RAMAN SPECTROSCOPIC ANALYSIS OF CELL DIFFERENTIATION AND DEATH MODES

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Raman spectroscopy provides opportunities for non-invasive, non-destructive, label-free analysis of cell states based on changes in the biochemical composition of cells. We are investigating the suitability of Raman spectroscopy to assess the stages of human embryonic stem cell (hESC) differentiation towards pancreatic insulin-positive cells. Raman microspectrometry analysis has revealed macromolecular composition differences over time that distinguished cell populations differentiating to pancreatic cell types, such as by an increase in the protein-to-nucleic acid signal ratio and to distinguish the presence of insulin. Added insight into these macromolecular changes were provided by principal component analysis (PCA) of the data. However, the application of PCA can be difficult to interpret. The usefulness of non-negative matrix factorization was explored to improve the interpretability of overlapping Raman bands. We demonstrated the utility of this procedure by analyzing spectra to determine the cellular insulin or glucagon content. Thus, Raman spectroscopy can detect such differences in cells to detect the desired product as well as the potential to detect residual hESCs or the emergence of unwanted cells.

We also investigated the suitability of Raman spectroscopy to detect the onset and types of cell death. Apoptotic, necrotic or autophagic Chinese Hamster Ovary cells were compared to uninduced cultures using Raman spectroscopy and PCA. Furthermore, uninduced cells were compared to cells sorted at different stages of apoptosis to determine how early the onset of apoptosis could be detected. Changes were observed in several peaks during the course of cell death, with repeated changes observed in nucleic acid- and lipid-associated peaks, enabling the distinction of cell death modes. Application of such death monitoring capabilities to cellular therapy cultures should be even more useful, given the need for more process analytical technologies to address the often more variable performance of these cultures, especially when adaptive control is needed for primary cell derived manufacturing.