CHAPTER

Single Cell Deposition 23

Jun Liu*,[1], Christopher Moraes†,[1], Zhe Lu*,[1], Craig Simmons‡, Yu Sun‡

**Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada*

†Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA

‡Institute of Biomaterials and Biomedical Engineering, Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada

[1]These authors contributed equally to this protocol

CHAPTER OUTLINE

Abstract

This chapter describes an experimental protocol for single cell pick-place using a general-purposed micromanipulator and a micropipette. The micromanipulation system uses computer vision and motion control approaches to accurately pick up a single cell, transfer it to a desired substrate/location, and deposit it at a specified location. A traditional glass micropipette having a diameter larger than the cell is used to aspirate a whole cell into the micropipette for pick-place. This protocol is designed to manipulate and position single cells within a variety of microenvironments. The

Methods in Cell Biology, Vol 112 0091-679X

http://dx.doi.org/10.1016/B978-0-12-405914-6.00023-8

system is broadly applicable to any type of substrate and can manipulate multiple cell types in an end-user customizable manner. The presented protocol is for aspirating and transferring single cells after trypsinization. Additional steps are required for pick-place cells in their adherent state, such as local trypsinization or mechanical cutting. The tool for pick-place of cells can be useful as a stand-alone or bolstering technology for single-cell studies.

1 PURPOSE

Population-based studies in biological experimentation are unable to probe the rich information available from the study of single cells (Ferrell & Machleder, 1998). Heterogeneity is a hallmark of cell biology, and is strongly evident in primary cell populations isolated from the same tissue (Chen, Yip, Sone, & Simmons, 2009). Furthermore, supposedly identical clonal cell populations have been shown to deviate in their genetic expression (Elowitz, Levine, Siggia, & Swain, 2002) and response to environmental stimuli (Bhola & Simon, 2009) over generations of cell division. This diversity has important implications in coordinating multicellular behavior, and is of critical importance in developmental biology, pathobiology, and tissue engineering. Therefore, studying single cells is necessary to understand the cellular basis for population behavior; and can also yield new methods for understanding signaling mechanisms and the biochemical basis for cellular function.

This protocol is designed to manipulate and position single cells within a variety of microenvironments. In this work, a general-purpose micromanipulator and glass micropipette are used to pick up single cells and deposit them on a microfabricated substrate. The system is broadly applicable to any type of substrate and can manipulate multiple cell types in an end-user customizable manner. This protocol consists of five steps. The first step is the fabrication of microwells. Cells are then randomly deposited on the microwells. A negative pressure is applied to aspirate a cell into the micropipette and keep the cell at a desired position. This cell is then deposited into a microwell by applying a fine positive pressure to the micropipette.

2 THEORY

Recent development of analytical techniques to investigate single-cell behavior (Brehm-Stecher & Johnson, 2004; Sims & Allbritton, 2007) has been complemented by (1) the development of high-throughput micro- and nanosystems (Moraes, Chen, Sun, & Simmons, 2010; Moraes, Wang, Sun, & Simmons, 2010) capable of precise and systematic manipulation of the cellular microenvironment and (2) the development of various systems to manipulate individual cells (Anis, Y. H. , Holl, M. R. , & Meldrum, D. R., 2008). The combination of these technologies is powerful for scientists to determine how single cells respond to a range of manipulated cues.

Single cell positioning has previously been achieved or attempted through using a few techniques. On the macroscale, an automated cell deposition system (CyClone,

Beckman Coulter Inc.) is designed for depositing single cells into standard multiwell plates. In addition to being an extra module for an already costly flow cytometer, the CyClone system requires large sample volumes. The positioning accuracy of this system is limited to 100 μm, making it unsuitable for many emerging microfluidic and bioMEMS technologies.

Micro- and nanotechnology-based approaches to manipulating single cells are growing in recent years. Micropatterning the substrate chemically or physically (Falconnet, Csucs, Grandin, & Textor, 2006) can be used to selectively allow cells to adhere to specified regions. By precisely patterning the size of these regions, parameters such as cell spreading area and the number of contacting cells can be controlled. However, reliably separating single cells requires the use of small micropatterned spots, which limits cell spreading area and alters cell function (Chen, Mrksich, Huang, Whitesides, & Ingber, 1997). Furthermore, this process lacks specificity and is stochastically driven, resulting in the loss of a large number of sites for single-cell analysis. The development of electrically and chemically switchable substrates (Yeo & Mrksich, 2006) has partially addressed this issue, but limited substrate chemistries that are presently available, and because of processing requirements, cannot be broadly and conveniently applied to microfabricated systems designed to screen for the effects of other microenvironmental parameters. Similar incompatibilities also exist in a number of other single cell manipulation techniques, including dielectrophoretic (DEP) trapping (Voldman, 2006), vacuum trapping arrays (Hosokawa, Arakaki, Takahashi, Mori, Takeyama & Matsunaga, 2009), and hydrodynamic localization (Skelley, Kirak, Suh, Jaenisch, & Voldman, 2009): all these methods require the incorporation of specific structures in a microdevice for cell manipulation purposes, which can interfere with or limit device operation. Other techniques such as optical trapping (Ashkin, Dziedzic, & Yamane, 1987; Mirsaidov, U., Scrimgeour, J., Timp, W., Beck, K., Mir, M., Matsudaira, P., et al. 2008) and acoustic wave manipulation (Gherardini, Cousins, Hawkes, Spengler, Radel, Lawler et. al., 2005) are also available, but dissipate power, and can potentially influence or damage biological material. Additionally, such methods are complex and require specialized equipment that is often unavailable in most wet labs.

