Methods for Purifying Stem Cell Derived Cardiomyocytes (SC-CMs)

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According to the American Heart Association(*1*), heart failure affects 5.7 million Americans with 670,000 newly diagnosed patients each year. Cardiomyocytes (CMs) are normally non-regenerative and their permanent loss due to aging or disease compromises cardiac functions. Heart transplantation is typically the last resort for end-stage heart failure patients. However, this option is hampered by a severe shortage of donor organs. Only 2,163 patients received a heart transplant in the United States in 2008 and 2,791 patients were on the waiting list as of 2009(*1*). Stem cells (SCs), either human embryonic or induced pluripotent, have the potential to act as an unlimited *ex vivo* source of CMs for cell-based therapies. Studies have achieved differentiation of SCs into CMs with efficiencies as high as 60%(*2*), but achieving higher purity SC-CM populations remains a challenge. It is important to purify SC-CM populations prior to their transplantation into patients to remove undesired, non-cardiac differentiated cells and undifferentiated tumorigenic(*3*) SCs. Furthermore, a study(*4*) showing the effectiveness of cardiovascular progenitors over vascular progenitors in improving heart function after myocardial infarction demonstrate the necessity of controlling the purity of cell populations in order to transplant the appropriate cell types for maximizing the benefit of cardiovascular cell therapy. The ability to obtain pure SC-CM populations would also significantly benefit fundamental biological and tissue engineering research, as well as CM drug discovery/testing.

Unfortunately, there is currently no practical method for purifying live SC-CM populations, mainly because CMs lack specific surface markers for convenient physical separation or enrichment. CMs can be identified by immunostaining for intracellular cardiac-specific proteins such as tropomyosin or α-actinin, but this requires cell fixation and permeabilization, which render the cells non-viable and non-recoverable. To date, there have been only a handful of approaches for selecting viable CMs from differentiating SCs, but each of these suffer from drawbacks and limitations(*5*). Ectopic expression of a fluorescence reporter protein under the transcriptional control of a CM-specific promoter achieved by lentiviral transduction has been used to identify SC-CM populations with >90% purity(*6*), but this method is not broadly applicable to many stem cell lines(*5*). Moreover, genetic modification of the cells complicates the clinical application of this method due to potential safety concerns of transplanting these cells into patients. CMs can be selected based on physical size separation on a Percoll gradient(*7, 8*), but purity is limited with this approach. A third method selects cardiac progenitors using a cell surface protein (kinase-insert domain-containing receptor; KDR)(*2*), but because these progenitors can also differentiate into smooth muscle cells and vascular endothelial cells, the purity of CMs is low.

Recently, a technique(*9*) demonstrated to be able to detect and sort for live SC-CMs uses a mitochondrial dye to detect the increased mitochondrial content of SC-CMs compared to undifferentiated SCs. The method was able to obtain SC-CM cell populations with a purity that did not result in tumor formation when transplanted into mice. *Other new methods such as this are urgently needed for accurately sorting pure populations of live SC-CMs*.

References

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