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Selection and Separation of Viable Cells Based on a Cell-Lethal Assay

Wei Xu[1], **Annadele Herman**[1], **Colleen Phillips**[1], **Jeng-Hao Pai**[1], **Christopher E. Sims**[1], and **Nancy L. Allbritton**[1,2,*]

[1]Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599, USA

[2]Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599 and North Carolina State University, Raleigh, NC 27695

Abstract

A method to select and separate viable cells based on the results of a cell-lethal assay was developed. Cells were plated on an array of culture sites with each site composed of closely spaced, releasable micropallets. Clonal colonies spanning multiple micropallets on individual culture sites were established within 72 h of plating. Adjacent sites were widely spaced with 100% of the colonies remaining sequestered on a single culture site during expansion. A laser-based method mechanically released a micropallet underlying a colony to segment the colony into two genetically identical colonies. One portion of the segmented colony was collected with 90% efficiency while viability of both fractions was 100%. The segmented colonies released from the array were fixed and subjected to immunofluorescence staining of intracellular phospho-ERK kinase to identify colonies that were highly resistant or sensitive to phorbol ester-induced activation of ERK. These resistant and sensitive cells were then matched to the corresponding viable colonies on the array. Sensitive and resistant colonies on the array were released and cultured. When these cultured cells were reanalyzed for phorbol ester-induced ERK activity, the cells retained the sensitive or resistant phenotype of the originally screened subcolony. Thus cells were separated and collected based using the result of a cell-lethal assay as selection criteria. These microarrays enabling clonal colony segmentation permitted sampling and manipulation of the colonies at very early times and at small cell numbers to reduce reagent, time and manpower requirements.

INTRODUCTION

Assays that result in the death of cells under study are both ubiquitous and of great importance in biological investigations. Techniques including polymerase chain reaction (PCR), Western blot, immunohistochemistry, mass spectrometry, chemical cytometry and others provide highly valuable information yet result in nonviable cells. PCR and other genomic assays identify the genetic make up of the cell, but cells must be lysed and their DNA purified for analysis. Proteomic studies using Western blot or mass spectrometry likewise require cell lysis to obtain the proteins for assay. Chemical cytometry, the chemical separation of the contents from single cells, requires cell lysis on a cell-by-cell basis in order to obtain intracellular analytes.[1] Immunohistochemical protocols require cells to be

*Corresponding author nlallbri@unc.edu. Fax: 919-962-2388, Phone: 919-966-2291.

SUPPORTING INFORMATION AVAILABLE

Further experimental results are given in Supplementary Data. This material is available free of charge via the Internet at http://pubs.acs.org.

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permeabilized and fixed so that the antibodies can access and bind intracellular targets. Thus, these techniques cannot easily be applied to choose cells with a given characteristic followed by culture and expansion of the cells. This practice, known as positive selection, is required to establish cell lines with unique characteristics for research studies or for cloning genetically engineered plants or animals.[2] In the not too distant future, it is expected that cloning techniques will play an important role in regenerative medicine as well.[3]

The implementation of these highly informative, yet destructive, molecular assays for screening cells to establish cell lines is essentially limited to two approaches-- limiting dilution and cloning rings. Both techniques are laborious, requiring significant time and manpower to expand single cells into large numbers of cells in multiple clonal populations which are then manually split and subjected to a destructive assay that identifies the population of interest. In limiting dilution, cells are placed in suspension at very low density and a volume of the cell suspension predicted to hold one or fewer cells is pipetted into individual wells, usually using 96- or 384-well plates.[4] Hundreds to thousands of wells are needed to provide adequate numbers of clonal colonies to achieve only a few target clones. The single cells are cultured for 1 – 2 weeks or longer in order to provide cell numbers adequate for splitting the expanded populations. One fraction of each sample is maintained in culture or frozen while its corresponding aliquot is subjected to the destructive assay. Similarly manpower and time intensive, isolation by cloning ring or pipette picking requires plating a dilute cell suspension in a multitude of Petri dishes.[5] Individual cells are then allowed to grow into isolated colonies that are hand picked, disaggregated, fractionated and placed in paired aliquots for identification of desirable colonies. A micro-scale technique for creating clonal populations that can be surveyed using destructive assays with minimal cell number could provide a valuable tool for positive selection and cell line creation that minimized reagent use, time, and manpower.