In this protocol, a robotic cell manipulation system, which is capable of manipulating multiple cell types, is used for manipulating single cells. This system is applicable to a variety of emerging microfabricated devices. Without modification, it can precisely deposit individual cells at desired locations on both flat and topologically complex microfabricated substrates. The key limitation to this technique is in the time required to deposit a large array of cells. Barring occasional situations in which the cell adheres to the micropipette tip, which necessitates quick replacement of micropipette, approximately 200 cells can be positioned within an hour. However, techniques such as microwell-assisted patterning via the BioFlipChip can deposit thousands of cells in a 4-h window (Rosenthal, Macdonald, & Voldman, 2007), at the expense of accuracy. In a nutshell, the automated robotic technology used in this protocol is an aspiration-based method, in which a micropipette is used to capture and transfer multiple types of single cells from site-to-site across a substrate, in a user-defined customizable manner.

3 EQUIPMENTS

3.1 Equipment Required

- Host computer
- Standard fluorescence inverted microscope (IX81, Olympus Microscopes)
- CMOS camera (601f, Basler)
- X–Y motorized stage (ProScan™, Prior Scientific Inc.)
- Pipette puller (P97, Sutter Inc.)
- Microforge (DeFonbrune-type, GlasswoRx)
- 3 degrees-of-freedom motorized micromanipulator (MP285, Sutter Inc.)
- Linear motorized stage (eTrack, Newmark Systems Inc.)
- Glass syringe (25 μL, Hamilton)
- Vacuum chamber and plate (F4203, Bel-Art Products)

3.2 Equipment Setup

As shown in Fig. 1, a fluorescence inverted microscope is connected with a CMOS camera. Cells are manipulated under a 20× objective. The X–Y motorized stage is mounted on the microscope. The travel range along both X and Y axes of the stage is 75 mm with a resolution 0.01 μm. The micropipette is pulled and forged using the pulling and microforge machines and is mounted on the micromanipulator that has a travel range of 25 mm and a 0.04 μm positioning resolution along three axes. The

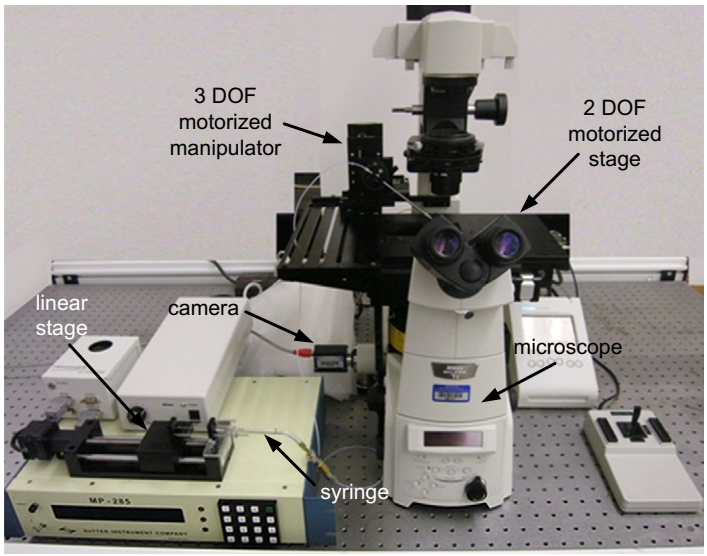

FIGURE 1

System setup. For color version of this figure, the reader is referred to the online version of this book.

micropipette is connected to the glass syringe using a polyethylene tube of 0.76 mm inner and 1.22 mm outer diameters. The syringe and tube are filled with mineral oil (M8410; Sigma–Aldrich; St. Louis, MO, USA), and mounted on a linear stage to control the movement of the syringe plunger to a resolution of 0.04 mm. A host computer coordinates control of the X–Y stage, micromanipulator and linear stage. The system is placed on a vibration isolation table (Kinetic Systems, Inc., Boston, MA, USA).

4 MATERIALS

Fibroblast cells isolated from porcine aortic valve leaflets are used for demonstration in this protocol. Thus, the culture medium used in this protocol is Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and antibiotics. During the preparation of cell subculture, trypsin and phosphate-buffered saline (PBS) are also used.

An SU-8 master mold is produced and is replica molded in silicone (PDMS) to produce an array of microwells. The SU-8 master is fabricated on glass microscope slides using conventional photolithography techniques. Other processes such as silicon etching or micromachining can also be used to create such a master. PDMS is then cast and cured over the master. The PDMS structure is peeled away from the master, and retains the features microfabricated into the master.

4.1 Solutions and Buffers—Step 1

Component	Final concentration	Stock	Amount/L
• Cell culture medium for fibroblast cells			
DMEM	89%	2–8 °C	222.5 mL
FBS	10%	–20 °C	25 mL
Antibiotics	1%	–20 °C	2.5 mL
• Trypsin-EDTA 1× (25200-072, Invitrogen Inc., Burlington, ON, Canada), storage at –20 °C			
• Dulbecco's Phosphate Buffered Saline (DPBS) 1× (14190-144, Invitrogen Inc., Burlington, ON, Canada), storage at 2–8 °C			

VIDEO 1

Cell positioning inside a micropipette.

