Rapid heart dissociation and subsequent purification of heart cells for cardiac tissue engineering

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Introduction

Engineered cardiac tissue offers the potential to replace infarcted myocardium and to investigate heart development and cardiomyogenesis in a simplified *in vitro* model. It is generally accepted that tri-culture of cardiomyocytes, fibroblasts, and endothelial cells enhances functionality of engineered cardiac tissues *in vitro* and improves survival *in vivo*. However, one of the major challenges is the availability of pure heart cells to generate *in vivo*–like cardiac tissues in a dish. Utilizing a semi-automated tissue dissociator (gentleMACS Dissociator; Miltenyi Biotec) we developed a rapid protocol for the dissociation of neonatal mouse or rat hearts, enabling the efficient immunomagnetic enrichment of cardiomyocytes, cardiac fibroblasts and endothelial cells.

Results

1 Rapid, standardized dissociation of neonatal hearts utilizing the Neonatal Heart Dissociation Kit and the gentleMACS Dissociator

Manual dissociation of neonatal hearts is a laborious procedure, which is difficult to standardize. In order to increase the reproducibility of enzymatic tissue dissociation and to simplify sample processing, we developed the Neonatal Heart Dissociation Kit. The protocol involves a semi-automated instrument for mechanical tissue dissociation, the gentleMACS Dissociator (fig. 1A). Neonatal hearts from mice and rats (P0–P3) were dissociated within one hour using the Neonatal Heart Dissociation Kit, resulting in high yields of single cells with excellent viability (fig. 1B). Flow cytometric analysis showed a high percentage of α-actinin-positive cardiomyocytes as well as vimentin-positive non-myocytes.

A

B

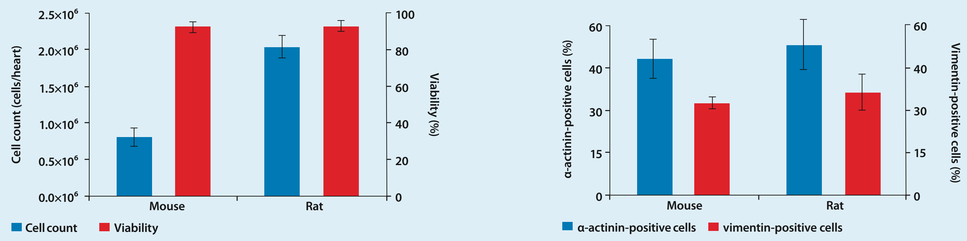
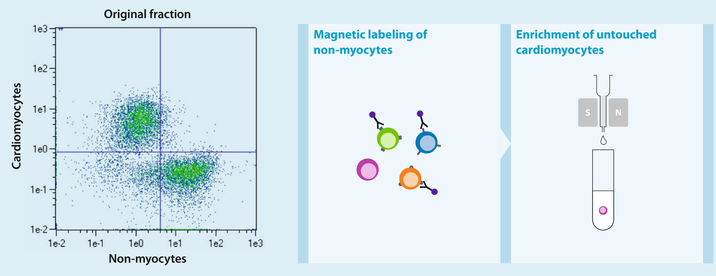


