Isolation of Cardiac Stem Cells and their Differentiation into Cardiomyocytes

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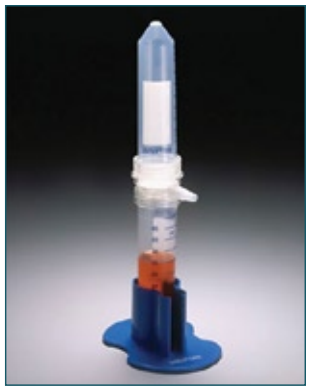
Abstract



Adult murine cardiomyocytes provide an excellent model system for the study of cardiovascular diseases, because the heart's striated muscle cells represent the functional contractile unit of the circulatory system. Methods to isolate cardiomyocytes have traditionally relied upon mechanical mincing and enzymatic treatment of ventricular tissues, followed by the use of cell strainers, but this risks introducing contamination to the sample. These methods often yield mixed populations of cells making analyses of discrete populations difficult. Here we report an improved method for the isolation and purification of cardiomyocytes from endogenous cardiac stem cells (CSCs). This technology provides a reliable and reproducible method for the isolation and culture of adult CSCs, and should be suitable for a wide array of biological applications.

Introduction

The isolation and expansion of cardiac stem cells opens new opportunities to the field of cardiac regenerative medicine. CSCs have recently been isolated from human and murine tissues based upon cell biomarkers (Sca-1, TERT, c-Kit, side population), demonstrating the presence of a non-circulating stem cell niche within the myocardium that is estimated to account for 11-14% of the total cell population[1]. These cells appear to be bi-potent in their capacity to form cardiomyocytes and vascular endothelial cells. Moreover, preliminary engraftment studies suggest that these cells are ideal candidates for future research on cardiac regeneration. However, the rarity of CSCs coupled with the complex isolation procedure is a significant challenge for the advancement of the field. To overcome this obstacle, we have developed an easy-to-use cell isolation kit capable of obtaining a high yield, pure population of CSCs. This advancement allows investigators to obtain a significantly greater number of CSCs for their studies without the need for time consuming, complex protocols and expensive cell sorting equipment.

Results

Isolation and purification of murine CSCs using the Millipore CSCs Isolation Kit (Cat. No. SCR061) begins with surgical resection of the heart from the thoracic cavity. Heart tissue is minced and pooled from five separate mice per collection and a cell suspension is created through gentle agitation in our dissociation buffer. A novel application of our isolation kit is the use of the self-contained Steriflip® filtration device, which allows for the separation of larger volumes of cellular material with the added advantage of maintaining a sterile environment

ensuring sample sterility, an option that open top gravity flow filter devices can not guarantee.

The study used a 100 µm nylon mesh vacuum driven Steriflip device that allows the passage of cells while retaining large tissue clumps. This improves recovery and lessens time to collect the isolated cells.

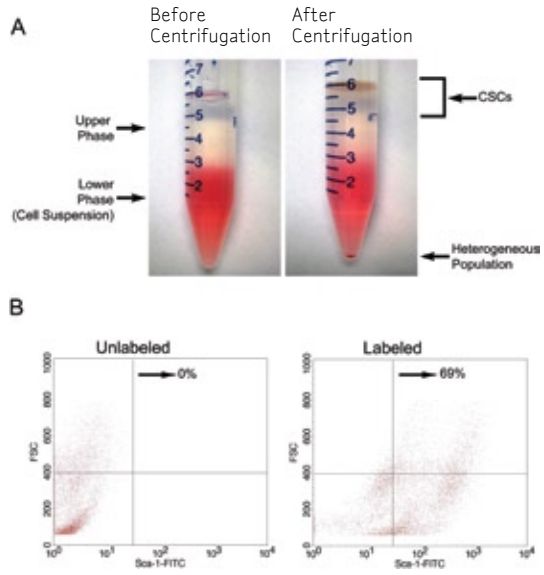


Figure 1. Discrete cell populations can be isolated from ventricular heart tissue through differential gradient centrifugation. (A) Representative photos depicting heterogeneous cell populations present in the lower phase before centrifugation and a pure CSC population present in the upper phase after centrifugation. (B) Purity of differential gradient isolated CSCs as determined by flow cytometry analysis for the stem cell marker Sca-1.

A major advantage that our system provides is the rapid purification of Sca-1 positive CSCs without the need for expensive cell sorting equipment. This is accomplished through the use of differential gradient centrifugation (Figure 1A). To validate the purification method, we harvested five C57/BL6 mouse hearts per triplicate isolation and performed the collections as described above. Purified samples were subsequently labeled with Sca-1-FITC conjugated antibodies and cells were analyzed using a FACSCalibur flow cytometer. Isolations yielded an averaged 1.2 x 10^6 cells/mL with a Sca-1 purity greater than 33 % (Figure 1B). This method appears far superior to the traditional method of dissociation and filtration[2] that yield a heterogeneous population composed of only 11-14 % Sca-1 positive cells.

