The glass micropallet array offers the same fundamental cell sorting and collection capabilities, but with a fully glass structure fabricated using glass machining methods. The creation of an all-glass micropallet array offers important advantages versus the previous micropallet array technology, for which photolithographic methods were employed to fabricate polymeric micropallets.

EXPERIMENTAL

Glass micropallets were fabricated by mechanical machining methods, as illustrated in Figure 1. A No. 1 glass coverslip was attached to a 2x2 in precision glass slide with ~200 µl of cyanoacrylate and application of pressure until cured. The glass micropallet array was then created by cutting two orthogonal sets of parallel channels into the glass sandwich (coverslip and substrate glass) using a K&S 780 dicing saw. The cut depth extended past the cyanoacrylate layer and into the substrate glass, creating glass pedestals with the top portion non-permanently attached by the cyanoacrylate layer. The array was hy-

drophobically modified by vapor deposition of a silane monolayer to support the air barriers that sequester cells to individual pallets during use. Finally, the array was coated with fibronectin immediately prior to use to support cell adhesion, as previously described [1]. Figure 2 shows a glass micropallet array.

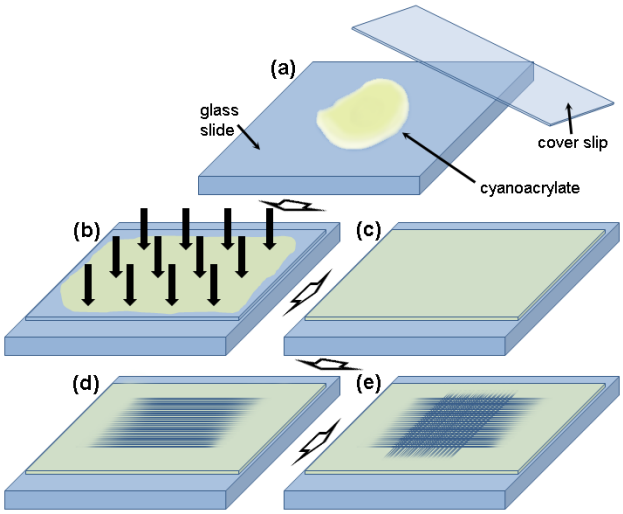

Figure 1. Fabrication of a glass micropallet array. (a) cyanoacrylate applied to substrate glass (b) coverslip glass placed atop substrate glass and pressure applied (c) non-permanently bonded glasses (d) a dicing saw cuts numerous parallel cuts into the glass, which extend through the full thickness of the coverslip (e) perpendicular cuts complete the creation of micropallets.

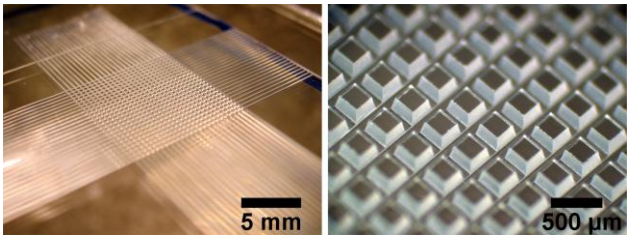

Figure 2. Two views of a glass micropallet array. All micropallets reported herein are ~220 µm on a side.

The optical transmission spectra of the various materials (SU-8, 1002F, glass) used to fabricate micropallet arrays were analyzed using a USB2000 spectrometer (Ocean Optics, Dunedin, FL) to measure the transmittance of light from a DT1000CEUV/vis light source (Analytical Instrument Systems, Flemington, NJ). An Olympus IX71 with built-in fluorescent module was used to characterize the autofluorescent properties of the materials.

For cellular imaging and collection experiments, 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% CO2 to allow them to adhere to the micropallets. Individual micro-pallets holding single cells were released using a pulsed laser focused at the micropallet and glass substrate interface, as previously described [2].

RESULTS AND DISCUSSION

Glass micropallet arrays are effective for the isolation and recovery of adherent mammalian cells and have several advantages over previous micropallet arrays made from polymeric materials. As seen in Figure 3, glass is more optically transparent than the SU-8 or 1002F photopolymers and, importantly for fluorescent cellular analyses, exhibits essentially zero autofluorescence, Figure 4. These attributes, along with excellent optical clarity, greatly improve the capability for morphologic and fluorescent analysis of cells held on the micropallets. The clarity of the glass micropallet allows much finer microscopic details of cells held on the micropallets to be resolved than possible with polymeric micropallets. We believe the clarity is improved due to the clearer micropallet material as well as a significant reduction of the refractive index mismatch between the micropallet and substrate materials, which contributes greatly to internal reflections, aberrations, and geometrical distortions. The clarity afforded by the glass micropallet is demonstrated in Figure 5. When examining cells with minor phenotypic differences, e.g., very low expression of specific surface markers or minor morphological differences, these improvements can be critically important. Additionally, glass is a proven substrate for adherent cell culture and is more familiar to scientists than SU-8 and 1002F, with established protocols for surface modification, antibody coupling, etc.

