Video Article

Silicon Microchips for Manipulating Cell-cell Interaction

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URL: http://www.jove.com/index/Details.stp?ID=268
DOI: 10.3791/268
Citation: Hui E.E., Bhatia S.N. (2007). Silicon Microchips for Manipulating Cell-cell Interaction. JoVE. 7. http://www.jove.com/index/Details.stp?ID=268, doi: 10.3791/268

Abstract

The role of the cellular microenvironment is recognized as crucial in determining cell fate and function in virtually all mammalian tissues from development to malignant transformation. In particular, interaction with neighboring stroma has been implicated in a plethora of biological phenomena; however, conventional techniques limit the ability to interrogate the spatial and dynamic elements of such interactions.

In Micromechanical Reconfigurable Culture (RC), we employ a micromachined silicon substrate with moving parts to dynamically control cell-cell interactions through mechanical repositioning. Previously, this method has been applied to investigate intercellular communication in co-cultures of hepatocytes and non-parenchymal cells, demonstrating time-dependent interactions and a limited range for soluble signaling [1].

Here, we describe in detail the preparation and use of the RC system. We begin by demonstrating the handling of the device parts using tweezers, including actuating between the gap and contact configurations (cell populations separated by a narrow 80-μm gap, or in direct intimate contact). Next, we detail the process of preparing the substrates for culture, and the multi-step cell seeding process required for obtaining confluent cell monolayers. Using live microscopy, we then illustrate real-time manipulation of cells between the different possible experimental configurations. Finally, we demonstrate the steps required in order to regenerate the device surface for reuse: toluene and piranha cleaning, polystyrene coating, and oxygen plasma treatment.

Protocol

Preparation of cell cultures:

1. Start with silicon parts coated with plasma-treated polystyrene.
2. Coat parts with appropriate extracellular matrix proteins to support attachment of desired cell type. For hepatocytes, incubate in 50 g/ml Collagen-1 solution at 37°C for 45 min. For 3T3 fibroblasts, no matrix is needed.
3. Lock parts with complementary parts in contact configuration.
4. Soak in 70% ethanol for a minimum of 10 min to sterilize. Rinse 2x in ddH_2O, and 1x in cell culture media.
5. Seed cells at a concentration of 500,000 cells/ml in the appropriate culture medium. Use 1 ml in each well of a 12-well culture plate.
6. Incubate for 1 hr, shaking every 15 min to re-suspend cells evenly.
7. If a confluent monolayer has not been achieved after 1 hr, aspirate the cell suspension and repeat seeding with a fresh suspension. Repeat until the desired cell density has been achieved.
8. Remove the complementary parts. Transfer parts to fresh wells and incubate overnight to allow cells to adhere and spread fully.
9. Form co-cultures by locking appropriate parts into either the gap or contact configuration. The configuration may be changed at any desired point during culture.
10. When changing media, take care to leave cells dry for as little time as possible, preferably just a second or two. Draw out fluid with one hand and immediately replace media using the other hand.

Processing silicon parts for reuse:

1. Strip cells by soaking in bleach and rinsing with water.
2. Allow parts to dry completely. Soak in toluene for 2 h to strip polystyrene.
3. Clean in piranha solution (1:2 H_2O_2:H_2SO_4) heated to 120°C.
4. Rinse for 15 min under a continuous flow of ddH_2O. If you are not going to deposit polystyrene immediately, store the parts in water.
5. Dissolve polystyrene in toluene at 100 mg/ml. Vortex in polypropylene conical for about 1 hr, or until fully dissolved. A little more than 1 ml of solution is required for every 10 parts.
6. Spin coat polystyrene solution on individual silicon parts at 2,400 rpm for 30 sec.
7. Bake for at least 5 h at 120°C.
8. Treat with oxygen plasma (200 mTorr, 200 W) for 1 min.

Discussion

This system is unique in that it enables the spatial organization of tissue to be dynamically manipulated at the cellular level. Consequently, this device has enabled a number of novel biological experiments, spanning topics such as intercellular signaling dynamics, contact-mediated versus soluble signaling, cell fate decisions, toxicology, and cellular crosstalk. This device should be widely generalizable since the culture substrate is standard tissue culture plastic, and the system is compatible with standard culture methods and assays. Hence, we believe that this platform will be of broad interest as a tool for studying cell-cell interaction among many different cells and tissues.

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Acknowledgements

The authors thank Salman Khetani, Jared Allen, Chris Flaim, and Austin Derfus for helpful discussions during the process of designing this device. This work was supported by the National Science Foundation Faculty Early Career Development Program, National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases, and the David and Lucile Packard Foundation. E.E.H. was supported by a Ruth L. Kirschstein National Research Service Award.

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