Microfabricated Devices for Cell Organization

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Primary CNF Tools Used: DWL2000 mask writer, ABM contact aligner

Abstract:

Different types of cells dynamically self-assemble and organize themselves in a spatiotemporal and context-dependent manner [1]. In this study, we report the spatiotemporal dynamics of cell organization of a binary cellular mixture (MDA-MB-231 and MCF10A cells) seeded in microfabricated microwells. The initial seeding ratio of binary cells determined the degree of encapsulation of MCF0A cells by MDA-MB-231 cells. When cells were free to grow, the differential proliferation rate of MDA-MB-231 (low growth rate) and MCF10A cells (high growth rate) resulted in a reversed core (MDA-MB-231)-shell (MCF10A) organization at seeding ratio of 1:1 (MDA-MB-231:MCF10A) and a side-by-side aggregate structure at seeding ratio of 4:1 after long-term culture.

Summary of Research:

Fabrication of Polydimethylsiloxane (PDMS) Microwell. The photomask was prepared using DWL2000 mask writer (Heidelberg Instruments). The silicon wafer was spin-coated with SU-8 2150 photoresist (MicroChem) at 500 rpm for 40 sec and then 2500 rpm for 30 sec. The wafer was covered with the photomask and exposed by a UV photolithography machine (ABM contact aligner) for 32 sec. After being developed and post-baked, the SU-8 master wafer was fabricated. The SU-8 master wafer was then used to create PDMS (Sylgard 184, Dow Corning) mold. A mixture (10:1) of Sylgard 184 silicone elastomer components was casted onto the master wafer and cured at 60°C overnight to prepare a PDMS microwell. Figure 1 is a microscopic image of PDMS microwells.

Formation of Cell Aggregates in PDMS Microwells. PDMS microwells were autoclaved, placed in a 24-well plate, and coated with 1% (w/v) Pluronic® F127 (Sigma) solution before cell seeding to prevent cell attachment on PDMS surface and facilitate formation of cell aggregates. To form cell aggregates, cell suspensions of MDA-MB-231/MCF10A mixture (MDA-MB-231:MCF10A=1:1 and 4:1, total 1.0×10^6 cells) were added to each well of 24-well plate with PDMS microwells inside. After four hours of static culture, the cells that were adhered to the interspace between microwells were removed by medium change. The cells that fell into the microwells formed cell aggregates after overnight culture. The cell aggregates were cultured in microwells for nine days. The mixed medium (MDA-MB-231 medium:MCF10A medium=1:1 and 4:1) was changed every two days. Figure 2 is a fluorescent image of cell segregation of MDA-MB-231 (red colour) and MCF10A (green colour) cells at 1:1 cell seeding ratio over nine days of culture. Figure 3 is a fluorescent image of cell segregation of MDA-MB-231 (red colour) and MCF10A (green colour) cells at 4:1 cell seeding ratio over nine days of culture.

In summary, the initial seeding ratio and cell proliferation have significant effects on the evolution of cell organization of binary cellular mixture over long-term culture. Depending on the initial seeding ratios, the cell organization is either a core-shell (1:1) or side-by-side (4:1) aggregate by the differential proliferation rates of MDA-MB-231 and MCF10A cells.

References:

[1] Yoshiki Sasai. Cytosystems dynamics in self-organization of tissue architecture. Nature 2013, 493, 318-326.

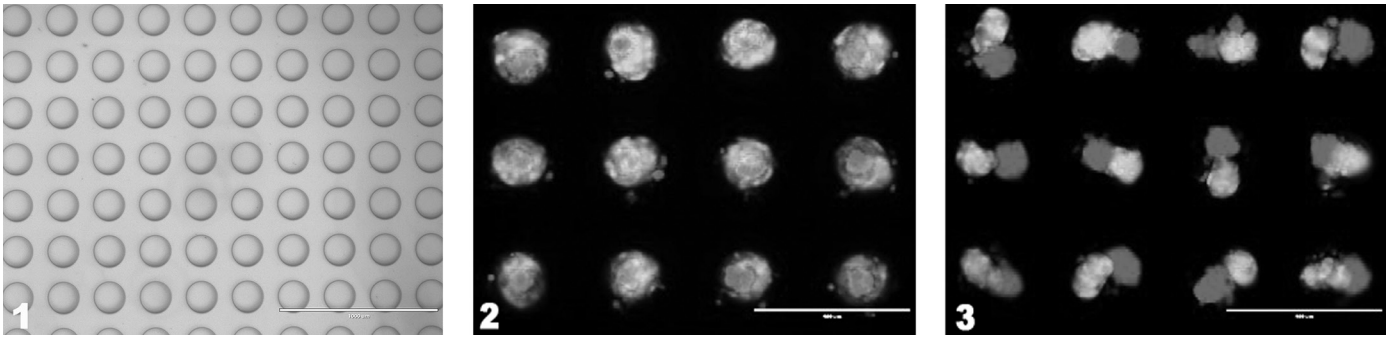

Figure 1, left: *A microscopic image of PDMS microwells. Scale bar: 1000 µm.* ***Figure 2, middle:*** *A fluorescent image of cell segregation of MDA-MB-231 (red colour) and MCF10A (green colour) cells at 1:1 cell seeding ratio over 9 days of culture. Scale bar: 400 µm.* *See full color version on pages xxviii-xxix.* ***Figure 3, right:*** *A fluorescent image of cell segregation of MDA-MB-231 (red colour) and MCF10A (green colour) cells at 4:1 cell seeding ratio over nine days of culture. Scale bar: 400 µm.* *See full color version on pages xxviii-xxix.*