The current work describes the use of a microengineered cell array that provides a novel bioanalytical means to isolate cells from within a microscopic colony. The unique design of the releasable elements on the array makes it possible to generate clonal colonies on the array and then sample and collect a portion of the cells from the colonies which are left adherent and viable. The sample cells are subjected to an assay that requires destructive biochemical analysis to identify the target colonies. The colonies identified in the assay to be of interest or “hits” are then released and collected for further analysis or expansion. In the current work, successful generation of clonal colonies on the array and colony sampling are demonstrated. An intracellular immunofluorescence assay of ERK kinase activation that requires cell fixation is used to identify clones with high and low activation levels of this important signaling molecule. These clonal colonies are then isolated and evaluated for ERK activation to demonstrate the feasibility and practicality of this technique.

EXPERIMENTAL SECTION

Materials

EPON resin 1002F phenol, 4,4’-(1-methylethylidene)bis-, polymer with 2,2’-[(l-methylethylidene)bis(4,1-phenyleneoxymethylene)]bis(oxirane) was obtained from Miller-Stephenson (Sylmar, CA). UVI-6976 photoinitiator (triarylsulfonium hexafluoroantimonate salts in propylene carbonate) was purchased from Dow Chemical (Torrance, CA). SU-8 developer (1-methoxy-2-propyl acetate) was purchased from MicroChem Corp. (Newton, MA, USA). All other photoinitiators and resins were from Sigma-Aldrich (St. Louis, MO) as was γ-butyrolactone (GBL). (Heptadecafluoro-1,1,2,2-tetrahydrodecyl) trichlorosilane was from Gelest Inc. (Morrisville, PA). Poly(dimethylsiloxane) (PDMS) (Sylgard 184 silicone elastomer kit) was purchased from Dow Corning (Midland, MI). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), 0.05% Trypsin-EDTA and penicillin/

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streptomycin were obtained from Invitrogen (Carlsbad, CA). Paraformaldehyde (16%) and ERK MAPK activation kit (including ERK Primary Antibody (rabbit), DyLight™ 488 Conjugated Goat Anti-Rabbit IgG, Hoechst Dye, Wash Buffer (10X), Blocking Buffer (10X), Permeabilization Buffer (10X) and Thin Plate Seal Assembly) were purchased from Thermo Scientific Inc. (Pittsburgh, PA). Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma-Aldrich (St. Louis, MO). Fibronectin, glass microscope slides and all other reagents were obtained from Fisher Scientific (Pittsburgh, PA). The microfabrication mask was drawn using TurboCAD (IMSI/Design, LLC, Novato, CA) and then printed and fabricated by Fineline Imaging (Colorado Springs, CO).

Laser-based release of microstructures from the array

A pulsed laser was used to effect release of the microstructures from the array as has been described in detail previously.[6] Briefly, a laser pulse (5 ns, 532 nm) of a Q-switch Nd:YAG laser (Minilite I, Continuum Electro-Optics Inc., Santa Clara, CA) was focused by a 40× microscope objective at the interface of the glass substrate and one of the microstructures making up a culture site (see Results). The focused pulse led to formation of a plasma and cavitation bubble. The expansion of the cavitation bubble at the base of the targeted microstructure mechanically dislodged it in an upward direction.[6] A threshold laser pulse energy was defined as the energy required to release 50% of targeted micropallets with one pulse.[6]

Cell culture and plating on the array

HeLa, a human ovarian carcinoma cell line, and NIH 3T3, a murine fibroblast cell line, were used in the current studies. Both wild type and molecularly engineered cell lines stably expressing the green fluorescent protein (GFP) were utilized. Cells on the array and those after collection were cultured in conditioned media. The base medium used was DMEM with 10% FBS, L-glutamine (584 mg/L), pencillin (100 units/mL) and streptomycin (100 μg/L). The arrays were sterilized by immersion in ethanol prior to use. The ethanol was removed by aspiration and the arrays were allowed to dry under sterile conditions. To enhance cell attachment and growth, the arrays were coated with fibronectin (20 μg/mL in phosphate buffered saline [PBS]: 135 mM NaCl, 3.2 mM $KHPO_4$, 0.5 mM KH_2PO_4 and 1.3 mM KCl; pH = 7.4) by incubation in the fibronectin solution for 2 h at room temperature. The arrays were washed with sterile deionized water twice before loading the cells. To plate cells on the array, a suspension of cells in media (2000 cells/mL for the arrays with 4000 culture sites and 3000 cells/mL for arrays with 8000 culture sites) was added to the chamber surrounding the array. Plated cells were cultured in a humidified, 5% CO_2 atmosphere at 37 °C.

