MICRODEVICE FOR STUDYING INTERCELLULAR MECHANICAL TRANSDUCTION

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ABSTRACT

This work reports the development of a polydimethylsiloxane (PDMS) microdevice for intercellular mechanical transduction study. Uni-axial strain with spatial gradient was generated by deforming a rectangular PDMS membrane. The strain distribution was experimentally measured. NIH 3T3 fibroblasts are cultured on the membrane and subjected to cyclic loading. Remarkable cell reorientation is observed in different strain regions. Analysis shows that cell morphological change in low strain region is due to intercellular mechanical transduction with the cells in the neighboring high strain region. Intercellular mechanical transduction can thus be quantitatively assessed.

KEYWORDS: Intercellular mechanical transduction, Biomedical microdevice, PDMS

INTRODUCTION

Cellular mechanotransduction refers to the process that cells transduce mechanical signals into biochemical responses. In a multicellular system, intercellular mechanical transduction allows neighboring cells to communicate with each other, and to coordinate their physiological activities. In order to investigate intercellular mechanical interaction, heterogeneous mechanical signal such as mechanical strain with spatially different magnitudes needs to be applied to a group of interconnected cells in culture. Current tools for cellular mechanical study, however, often apply the same magnitude of mechanical strain to all the cells in culture [1]. The lack of spatial variation of mechanical signals makes these tools inappropriate for intercellular study. Tools that can apply localized mechanical stimulus, e.g. atomic force microscopy [2], often require complex configuration and precise tip manipulation, and are not well compatible with cell culturing. Therefore, there are imperative needs for tools that can deliver controllable spatial variation of mechanical signals to selected cells in culture where the heterogeneous strain allows systematic investigation of intercellular mechanical interaction. In this work, we developed a polymeric microdevice, where an array of cell loading sites is arranged. Each site can be independently controlled without the need of precise positioning of the stimulating tip. A continuous heterogeneous strain field is applied to the cells in culture. Quantitative study of intercellular mechanical transduction is thus enabled.

FABRICATION

The microdevice consists of an array of rectangular PDMS membranes. Each membrane is 7500μm in length, 500μm in width, and 50μm in thickness. This device was fabricated using a double-side replica molding process (Figure 1). The top surface of each rectangular membrane serves as a cell loading site. The bottom side of the membrane is connected to a microfluidic channel, where fluid can be delivered to generate positive or negative differential pressure. In this work, each microfluidic channel is independently driven. Once the membrane is deflected by the differential pressure, the cells cultured on the top surface of the membrane are loaded. In particular, the cells are subjected to a heterogeneous uni-axial strain, which has a spatial gradient along the stretching direction (along the short edge of the rectangle).

To examine the actual strain delivered to the cells, an array of microdots (5μm in diameter; single spaced) is patterned on the top surface of the membranes [3]. The displacements of these microdots upon membrane deformation are optically tracked, where the in–plane strain field is derived using the classic large strain/displacement equations with Lagrangian

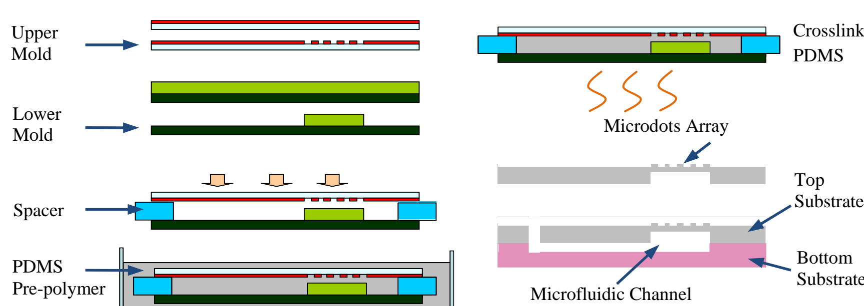


Fig. 1: *Fabrication of the polymeric device.*

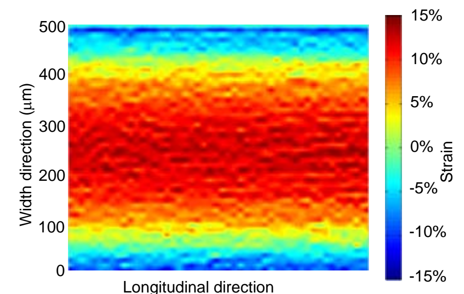


Fig. 2: *Experimentally determined strain field at the maximum membrane center deflection at 70 μm.*

operator (Figure 2). The result confirms that the strain field is uni-axial and non-uniform, *i.e.* some cells on the membranes are subjected to much higher strains than other cells. For example, when the vertical deflection at the membrane center is about 70 μm, the cells cultured in the region within 150 μm from the membrane center are subjected to tensile strain. The maximal tensile strain occurs at the membrane center and is about 15%. The cells close to the membrane edge are subjected to compressive strain. The maximal compressive strain occurs at the edge is about 10%. Between the tensile strain region and the compressive strain region, the cells cultured in the region 150-200 μm from the membrane center are subjected to relatively small strain with the absolute strain magnitude below 4%, which refers to "near zero" strain region. Experiment examination showed that the boundaries of the three strain regions do not shift during dynamic loading.

