Dynamic Label-free Imaging of Live-cell Adhesion Using Photonic Crystal Enhanced Microscopy (PCEM)

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Abstract: We demonstrate label-free imaging of cell attachment upon a photonic-crystal biosensor surface. Newly-implemented PCEM image-analysis software is used to dynamically visualize individual live-cell movement and demonstrate the spatiotemporal-distribution of cellular material during adhesion and motion.

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1. Introduction

Live cells are integrated not only structurally, but also functionally with their surrounding extracellular matrices (ECMs) in highly organized processes. The complex interaction between cells and the ECM molecules is an essential element of the regulation and mediation of various cellular activities, including adhesion and migration. Fluorescence dyes have been used to assist visualiziation of the cell adhesion process. In this work, we utilized a near real-time label-free imaging modality using a photonic crystal biosensor surface and a newly developed label-free microscopy approach to dyamically image live cell adhesion and migration over extended time periods.

2. Methods

We employed an imaging approach, photonic crystal enhanced microscopy (PCEM), to detect the live-cell adhesion on a label-free optical biosensor [1-4]. This imaging system incorporates the photonic crystal (PC) biosensor with an ordinary microscope system and a spectrometer to generate the reflection spectrum of the PC, while the output spectrograph is collected with a CCD camera. The PCEM system utilizes the evanescent field atop of the PC as the sensing zone (with a penetration depth of ~200 nm) and can quantitatively measure the dynamic cellular material change and cell–ECM interactions (whinin the sensing zone). It can also be applied with substrates functionalized with different types or concentrations of ECM molecules, and thus can be broadly used in many extracellular conditions. Furthermore, as a label-free imaging system, the PCEM does not suffer from the limitations of fluorescence photobleaching, and therefore enables long-term monitoring of cell attachment over substantial time scales (such as hours, or up to days).

3. Results and Discussion

We demonstrate the dynamic detection of a single stem cell attachment using PCEM imaging system. Murine dental stem cells (mHAT) were cultured and seeded onto a PC biosensor surface prepared with fibronectin extracellular matrix coating (concentration of 40 μg/mL). As shown in Fig. 1 (top row), a sequence of peak wavelength value (PWV) images (acquired at 10 minute intervals) represents mHAT cells progressively spreading and actively attaching to the PC surface, and the intensity changes of the PWV images indicate the distribution of the cell body mass during adhesion. As the cell attaches and spreads on the PC biosensor surface, dynamic PWV shifts (comparing to background pixels) are observed and can be interpreted as an accumulation of the cellular material in the evanescent field atop of the PC. This indicates the cell mass actively entering the sensing zone of the biosensor, which may be related to the binding between an integrin and the fibronectin layer. Generally, the live cell attachment profile starts with a small circular/elliptical area, then gradually grows to a bigger area, and finally exhibits a large irregular shape due to the formation of lamellipodia or filopodia.

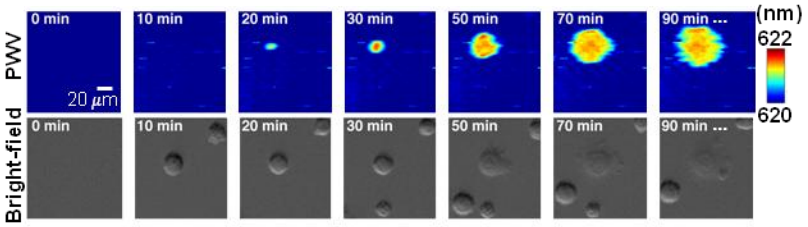

Fig.1 Label-free Imaging of Dynamic Live-cell Adhesion Using Photonic Crystal Enhanced Microscopy (PCEM). Top row: Peak Wavelength Value (PWV) images of an individual stem cell attaching on a PC biosensor surface; Bottom row: Bright-field microscope images.

One phenomenon observed from the PWV images is that the distribution of the cell mass in the sensing zone is non-uniform and dynamically changing. Typically, higher concentration of cellular material appears in the periphery (Fig.2 (a)) which can be attributed to accumulated adhesion or cytoskeleton proteins for cell morphology