Purified CSCs can maintain a mesenchymal stem cell like phenotype (Figure 2A) during *in vitro* culture. Moreover, when regular growth media is replaced with differentiation media, CSCs undergo a phenotypic change indicative of mature cardiomyocytes (Figure 2A). Labeling cells for filamentous actin (F-Actin) highlights the transition from stem cell to cardiomyocyte, with long striated myofibrils present in the differentiated population (Figure 2B). Both isolation of adult cardiomyocytes and differentiation of embryonic stem cells into cardiomyocytes have been problematic due to the limited number of cells one can obtain. The Millipore CSC isolation kit provides the advantage of not only purifying greater number of cells, but also allowing expansion of the original population as needed. Isolated cells can be expanded in culture using defined growth media for periods of up to two weeks before quiescence (Figure 2C), providing the opportunity for additional experimental analysis.

Isolated cells were cultured for 1 week and characterized. Immunocytochemical staining demonstrated a ubiquitous Sca-1 labeling, as previously shown through FACS analysis. Moreover, these cells concurrently stained for the stem cell marker, telomerase, suggesting the retention of their stem cell characteristics (Figure 3A). Furthermore, CSCs maintained in culture continued to proliferate and stained positive for Ki67 (Figure 3A).

To establish the differentiative capacity of isolated CSCs, we plated cells on poly-L-ornithine coated slides and maintained them in differentiation media for a period of 12 days. Samples were then fixed and stained with markers for mature cardiomyocytes. Cells showed strong immunoreactivity to cardiomyocyte markers (Cat. No. SCR059), Troponin I, Desmin, and Actinin (Figure 3B). Collectively, these results demonstrate an efficient isolation of CSCs that are capable of expansion and differentiation when treated with the appropriate media *in vitro*.

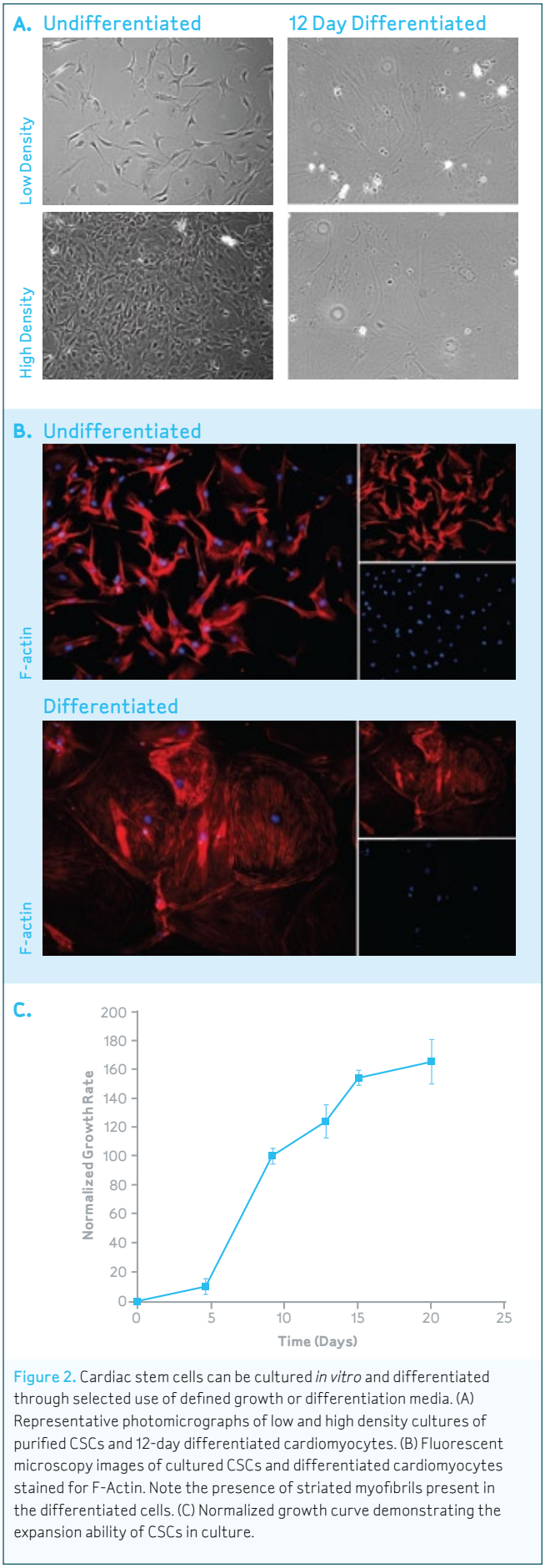


Figure 2. Cardiac stem cells can be cultured *in vitro* and differentiated through selected use of defined growth or differentiation media. (A) Representative photomicrographs of low and high density cultures of purified CSCs and 12-day differentiated cardiomyocytes. (B) Fluorescent microscopy images 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 expansion ability of CSCs in culture.

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 enables researchers to obtain significantly greater numbers of cells for use in their experimental applications. Our novel isolation and purification approach, coupled with defined growth media, permits pure CSCs to be isolated from ventricular tissue and expanded as needed. Using our defined differentiation media, 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.