Cell collection and culture after release

The array was rinsed with fresh pre-warmed culture medium (37 °C) ×3 before release experiments. After laser-based release, micropallets were transferred to a multiwall collection plate as previously reported.[7] The collected cells were maintained in freshly prepared conditioned media for expansion. To identify and track the released micropallets, the array was fabricated with numbered micropallets.

Viability Assay

After cleavage and sampling of the colony, the media on the array and collection plate were replaced with an extracellular buffer (ECB: 135 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, and 2 mM $CaCl_2$, pH 7.4) containing the viability dye Calcein Red-Orange (2 μM, Invitrogen, Carlsbad, CA). Dye loading was performed according to manufacturer

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protocol and the cells were imaged by brightfield and fluorescence microscopy. Cells were scored as viable (fluorescent) or non-viable (non-fluorescent).

Cell isolation for identification of target colonies and subsequent expansion of targets

Clonal colonies were established by plating cells suspended in conditioned media on an array at ½ - ⅓ the total number of individual culture sites. The gap between individual culture sites was 50 μm, and the gap between the micropallets within the site was 10 μm unless otherwise specified. The size of the micropallets varied depending on experimental conditions as described in the Results. Each culture site could be identified by its unique column number and row number on the array. Plated cells were cultured for up to 120 h under standard tissue culture conditions using conditioned media. The media was exchanged every 24 h.

Immunofluorescence assay of ERK MAP kinase translocation

Cells were placed in serum-free media for 24 h. The cells were then exposed to PMA (500 ng/ml) at 37 °C for 30 min. The cells were immediately fixed, permeabilized and labeled with primary and secondary antibodies per manufacturer protocol and imaged by fluorescence microscopy. The recorded fluorescence values were normalized so that the 10% of cells with the greatest intranuclear fluorescence possessed an average fluorescence value of one.

RESULTS AND DISCUSSION

Design of the culture sites for colony expansion and sampling

An array of culture sites was designed such that each site was comprised of closely spaced micropallets across which a single cell could expand into a colony. A variety of different micropallet shapes, sizes and orientations within a culture site were assessed. A gap of 50 μm between the culture sites was used to prevent the expansion of cells between sites.[8] In most cases, the gap between the micropallets within a culture site was 10 μm to enable spread of cells within one unit as colony expansion occurred. For each design, an array composed of 4000 culture sites was fabricated on a glass substrate. To test the designs, 2000 HeLa cells were loaded onto each array. Release tests were performed after 5 days of culture in conditioned media.

In a first design (Supplemental Data Fig. S1A), the unit was composed of a larger (110 μm) micropallet surrounded by small (30 μm) sampling micropallets along its side. When the sampling micropallet was released, a large fraction (50% ± 18%) of the sampling micropallets failed to retain cells on their surface. Of those sampling micropallets with cells, only 35% ± 10% had more than one morphologically intact cell from the colony (release energy 8 μJ). To overcome these issues, a second design was tested. In a second design (Supplemental Data Fig. S1B), each unit was comprised of two micropallets: a larger (150 μm × 150 μm) micropallet with a portion composed of a smaller triangular sampling micropallet (100 μm × 60 μm × 60 μm). However, it was found that 4 to 5 laser pulses (8 μJ -10 μJ) were typically required to break the cell-cell connections along the two edges of the triangular micropallet resulting in a success rate for colony cleavage of 75% ± 15%. The multiple laser pulses also led to damage of cells so that only 60% ± 10% of the released micropallets contained at least one morphologically undamaged cell. A third design was composed of two micropallets of equal dimensions (100 μm × 150 μm) and shape (Supplemental Data Fig. S1C). In culture on the array, each colony increased in cell number at different rates, so that the number of cells crossingthe narrowed bridging section varied from colony-to-colony. Consequently, the laser energy required to release one of the micropallets in the pair varied from colony-to-colony depending on the number of bridging

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cells (Supplemental Data Fig. S1D). Successful cleavage of the colony then required an estimation of the release energy before sampling. The use of relatively high release energy (*e.g.* 20 μJ) could provide a 100% cleavage rate, but resulted in fewer released micropallets containing at least one intact cell (30% ± 10%).