4.2 Solutions and Buffers—Step 2

- 3″ × 2″ Microscope slides soaked in acetone
- SU-8 2025 negative photoresist (Microchem)
- SU-8 Developer (Microchem)
- Polydimethylsiloxane Sylgard 184 kit (monomer and crosslinker; Dow Corning)
- (Tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane

4.3 Solutions and Buffers—Step 3

- Dulbecco's Phosphate Buffered Saline (DPBS) 1× (14190-144, Invitrogen Inc., Burlington, ON, Canada), storage at 2–8 °C

5 PROTOCOL

Duration		Time
	Preparation	1 h
	Protocol	2 days

Preparation In the preparation step, a micropipette is pulled and forged using the pipette pulling machine and microforge machine. The micropipettes are pulled from the thin-walled B100-75-10 borosilicate glass capillaries without microfilament (100 mm length, 1.0 mm outer diameter, 0.75 mm inner diameter, Sutter Inc.). The glass capillary is placed in a heating chamber with the middle part surrounded by an electrical heating part. Then, a horizontal force is applied to pull the heated glass capillary to form a tapered needle. During the end-stage of pulling, compressed air is blown to cool down the glass heating chamber. Heating temperature, air pressure, pulling force, pulling velocity and cooling time are critical parameters for producing needle of desired shape and tip diameter.

The pulled pipette is forged by a microforge machine. The pipette is mounted on the DeFonbrune-type forge machine that has an integrated microscope. A reticle with a linear scale on the forge machine is used to select the appropriate point to forge the pipette.

Caution Since the pipette tip size is important for cell pick-place, it is necessary to choose an appropriate recipe for pulling pipettes. Heating temperature of the pipette pulling machine typically ranges from 400 to 700 °C depending on the shape of the heating part, the distance between the heating part to the glass capillary, and the glass type of the capillary.

Tip The parameters used for pulling pipettes in our experiment are Heating = 520, Pull = 60, Velocity = 70, Time = 200, and Pressure = 200. Note that the heating temperature may vary slightly across machines of the same brand/model. The first thing for setting the parameters is to do a ramp test that determines the melting point of the glass capillary. Additionally, users should use glass capillaries without microfilaments for cell pick-place use. Finally, instead of using self-pulled pipettes, commercial micropipettes (e.g. Humagen, Charlottesville, VA, USA) with accurately controlled tip diameters are available.

See Fig. 2 for the flowchart of the complete protocol.

5.2 Step 1—Device Fabrication

Overview An array of micropillars is fabricated using SU-8 photolithography, and a negative replica of microwells for cell deposition is produced using PDMS soft lithography.

Duration 2 days.

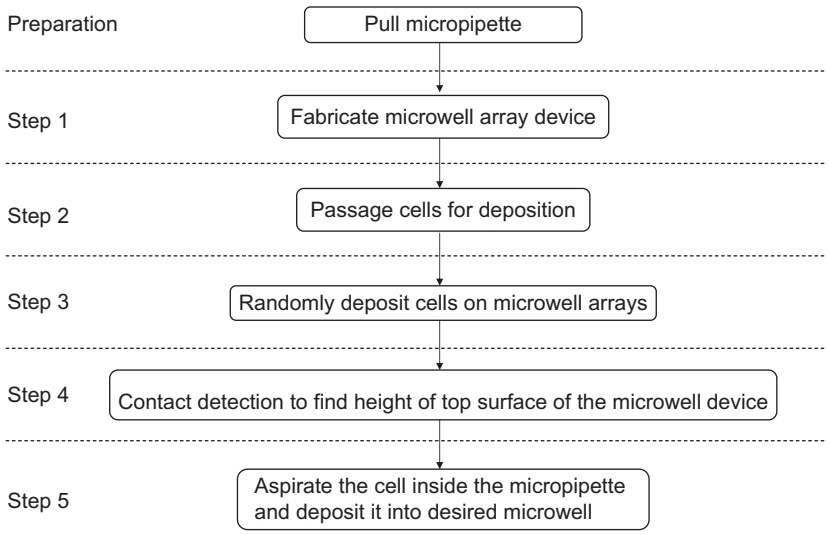

FIGURE 2

Flowchart of the complete protocol.

Procedure

1.1	Remove glass slides from acetone bath, rinse with isopropanol and blow dry with nitrogen gas.
1.2	Place on hotplate at 200 °C for at least 20 min to dehydrate the slide, allow samples to cool.
1.3	Transfer 1–2 mL of SU-8 2025 negative photoresist onto the substrate.
1.4	Place a slide in spin coater and spin for 30 s at 2000RPM, to achieve a thickness of approximately 25 μm.
1.5	Place the spin-coated sample on a hotplate at 65 °C for 5 min.
1.6	Place the spin-coated sample on a hotplate at 95 °C for 15 min.
1.7	Expose the sample to UV irradiation through a photomask for 15 s at 16.2 mW/cm^2, using a Karl Suss mask aligner.
1.8	Bake the sample for 2 and 7 min at 65 °C and 95 °C, respectively.
1.9	Soak the sample in SU-8 developer (Microchem) for 15 min while agitating the sample to develop the features.
1.10	Silanize the master by placing it in a vacuum desiccator with a few drops of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane, under vacuum overnight.
1.11	Thoroughly mix PDMS monomer and crosslinker in a 10:1 ratio by weight.
1.12	Pour PDMS over the silanized master mold, and degas under low vacuum.
1.13	Once bubbles are removed, place sample in an oven at 60 °C and cure overnight.

1.14	Carefully peel the PDMS replica away from the SU-8 master. The master can be reused to replicate more PDMS microwells.
Tip	The life of an SU-8 master can be extended by hard-baking the master after fabrication for 2–3 days in an oven at 60 °C, before silanization.

See Fig. 3 for the flowchart of Step 1.

