Isolation of Cardiac Stem Cells and their Differentiation into Cardiomyocytes.

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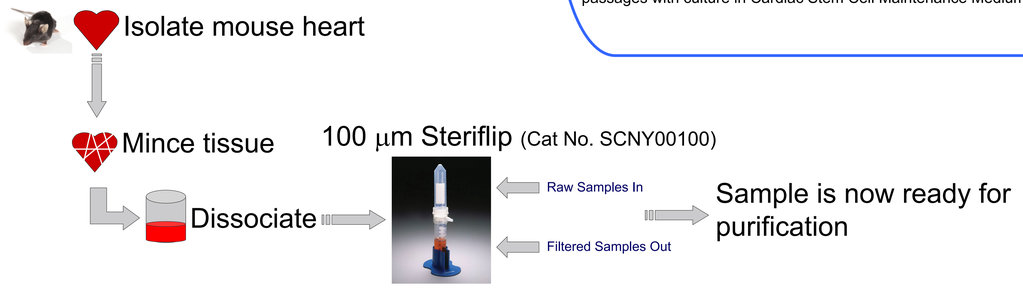
Abstract/Introduction

The striated muscle cells of the heart represent the functional contractile unit permitting blood flow throughout the circulatory system. Adult murine cardiomyocytes provide an excellent model system for the study of cardiovascular diseases. Methods to isolate cardiomyocytes have traditionally relied upon mechanical mincing and enzymatic treatment of ventricular tissues followed by the use of cell strainers, which risks introducing contamination to the sample. These methods often yield mixed populations of cells. Cell heterogeneity makes analyses of discrete cell populations difficult. Here we report a novel method for the isolation and purification of cardiomyocytes from endogenous cardiac stem cells (CSCs). Ventricular heart tissues were isolated using sterile tissue dissection techniques from C57/BL6 mice and transferred to CSC Isolation Buffer. These tissues were subsequently minced and transferred to a Cardiac Tissue Dissociation Buffer. Following incubation and tituration, the cell suspension was filtered through a Steriflip unit, a self-contained, vacuum-assisted apparatus with a modified 100 μm nylon mesh membrane. The cell mixture was further purified using gradient centrifugation. Freshly isolated CSCs can either be further expanded for up to 2 weeks in culture in CSC Maintenance Medium or differentiated into cardiomyocytes by culture in Cadiomyocyte Differentiation Medium. Characterization of the differentiated cardiomyocytes was accomplished using a panel of antibodies which include, tropomyosin, desmin, actinin, troponin I. Collectively our results demonstrate a reliable and reproducible method for the isolation and culture of ventricular cardiomyocytes and endothelial cells and should facilitate the use of these purified cells for a wide array of biological applications.



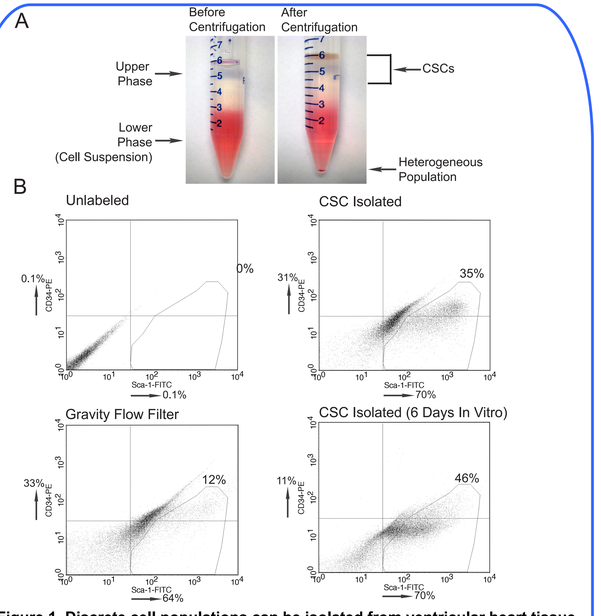


Figure 1. Discrete cell populations can be isolated from ventricular heart tissue through differential gradient centrifugation.
(A) Representative photos depicting heterogeneous cell populations present in the lower phase before centrifugation and a pure CSC population present in the upper phase after centrifugation.
(B) Purity of freshly isolated CSCs as determined by flow cytometry analysis for the cardiac stem cell marker Sca-1 and low for the hemopoetic stem cell marker CD34 (Sca-1+, CD34low). Cells purified using CSC isolation kit (SCR061) contain almost three times the percentage of CSCs. The population can be further expanded for 10 passages with culture in Cardiac Stem Cell Maintenance Medium (Cat No. SCR101).