To address the weaknesses of the prior designs, a tripartite pattern (Fig. 1A) composed of discrete microstructures was tested. The two larger micropallets provided adequate surface on which cells could adhere and grow into an adequate colony size for collection or assay. The small intervening micropallet served as a bridge for the cells to move between the two larger micropallets as well as acting as a "cutting" micropallet to cleave the colony into two genetically identical portions (Fig. 1B). To test spread of colonies on the arrays in these experiments, HeLa cells (3000) were plated onto an array containing 8000 culture sites such that ≤1 cell was present in any single culture site. The arrays were observed under brightfield immediately after plating and at 24 h intervals to identify the percentage of culture sites with cells growing across the cutting micropallet to span all three micropallets of the culture site. Initially, the dimensions of the structures making up the culture site were: larger micropallet 60 μm on a side; cutting micropallet mid-portion length 20 μm; and gap between structures 15 μm (Fig. 2A). By varying the length of the widened mid-portion of the cutting micropallet, the percentage of culture sites with colonies crossing the bridge was found to be the same at all time points evaluated when this dimension was ≥20 μm (Fig. 2B). With a fixed mid-portion of 40 μm, but a variable gap between the cutting micropallet and the larger micropallets, a 10 μm gap provided the shortest time in which the majority of colonies extended over the individual micropallets within a culture site (Fig. 2C). Using these optimized dimensions for cutting micropallet and gap, the effect of varying the size of the larger micropallets on the time for colony expansion across the culture site was then evaluated. Sets of arrays were prepared possessing 4000 culture sites composed of 60 μm, 70 μm, 100 μm or 120 μm micropallets. The arrays were plated with HeLa cells (2000) and observed as described above. The results demonstrated that the larger the micropallet, the slower the extension of the colony across the elements of the culture site (Fig. 2D). This finding is likely due to the larger culture surface area on the individual micropallets which allowed the colonies to expand for longer periods before needing to spread across the narrow gap between the microstructures within a culture site.

Colonization of culture sites by single cells

To further characterize occupation of the culture sites by a single cell, HeLa cells (3000) were plated on arrays composed of 8000 culture sites so that the majority of sites contained either 1 or no cells.[8] After 2 h, the plate was examined by brightfield microscopy to confirm that the individual culture units contained single cells (Fig. 3A). The array was returned to culture and imaged every 24 h to assess colony formation. As the colonies expanded, the cells migrated across the narrow gap between the three elements making up the culture site. The cutting micropallet functioned as a bridge to enable cells to expand to the larger micropallets within a culture site. When using the fast growing HeLa cell line, the gap (50 μm) between the culture sites prevented spread of a colony to other sites for up to 3 days in culture. After 72 h, 80% ± 10% of colonies (n = 50 culture sites, 3 independent experiments) had expanded over all of the elements within the culture site (Fig. 3A). After 96 – 120 h in culture, 100% ± 0 colonies had spread to fill their culture sites. Wild-type NIH 3T3 and MCF-7 cells were also tested with similar results (Supplementary Data Fig. S2A&B).

Clonal expansion of colonies

To determine whether the colonies growing on each culture site were truly clonal, a 10:1 mixture of wild-type HeLa cells and HeLa cells stably transfected with a GFP-histone was plated on the array (3000 total cells). The array was then imaged by both brightfield and

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fluorescence 2 h after plating to identify culture sites containing a single fluorescent HeLa cell with wild-type cells in adjacent culture sites (Fig. 3B&C). These sites were again examined at 72 h. No non-fluorescent cells were observed within the GFP-expressing colonies (n = 10) and no fluorescent cells were present in wild-type colonies (n = 10). These data demonstrate that the culture sites seeded with a single cell maintained their clonality during expansion for a period of up to 3 days, the longest period tested. These results compare favorably with other literature in which cell arrays based on patterned chemical surface modifications sequestered HeLa cells in localized colonies for less than 72 h.[9]

Colony cleavage and sampling

The procedure for sub-sampling colonies is shown schematically in Figure 1B. To segment a colony, a laser pulse was fired at the interface of the cutting micropallet and the glass substrate of the array. The energy of the laser pulse used in these experiments was varied over a range of 2 – 12 μJ (Supplementary Data Fig. S2C). It was observed that although the threshold laser pulse energy of 3.3 μJ was adequate to dislodge the cutting micropallet, this energy was not sufficient to cleave the colony. Only when the laser energy was increased by 3-fold (10 μJ) was adequate kinetic energy imparted to the cutting micropallet to segment the colony. With colonies formed from HeLa cells, the cutting micropallets efficiently separated the colonies into two portions (100% ± 0, n = 50) (Fig. 4A&B). In each case, the remaining micropallets retained morphologically intact cells on their culture surface. Wild-type NIH 3T3 and MCF-7 cells were also tested with similar results (Supplementary Data Fig. S2C).