5.3 Step 2—Cell Preparation

Overview	This step prepares cell culture medium and uses the medium for passaging cells.
Duration	20 min

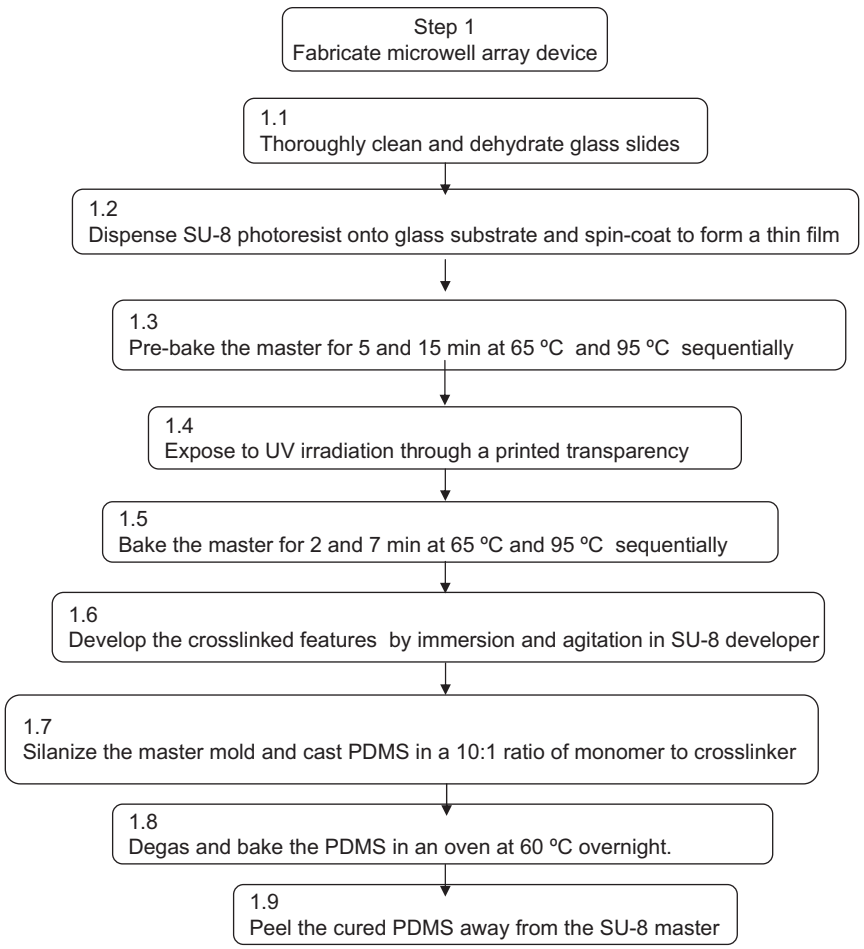

FIGURE 3

Flowchart of Step 1.

Procedure	
2.1	Thaw FBS and antibiotics in a water bath at 37 °C.
2.2	Add 222.5 mL of DMEM to a sterilized glass bottle.
2.3	Once FBS and antibiotics are thawed, add 25 mL of FBS and 2.5 mL of antibiotics to the DMEM.
2.4	Warm Trypsin, supplemented DMEM, and PBS in the water bath.
2.5	Flip the cell flask upside down and aspirate the old medium from the flask.
2.6	Add 5 mL of DPBS to the cells in a 25-mL flask and let it stay for 20 s.
2.7	Aspirate DPBS from the flask.
2.8	Add 2 mL of trypsin to the flask and put the flask in the incubator for 7 min.
2.9	Take the flask out of the incubator and add 4 mL of fresh supplemented DMEM.
2.10	Transfer the cell-trypsin-medium into the 1.5-mL vials and centrifuge these vials at 1200 rpm for 7 min.
2.11	Take out the vials from the centrifuge and remove the supernatants.
2.12	Resuspend the cells using fresh supplemented DMEM.
2.13	Put some of cells into a standard 35 mm petri dish for experimental use and put the rest of the cells back to a new 25-mL flask.
2.14	Label the petri dish and the flask with the experimenter's name, cell type, passage number and date; then put it back in the incubator.
2.15	Put the DPBS and DMEM back to the fridge, and freeze trypsin at –20 °C.
Caution	Step 2.6 is necessary to wash remaining serum since serum contains trypsin inhibitors. In Step 2.11, make sure not to aspirate the cells on the bottom when removing the supernatants.

See Fig. 4 for the flowchart of Step 2.

5.4 Step 3—Random Deposition of Cells

Overview	Placing cells randomly on microwell arrays before single-cell manipulation makes most of microwells occupied by single cells, which will effectively reduce the workload of cell pick-place.
Duration	20 min.
Procedure	
3.1	Add 4 mL of DPBS to a standard 35 mm petri dish.
3.2	Submerge the PDMS microwell device in the DPBS.
3.3	Put the petri dish with microwell device and DPBS inside in a low vacuum chamber and turn on the vacuum chamber to eliminate air bubbles from within each microwell.
3.4	Take out the petri dish and remove the excess DPBS using a standard pipette.
3.5	Add 200 μL of well-mixed cell suspension on the microwell device and allow the cells to sediment for 15 min.
3.6	Remove the excess cells with a standard pipette.