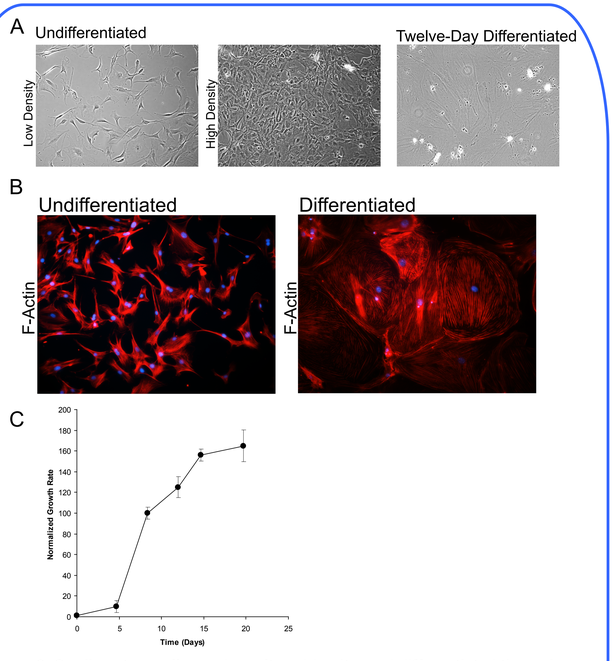


Figure 2. Cardiac stem cells can be cultured *In vitro* and differentiated through selected use of growth or differentiation media.
(A) Representative photomicrographs of low and high density cultures of purified CSCs and 12-day differentiated cardiomyocytes.
(B) Fluorescent microscopy images of cultured CSCs and differentiated cardiomyocytes stained for F-Actin. Note the presence of striated myofibrils present in the differentiated cells.
(C) Normalized growth curve demonstrating the proliferative ability of CSCs in culture.

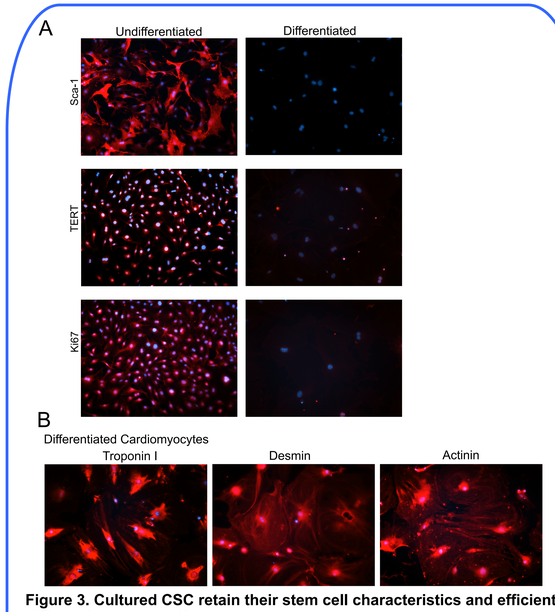


Figure 3. Cultured CSC retain their stem cell characteristics and efficiently differentiate into cardiomyocytes.
(A) One-week cultures of purified CSCs ubiquitously express stem cell markers Sca-1 and telomerase, while remaining in a proliferative state as determined by Ki67 immunoreactivity.
(B) Differentiated CSCs express mature markers for cardiomyocytes.

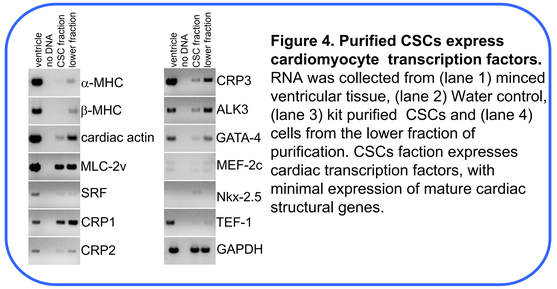


Figure 4. Purified CSCs express cardiomyocyte transcription factors. RNA was collected from (lane 1) minced ventricular tissue, (lane 2) Water control, (lane 3) kit purified CSCs and (lane 4) cells from the lower fraction of purification. CSCs faction expresses cardiac transcription factors, with minimal expression of mature cardiac structural genes.

Conclusion

Efficient high throughput isolation of cardiac stem cells has remained elusive.This obstacle has slowed progress on the development of therapeutic applications of endogenous CSCs. We show here how Millipore's CSC Isolation Kit (Cat No. SCR061) enables researchers to obtain significantly greater numbers of cells for use in their experimental applications. Our novel isolation and purification approach, coupled with Cardiac Stem Cell Maitenance Medium (Cat No. SCR101), permits pure CSCs to be isolated from ventricular tissue and expanded as needed. Using our Cardiomyotcyte Differentiation Medium (Cat No. SCR102), cardiomyocytes can be efficiently generated. The dynamic capabilities of this kit enable advancement of cell and molecular biology related to both cardiac stem cell and cardiomyocytes.

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