EVENT ABSTRACT

Tracing of individual hematopoietic stem cell specification events usir Raman Spectroscopy and photonic crystal enhanced microscopy

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Introduction: Hematopoietic stem cells (HSC) are rare adult stem cells residing in the bone marrow that are responsible for life-lo hematopoiesis. To enhance our understanding of the underlying mechanisms of HSC regulation and facilitate the clinical use of HS desirable to engineer their specific fate decision events (self-renewal vs. differentiation) in vitro. Such efforts are limited by the lac markers that enable non-invasive, dynamic analysis of individual HSCs in situ. Raman Spectroscopy is a promising chemical imagi provides unique molecular fingerprints of individual, live cells in situ in a label-free manner. Similarly, photonic crystal enhanced n (PCEM) is a label-free imaging platform that enables dynamic quantification of single HSC adhesion profiles. Here, we demonstrat of applying Raman Spectroscopy and PCEM for screening HSC phenotype via the identification of primary hematopoietic cell pop segmentation of primitive hematopoietic progenitor cell populations during their differentiation to granulocytes.
Materials and Methods: We isolated primary hematopoietic cell populations (long-term HSCs: LT-HSC, short-term HSCs: ST-HS granulocytes) from C57BL6 mouse bone marrow and analyzed individual cells with Horiba Raman confocal imaging microscope. analyzed individual 32D cells (a myeloid progenitor cell line) during differentiation towards granulocytes. All cells were seeded on substrates decorated with or without a protein-immobilized hydrogel layer. For PCEM, cells were seeded on fibronectin-coated pho and wavelength shifts in the transmitted light from accumulation of cellular materials on cell-surface interface were quantified to vi adhesion profiles.

Figure 1

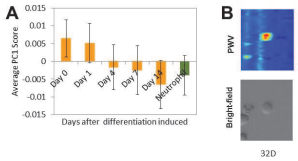

Figure 1.
A. PC1 scores generated from principal component analysis of Raman peaks collected from individual hematopoietic progenitor cells (32D cell line) during differentiation to granulocytic neutrophils successfully segmented them to the extent their specification. Positive scores indicate undifferentiated (Day 0) cells while negative scores indicate specification towards differentiation.
B. A representative adhesive profile of a 32D cell quantified from wavelength shifts in transmitted light (PWV) compared to its brightfield image.

Results and Discussion: Multivariate analysis of Raman peaks from individual hematopoietic cells revealed that Raman spectra-ge molecular signatures could be used for their identification with less than 4% false identification rates. Notably, subsets of primitive (LT-HSCs, ST-HSCs) whose nuanced functional differences are difficult to segment could be easily distinguished from each other a mature downstream cells (B cells, granulocytes). Additionally, cells seeded on soft vs. stiff hydrogels could be analyzed in a simila indicating that Raman Spectroscopy is a promising approach for in situ screening of HSCs. Moreover, principle component analysis peaks from individual hematopoietic progenitor cells (32D cell line) was able to segment discrete stages of granulocyte specificatio Ongoing work with PCEM suggest that adhesive phenotype (adhesion strength, migration speed, and total displacement) of individ during differentiation may provide unique adhesive signatures of individual cells indicative of their specification state (Fig.1B).
Conclusions: Our results show that Raman imaging and PCEM can generate unique molecular and adhesive fingerprints of individ

could be used as novel markers reflecting their functional phenotype. We therefore envision these methods may provide a means to screen HSC fate specification events in situ in real time for future bioengineering and biomanufacturing applications.

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