EXPERIMENTAL AND RESULTS

Cellular response to the spatial varying strain field is examined using NIH 3T3 fibroblasts (Figure 3). The cells on the membranes were loaded with six-hour cyclic strain. The maximal tensile strain at the membrane center is about 15%. The loading frequency is 0.5Hz. Cell morphological change is examined using optical microscopy. As shown in Figure 3, remarkable cell reorientation is observed upon cyclic loading. The cells appear to align themselves along the longitudinal direction of the membrane (normal to the uni-axial strain). This is confirmed by the statistical analysis (Figure 4a-c). Specifically, before loading the cells on the membrane randomly orient. The average angle between the longitudinal axis of the cells and longitudinal direction of the membrane is 49.2°, where only 20% cells orient themselves along the longitudinal direction (less than 15° to the longitudinal direction) (Figure 4a). After three-hour cyclic loading, about 48% cells orient themselves along the longitudinal direction (Figure 4b). The average angle between orientation of the cells and membrane longitudinal direction significantly decreases to 22.5°. After six-hour cyclic loading, about 60% cells orient themselves along the longitudinal direction (Figure 4c). The average angle between the long axis of the cells and the longitudinal direction of the membrane decreases to 18.1°. Besides cell orientation, the length-to-width ratio also exhibits significant increase upon cyclic loading, (Figure 4d). The average length-to-width ratio of the cells before loading is about 1.8, which increases to about 2.3 after three hours and 3.1 after 6 hours. It is also noted that, under large strain magnitude condition, the cell reorientation and length-to-width ratio change were observed not only in the regions with high strain magnitude, but also in the unloaded region. Comparison of cell morphological changes in the membrane center (tensile strain region), membrane edge (compressive strain region) and the unloaded region (near zero strain region) shows no significant difference (Figure 5).

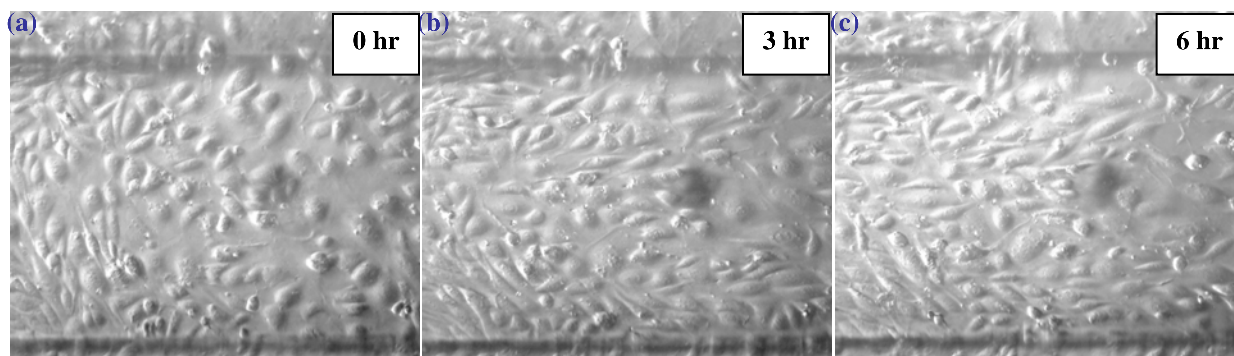


Fig. 3: *Morphological change of 3T3 fibroblasts on a membrane: (a) before loading (0hr); (b) after 3hr cyclic loading; and (c) after 6hr cyclic loading. (0.5Hz, maximum strain 15%)*