References

1. Oh, H., Bradfute, *et al.* (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci.* **100**:12313-12318.
2. Barile, *et al.* (2007) Endogenous cardiac stem cells. *Prog. Cardiovasc. Dis.* **50**:31-48.

Millipore Products

Description	Cat. No.
Cardiac Stem Cell Isolation Kit, 5 isolations	SCR061
Cardiomyocyte Characterization Kit, 1 kit	SCR059
Steriflip 100 µm Nylon Net, 25/pk	SCNY00100
Steriflip 60 µm Nylon Net, 25/pk	SCNY00060
Steriflip 40 µm Nylon Net, 25/pk	SCNY00040

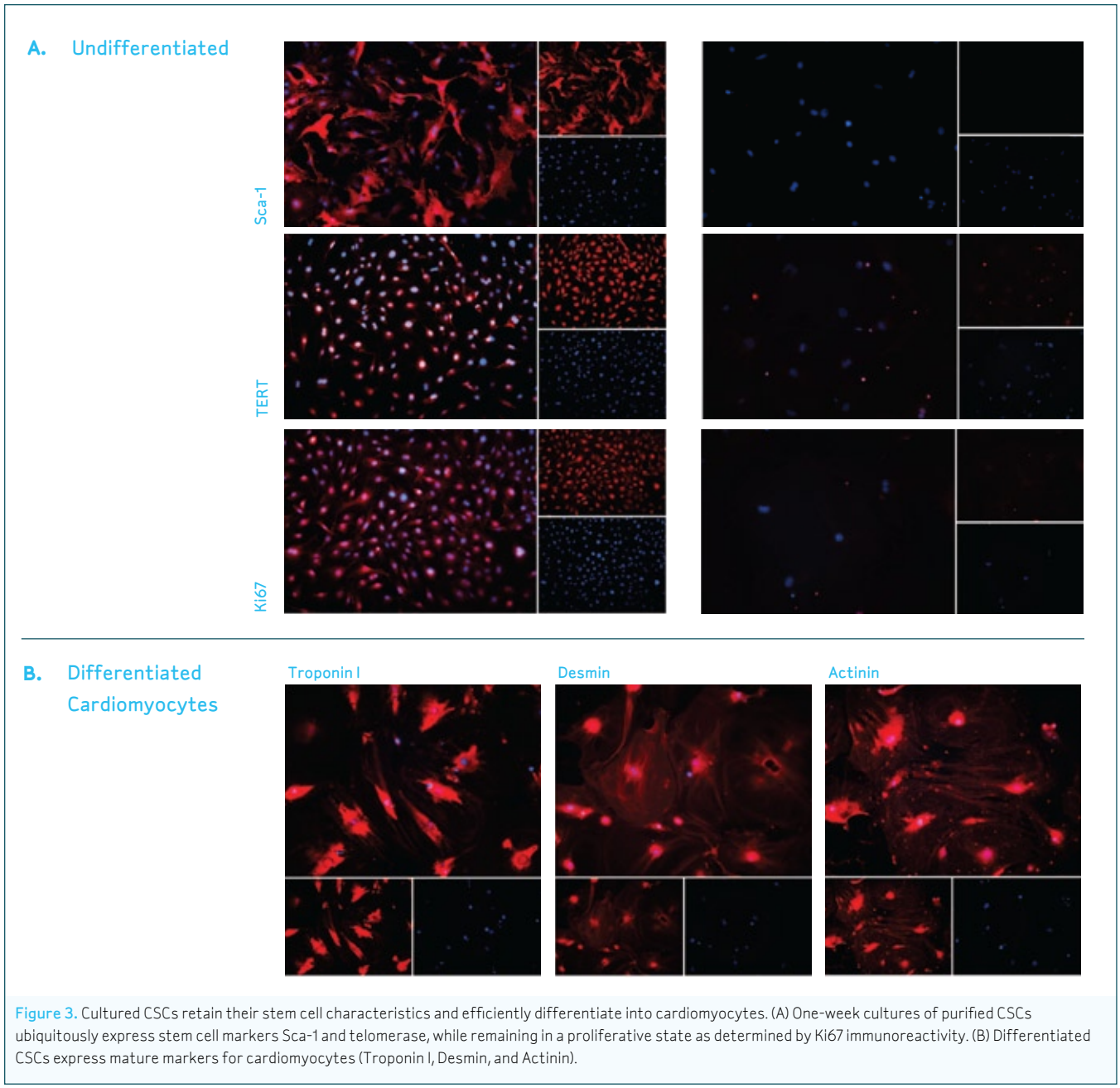


Figure 3. Cultured CSCs retain their stem cell characteristics and efficiently differentiate into cardiomyocytes. (A) One-week cultures of purified CSCs ubiquitously express stem cell markers Sca-1 and telomerase, while remaining in a proliferative state as determined by Ki67 immunoreactivity. (B) Differentiated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

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