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Neuromuscular junction formation between human stem cell-derived motoneurons and human skeletal muscle in a defined system

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ABSTRACT

Functional in vitro models composed of human cells will constitute an important platform in the next generation of system biology and drug discovery. This study reports a novel human-based in vitro Neuromuscular Junction (NMJ) system developed in a defined serum-free medium and on a patternable non-biological surface. The motoneurons and skeletal muscles were derived from fetal spinal stem cells and skeletal muscle stem cells. The motoneurons and skeletal myotubes were completely differentiated in the co-culture based on morphological analysis and electrophysiology. NMJ formation was demonstrated by phase contrast microscopy, immunocytochemistry and the observation of motoneuron-induced muscle contractions utilizing time-lapse recordings and their subsequent quenching by D-Tubocurarine. Generally, functional human based systems would eliminate the issue of species variability during the drug development process and its derivation from stem cells bypasses the restrictions inherent with utilization of primary human tissue. This defined human-based NMJ system is one of the first steps in creating functional in vitro systems and will play an important role in understanding NMJ development, in developing high information content drug screens and as test beds in preclinical studies for spinal or muscular diseases/injuries such as muscular dystrophy, Amyotrophic lateral sclerosis and spinal cord repair.

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1. Introduction

For centuries, animals and animal-derived tissues have been the major tools for understanding biological systems, human diseases, the development of therapeutic strategies and for screening drugs. However, translating the animal data to clinical applications has been problematic and has led to fewer drugs being approved and an increasing cost in the drug discovery process [1]. Human-based functional in vitro systems in defined, serum-free medium are the logical next step in bridging the gap between discovery research and clinical application as well as for the next generation of systems biology tools [2].

While some functional in vitro systems composed of human cells have been reported for liver [3], skin [4,5], cardiomyocytes [6,7] and for motoneurons [8,9], no functional systems derived from human stem cells has been reported for the neuromuscular junction. Systems based on functional Neuromuscular Junctions (NMJ) are of particular interest due to the fact that NMJs represents a synapse-based model that would be clinically applicable to spinal cord injury, muscle and motoneuron-related diseases such as Amyotrophic lateral sclerosis (ALS) [10], spinal muscular atrophy [11] and muscular dystrophy [12]. An in vitro co-culture system composed of human motoneurons (MNs) and skeletal muscle (SKM) would be useful for studies ranging from understanding NMJ synaptogenesis, investigating pathogenesis for NMJ related diseases, screening therapeutic candidates and conducting drug efficacy/toxicology evaluation. The obvious advantages of human-based in vitro systems compared to in vivo systems reside in that they are much simpler and therefore it is straightforward to manipulate the system variables, to dissect the mechanisms or pathways and to analyze the results.

To date, multiple motoneuron-muscle co-cultures have been described in Xenopus [13,14], chick [15–17], mouse [18,19] and rat [20,21], as well as cross-species experiments with mouse MN-chick