Experiments were then performed to assess the ability to release and collect a portion of a colony segmented as described above. After releasing the cutting micropallet, one of the two remaining micropallets in the site was chosen for release and collection of cells (n = 30 samples per array, 3 arrays). This step of release and collection was termed colony sampling. A single laser pulse (5 – 6 μJ) was used to release one of the pair of micropallets which was then collected. The collected micropallets and the micropallets remaining on the array were subsequently monitored by brightfield imaging. 100% ± 0 (n = 30) of the micropallets on the arrays and 90% ± 10% (n = 30) of the collected micropallets retained their cells. To determine whether the colony sampling process affected the viability of the retained colonies on the array, the short-term and long-term viability of the cells was evaluated. Immediately after colony cleavage and collection, both the cells on the array and those collected were stained with the viability dye Calcein Red-Orange (Supplementary Data Fig. S3A&B). Both the colonies remaining on the array and on the collected micropallets were comprised of living cells (100% ± 0, n = 20). To evaluate the long-term viability of sampled colonies, arrays were returned to culture after sampling and observed for 48 h. The colonies continued to expand in culture (100% ± 0, n = 30) indicating their continued viability after the sampling procedure (Fig. 4C&D). To test the viability of the sampled portion of the colonies after release and collection, the collected micropallets were placed in cell culture for 72 h and then observed by brightfield imaging. In these experiments (n = 3), 100% ± 0 of the collected samples continued to expand (Supplementary Data Fig. S4A&B). These results clearly indicated that this approach could be used as an efficient method for sampling colonies with both the sample and the remaining colony retaining viability and the potential for expansion.

Cell selection based on a destructive assay

The activation state of signal transduction pathways is of widespread interest. The MAP kinase pathways are of particular importance due to their central role in promoting cell growth and survival.[10, 11] These pathways are also the target of a number of pharmaceutical inhibitors for a variety of diseases including various cancers and autoimmune disorders.[12-18]

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For these reasons, it would be of value to assay the activity of a MAP kinase such as ERK as a means of cell selection. Unfortunately, the current assays for ERK and other MAP kinases require cell fixation or lysis to assess activity by immunofluorescence, Western blot, or other means. Thus, clonal populations must be individually established, then grown to an adequate number of cells (typically $10^6 – 10^7$) to perform the assay while continuing to culture the clones for further use.[4, 5, 19] In the current experiment, efficient sampling of small clonal colonies co-cultured on an array was combined with immunofluorescence-based assay of ERK activity to identify clones that were insensitive or highly sensitive to PMA-induced activation of ERK.

To accomplish this experiment, wild-type HeLa cells were plated as single cells on the array's culture sites and allowed to grow into clonal colonies as described above. After 72 h in culture, 200 colonies having at least 2 cells present on each of the two larger micropallets in the culture site were chosen as candidates for sampling. The colonies were segmented and one of the micropallets in each culture site was released and collected for assayed for PMA-induced ERK activity. While the assay was being performed, the array was returned to an incubator for 24 h to maintain the arrayed colonies in culture. ERK resides in the cytoplasm when not activated, but upon activation translocates to the nucleus.[20, 21] Immunofluorescence staining of individual cells for the presence and intensity of intranuclear fluorescence has been established as a validated means for assaying ERK activity in response to stimulation.[20, 22] The 10% of samples possessing the highest (1.0 ± 0.2) and the lowest (0.6 ± 0.1) intranuclear fluorescence in response to PMA were identified (Fig. 5A,B). Within the population of cells on the array, there was nearly a 2-fold difference in the amount of PMA-induced ERK activity. The colony fragments with high or low ERK activity were matched back to the viable parent colony on the array. This parent colony was then released, collected and maintained in culture for an additional 50 hours. To determine whether the parent colony exhibited the same phenotype as the subfragment used for the selection, the cultured parent colonies were also assayed for PMA-induced ERK activity. The average intranuclear fluorescence of the colonies selected for high ERK activity was 1 ± 0.2 while the fluorescence of the colonies selected for low ERK activity was 0.5 ± 0.1. Thus, the difference in the degree of PMA-induced ERK activation in the high and low populations was preserved. These data demonstrate that cells can be selected and separated based on a cell-lethal assay by initially removing a very small number of cells (as few as two cells) for the assay. After identification of cells with the appropriate phenotype, the remainder of the colony can be retrieved for culture and future analyses.

