A patterned superhydrophobic surface array using nanoparticle coating for screening collective cell migration

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Statement of Purpose: Collective cell migration, which cells migrate and communicate as a group, is a fundamental biological phenomenon. Wound healing , manually scratching the confluence cells to generate a cell free area, is the most widely used method to study collective cell migration. However, stretch based assay leads to cell death and alters the surface properties of the substrate. Manually scratching is also poor in uniformity. In addition, scratching in a multiple well plate is not convenient, which limits its application in high throughput research. Superhydrophobic surface, on which the water contact angle is greater than 150 degrees, has been recently used to generate patterned arrays. Independent cell culture areas can be generated on a single piece substrate that a patterned 2D surface can serve as a conventional multiple well plate. The superhydrophbic patterned 2D well plate is easier to manipulate and low cost. However, so far there is no report on superhydrophobic patterning for cell migration study. In the current project, we developed a pattern superhydrophobic array in a regular Petri dish using controlled nanoparticle coating and generated circular cell free areas in each 2D well using sub millimeter silicone disk covering. This system enables studying collective cell migration in high throughput.

Methods:1 ***Generate super hydrophobic surface separated 2D multiple wells****:* Regular Petri dishes(ranging from 35mm to 100mm) were cover with an array of 3mm diameter circular masks as shown in Fig 1-A. Then the dishes were coated with silica nanoparticles to generate superhydrophobic surface outside the masks (Fig 1-B). The masks were removed after the superhydrophobic surface has developed and multiple 2D wells, separated by superhydrophobic surface, were generated on the Petri dish surface. **2.** ***Generate cell free areas***: in order to generate cell free area within each 2D well for collective cell migration study, sub mm diameter silicone disks were placed in the center of each none coated 2D wells (Fig 1-C). Mouse macrophage cell suspension was then pipetted to each 2D well manually or using a liquid robot (Fig 1-D). After overnight culture, the silicone disks were removed and cells were further cultured for 24h (Fig 1-E&F). ***3. Image acquisition and processing***: phase contrast images of each well were taken immediately at the removal of the silicone disks (0h) and after 24h. Acquired images were binaried and total pixels in the cell free area at 0h(P0) and 24h(P24) were calculated using an in-house developed software in Matlab. The migration rate was calculated using the following equation: (P0-P24)/P0*100%.

Results: 1 Characteristics of superhydrophobic 2D wells: media added in each individual 2D well were completely separated from each other. Media remained within the none coated 2D wells even when the Petri Dish was placed vertically or shaken in a orbital shaker at 120 RPM. Therefore, the superhydrophobic 2D wells are fully suitable for normal laboratory handling. Media can be added up to about 3mm in hight in the 2D wells, which provided enough nutrition support for the cells. **2** For **high throughput** purposes, as shown in the pattern in Fig.2, a total of 185 2D wells were generated in a regular 100mm Petri dish(actual inner diameter is about 86.5mm). **3 Collective cell migration**: cell grew confluent after over night culture (transparent red color covered area in a representative image in Fig. 3-A). After the silicone disk was removed, a circular cell free area was generated (gray color area in Fig. 3-A). There was no cell damage nor surface propensities altering observed after the removal of the disks. Cells then began to migrate towards the cell free area collectively and representative image shows 70% migration rate after 24h in Fig 3-B.

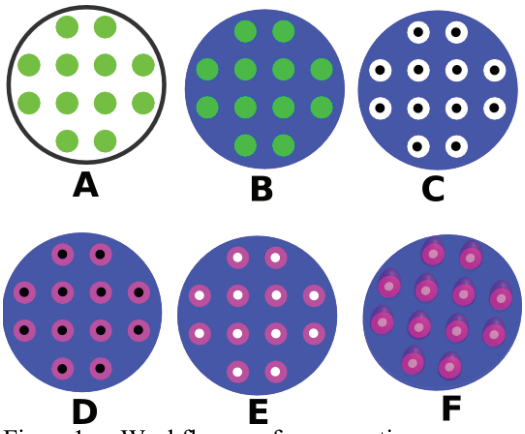

Fig. 1. Workflow of generating nanoparticle coated superhydrophobic surface array (A&B), and generating cell free area in each 2D well (C-F). F is a 3D view of the array. Color codes: *white*: Petri dish(polystyrene), *green*: mask, *blue*: silica nanoparticle coating, *black dots*: silicone disks, *purple*: cells, *light purple*:cell culture media.

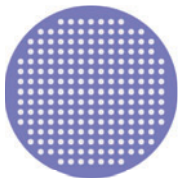

Fig. 2 185 2D wells were patterned in a Petri Dish.

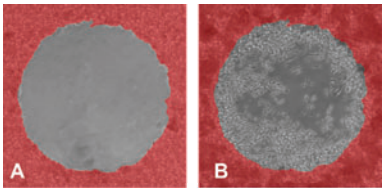

Fig. 3 Representative images of cell free area (A) and collectively migrated cells after 24h.

Conclusions: Here we demonstrated a novel high throughput system for studying collective cell migration using patterned superhydrophobic surface array. Our system uses novel 2D wells, which provides easier manipulation comparing with conventional well plates. Our cell free generation technique does not damage cells or alter the surface properties. This system will provide a versatile platform for studying cell migration in many research areas.