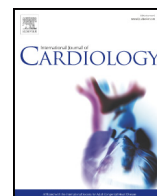




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A simplified protocol for culture of murine neonatal cardiomyocytes on nanoscale keratin coated surfaces

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ABSTRACT

Objective: We aim to develop a simple, efficient and cost-effective protocol for culturing the neonatal cardiomyocytes using keratin derived from human hair, which can be used for studying cardiac hypertrophy *in vitro*.
Methods: Keratin was extracted from human hair and applied as nanoscale coating onto the culture dishes. Physical parameters such as surface morphology and roughness of the coating were studied by SEM and AFM. Cardiomyocyte specific markers were assessed by immunofluorescence. Signaling pathways activated in hypertrophy were analyzed by western blotting and changes in the expression of fetal genes were analyzed by qPCR. The changes in the calcium fluxes were observed microscopically using Fluo-4.

Results: Keratin coated surfaces displayed a uniform coating and comparable roughness across dishes. Our optimized protocol for isolating cardiomyocytes yielded up to $\sim 10^6$ cells per heart. Characterization of cardiomyocytes with specific markers revealed that they can attach, grow and show spontaneous contractions on keratin-coated substrates similar to fibronectin-coated surfaces. Phenylephrine (PE) treated cardiomyocytes grown on keratin-coated substrates exhibited increased cell size, sarcomere organization and perinuclear ANP expression indicating the development of cardiac hypertrophy. In addition, we observed increased activation of Akt and ERK pathways, induction of the fetal genes and increased protein synthesis upon PE treatment, which are characteristics of cardiomyocyte hypertrophy. The protocol was extended to mouse cardiomyocytes and found to show similar results upon examination.

Conclusion: We demonstrate that keratin can act as an efficient yet cost effective alternative substrate for the attachment, growth and differentiation of neonatal murine cardiomyocytes.

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1. Introduction

Neonatal murine cardiomyocyte cultures have been widely used as a model for studying the spontaneous contractions, arrhythmia, hypoxia and ischemia at the cellular level. It not only permits the use of biochemical, morphological and electrophysiological approaches to understand the cardiovascular function but also offers great potential to study toxicological and pharmacological effects of drugs [1]. Besides, the primary cardiomyocyte culture has been extensively used to gain insights into the molecular mechanisms governing defects in cardiac regeneration and wound healing [2]. It is a validated and well accepted model to study the pathophysiology of the heart *in vitro*.

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The isolation of primary cardiomyocytes was first reported in 1963 by Harary and Farley and since then many modifications of the original protocol have been reported [3–6]. However, it is still a challenge to obtain superior quality cardiomyocytes in terms of viability, spontaneous contractions and increased yield of cardiomyocytes *in vitro* [6]. From previous studies, it is well known that substrates on which cardiomyocytes are grown play a crucial role in enhancing cell attachment, growth and differentiation [7]. For this purpose, a variety of extracellular matrix (ECM) proteins such as collagen, fibronectin, laminin and gelatin have been shown to be useful, most of which are sourced from slaughtered animals while fibronectin is purified from human plasma [7–10]. While the skin, tendons, ligaments, cartilages or bones are treated chemically to obtain gelatin, their tails and other tissues are used for obtaining collagen or laminin [10,11]. Besides the sacrifice involved, products prepared from animal sources carry a significant risk of contamination and heterogeneity.

Recent research has focused on the alternatives for these conventionally used substrate coatings. Over the past decade, a family of intermediate filament proteins, namely keratins has been gaining substantial interest as a substrate for surface coating [12,13]. Even though they are

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