CONCLUSION

The sampling procedure described in the current paper enables the identification and selection of colonies from a mixed population at a stage when the colonies contain far too few cells to be manipulated by traditional means. In the current studies, colonies with as few as six cells were easily sub-sampled. The technique also provides the ability to manipulate the cells so that an analysis can be performed much earlier than with conventional approaches, such as limiting dilution and cloning rings, thus reducing reagent and manpower costs in culturing the cells. Furthermore, colonies can be sampled in two simple steps while maintaining the viability of both the parent colony. The segmentation and collection of a clonal colony permitted identification of the colony phenotype by a cell-destructive assay. This ability greatly broadens the selection criteria applied to a mixed population for subsequent cell separation relative to traditional technologies, for example fluorescence activated cell sorting. The technology enables the possibility of cell separation based on a genotype or phenotype identified by PCR techniques, immunohistochemistry, cell cytometry and other cell-destructive assays. Furthermore, the technique enables numerous colonies to be efficiently sampled, so that colonies of interest can be segregated from colonies of no

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further value after only a short period in culture. A future goal will be to implement automated laser-based segmentation of colonies and release of colony fragments for large scale array screening by assays requiring cell destruction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Dovichi NJ, Hu S. Curr Opin Chem Biol 2003;7:603–608. [PubMed: 14580565]
2. Taylor TB, Nambiar PR, Raja R, Cheung E, Rosenberg DW, Anderegg B. Cytometry A 2004;59:254–261. [PubMed: 15170605]
3. Teo AK, Vallier L. Biochem J 2010;428:11–23. [PubMed: 20423328]
4. Mishell, BB.; Shiigi, SM., editors. Selected Methods in Cellular Immunology. W.H. Freeman; New York: 1980.
5. Freshney, RI. Culture of Animal Cells. Wiley-Liss; New York: 2000.
6. Quinto-Su PA, Salazar GT, Sims CE, Allbritton NL, Venugopalan V. Analytical Chemistry 2008;80:4675–4679. [PubMed: 18489124]
7. Wang Y, Young G, Bachman M, Sims CE, Li GP, Allbritton NL. Anal Chem 2007;79:2359–2366. [PubMed: 17288466]
8. Shadpour H, Sims CE, Thresher RJ, Allbritton NL. Cytometry A 2009;75:121–129. [PubMed: 19012319]
9. Rozkiewicz DI, Kraan Y, Werten MW, de Wolf FA, Subramaniam V, Ravoo BJ, Reinhoudt DN. Chemistry 2006;12:6290–6297. [PubMed: 16741908]
10. Huang C, Jacobson K, Schaller MD. J Cell Sci 2004;117:4619–4628. [PubMed: 15371522]
11. Kavurma MM, Khachigian LM. J Cell Biochem 2003;89:289–300. [PubMed: 12704792]
12. Sebolt-Leopold JS. Oncogene 2000;19:6594–6599. [PubMed: 11426644]
13. Dalmas DA, Tierney LA, Zhang C, Narayanan PK, Boyce RW, Schwartz LW, Frazier KS, Scicchitano MS. Toxicol Pathol 2008;36:958–971. [PubMed: 19126791]
14. Brown KK, Heitmeyer SA, Hookfin EB, Hsieh L, Buchalova M, Taiwo YO, Janusz MJ. J Inflamm (Lond) 2008;5:22. [PubMed: 19055838]
15. Grande MT, Lopez-Novoa JM. Curr Med Chem 2008;15:2054–2070. [PubMed: 18691056]
16. Mizuno H, Cho YY, Ma WY, Bode AM, Dong Z. Mol Carcinog 2006;45:1–9. [PubMed: 16302268]
17. Zawada WM, Meintzer MK, Rao P, Marotti J, Wang X, Esplen JE, Clarkson ED, Freed CR, Heidenreich KA. Brain Res 2001;891:185–196. [PubMed: 11164822]
18. Henry JR, Rupert KC, Dodd JH, Turchi IJ, Wadsworth SA, Cavender DE, Schafer PH, Siekierka JJ. Bioorg Med Chem Lett 1998;8:3335–3340. [PubMed: 9873730]
19. Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular Cloning. 3rd ed.. Cold Spring Harbor Laboratory Press; Plainview, NY: 2001.
20. Chen RH, Sarnecki C, Blenis J. Mol Cell Biol 1992;12:915–927. [PubMed: 1545823]
21. Lenormand P, Sardet C, Pagès G, L'Allemain G, Brunet A, J. P. J Cell Biol 1993;122:1079–1088. [PubMed: 8394845]
22. Henriksen U, Fog J, Loechel F, Praestegaard M. Comb Chem High Throughput Screen 2008;11:537–544. [PubMed: 18694390]

