Surface Attachment Profiling of Stem Cell Differentiation Using Photonic Crystal Enhanced Microscopy (PCEM)

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The ability for stem cells to differentiate into special types of cells can maintain normal multi-cellular-organism development, and thus is critical for tissue repair or embryonic development. Due to modified gene-expression, many characteristics of a stem cell body can undergo significant changes during differentiation, including morphology, metabolic activity and responsiveness to certain environment signals that are reflected in features, such as shape, strength, and spatiotemporal distribution of their surface attachment profile. These features can be used to distinguish and characterize the differentiation stage. Cell-extracellular matrix (ECM) interaction is one critical factor for the regulation and mediation of various cellular activities including differentiation. However, the underlying interacting mechanisms for intra-cellular regulation during differentiation remain unclear due to the lack of tools that enable long-term label-free quantitative observation of cell-surface interactions. To address these challenges, we designed a new form of microscopy, termed Photonic Crystal Enhanced Microscopy (PCEM) that utilizes a photonic crystal (PC) optical biosensor surface to image live cell attachment during differentiation. In PCEM, the reflected color of the PC surface is locally tuned by the attachment of cell components within a ~200 nm deep evanescent electric field. The PC structure prevents lateral light propagation, enabling the PCEM system to gather high resolution images (0.5 micron pixels) of cell attachment in near-real-time (10 seconds per image) for extended time periods, using low intensity broadband illumination.

We performed an osteogenic-differentiation assay for adipose stem cells (ASCs) on a PC biosensor surface and measured the peak resonance wavelength variation using the PCEM system. The ASCs were treated with osteogenic induction medium and seeded on the PC biosensor surface to dynamically visualize their spatiotemporal attachment performance. From the sequence of peak wavelength value (PWV) images measured from PCEM, we could see that the attachment profile of ASC was more elongated with a thin spindle shape in the beginning, then gradually became more rectangular, finally demonstrated a rectangular morphology with lamellipodia or filopodia (similar to an osteoblast) at the end of the differentiation.

We also studied the differentiation of 32D stem cell, an interleuin-3 (IL-3) dependent murine myeloid progenitor cell line, into neutrophilic granulocytes. The 32D cells demonstrated strong adhesion in the beginning, then gradually reduced to weak adhesion, and finally returned to a strong adhesion profile at the end of the differentiation. These label-free imaging results illustrate that the PCEM can successfully measure the spatiotemporal variation of cell attachment during differentiation.