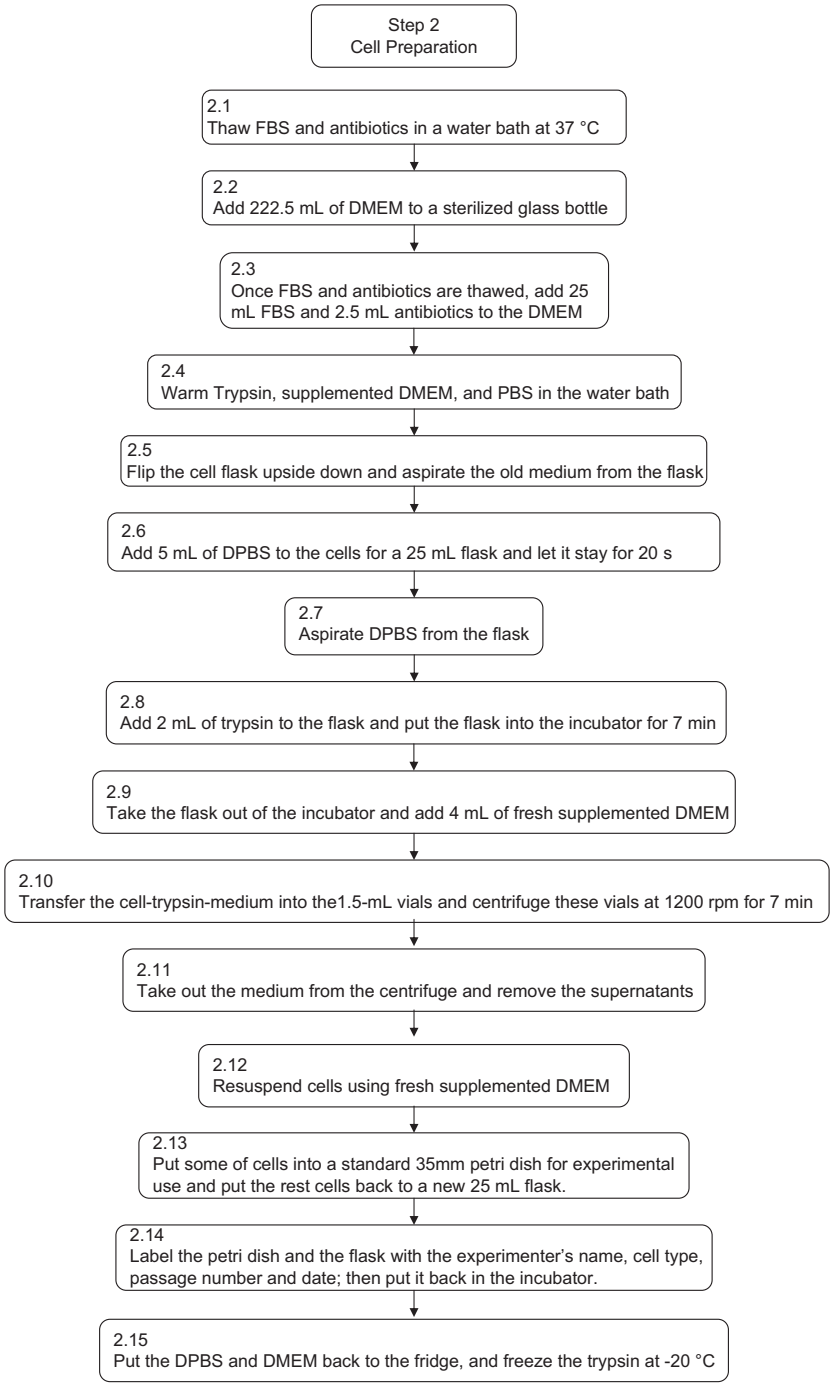

FIGURE 4

Flowchart of Step 2.

3.7	Add 2 mL of DPBS on the microwell device to wash the cells.
3.8	Gently shake the microwell device to allow single cells to settle down into vacant microwells.
3.9	Remove the excess DPBS and cells.
3.10	Repeat Steps 3.7–3.9 for three times.
Caution	Step 3.3 is necessary to eliminate the air bubbles from each microwell.
Tip	Excess medium and cells should be removed at the side of petri dish to avoid aspirating away the cells in microwells.

See Fig. 5 for the flowchart of Step 3.

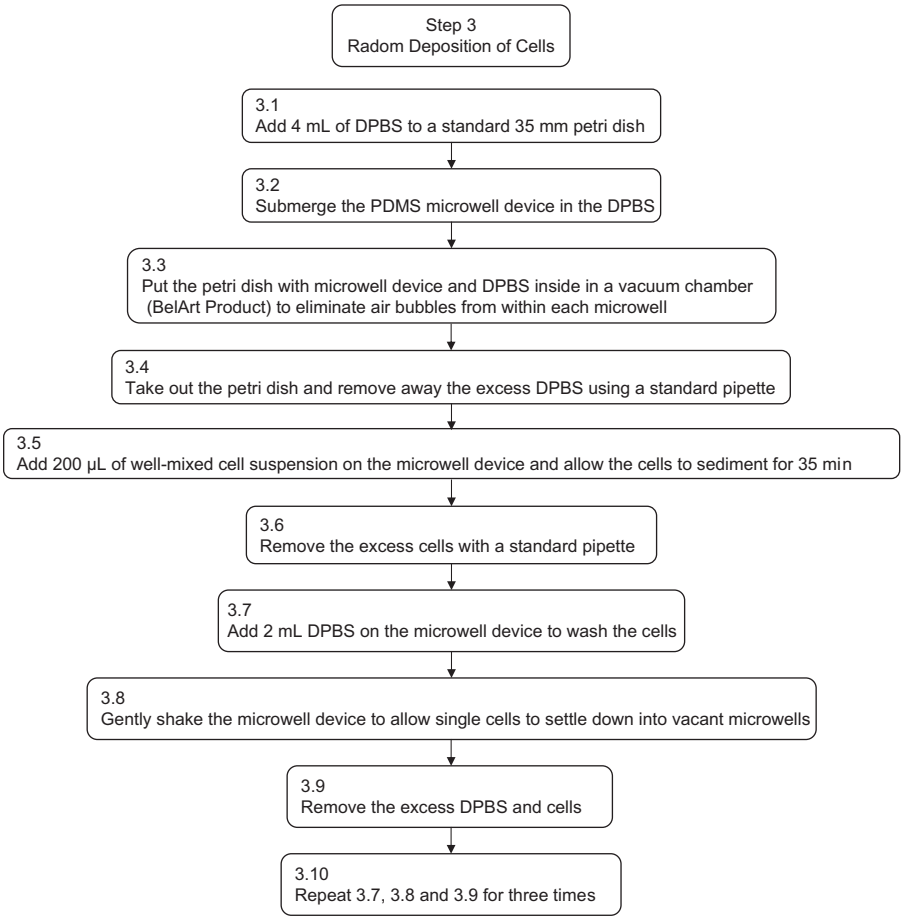

FIGURE 5

Flowchart of Step 3.