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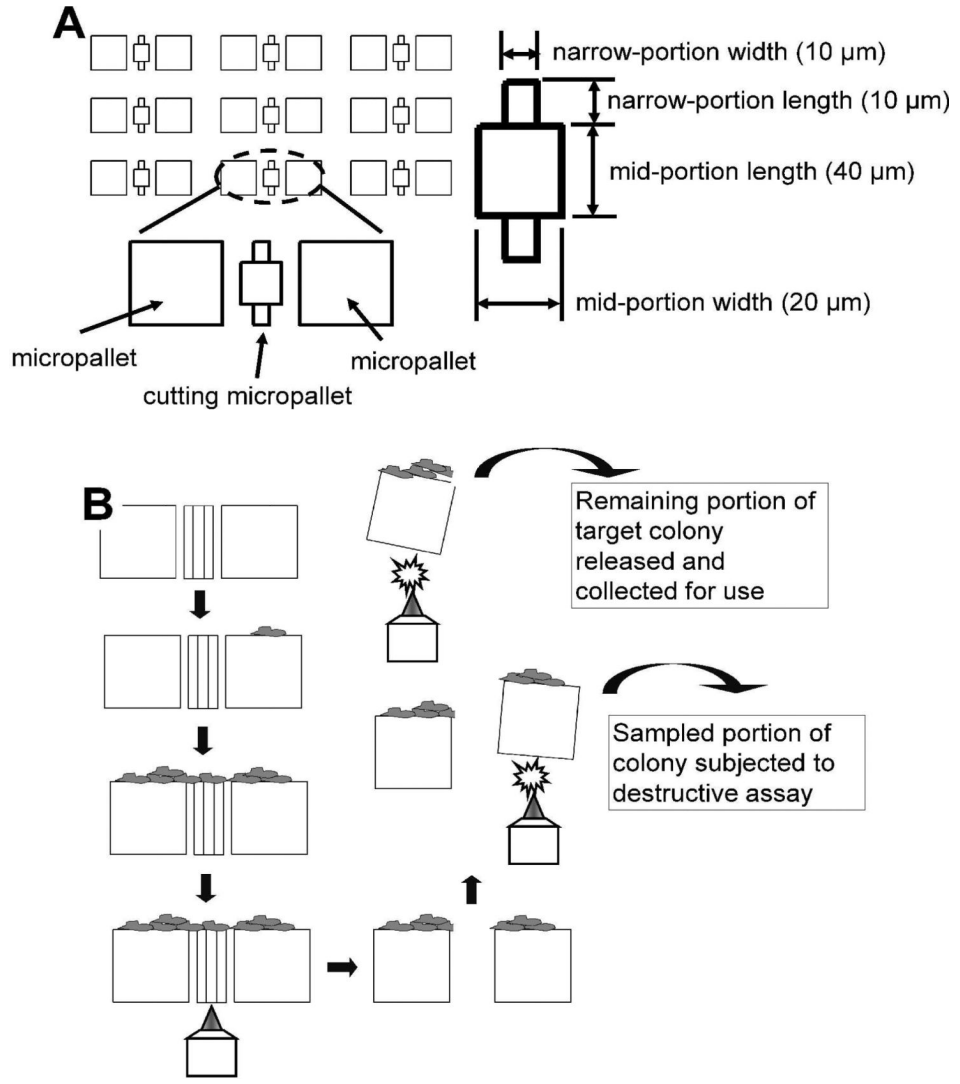

Figure 1.

Conceptual design and use of the colony sampling array. (A) Schematic of the array. In this drawing, each cell culture site is composed of three micropallets: two larger micropallets and one cutting micropallet. (B) Schematic of the sampling and identification process using the culture sites composed of micropallets.

