CLASSIFICATION OF CELL TYPES USING MECHANICAL AND ELECTRICAL MEASUREMENT ON SINGLE CELLS

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ABSTRACT

This paper presents a microsystem for single-cell characterization by aspirating cells continuously through a constriction channel while cell impedance profiles and images are measured to quantify transit time, the impedance amplitude ratio, and cell elongation simultaneously. The system demonstrated that osteoblasts, compared with osteocytes, have a larger cell elongation length, longer transit time, and a higher amplitude ratio. The system also classified EMT6 and EMT6/AR1.0 cells with success rates of 51.3% (cell elongation), 57.5% (transit time), 59.6% (amplitude ratio), and 70.2% (both) using neural network, further verifying the device’s capability for performing both electrical and mechanical measurements on single cells.

KEYWORDS: Microfluidics, Cellular Biophysics, Single Cell Analysis, Impedance Spectroscopy, Constriction Channel, Neural Network

INTRODUCTION

The electrical properties of the cell membrane and cytoplasm and the mechanical properties of the cytoskeleton determine the overall biophysical properties of a cell, which have been correlated with pathophysiological states in diseases, such as cancer [1,2]. Existing microdevices for studying single-cell biophysics can only measure either electrical [3] or mechanical [4] properties of cells. The only reported microdevice performing both electrical and mechanical characterization has limited throughput, incapable of collecting statistically significant data [5].

This paper presents a microfluidic system for single-cell mechanical and electrical characterization using constriction channel and impedance spectroscopy (see Figure 1(a)). Cells are aspirated continuously through a constriction channel while cell elongations and impedance profiles are measured simultaneously. Transit time and the impedance amplitude ratio are quantified as cell’s mechanical and electrical property indicators while cell elongation length inside the channel is used as a measure of cell size.

METHODS

The PDMS device was replicated from a double-layer SU-8 mold and bonded to a glass slide. It was first filled with culture medium, followed by a droplet of cell suspension pipetted to the entrance of the cell loading channel. A negative pressure of 10 kPa aspirated cells continuously through the constriction channel. Cell images were taken by an inverted microscope and impedance data were recorded by an impedance analyzer.

Figure 1: (A) Schematic of the microfluidic system for electromechanical characterization of single cells using impedance spectroscopy and constriction channel. Cells are aspirated continuously through the small constriction channel with impedance data (transit time and impedance amplitude ratio), and cell elongation length measured simultaneously. (B) Impedance measurement of single cells (amplitude vs. time). Transit time indicates cellular mechanical properties and impedance amplitude ratio indicates cellular electrical properties. (C) A cell aspirated in the constriction channel. As an indicator of the cell size, the elongation length was measured from image processing approaches.
When a cell is aspirated through the constriction channel, it blocks electric fields, leading to higher impedance amplitude values. The time duration for this increased amplitude is interpreted as transit time, as a mechanical property indicator (Figure 1(b)). The ratio between the highest impedance amplitude value captured during cell’s squeezing through the channel and the basal impedance amplitude value is defined as the impedance amplitude ratio, as an electrical property indicator (Figure 1(b)).

To measure cell elongation length inside the constriction channel, image processing steps including frame differencing, thresholding, particle removal using erosion, and edge detection along the channel (Figure 1(c)) were used. A two-layer back propagation neural network was used for pattern recognition with three groups of parameters as the input data (transit time, impedance amplitude ratio, and cell elongation length). The complete dataset was divided into training data (70%), validation data (15%), and testing data (15%). In order to avoid the inappropriate selection of the number of neurons, a loop function was used to enumerate the neuron number from 5 to 200 with the highest cell classification success rate recorded.