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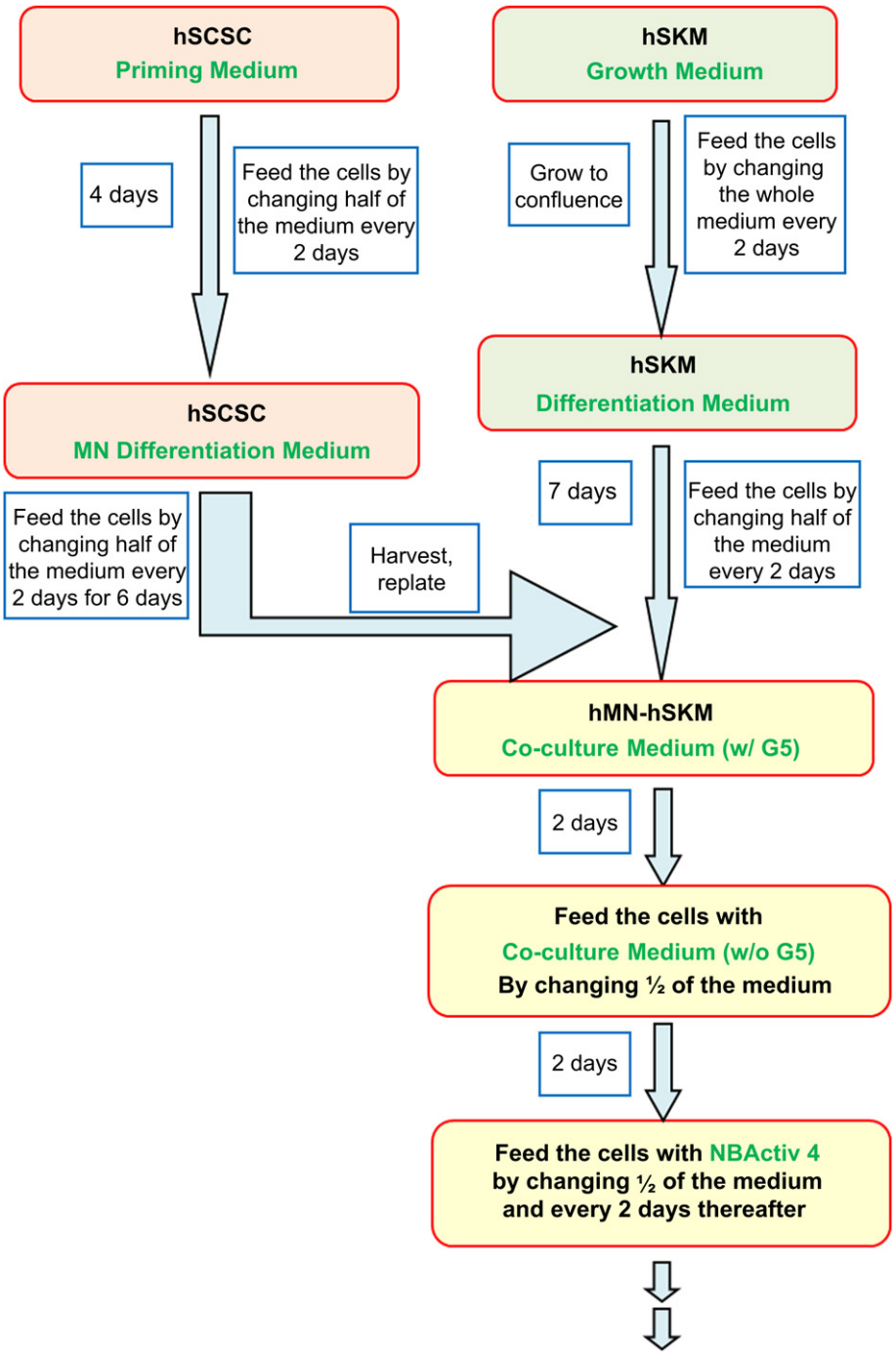

Fig. 1. Schematic diagram of the culture protocol and timeline.

muscle [19,22] and human embryonic stem cell (hESC)-derived MNs-C2C12 myotubes [23]. However, all of these in vitro motoneuron-muscle co-culture systems use serum containing media and a biological substrate [15–17,20,21]. Serum brings in unknown variable that is not amenable for reproducible assays. Moreover, serum contains many factors which can confound the elucidation of a drug's effect on single cell analysis or with functional constructs. In addition, a recent report suggested inhibition of full functional development of the myelination of motoneurons by serum in vitro [24]. Thus, some serum-free systems have been developed in an attempt to eliminate the inherent variability with serum [25] and NMJ formation in serum-free media has been demonstrated in rat [26] and cross species between human MN and rat muscle [27]. In general, in vitro systems composed of animal-derived components have provided the scientific community with readily available models for understanding NMJ synaptogenesis and NMJ-related diseases. However, due to species-specific differences, there is the problem of extrapolating the findings from animal systems to human systems especially for drug discovery and toxicology leading to clinical applications.

The major hurdles in building in vitro biological systems consisting of human components are the limitations due to tissue source. The emergence of stem cell biology in recent years however provides an avenue to not only have an unlimited supply of human

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