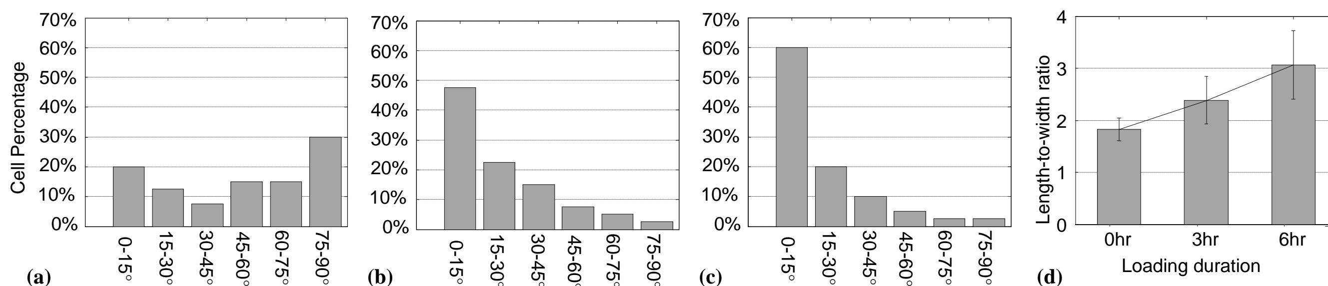
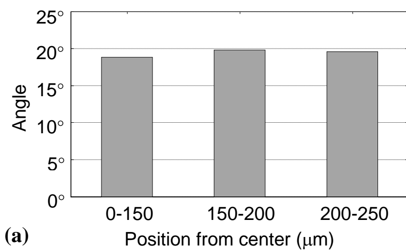


Fig. 4: *Effect of mechanical strain on cell reorientation and length-to-width ratio: (a) cell orientation before loading (0hr); (b) cell orientation after 3hr cyclic loading; and (c) cell orientation after 6hr cyclic loading); and (d) the length-to-width ratio change during loading. 0° denotes the longitudinal direction of the loading membrane.*



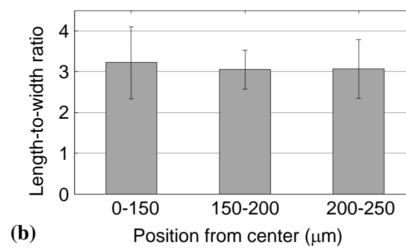


Fig. 5: *Morphological profile in different strain regions. (a) cell re-orientation and (b) length-to-width ratio. According to Fig. 4(b), 0-150μm from membrane center is tensile strain region; 150-200μm from membrane center is "near zero" strain region; 200-250μm from membrane center is compressive strain region.*

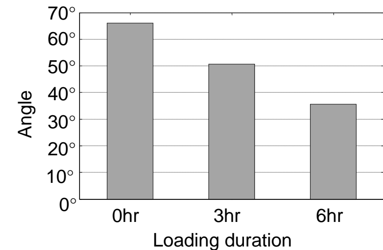


Fig. 6: *Cell reorientation in the off-membrane region (about 50μm from the membrane boundary).*

As a control, a reference membrane is loaded with the maximal tensile strain at the membrane center about 4%. This is to examine whether a low strain alone can induce cell morphological change. After six-hour loading, no significant cell reorientation or increase in length-to-width ratio is observed. This result suggests that the cell morphological changes in the "near zero" region are not due to the cyclic loads with low strain magnitudes. It is plausible to conclude that the cell morphological changes are primarily due to the intercellular mechanical transduction with cells in the neighboring "tensile strain region" or "compressive strain region".

To further confirm the presence of intercellular transduction, the cells in the off-membrane region (about 50 μm from the membrane boundary) is also examined (Figure 6). Although not as significant as those on the membrane, the cells off-membrane also exhibit obvious reorientation. Such reorientation diminishes as the distance from the membrane boundary increases. It can thus be concluded that spatial variation of mechanical signals can induce intercellular transduction to affect neighboring cells. The dependency of such transduction on the spatial strain gradient deserves further exploration.

CONCLUSION

This work reports the development of a PDMS based microdevice for intercellular mechanotransduction study. In this device, mechanical strain with spatial variation can be applied to a selected group of cells by deflecting the rectangular PDMS membrane using hydraulic differential pressure. The actual in-plane strain distribution on the membrane top surface upon deflection is experimentally examined, showing that the delivered strain is uni-axial and non-uniform. Tensile strain, compressive strain, and transitional "near zero" strain can be applied at same time on different regions of the membrane. Experimental results validate the efficacy of the device in delivering uni-axial strain, and show that significant cell morphological changes occur not only in high strain region (15% in tensile or 10% in compressive), but also in the low strain region (less than 4% in absolute value) after cyclic loading, while the low strain alone is not sufficient to induce dicernable morphological changes. With the capacity of providing a controllable heterogeneous strain field to load interconnected cells at the same time, the device is expected to serve as a universal platform for investigating intercellular mechanical transduction in a broad array of fundamental and applied biomechanical studies.

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