Table 1. Cell classification success rates

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell elongation</th>
<th>Transit time</th>
<th>Amplitude ratio</th>
<th>Transit time + amplitude ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoblast vs. Osteocyte</td>
<td>90.5%</td>
<td>69.8%</td>
<td>85.3%</td>
<td>93.7%</td>
</tr>
<tr>
<td>EMT6 vs. EMT6/AR1.0</td>
<td>51.3%</td>
<td>57.5%</td>
<td>59.6%</td>
<td>70.2%</td>
</tr>
</tbody>
</table>

EXPERIMENTAL RESULTS

Microdevices with a constriction channel of 6 µm×6 µm were used to characterize osteoblasts (n=206) and osteocytes (n=217) (impedance measurement frequency: 100 kHz, aspiration pressure: 10 kPa, see Figure 2). Compared with osteocytes, osteoblasts have a larger cell elongation length (64.51±14.98 µm vs. 39.78±7.16 µm), longer transit time (1.84±1.48 sec vs. 0.94±1.07 sec), and a higher impedance amplitude ratio (1.198±0.071 vs. 1.099±0.038).

Neural network-based cell classification resulted in cell classification success rates of 69.8% (transit time), 85.3% (impedance amplitude ratio), and 93.7% (both transit time and impedance amplitude ratio), suggesting that biomechanical (transit time) and bioelectrical (impedance amplitude ratio) parameters, when used in combination, could provide a higher cell classification success rate than using electrical or mechanical parameter alone (see Table 1). Interestingly, using cell elongation length data only, cell classification success rate was as high as 90.5%. This is due to the fact that significant size differences exist between osteoblasts and osteocytes. This size difference may also account for their differences in transit time and impedance amplitude ratio.

To classify cells with insignificant differences in size distributions, the microdevice was applied to test EMT6 (n=747) and EMT6/AR1.0 (n=770) cells (impedance measurement frequency: 100 kHz, aspiration pressure: 10 kPa, constriction channel cross-section: 8 µm×8 µm). EMT6/AR1.0 cells are from drug treated EMT6 cells, having almost the same size distributions. Figure 3(a) shows a scatter plot of transit time vs. cell elongation length, indicating that there is a higher number of EMT6/AR1.0 cells with transit time less than 0.1 sec compared to EMT6 cells. Figure 3(b) reveals a linear trend between cell elongation length and impedance amplitude ratio with different slopes (0.0022 µm⁻¹ vs. 0.0028 µm⁻¹) and different y-axis intersections (0.990 vs. 0.967) for EMT6 and EMT6/AR1.0.

The success rate of classifying EMT6 vs. EMT6/AR1.0 cells using cell elongation length alone is only 51.3% (EMT6 vs. EMT6/AR1.0, Table 1), due to the insignificant difference in cell size (cell elongation length: 51.47±11.33 µm vs. 50.09±9.70 µm) for EMT6 and EMT6/AR1.0 cells. Cell classification success rates of 57.5% (transit time), 59.6% (impedance amplitude ratio), and 70.2% (both transit time and impedance amplitude ratio) suggest that biomechanical (transit time) and bioelectrical (impedance amplitude ratio) parameters, when used in combination, could provide a higher cell classification success rate than using electrical or mechanical parameter alone.
CONCLUSION

This paper presented a microfluidic measurement system for electromechanical characterization of single cells using constriction channel and impedance spectroscopy. The device was used to test osteoblasts and osteocytes, demonstrating that osteoblasts, compared with osteocytes, have a larger cell elongation length, longer transit time, and a higher impedance amplitude ratio. The microdevice was also used to distinguish EMT6 from EMT6/AR1.0 cells with comparable size distributions. Neural network-based pattern recognition for EMT6 and EMT6/AR1.0 produced cell classification success rates of 51.3% (cell elongation), 57.5% (transit time), 59.6% (amplitude ratio), and 70.2% (both transit time and amplitude ratio). These preliminary cell classification results suggest that biomechanical and bioelectrical parameters, when used in combination, could provide a higher cell classification success rate than using electrical or mechanical parameter alone.

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REFERENCES


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