Keeping the beat. Focus on “Enrichment of neonatal rat cardiomyocytes in primary culture facilitates long-term maintenance of contractility in vitro”

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Despite 20–30 years of extensive research into the cellular and molecular mechanisms underlying the development of cardiovascular disease (CVD), CVD remains the leading cause of death (25% all-cause mortality) in men and women. Nevertheless, advances in CVD therapeutics, coupled with improved specialty CVD care, have resulted in more CVD patients progressing to congestive heart failure (CHF). In fact, CHF has reached an incidence that ranges from 0.1% to 0.5% (400,000 new CHF patients per year) and up to 10% of patients 80 years of age or older in developed countries (5). This latter point clearly illustrates that CVD is a progressive, continuously evolving condition whose pathological trajectory depends largely on the interaction of disease etiology, treatment strategies, and both environmental and genetic factors. Consequently, elucidation of how the underlying cellular and molecular mechanisms are changing concurrent with the clinical sequelae becomes tantamount to the identification and development of appropriate treatment regimens.

Success for this type of CVD research largely depends on the ability to establish longitudinal in vivo and in vitro paradigms for studying the cardiovascular tissues and cell types, in particular the cardiac cells. It can be argued that, up to this point, only animal models (large and small; surgical and transgenic) are the most suitable models for the type of longitudinal and translational research required for the study of CVD. Still, many investigators have successfully utilized myocardial cell populations isolated from whole hearts to study specific issues related to the molecular, cellular, and physiological mechanisms of cardiac biology that cannot be achieved using an animal model (9). Currently, primary cultures of neonatal rat cardiomyocytes (NRCMs) are the in vitro model of choice. However, the major limitation to the use of NRCMs is that the restricted time frame (usually 2–3 wk) before NRCMs lose their contractile and myocardial cell phenotype due to dedifferentiation and cytoskeletal remodeling.

Consistent with previous studies, a recent analysis identified that the adult murine and rat myocardium is composed of approximately 56% contractile myocytes, 27% fibroblasts, 7% endothelial cells, and 10% vascular smooth muscle cells (2). The proliferating non-myocardial cells are the primary cause of dedifferentiation of the population (1,8). Therefore, investigators have employed several techniques to minimize the cellular heterogeneity following enzymatic digestion of the intact heart, such as preplating to remove adherent non-myocardial cells and/or Percoll gradients to separate out lower-density, non-myocardial cells. Still, these enrichment strategies remain insufficient for maintaining long-term culture of NRCMs with a contractile phenotype.

Applying an alternative approach, Hattori et al. (4) were able to exploit the utility of a functional mitochondria-specific fluorescent dye, tetramethylrhodamine methyl ester perchlorate (TMRM) and fluorescence-activated cell sorting (FACS), to isolate high-purity cultures of mitochondria-rich embryonic stem cell-derived cardiomyocytes (4). The advantage of this approach is that the isolation is achieved without genetic manipulation and therefore has the potential to be applied to several species including rat and human.

In this issue of the American Journal of Physiology-Cell Physiology, Nguyen et al. (7) show that this procedure can be employed to generate a myocyte population from neonatal rat hearts that remains contractile for long periods. The authors demonstrate that highly TMRM-fluorescent cells express sarcomeric proteins including α-actinin, myosin heavy chain, sarcomeric actin, and troponin I, all markers of cardiomyocytes. This is in contrast to the low expression of vimentin, a marker of cardiac fibroblasts and myofibroblasts. More importantly, these cells maintain consistent and synchronous contractility up to at least 160 days. These NRCMs also show expected Ca\(^{2+}\) cycling and dose-dependent responses to both β-adrenergic (isoproterenol) and cholinergic (carbachol) agonists.

Two key findings from this work are that NRCMs isolated using this method of myocyte enrichment maintain 1) the cellular phenotype at the protein level, and 2) the contractile phenotype at the physiological level, well beyond the typical 2- to 3-wk time frame of previous in vitro studies with NRCMs. In addition, this latter finding indicates the maintenance of cell-to-cell communication mechanisms similar to an intact heart. The net result is an in vitro model that will allow more longitudinal studies on the molecular, cellular, and physiological remodeling that occurs under conditions of prolonged pathological stimulation. In this respect, high throughput screening of drug libraries for off-target applications, including the timing of interventional therapies throughout CVD disease progression, will benefit from access to a relatively homogeneous population of contractile myocytes. For example, treatment of NRCMs with phenylephrine induces a pathological hypertrophic response within 2–3 days but the long-term impact in NRCM cultures has not been studied. Using this new methodology, NRCMs can be treated for 4–5 mo and subjected to therapeutic interventions at various time points using high-content screening platforms in attempts to reverse the pathological phenotype (3) (Fig. 1).

There remain some notable limitations with the use of this in vitro system. Based on the current study, NRCMs isolated using this technique continue to undergo morphological alterations such as elongation, enlargement, and the development of multiple processes over the 160-day culture period. At the same...
Fig. 1. Cells dispersed from neonatal rat hearts are loaded with a mitochondrial membrane potential-sensitive fluorophore tetramethylrhodamine methyl ester perchlorate (TMRM). The cells are sorted based on the TMRM signal, and those with high fluorescence are selected for use. These contractile myocytes can then be plated onto multiwell culture dishes and maintained and treated for extensive periods before analysis for changes in phenotype relative to nontreated cells. It is envisioned that these cells also could be used for generation of contractile patches for repair of cardiac infarction. Importantly, the potential exists to utilize this approach for isolation of contractile cells dispersed from the hearts of many species.

time, non-myocyte populations continue to grow, as indicated by the increase in vimentin expression. In addition, interactions between myocyte and non-myocyte populations impact on both physiological and pathophysiological adaptations in the intact heart, which is lost in these enriched cardiomyocyte populations. Yet this points towards another potential for implementing the contractile population for use in tissue bioengineering. Recent studies have shown that cardiac fibroblasts can be grown on synthetic substrates and used as a patch to assist electrical conduction after myocardial infarction (6, 10). However, the absence of contractile force generation limits recovery of function. Access to a relatively homogeneous population of contractile myocytes for organized three-dimensional scaffolding may provide an approach to improve on this emerging technology.

In sum, a simple approach to access a population of contractile cardiac myocytes, as described by the study in this issue of AJP-Cell, holds great promise for advancing our understanding of myocyte alterations during chronic insult and for a wide range of potential technical applications.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
J.P.K. and R.M.L. conception and design of the research; J.P.K., S.M.B., and R.M.L. prepared the figures; J.P.K., S.M.B., and R.M.L. drafted the manuscript; J.P.K., S.M.B., and R.M.L. edited and revised the manuscript; J.P.K. and R.M.L. approved the final version of the manuscript.

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