maintenance and lamellar extension. To investigate the spatiotemporal redistribution of cell material along the boundary of plasma membrane, PCEM-based image-analysis software is developed to dynamically visualize and quantitatively analyze the PWV images acquired during the cell adhesion and migration. The temporal cell boundary-evolution traction results from a single mHAT cell adhesion are shown in Fig. 2 (b): the color (from blue to red) represents the cell boundary evolution time (from 0 to 220 minutes). The resulting spatiotemporal cellular material distribution map (map I) along the cell boundary is shown in Fig. 2(c), where the x-axis represents time (in minutes) and the y-axis represents the normalized relative location along cell boundary (in absence of units). The map intensity represents the measured PWV (in nm) which corresponds to cell mass (blue color – less mass, red color – more mass). Similarly, the spatiotemporal cell boundary-evolution map (map II) is shown in Fig. 2(d), while the map intensity represents the cumulative displacement measured as the distance (along the boundary evolution path) from the position at the initial time frame. The blue color corresponds to short migration distances while the red color represents long migration distances. From the boundary evolution map, we can see that the mHAT cell attaches and spreads during first ~70 minutes, so the boundary movement distance is relatively short (lower than 5 μm) as indicated by the blue color in the evolution map. Then, the mHAT cell starts to migrate from left to right after ~70 minutes, so the boundary movement distance starts to directionally increase and reaches the longest migration distance (around 14 μm) after 140 minutes. An interesting correspondence between the two maps (Fig. 2(c) and (d)) is observed: this stem cell mass seems to be accumulated on the two sides of cell body as two anchoring points (points C and D) before migrating forward (towards to point A).

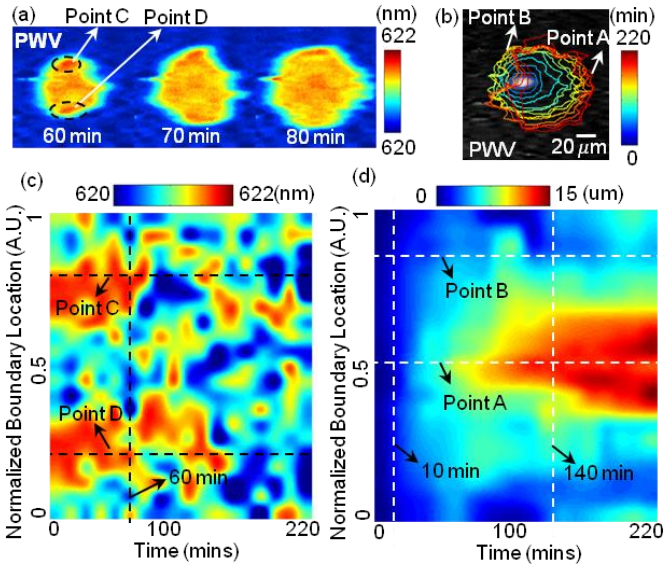

Fig. 2 PCEM-based Image-analysis Software to Analyze Stem Cell Adhesion and Migration. (a) A single stem cell progressively adheres on the PC biosensor surface. (b) Stem cell boundary-evolution extracted from PWV images along temporal dimension (color bar demonstrates the time for cell attaching and spreading). (c) Two-dimensional map represents the spatiotemporal change of the redistribution of cellular material along cell boundary. (d) Two-dimensional map represents the spatiotemporal change of cell morphological behavior along cell boundary.

Fig. 3 demonstrates two representative cross-section curves along the temporal (Fig. 3(a)) and the spatial (Fig. 3(b)) direction of map II. In Fig. 3(a), the blue curve represents point A and red curve represents point B at the cell boundary in Fig. 2(b). The two curves begin with similar migration distance for the first 50 minutes during attachment, after which the red curve accelerates and reaches a plateau (around 14 μm) after 140 minutes. Our work demonstrates that PCEM can be used to track the evolution of cell attachment characteristics that include attachment strength, periphery, lateral translation, and shape.

4. Conclusions

In conclusion, we report the label-free detection and image-analysis of live cell attachment and movement by PCEM. Future work will focus on uncovering the underlying mechanism of cellular material redistribution during cell adhesion and migration, and extension of the software for simultaneous analysis of multiple cells within the imaging field of view.

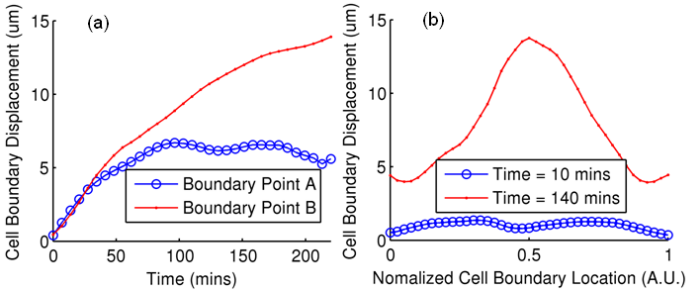

Fig. 3 Quantitative Measurement of Stem Cell Movement Using PCEM Image-analysis Software. (a) Stem cell boundary displacement at two different boundary locations along time dimension. (b) Stem cell boundary displacement at two different time points along normalized cell boundary

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