Any individual glass micropallet can be released from the array using mechanical dislodgement or via laser ablation of the cyanoacrylate adhesion layer, using methods similar to those previously described [1]. Released micropallets can be collected [1], enabling the recovery of individual targeted cells from a bulk population. Figure 6 shows our ability to release and recover specific glass micropallets that hold adherent cells.

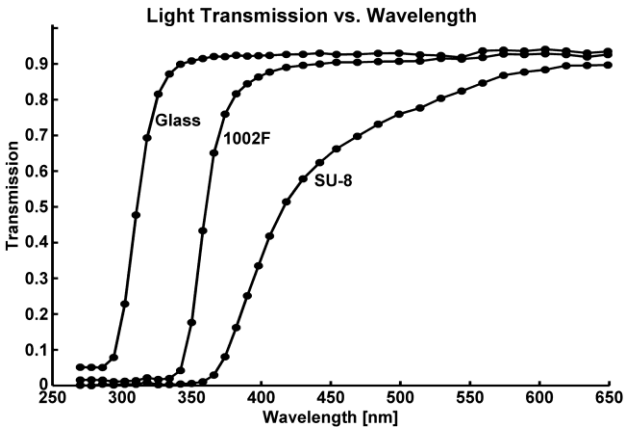

Figure 3. Light transmission of the different materials used to fabricate micropallets. Light transmission was measured through a 150 µm thick specimen of each material and five measurements were averaged for each data point. Glass offers improved light transmission, especially at shorter wavelengths (useful for applications requiring UV excitation) vs. 1002F and SU-8.

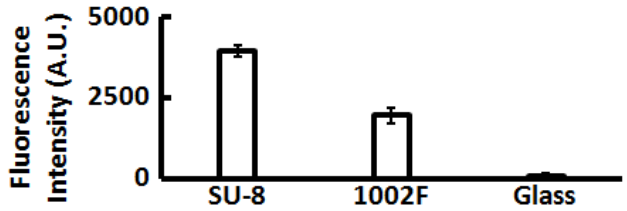

Figure 4. Autofluorescence of the different materials used to fabricate micropallets (FITC filter set; autofluorescence is most greatly exhibited in green). The essentially zero autofluorescence of glass greatly improves the ability to detect minimally-expressed markers using fluorescent labeling methods.

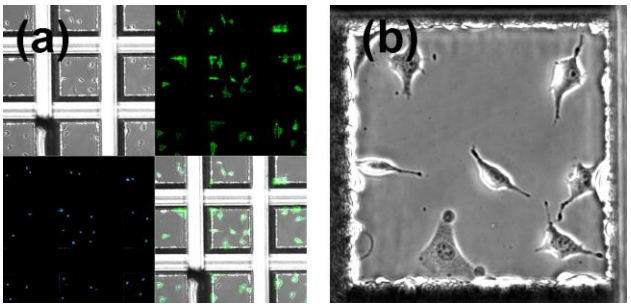

Figure 5. Superior imaging ability is afforded by using glass as the micropallet structural material. (a) NIH 3T3 cells stably transfected with rat neu growing on glass micropallets with fluorescently labeled rat neu surface marker (AF-488; green) and fluorescent nuclear stain (Hoechst 33342; blue). (b) Enlargement of center micropallet to show detail (phase contrast channel).

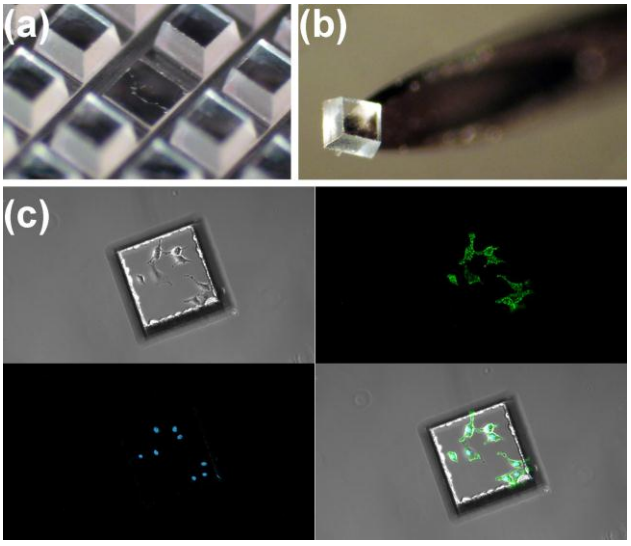

Figure 6. The weak cyanoacrylate bond enables the release of individual micropallets. (a) A glass micropallet array that has had a single micropallet selectively dislodged. (b) A recovered glass micropallet held on the tip of a 26 gauge needle. (c) A recovered glass micro-pallet, which holds a small cell colony on its surface.

CONCLUSION

In summary, the glass micropallet array eliminates previous shortcomings of the base micropallet array technology and provides an elegant solution for sorting single adherent cells. We have employed a unique approach to the fabrication of large areas with many microscale features, namely the use of mechanical machining methods, which was made possible by the repetitive, orthogonal layout of the micropallet array, combined with the precise capabilities of dicing saw technology that has been adapted from the integrated circuit industry. We demonstrated the glass micropallet array's improved optical properties over the polymer micropallet array and its use for the recovery of selected adherent cells from a large background population.

REFERENCES

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