European Journal of Cell Biology 95 (2016) 69–88

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Compartmental microfluidic system for studying muscle–neuron communication and neuromuscular junction maintenance

Ariel Ionescu [1], Eitan Erez Zahavi [1], Tal Gradus, Keren Ben-Yaakov, Eran Perlson *

Department of Physiology and Pharmacology, Sackler Faculty of Medicine, and the Sagol School of Neuroscience, Tel Aviv University, Tel Aviv 69978, Israel

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 1 November 2015
Accepted 19 November 2015

Keywords:
Microfluidic-chamber
Neuromuscular Junction
Axonal transport
Neurotrophic factors
Motoneuron

ABSTRACT

Molecular communication between the motoneuron and the muscle is vital for neuromuscular junction (NMJ) formation and maintenance. Disruption in the structure and function of NMJs is a hallmark of various neurodegenerative processes during both development and pathological events. Still due to the complexity of this process, it is very difficult to elucidate the cellular mechanisms underlying it, generating a keen interest for developing better tools for investigating it. Here we describe a simplified method to study mechanisms of NMJs formation, maintenance and disruption. A spinal cord explant from mice expressing the *Hb9*::GFP motoneuron marker is plated on one side of a compartmental chamber, and myotubes derived from muscle satellite progenitor cells are plated on the other. The GFP labeled motoneurons extend their axons via microgrooves in the chamber to innervate the muscle cells and to form functional in-vitro NMJs. Next we provide procedures to measure axon growth and to reliably quantify NMJ activity using imaging of both muscle contractions and fast intracellular calcium changes. This platform allows precise control, monitoring and manipulation of subcellular microenvironments. Specifically, it enables to distinguish local from retrograde signaling mechanisms and allows restricted experimental intervention in local compartments along the muscle–neuron route.

Introduction

Motoneurons (MN) extend axons over long distances and through varying extracellular microenvironments to form synapses with muscles. The formation and maintenance of these neuromuscular junctions (NMJs) depends on both internal and external signals that need to integrate with specificity and fidelity over space and time (Wu et al., 2010; Shi et al., 2012). This MN-muscle communication is vital for NMJ formation and maintenance, as well as for MN survival and proper function. Alterations in such intercellular communication can lead to synapse disruption as well as axon degeneration, and is a critical step in neurodegenerative diseases. This bidirectional communication process is conducted by both adherent and secreted factors, and mediated via ligand-receptor mechanisms. Still the nature of the signals and the specific molecular mechanisms regulating NMJs structure/function are yet to be fully understood. In particular, little is known of the mechanism of retrograde and anterograde signaling between the neuron and the muscle. Much of the difficulties in deciphering these mechanisms are due to the technical challenges of studying these complex intra- and intercellular communications at the subcellular level.

In-vitro compartmental systems that separate neuronal cell bodies from their axons and synapses are becoming a progressively useful tool for researchers. This platform enables the precise control, monitoring and manipulation of cellular microenvironments. Unlike traditional compartmentalized Campenot chambers which were used in studying NMJ remodeling (Campenot, 1977), silicon based microfluidic cell-cultures (El-Ali et al., 2006; Park et al., 2006; Taylor et al., 2003, 2005) are used to direct and image axon growth in the CNS, as well as motor neurons, with better resolution (Restani et al., 2012; Eleftheriadou et al., 2014), providing a highly adaptable system to model many aspects of neurodegeneration. Until now, however, this platform has not been useful for studying mechanisms of NMJ formation and maintenance. Recent attempts to build such a system turned out to be very complex, lacked the proof of functional NMJs, and did not demonstrate any specific utility for this type of platform (Southam et al., 2013; Park et al., 2013). Here we describe a simplified and efficient procedure to establish NMJs in a microfluidic compartmentalized culture system with MN cell bodies on one side and primary muscle cells on the other. This system is optimized for NMJ cell biology, and allows visualizing and manipulating NMJs at the pre- and postsynaptic cell compartments independently.

* Corresponding author. Tel.: +972 3 6408743.
E-mail address: eranpe@post.tau.ac.il (E. Perlson).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejcb.2015.11.004
0171-9335/

Application

The protocol detailed here can be employed to investigate cellular and molecular mechanisms involved in the biology of motoneuron and muscle communication including, but not exclusively, axon growth and guidance, synapse formation and neuronal stimulation of muscle contractile activity. The compartmentalization of muscles and neurons in our system simplifies localized application of treatments, such as genetic manipulation using viral vectors or pharmacological treatments, growth factor or other media constituent in the different compartments. As many motoneuron and muscle degenerative diseases remain untreatable and poorly understood at their underlying molecular basis, new in-vitro models of the neuromuscular system can be useful in dissecting the molecular factors contributing to the pathology and to test potential treatments in a simplified experimental design.