5.5 Step 4—Contact Detection

Overview	This step is for the micropipette to locate the device surface through lowering the micropipette until the pipette contacts the device surface. A self-pulled micropipette with a small opening is used. At first, the micropipette mounted on the micromanipulator is brought into the field of view under the microscope manually. Then, vision-based contact detection is applied to automatically determine the vertical positions of micropipette and the microwell-device surface.
Duration	5 min.
Procedure	
4.1	A micropipette is inserted into a pipette holder that is mounted on the micromanipulator.
4.2	Adjust the horizontal angle of micropipette to 30°.
4.3	Place the microwells device under the microscope (20× magnification).
4.4	Open the single-cell deposition program on the host computer.
4.5	Bring the pipette tip into the field of view under microscope.
4.6	Double click the micropipette tip on the screen of host computer. The system automatically lowers the micropipette until the pipette tip slips on the device surface. The position of device surface is then recorded by the computer.
4.7	The micropipette tip is set to 30 μm above the microdevice surface and 20 μm left to the center of the field of view of the microscope.
Caution	If contact detection is conducted manually, extreme care must be taken to avoid breaking the micropipette when lowering it.

See Fig. 6 for the flowchart of Step 4.

5.6 Step 5—Cell Aspiration and Deposition

Overview	In this step, the motorized X–Y stage and micromanipulator are controlled to move the micropipette to approach a selected cell. When the micropipette tip is close to the cell, a negative pressure is applied to aspirate the cell into the micropipette and precisely control the position of the cell inside the micropipette.
Duration	30 s per cell.
Procedure	
5.1	Locate the cells under the microscope's field of view by controlling the position of the X–Y stage.
5.2	Select an appropriate cell for transfer via computer mouse clicking. The system then recognizes the cell via image processing and brings the cell to the center of the field of view.

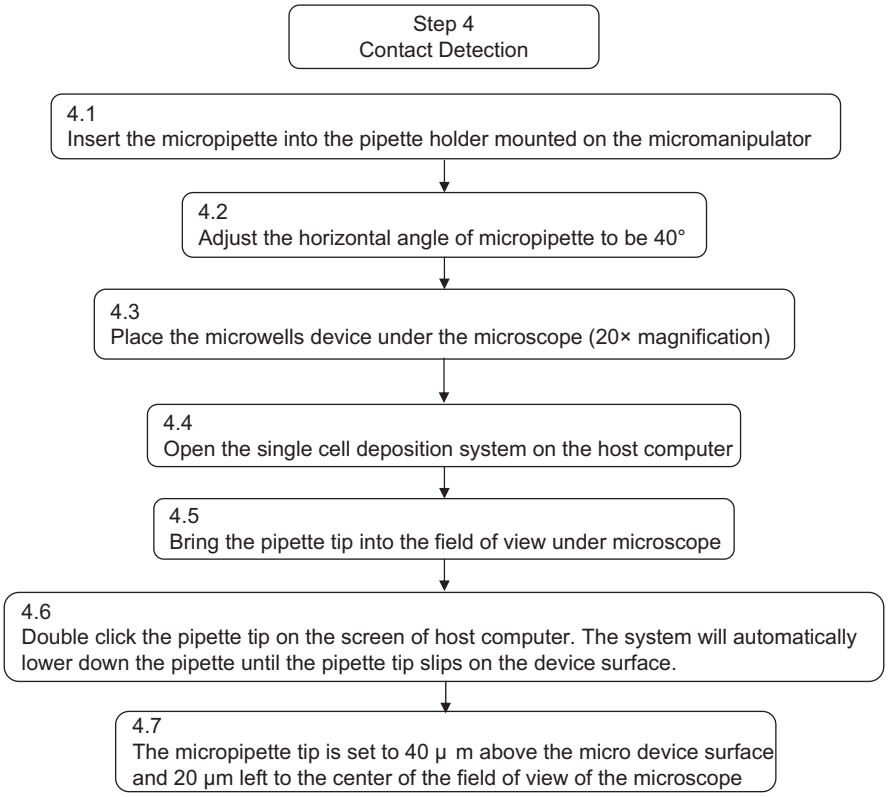

FIGURE 6

Flowchart of Step 4.

5.3	Control the micropipette to move downward to vertically align with the target cell.
5.4	Apply a negative pressure to aspirate the cell inside the micropipette. Figure 7 shows a cell aspirated into the micropipette.
5.5	Raise the micropipette 30 μm above the microdevice surface.
5.6	Move a vacant microwell to the center of field of view.
5.7	Lower the micropipette to the bottom of the microwell device.
5.8	Apply a fine positive pressure to deposit the single cell into the target microwell.
5.9	Repeat Steps 5.1–5.8 for pick-place of more cells.