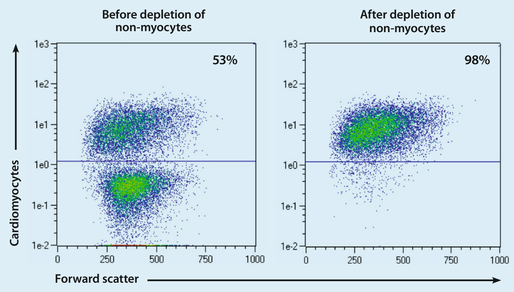
Figure 1

2 Depletion of cardiac non-myocytes results in a highly enriched cardiomyocyte population

A



B



C

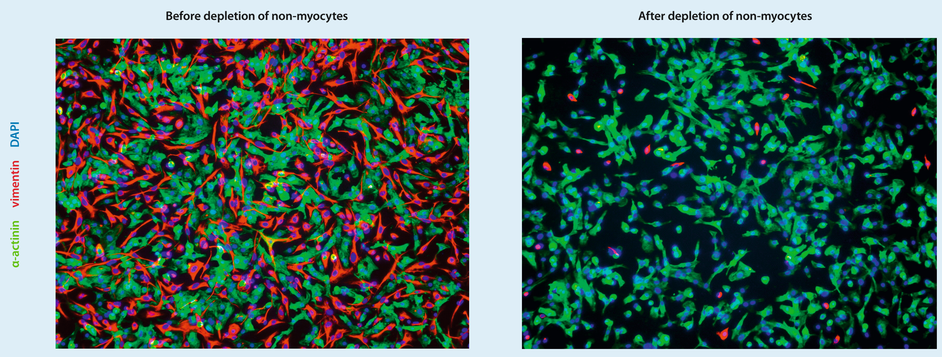


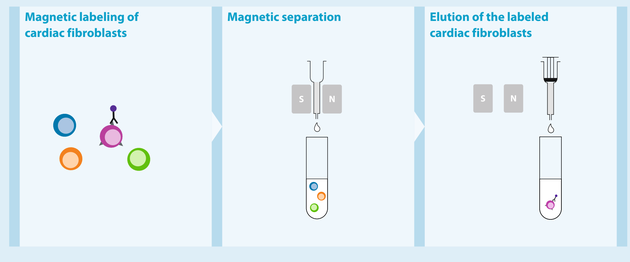
Figure 2

In order to develop a procedure for the selective enrichment of cardiomyocytes from dissociated neonatal mouse hearts, we screened for specific cell surface markers. Several candidate markers were identified, which were exclusively expressed by non-myocytes. Based on these markers, we defined an antibody cocktail and a strategy for the magnetic depletion of non-myocytes by MACS Technology, enabling the enrichment of cardiomyocytes (fig. 2A). Utilizing our depletion strategy we efficiently isolated cardiomyocytes with purities of up to 98% (fig. 2B). Cultures of enriched cardiomyocytes showed synchronous beating activity and the expression of sarcomeric proteins (α-actinin) in a cross-striated pattern. Vimentin-positive non-myocytes were just barely detectable (fig. 2C).

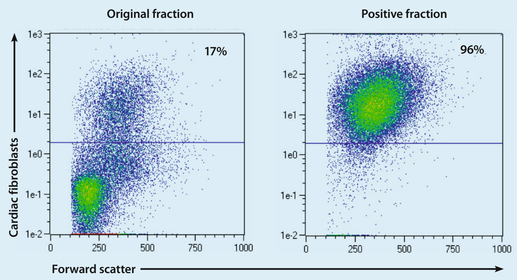
3 Enrichment of cardiac fibroblasts from dissociated neonatal hearts

Cardiac fibroblasts contribute to structural, biochemical, mechanical, and electrical properties of the myocardium. In order to isolate cardiac fibroblasts from dissociated neonatal hearts, we developed an enrichment strategy based on MACS Technology (fig. 3A). Enrichment resulted in cardiac fibroblast populations with purities of up to 96% (fig. 3B). Immunofluorescent staining of enriched and subsequently plated cardiac fibroblasts showed homogenous expression of vimentin with virtually no contaminations of α-actinin-positive cardiomyocytes (fig. 3C).

A



B



C

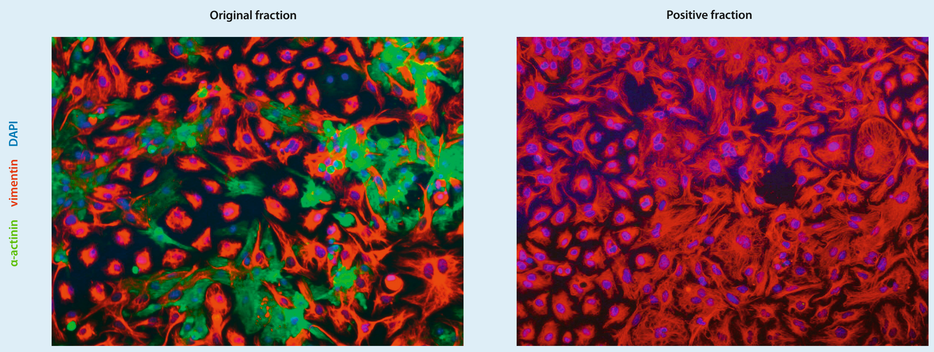


Figure 3

4 Enrichment of endothelial cells from dissociated neonatal hearts

Tissue-engineered myocardium with endothelial networks that can be easily connected to host vessels may contribute to the development of tissue grafts with a high potential for functional cardiac repair. Moreover, neovascularization might contribute to improved cardiac function after transplantation of engineered cardiac tissues. Highly enriched endothelial cell populations are a prerequisite to facilitate vascularization in tissue-engineered myocardium. Therefore, we established a protocol for the immunomagnetic isolation of endothelial cells from dissociated neonatal hearts to obtain purities of up to 97% (fig. 4).

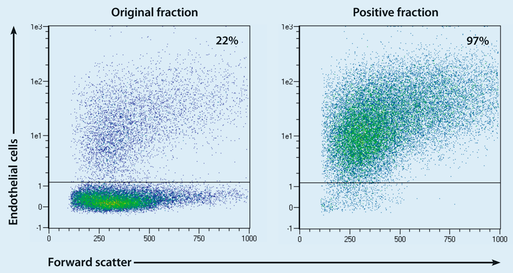


Figure 4

Conclusion

- Neonatal Heart Dissociation Kit and gentleMACS Dissociator enable the standardized generation of single-cell suspensions from neonatal hearts within one hour.
- Depletion of cardiac non-myocytes with a cocktail of specific antibodies yielded neonatal cardiomyocyte populations with high purities.
- Cardiac fibroblasts and endothelial cells were efficiently enriched and contaminating cardiomyocytes eliminated by MACS Technology.
- Taken together, we established a semi-automated procedure for the dissociation of neonatal hearts, enabling the subsequent immunomagnetic enrichment of cardiomyocytes, cardiac fibroblasts, and endothelial cells, which can readily be used to generate *in vitro* heart muscle models and surrogate tissue for myocardial repair.