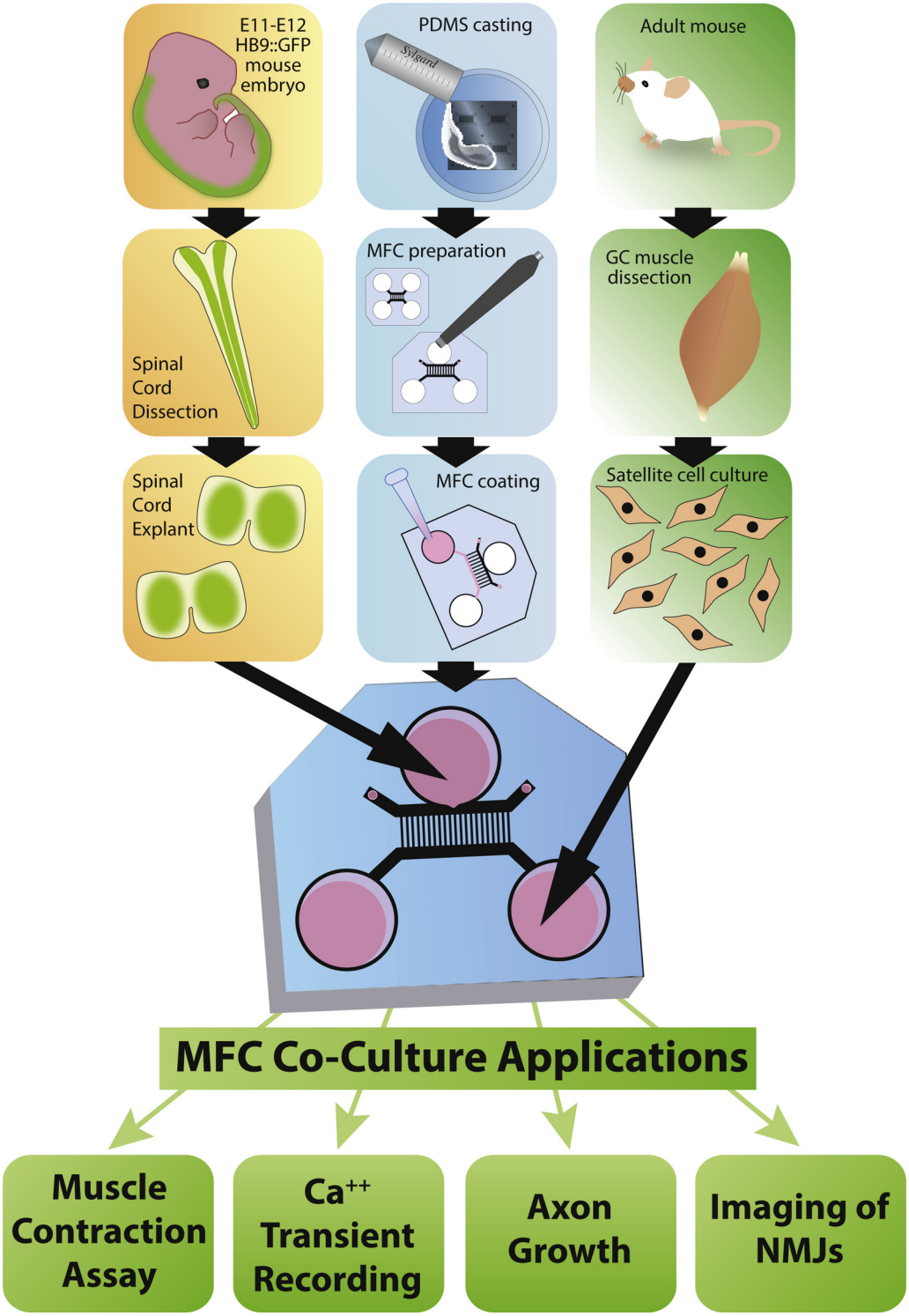

Fig. 1. Scheme of microfluidic neuromuscular co-culture. A co-culture of *Hb9*::GFP labeled motoneurons (as embryonic dissociated cells or spinal cord tissue explant) and adult derived myocytes is grown in compartmentalized microfluidic system enabling innervation by axons crossing from the neuronal to the muscle compartment. Following innervation, formation and function of in-vitro NMJ can be characterized and measured by labeling and live imaging of muscle contractions and calcium transients.