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Image Analysis for Characterization of Microtubule Protrusions

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Abstract

The dynamics of microtubules is central to how cells probe their environment and adopt both morphology and phenotypic fate. The length and dynamics of the protrusions that microtubules drive may be affected by the growth environment of a cell. This independent study project aimed to obtain data on the relationship between environment and microtubule disposition through integrated experiments and analysis. PDMS models in different sizes were fabricated by photolithography technology. The diameters of the PDMS model used in this experiment were 50, 75, 100, and 200 microns, respectively. A single 3T3 cell was grown in the PDMS model. After the 3T3 cell was grown and established, a three-dimensional picture of the 3T3 cell was taken by a microscope, and the length of the protrusions was obtained by measuring by software, and then the data was analyzed by MATLAB. The experimental result showed that the cells grown in 200-micron circles had a protrusions like that of cells grown in a normal environment and longer than those of cells grown in 100-, 75- and 50-micron PSMD models.

Introduction

The extracellular matrix is a macromolecular substance synthesized and secreted by animal cells, mainly distributed on the cell surface and between cells. The extracellular matrix is mainly composed of polysaccharides and proteins or proteoglycans. The complex lattice structure of these substances has an impact on the physiological activities of cells. For example, the extracellular matrix controls the speed and direction of cell migration and provides support for cell movement. When a cell moves in the extracellular matrix, it needs to form protrusions to move its front ends. The purpose of this paper was to investigate whether different 3D growth environments would influence the protrusions growth of cells.

Previous studies have found that the contractility of cell protrusions restricts the growth of microtubules by exerting compressive forces on the microtubules [1]. Since this study only

wanted to explore the relationship between cell protrusions and the 3D environment in which cells grow, we did not expect the shrinkage of protrusions to affect the results of the experiment. Cytochalasin D (CytoD), an organic compound derived from fungi, was used in the experiments to study actin dynamics. CytoD binds with high affinity to the barbed end of actin filaments and inhibits the polymerization and depolymerization of actin subunits at this end [2]. Thereby removing the effect of the contractile force on the microtubules allows for better growth of protrusions.

Methods

The first step in this experiment was to make the PDMS model which is used for cell growth. Gels were created by first creating a 10:1 ratio of Sylgard 184 to Quri. Pour the gel onto a mold and heat until set, remove the gel. At this point, the PDMS model is complete. Figure 1 shows the completed PDMS model. Then, putting the PDMS model in a glass-bottom dish, cells are grown in this dish, which is shown in figure 2.

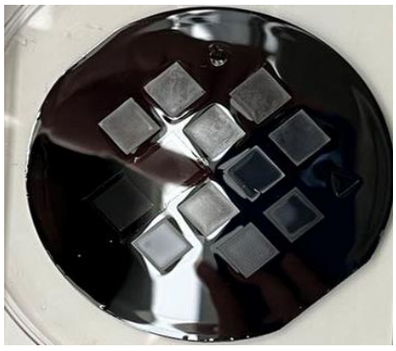

Figure 1: PDMS model

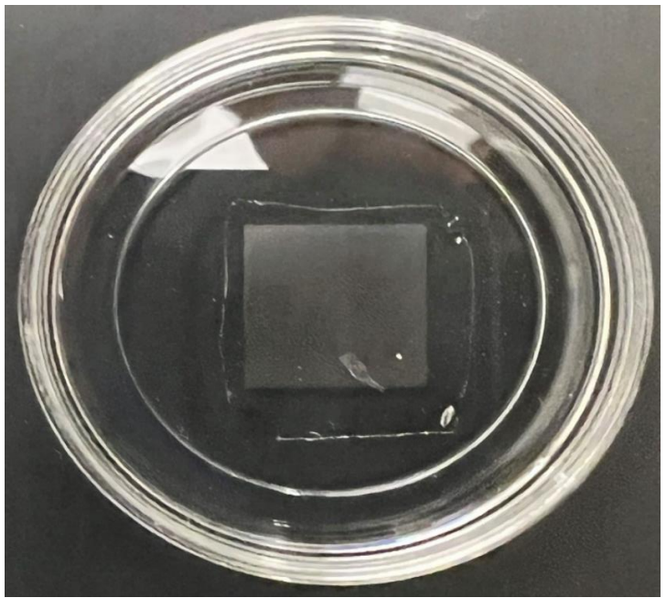

Figure 2: glass-bottom dish with PDMS model

Next, drop the prepared CytoD treated cell-collagen mixture in an incubator for 20 hours to allow cells to have enough time to grow protrusions. After 20 hours, use paraformaldehyde to fix cells. Paraformaldehyde (PFA) in PBS is one of the widely used fixatives for fluorescent protein-labeled samples [3]. Then, use 0.5% goat serum and Tubulin to dye fluorescent and incubate overnight.

On the second day, the confocal microscope is used to find cells with protrusions and take 3D photos. After taking enough photos, using ZEN Blue and ImageJ to measure the length of each protrusion. It is easy to look at the structure of cells with protrusions and use the 3D measurement function in ImageJ to measure the length of each protrusion. Because protrusion grows into a curve and bends shape rather than straight in a 3D environment which is hard to see without ZEN Blue.

