COLLECTIVE MIGRATION OF SMALL-SIZED MULTI-CELLULAR CLUSTERS STUDIED BY DYNAMIC CELL MICRO-PATTERNING BASED ON A CELL-FRIENDLY PHOTORESIST

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ABSTRACT

Variation in numbers may have minimal effects on the behavior of multi-cellular clusters composed of tens of cells, but would have profound effects on groups with less than ten cells. Herein, we report a new dynamic cell micro-patterning method using a cell-friendly photoresist PDMP film. Using this method, we fabricated multi-cellular clusters composed of two, four, and eight cells; then, systematically investigated the effect of multi-cellular cluster sizes on their motility behaviors. The behavior of multi-cellular clusters composed of less than ten cells was not sensitive to initial configuration, but rather was determined by dynamic force balances among the cells.

KEYWORDS: Dynamic patterning, Photoresist, Multi-cellular clusters, Microscope projection photolithography, collective migration

INTRODUCTION

Dynamics of small-sized multi-cellular clusters is important for many biological processes including embryonic development and cancer metastasis [1, 2]. Previously developed microfabrication methods are not suitable for the fabrication of multi-cellular clusters composed of less than ten cells with precisely controlled numbers because they are based on stochastic adhesion and proliferation of cells [3-6]. Here, we devised a new dynamic cell micro-patterning technique overcoming the aforementioned issue using a cell-friendly photoresist [7, 8]. We first fabricated single cell arrays of partially spread cells. Then, by merging neighboring cells, we successfully fabricated multi-cellular clusters with precisely controlled number, composition, and geometry. Using this method, we fabricated multi-cellular clusters composed of two, four, and eight cells; then, systematically investigated the effect of multi-cellular cluster sizes on their motility behaviors. We found out that the behavior of multi-cellular clusters composed of less than ten cells was not sensitive to initial configurations, but rather determined by dynamic force balances among the cells. Initially, the multi-cellular clusters became rounded and exhibited minimal translocation probably due to tension at the periphery of the clusters. For 2- and 4-cell clusters, single leaders emerged over time and entire groups aligned and co-migrated as single super-cells. Such coherent behavior did not occur in 8-cell clusters, indicating critical group size led by a single leader may exist. Pharmacological inhibitor study revealed that myosin light chain kinase (MLCK)-mediated peripheral tension as well as Rac-mediated protrusion was essential for corporative migration of 2- and 4-cell clusters led by single leaders.

EXPERIMENTAL

Multi-cellular clusters with precisely controlled shapes and cell numbers were fabricated as schematically shown in Figure 1A. Glass coverslips coated with gelatin, which promotes cell adhesion, were spincoated with a cell-friendly photoresist poly(2,2-dimethoxy nitrobenzyl methacrylate-r-methyl methacrylate-r-poly(ethylene glycol) methacrylate) (PDMP) and loaded in a chamber filled with phosphate buffered saline (PBS). First, microscope projection photolithography (MPP) was performed with a photomask containing arrays of circles with projected diameter, which is slightly smaller than the diameter of HeLa cells (14.6 ± 1.6 μm) or Madin-Darby Canine Kidney (MDCK) cells (15.2 ± 1.7 μm) in suspension. Since PDMP thin films spontaneously dissolve in PBS upon illumination of UV at 365 nm [7, 8], circular patches of gelatin layers surrounded by PDMP films were formed (Figure 1A(iii)). Then, cells suspended in culture media containing cell-cycle inhibitors (2 mM hydroxyurea and 1 μg/ml aphidicolin) were applied to the surface, incubated for two hours, and rinsed to remove non-adhering cells (Figure 1A(iv)). Media containing cell-cycle inhibitors was used throughout the experiments to prevent cell division. Since PDMP does not allow cell adhesion, and the gelatin island size is slightly smaller than the size of cells used, single cell arrays of cells were formed. Subsequently, a single cell array-containing chamber was mounted to the microscope stage equipped with environmental chambers maintaining cell culture conditions (37°C of temperature and 5% of CO2). A second photomask containing features that could cover each cell array was inserted into the field diaphragm, registered with the cell array, and the second MPP was performed (Figure 1A(v)). Then, the initially adhered cells spontaneously spread to fill area of freshly exposed gelatin layer resulting in multi-cellular cluster formation (Figure 1A(vii)). Successfully fabricated multi-cellular clusters composed of two, three, and four cells in different geometries from single cell arrays are shown in Figure 1B. This method can be extended readily to fabricate multi-cellular clusters composed of multiple types of cells by repeating steps (iii) and (iv) multiple times and finally merging them. Next, successfully formed multi-cellular clusters composed of a defined number of cells were confined in PDMP for 6 h to stabilize their adherens junction, and released by brief exposure to UV without a photomask to dissolve the PDMP films around them (Figure 2A). Three distinct behaviors emerged when the released multi-cellular clusters were observed by time-lapse microscopy over 36 h.
RESULTS AND DISCUSSION

Cells in 2- and 4-cell clusters maintained epithelial characteristics, rounded morphologies with junctional integrity, and all the cells in the clusters aligned and exhibited coordinated motion in following one ‘leader’ cell. In sharp contrast, cells in 8-cell clusters became thin and elongated, lost junctional integrity, and individual cells migrated without coordinating with neighboring cells, even while maintaining thin fibrous connections, which are characteristics of epithelial-mesenchymal transition.

Figure 1: Fabrication of multi-cellular clusters from single cell arrays. A. Schematic of multi-cellular cluster fabrication using single cell arrays. B. Representative differential interference contrast (DIC) images of multi-cellular clusters of HeLa cells with various numbers and geometries. C. Representative overlay images of DIC/red fluorescence of unlabeled and labeled HeLa cells.

Figure 2. Characteristics of motile population of multi-cellular clusters. A. Experimental scheme. B. Representative time-lapse images of motile MDCK clusters of various numbers. C. Single cell area of leaders and followers in 2-cell and 4-cell clusters of MDCK cells. D. Representative time-lapse images of 4-cell and 8-cell clusters with linear initial configuration.
CONCLUSION
Multi-cellular clusters composed of various numbers of cells in different geometries were fabricated previously, but the number of cells in these clusters was stochastically determined, rather than precisely controlled. In contrast, we started with single cell arrays, and by merging neighboring cells within single cell arrays, we could fabricate multi-cellular clusters composed of less than ten cells with precisely defined number, geometry, and composition. Compared with clusters of more than ten cells, the effect of initial geometry was minimal, and the behavior of multi-cellular clusters was more dynamically determined by force balancing among cells. Peripheral contractile force and intracellular adhesion force drove rounded shapes of multi-cellular clusters with minimal translocation, denoted by stationary clusters. Protrusive force played a critical role in leader formation, and peripheral contraction was required to maintain leadership of groups. Coherent migration led by a single leader was observed for 2-cell and 4-cell clusters, but such coordination did not occur in 8-cell clusters, and eventually multiple leaders emerged. These results will provide new insights into the mechanisms of collective migration and multi-cellular dynamics.

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REFERENCES

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