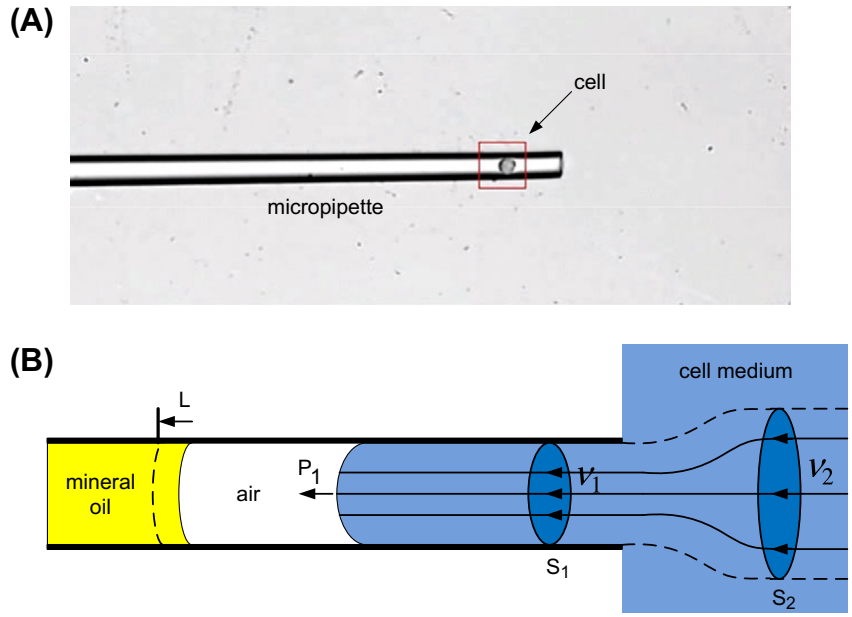

FIGURE 7

Cell aspiration using micropipette. (a) Cell inside a micropipette. (b) Fluid dynamics in the micropipette tip. For color version of this figure, the reader is referred to the online version of this book.

Caution	Failure can be caused by the micropipette wall that blocks a cell from entering the micropipette. For example, for a micropipette with a 30 μm inner diameter, the wall thickness of the micropipette tip is ~5 μm. When the micropipette tip is positioned on the surface of a microdevice, a cell (8–18 μm) is sometimes blocked by the outer wall of the micropipette tip. In about 20% of the experimental situations, a low fluid velocity was not able to overcome the blocking force, while a high fluid velocity caused the cell to disappear far into the micropipette, both resulting in failures.
Tip	During cell deposition, fine control of fluid velocity is necessary before the cell exits the micropipette, in order for the cell to be accurately deposited at a target location. Experimentally, the speed was controlled to be lower than 50 μm/s. It took an average of 9 s to dispense a cell and position it into a desired microwell.

See Fig. 8 for the flowchart of Step 5.

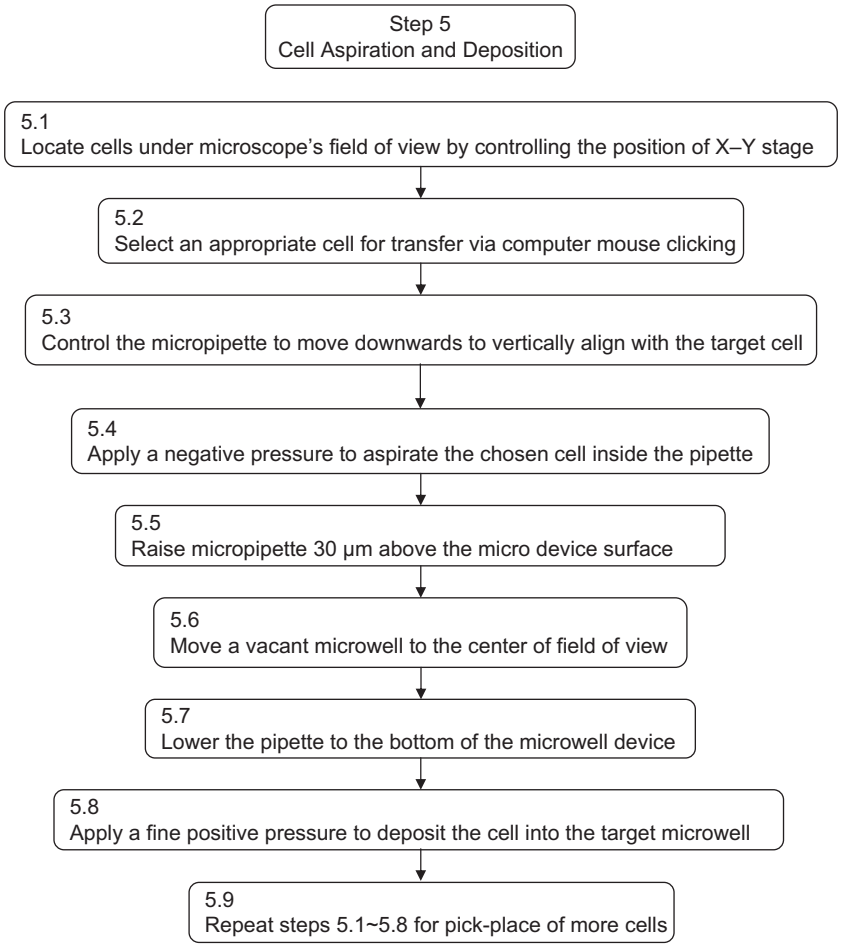

FIGURE 8

Flowchart of Step 5.

