APPLYING ELECTRIC CELL-SUBSTRATE IMPEDANCE SENSING (ECIS) TO STUDY CELL ADHESION AND CELL SPREADING OF AN INDIVIDUAL CELL

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ABSTRACT
This work presents a novel application of Electric Cell-substrate Impedance Sensing (ECIS) to study cell adhesion and cell spreading. Specifically, the measurement of the cell substrate separation \( h \) and the projected area \( A_{cell} \) of the cell from the ECIS technique was used to discriminate two individual adherent cell populations that were present during the initial cell adhesion and cell spreading processes. Experimental results from ECIS measurement demonstrated good agreement with those obtained from conventional methods.

KEYWORDS: ECIS, Cell adhesion, Cell spreading, Single cell analysis

INTRODUCTION
Cell adhesion and cell spreading are essential cellular processes, crucial to the body’s defense system, the formation of tissues, as well as the signal transduction pathways of a cell [1]. Cell adhesion and cell spreading are distinct cellular processes although they are very related. In many cases cell spreading follows the initial cell adhesion on the substrate.

The dynamics of both cell adhesion and cell spreading have been characterized by observing a temporal behavior of several properties of the individual cell including the cell substrate separation \( h \) and the projected area \( A_{cell} \) of the cell [1,2]. Therefore, both \( h \) and \( A_{cell} \) can be used as two metrics for studying cell adhesion and cell spreading processes. Figure 1 shows the typical observed behaviors of \( A_{cell} \), \( h \), and cell morphology during cell adhesion and cell spreading processes.

This work demonstrates a novel application of Electric Cell-substrate Impedance Sensing (ECIS) to study cell adhesion and cell spreading. Specifically, the measurement of \( h \) and \( A_{cell} \) was used to discriminate two individual adherent cell populations present during the initial cell adhesion and cell spreading processes. The first population refers to the cells that have just landed on the substrate (the spherical cell at the beginning of the initial cell adhesion process). The second population refers to the cells that have polarized and spread (the stretchy cell during cell spreading process).

EXPERIMENTAL
Two individual adherent cell populations were selected based on how much time the cell was allowed to associate with the substrate. The first population belonged to the cells that had very little time (the maximum time of 10 minutes) to associate (touch, attach, crawl) with the substrate. The second population represented the cells that had more time (more than 5 hours) to associate with the substrate. These two cell populations had distinct cell morphologies (Figure 2). From optical images the first population typically showed a lower mean value of \( A_{cell} \) compared to that of the second population. In addition, the cell circularity in the first cell population was
closer to 1 whereas the second cell population possessed a variety of cell circularities. According to published literature, the value of $h$ in the first population was expected to be higher than that of the second population \[3,4\]. Impedance spectra of individual cells from these two populations were recorded and analyzed to obtain $h$ and $A_{cell}$ from ECIS measurement as described in \[1,2\].

Figure 1. The typical temporal behavior of cell morphology (top view and side view), $h$, and $A_{cell}$ of an individual cell upon adhesion and spreading on the substrate. Cell shape changes from spherical to discoid during the initial cell adhesion process. Then, the cell polarizes and the cell shape become stretchy and irregular. The value of $h$ decreases during the initial cell adhesion process, but is unknown during the cell spreading. The value of $A_{cell}$ increases slightly during the initial cell adhesion process, but increases significantly during cell spreading and later becomes saturated.

RESULTS AND DISCUSSION

Figure 3 demonstrates that two individual cell populations were discriminated using the measured values of $h$ and $A_{cell}$ from ECIS measurement. The values of $h$ were higher and the values of $A_{cell}$ were lower in the first cell population compare to those in the second cell population as expected. These results agreed well with the typical temporal behaviors of an individual cell as presented in Figure 1. The mean values of $h$ obtained from ECIS in the first and second populations were 3.29 $\mu$m and 358 nm, respectively. The mean value of $h$ in the first population was similar to the calculated value of $h$ assuming that the cell shape was spherical (Figure 4). The mean values of $A_{cell}$ obtained from ECIS in the first and second population were 201 $\mu$m$^2$ and 356 $\mu$m$^2$, respectively. These mean values were in the expected range of $A_{cell}$ observed optically. These results implied that when a spherical cell was given time to associate with the substrate, the cell made the initial contact to the substrate, flattened, became discoid, and finally spread.
CONCLUSIONS
The ECIS technique has been applied to discriminate two cell populations that have distinct differences in both $h$ and $A_{cell}$. These two populations were important in the cell adhesion and cell spreading processes. It is believed that if the cell was followed over time during its initial adhesion and later spreading periods, the ECIS technique developed in this work would have the sensitivity to observe the dynamics of the cell during cell adhesion and cell spreading processes. Thus, the results obtained provide a step forward in applying ECIS to study these two cellular processes.

<table>
<thead>
<tr>
<th>Individual JEBG cells association with substrate</th>
<th>&lt;10 minutes</th>
<th>5 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{cell}$ (Mean, std., histogram, $n=100$)</td>
<td>274, 58 $\mu$m$^2$</td>
<td>308, 90 $\mu$m$^2$</td>
</tr>
<tr>
<td>Cell circularity (Mean, std., histogram, $n=100$)</td>
<td>0.92, 0.02</td>
<td>0.8, 0.11</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Round, spherical</td>
<td>Not round, flat, spread</td>
</tr>
<tr>
<td>Typical optical images of a cell on gold substrate</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 2. Cell morphologies of two populations of individual keratinocytes from an individual with junctional epidermolysis bullosa gravis (JEBG cells) measured at time less than 10 minutes and time equals 5 hours. $A_{cell}$ and cell circularity were calculated by analyzing optical images (light microscopy) using a free software package, ImageJ. When a cell is round, cell circularity equals one. $n$ is number of individual cells used in the experiment.

ACKNOWLEDGEMENTS
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REFERENCES
Figure 3. Both $h$ and $A_{cell}$ of two cell populations ($n$ is number of individual cells used in the experiment, $n=8$ for the first cell population and $n=3$ for the second cell population) were obtained from ECIS measurement. Error bars indicated variations in $h$ when the measurement was repeated on the same cell. Dotted lines represented the average values of $h$ for both cell populations.

Figure 4. Approximation of $h$ based on the shape of individual cells in the first cell population. A) Typical cell shape was close to a sphere when the cell had very little time to associate with the substrate. B) By approximating an area under the spherical cell ($a_1$) with an area of a rectangular ($a_2$) and substituting $r_c$ equals 9.35 µm for JEBG cells, $h$ of a JEBG cell was approximated to 2 µm.