

In Situ Purification of Human ES/iPS Cell Colonies and their Differentiated Progeny via Laser-Based Processing

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Purification of human embryonic and induced pluripotent stem cells (ESC and iPSC) is very challenging with conventional flow cytometry sorting due to their inherent sensitivity to manipulation and single cell dissociation, but is required to derive genetically modified and new stem cell lines. Similarly, purification of homogeneous populations of differentiated progeny from ESCs/iPSC is critical for use of stem cells in drug discovery, toxicity studies, and therapeutic applications, but these complex adherent cell cultures do not lend themselves to conventional cell purification methods. A few studies have reported successful sorting of ESC (some with the use of ROCK inhibitor), but with very low recovery. In addition, different ESC/iPSC lines vary in their response to single cell dissociation and ROCK inhibition, making the establishment of standardized protocols for sorting these cells difficult. A novel in situ approach for high-yield purification of intact ESCs/iPSC colonies and their differentiated progeny has been developed using the LEAP Cell Processing Workstation. Brightfield and fluorescent imaging was combined with laser-mediated processing to provide rapid identification and purification of colonies directly in well plates. Results showed that different ESC/iPSC lines stained with typical stem cell markers (SSEA3, SSEA4, TRA1-81, TRA1-60 and SSEA1), under both feeder-dependent and feeder-independent growth conditions, were isolated with high purity and viability. Expression of these markers was also used to eliminate unwanted differentiated areas from stem cell cultures. Laser-mediated purification was used to derive stable genetically modified ESC lines and create multiple human iPSC lines. After laser-mediated purification, ESC and iPSC expanded normally, expressed markers for pluripotency, differentiated into all three germ layers, and were genetically stable. In addition, LEAP was used to successfully isolate human ESC-derived cardiomyocytes with high yield and purity. These results demonstrate the effectiveness of in situ laser-mediated processing for purification of ESC/iPSC colonies and their differentiated progeny, based on cell surface marker expression and/or cell morphology, without the need for single cell dissociation or detachment from the culture substrate.