Keywords

Keyword Class	Keyword	Rank	Snippet
Methods List the methods used to carry out this protocol (i.e. for each step).	Device fabrication		Step 1 Device fabrication
	Passaging cells		This step prepares … for passaging cells.
	Random deposition		Step 3 random deposition of cells
	Contact detection		Then vision-based contact detection is applied to…
	Cell deposition		During cell deposition, fine control of fluid velocity is…

Keyword Class	Keyword	Rank	Snippet
Process List the biological process(es) addressed in this protocol.	1		
	2		
	3		
	4		
	5		
Organisms List the primary organism used in this protocol. List any other applicable organisms.	1		
	2		
	3		
	4		
	5		
Pathways List any signaling, regulatory, or metabolic pathways addressed in this protocol.	1		
	2		
	3		
	4		
	5		
Molecule Roles List any cellular or molecular roles addressed in this protocol.	1		
	2		
	3		
	4		
	5		
Molecule Functions List any cellular or molecular functions or activities addressed in this protocol.	1		
	2		
	3		
	4		
	5		
Phenotype List any developmental or functional phenotypes addressed in this protocol (organismal or cellular level).	1		
	2		
	3		
	4		
	5		
Anatomy List any gross anatomical structures, cellular structures, organelles, or macromolecular complexes pertinent to this protocol.	1		
	2		
	3		
	4		
	5		
Diseases List any diseases or disease processes addressed in this protocol.	1		
	2		
	3		
	4		
	5		

Keyword Class	Keyword	Rank	Snippet
Other List any other miscellaneous keywords that describe this protocol.	1		
	2		
	3		
	4		
	5		

References

Source article(s) used to create this protocol

Lu, Z., Moraes, C., Ye, G., Simmons, C. A., & Sun, Y. (2010). Single cell deposition and patterning with a robotic system. *PLoS ONE*, *5*(10), e13542.

Lu, Z., Moraes, C., Zhao, Y., You, L., Simmons, C. A., Sun , Y. (2010). A micromanipulation system for single cell deposition. IEEE International Conference on Robotics and Automation (ICRA2010) Alaska, May 3–8, 2010.

Referenced literature

Ashkin, A., Dziedzic, J. M., & Yamane, T. (1987). Optical trapping and manipulation of single cells using infrared-laser beams. *Nature*, *330*, 769–771.

Bhola, P. D., & Simon, S. M. (2009). Determinism and divergence of apoptosis susceptibility in mammalian cells. *Journal of Cell Science*, *122*, 4296–4302.

Brehm-Stecher, B. F., & Johnson, E. A. (2004). Single-cell microbiology: tools, technologies, and applications. *Microbiology and Molecular Biology Reviews*, *68*, 538–559.

Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M., & Ingber, D. E. (1997). Geometric control of cell life and death. *Science*, *276*, 1425–1428.

Chen, J. H., Yip, C. Y., Sone, E. D., & Simmons, C. A. (2009). Identification and characterization of aortic valve mesenchymal progenitor cells with robust osteogenic calcification potential. *American Journal of Pathology*, *174*, 1109–1119.

Elowitz, M. B., Levine, A. J., Siggia, E. D., & Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science*, *297*, 1183–1186.

Falconnet, D., Csucs, G., Grandin, H. M., & Textor, M. (2006). Surface engineering approaches to micropattern surfaces for cell-based assays. *Biomaterials*, *27*, 3044–3063.

Ferrell, J. E., Jr., & Machleder, E. M. (1998). The biochemical basis of an all-or none cell fate switch in *Xenopus oocytes*. *Science*, *280*, 895–898.

Gherardini, L., Cousins, C. M., Hawkes, J. J., Spengler, J., Radel, S. Lawler, H, et al., (2005). A new immobilisation method to arrange particles in a gel matrix by ultrasound standing waves. *Ultrasound in Medicine and Biology*, *31*, 261–272.

Hosokawa, M., Arakaki, A., Takahashi, M., Mori, T., Takeyama, H. & Matsunaga, T. (2009). High-density microcavity array for cell detection: single-cell analysis of hematopoietic stem cells in peripheral blood mononuclear cells. *Analytical Chemistry*, *81*, 5308–5313.

Mirsaidov, U., Scrimgeour, J., Timp, W., Beck, K., Mir, M. Matsudaira, P., et al. (2008). Live cell lithography: using optical tweezers to create synthetic tissue. *Lab on a Chip*, *8*, 2174–2181.

Moraes, C., Chen, J. H., Sun, Y., & Simmons, C. A. (2010). Microfabricated arrays for high-throughput screening of cellular response to cyclic substrate deformation. *Lab on a Chip*, *10*, 227–234.

Moraes, C., Wang, G., Sun, Y., & Simmons, C. A. (2010). A microfabricated platform for high-throughput unconfined compression of micropatterned biomaterial arrays. *Biomaterials*, *31*, 577–584.

Rosenthal, A., Macdonald, A., & Voldman, J. (2007). Cell patterning chip for controlling the stem cell microenvironment. *Biomaterials*, *28*, 3208–3216.

Sims, C. E., & Allbritton, N. L. (2007). Analysis of single mammalian cells on-chip. *Lab on a Chip*, *7*, 423–440.

Skelley, A. M., Kirak, O., Suh, H., Jaenisch, R., & Voldman, J. (2009). Microfluidic control of cell pairing and fusion. *Nature Methods*, *6*, 147–152.

Voldman, J. (2006). Electrical forces for microscale cell manipulation. *Annual Review of Biomedical Engineering*, *8*, 425–454.

Yeo, W. S., & Mrksich, M. (2006). Electroactive self-assembled monolayers that permit orthogonal control over the adhesion of cells to patterned substrates. *Langmuir*, *22*, 10816–10820.

Related literature

Anis, Y. H., Holl, M. R., & Meldrum, D. R. (2008). Automated vision-based selection and placement of single cells in microwell array formats. *Proceedings IEEE International Conference on Automation Science and Engineering*, 315–320.

Di Carlo, D., Wu, L. Y., & Lee, L. P. (2006). Dynamic single cell culture array. *Lab on a Chip*, *6*, 1445–1449.

Faley, S., Seale, K., Hughey, J., Schaffer, D. K., VanCompernolle, S., McKinney, B., et al. (2008). Microfluidic platform for real-time signaling analysis of multiple single T cells in parallel. *Lab on a Chip*, *8*, 1700–1712.