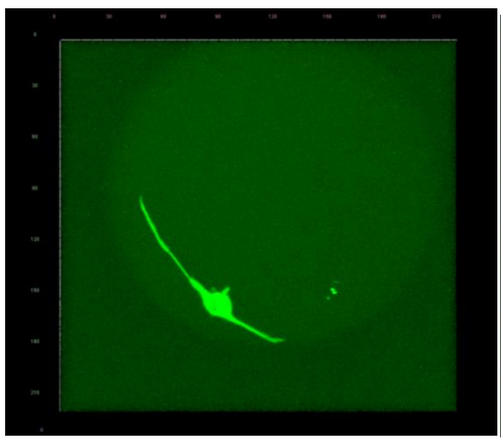

Figure 3: Cell's 3D image in Zen Blue

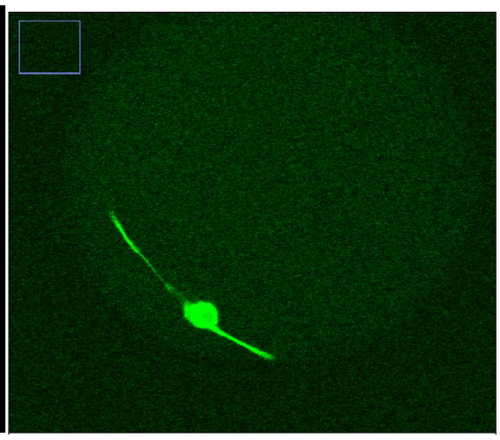

Figure 4: Cell image in ImageJ

After measurement of all lengths of protrusions, using MATLAB to plot P-value of all

lengths of protrusions in each cell. P-value is the probability of an observed result being more extreme than the resulting sample when the null hypothesis is true. When a p-value is less than 0.05, it means that the independent variable is important because this independent variable is related to the output. For calculating the p-value of each sample, the mean, standard deviation, and sample size should be known.

The equation is used to calculate the standard deviation s:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

Where s_1 and s_2 are the standard deviations of the two samples with sample sizes n_1 and n_2.

The standard error (SE) is calculated as:

$$se(\bar{x_1} - \bar{x_2}) = s \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

The P-value or significance level is calculated using the t-test and the equation is shown as:

$$t = \frac{\bar{x_1} - \bar{x_2}}{se(\bar{x_1} - \bar{x_2})}$$

When the P-value is less than 0.05($P<0.05$), this means that the two means are significantly different [4].

Result

After measurement of all data, the result is shown below (Chart 1). In the chart, green data shows P-value is larger than 0.05 which is mean the compared examples do not have a significant relationship. Conversely, black figures show that the two compared examples have a significant relationship. The X-axis shows the sizes of the PDMS model. "∞" means cells grow in a glass-bottom dish. 200, 100, 75, and 50 mean cells grow in different sizes PDMS models.

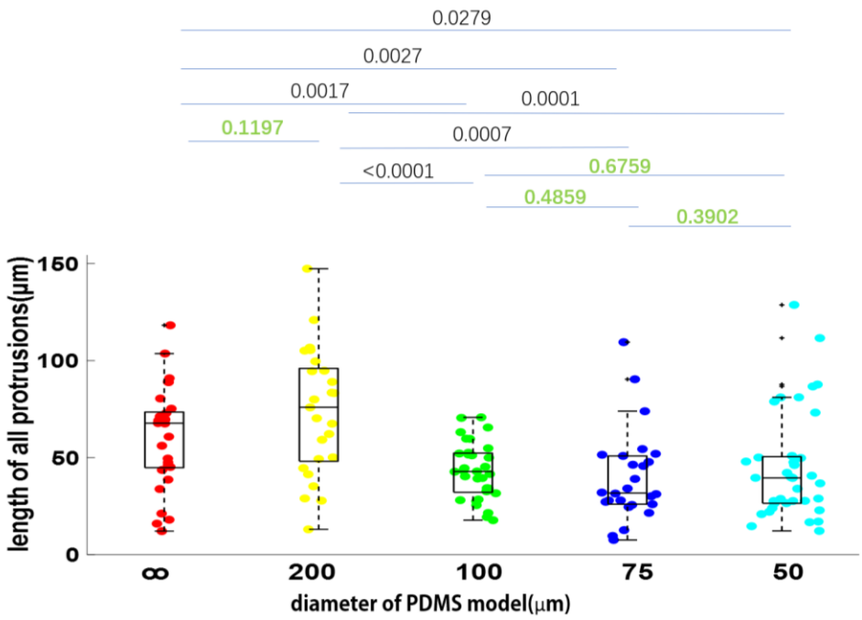

Chart1: P-value between the length of all protrusions and sizes of PDMS model

Discussion

The result is as excepted. This will provide a reference for in-depth research of cell protrusions in 3D environments. Some questions could be discussed in the future. For example, how does the cell control the direction of the protrusion growth, and why the length of protrusions in the same cell is different?

Conclusion

Overall, the protrusion length of the cell is related to the size of the PDMS model in which the cell grows. According to the result, the average in control is similar to that in 200 microns and they are significant to the data in 100, 75, and 50 microns which areas are excepted. However, p values in 100, 75, and 50 microns do not have a relationship.

References

[1] Delaram Shakiba., et al. “The Balance between Actomyosin Contractility and Microtubule Polymerization Regulates Hierarchical Protrusions That Govern Efficient Fibroblast–Collagen Interactions.” ACS Nano 2020, 14, 7, 7868-7879, April 14,2020.

[2] Kazuya Shoji., et al. “Cytochalasin D acts as an inhibitor of the actin–cofilin interaction.” Biochemical and Biophysical Research Communications, vol. 424, no.1, 2012, pp. 52-57.

[3] Share biology. 4% paraformaldehyde (PFA) solution preparation. https://sharebiology.com/4-paraformaldehyde-pfa-solution-preparation/

[4] MedCalc Software Ltd. Comparison of means calculator. https://www.medcalc.org/calc/comparison_of_means.php (Version 20.109; accessed May 9, 2022