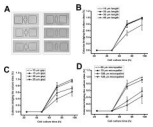

Figure 2.

Optimization of tripartite culture sites. (A) Brightfield images of culture sites with cutting micropallets of different mid-portion length (left) and different gap (right). (B-D) Plots of the percentage of culture sites with colonies expanding across the individual structures in a single culture site with respect to culture time. The dimensions of the geometry of cutting micropallet (B), gap between cutting and larger micropallets (C), or size of the two larger micropallets (D) were varied.

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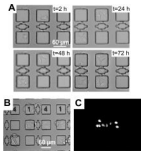

Figure 3.
Colonization of culture sites by single cells. (A) Brightfield images of two colonies created from single cells as the nascent colonies grow over culture sites during a 72 h period. Brightfield (B) and fluorescence (C) images of a region of an array with a culture site containing a clonal colony of HeLa cells possessing fluorescent nuclei amid colonies of non-fluorescent cells on adjacent culture sites.

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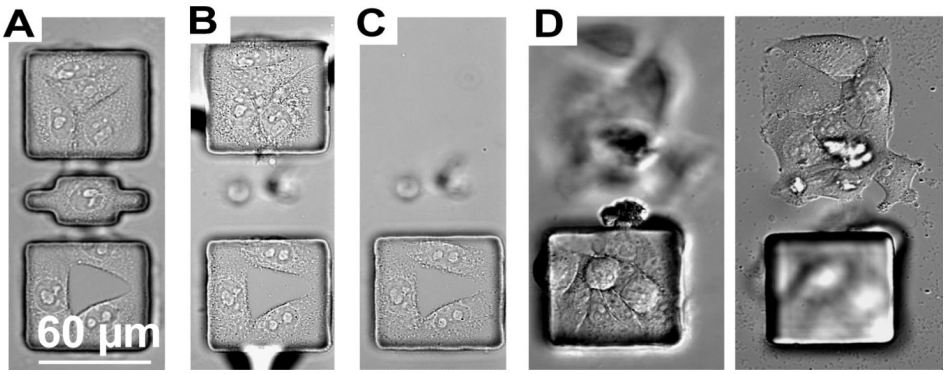

Figure 4.

Colony sub-sampling. (A) A colony of HeLa cells on a culture site after 72 h in culture. (B) The colony has been divided into two portions by release of the cutting micropallet. The cells remaining on the array after colony division remain attached and appear morphologically intact. (C) One of the micropallets has been released and collected leaving a genetically identical colony on the array. (D) Image of the same colony in “C” after an additional 48 h in culture demonstrating continued expansion of the colony. The unreleased portion has grown and migrated off the micropallet into an unsilanized region of the glass substrate free of the virtual wall.

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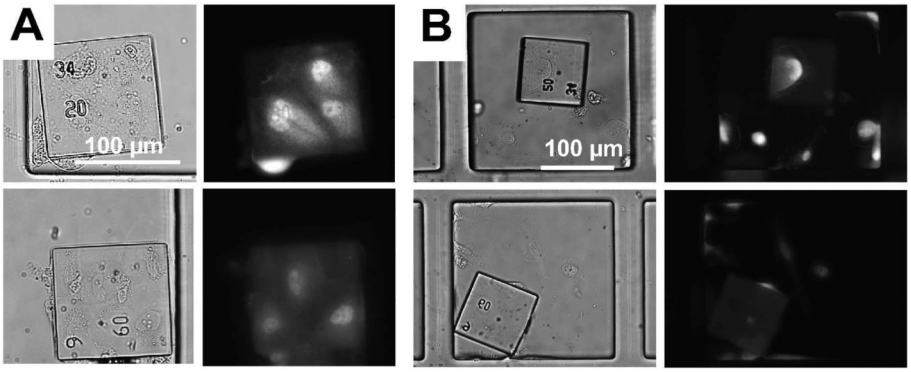

Figure 5.

ERK activation assay. (A) Brightfield and fluorescence images of the sampled portion of colonies demonstrating cells with high (upper panels) and low (lower panels) ERK activation based on nuclear fluorescence intensity. (B) Brightfield and fluorescence images of the parent colonies remaining on the array following release and culture of these colonies. The upper panel is a representative sample from the high ERK activation group and the lower panel is of the low ERK activation group.