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Non-invasive, label-free spectroscopic separation of human embryonic stem cells (hESCs) and their cardiac derivatives

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Background: Self-renewable, pluripotent human embryonic stem cells (hESCs) can provide an unlimited source of cardiomyocytes (CMs) for transplantation therapies. However, the presence of undifferentiated hESCs in a graft may lead to tumors. Unlike certain lineages such as hematopoietic cells, CMs lack specific surface markers for convenient physical separation or enrichment. Immunostaining of cardiac-specific proteins such as troponin requires permeabilization, which renders the cells unviable and non-recoverable. Ectopic expression of a reporter protein under the transcriptional control of a heart-restricted promoter for identifying hESC-derived CMs (hESC-CMs) is useful for research but complicates potential clinical applications. Methods and Results: Micro-Raman spectroscopy is a laser-based, label-free, and noninvasive method that measures the inelastic scattering of incident photons by intrinsic molecular bonds. Since DNAs, RNAs, proteins, lipids and carbohydrates exhibit multiple unique spectral markers, here we hypothesize that Raman spectra can function as "optical fingerprints" for separating or enriching hESCs and their cardiac derivatives. By applying a combination of spectroscopic and multivariate statistical methods, our results indicate that hESCs, human fetal left ventricular CMs and hESC-CMs can be identified with an accuracy of 96%, 98% and 66%, respectively. Conclusions: The present study lays the groundwork for developing a systematic, high-throughput, and automated method for noninvasively sorting i) high-quality hESCs for expansion, and ii) *ex vivo* CMs (derived from embryonic or adult stem cells) for cell-based heart therapies.

***Keywords*:** human embryonic stem cells, cardiomyocytes, micro-Raman spectroscopy, non-invasive
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