The earliest reports for the successful dissociation and culture of rodent heart cells dates back to the 1960's 1,2. Even then, Harary and Farley noticed that cultured cardiomyocytes "may provide a unique system for the study of the requirements of the periodic contractility [, and may] provide a means of determining the contribution of various metabolic pathways for the [beating] process". Although Harary and Farley isolated and cultured cardiomyocytes from young rats, and the original protocol has been adapted and modified by many scientists over the years, the general isolation and culturing procedure has not greatly changed. However, better enzymes 3, standardized solutions 4,5, and the addition of the reversible channel and myosin ATPase inhibitor BDM to protect cells during the isolation procedure 6-9 has significantly improved cell-yield and viability.

Adult vs. neonatal cardiomyocytes

Cardiomyocytes isolated and cultured from neonatal mice or rats have several advantages over cultures of adult cardiomyocytes. Foremost, the isolation procedure for neonatal mouse or rat hearts is easier and less costly, when compared to the isolation of cardiomyocytes from adult mouse or rat 10. Neonatal cardiomyocytes are far less sensitive to reintroduction into a calcium-containing medium after dissociation, greatly increasing cell-yield. Another big advantage is that neonatal mouse cardiomyocytes undergo a more rapid dedifferentiation - redifferentiation cycle that typically results in spontaneously beating cells 20 hr after plating, while adult cardiomyocytes typically require pacing to induce contraction. Neonatal cardiomyocytes are also more readily transfectable with liposomal transfection methods, whereas adult cardiomyocytes require viral vectors for successful delivery of transgenic DNA. In contrast to neonatal cardiomyocytes, culture of adult rodent cardiomyocytes 11-13 allows for investigations of myofibrillar degradation and eventual reestablishment of the contractile apparatus. These characteristic morphological changes in adult cardiomyocytes occur over periods of 1-2 weeks. The dedifferentiation - redifferentiation cycle is accompanied by reexpression of the fetal gene program, thereby mimicking pathological changes observed in human cardiomyopathies 14. Another advantage of adult rat cardiomyocytes over the culture of neonatal cardiomyocytes is the ability to culture these cells for long periods of time.

Rat vs. mouse cardiomyocytes

The isolation and culture of rat neonatal cardiomyocytes has some benefits over that of mouse neonatal cardiomyocytes, including higher yields of viable cells and increased transfection rates. However, the wide usage of genetically modified mouse models for cardiac diseases (e.g. the muscle lim protein knockout mouse as model for dilated cardiomyopathy 15) has led to the adaptation of the isolation procedure for cardiomyocytes derived from neonatal mice. Although the protocols used to isolate neonatal rat and mouse cardiomyocytes are nearly identical, greater care must be taken in the selection of an appropriate enzyme mix for the latter. Indeed, neonatal mouse cardiomyocytes are generally more susceptible to overdigestion, resulting in a reduced cell-yield and viability. Moreover, the plating density should be adjusted, because cardiomyocytes derived from neonatal mice are somewhat smaller compared to cells derived from neonatal rat hearts.

With many uses for the investigation of morphological, electrophysiological, biochemical, cell-biological and biomechanical parameters as well as for the process of myofibrillogenesis, cultured neonatal cardiomyocytes have become one of the most versatile systems for the study of cardiac cell functions in vitro. The first step to a successful assay however, depends on an easy and reliable methodology to isolate neonatal mouse cardiomyocytes. Our protocol draws its methodology from many sources and was optimized for reproducibility and robustness. We discuss factors that influence cardiomyocyte-yield and viability, and provide a variety of options for the optimization of isolation and culture conditions.