A NEW PLATFORM FOR PATIENT-BASED PRECLINICALTESTS IN MUSCULAR DYSTROPHIES: FIRST STEPS TOWARDS AN OPTOGENETIC ENGINEERED 3D ARTIFICIAL MUSCLE

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Background: Duchenne muscular dystrophy (DMD) is a severe X-linked disorder, characterized by the absence of dystrophin, that causes progressive muscle wasting. Currently there is no cure and pre-clinical studies in mouse models are ongoing to establish new therapeutic strategies. We are presently working to obtain an in vitro functional organoid model for DMD and others primary myopathies that may serve as fast, ethical and predictable platform to investigate pathology mechanisms and for fast drug screening. Previous attempts of building an artificial muscle gave modest results, as the organoid lacks a significant level of differentiation, while being poorly characterized for metabolic and contractile profile in vitro (Tedesco et al., 2018). Our novel approach is to obtain in vitro a fully differentiated and functional artificial muscle by engineering muscle precursors with an optogenetic construct, a technique which uses light pulses to trigger electric impulses on excitable tissues. This in order to mimic nerve-mediated activity in vitro, by means of non-invasive and finely tuned light pulses.

Methods: Stbl4cells were transformed with the optogenetic construct pcDNA3.1/hChR2-mCherry, to express avariant of the blue light sensitive ion channel, channelrhodopsin-2. After transformation, satellite cell derived H2K-2B4wild-type muscle cells were transfected. The dystrophic satellite cell derived SF1, obtained from mdx mice were transfected in parallel. The transfected clones were selected to obtain a 3-D muscle on a Matrigel scaffold and to undergo in vitro differentiation via light-induced stimulation of muscle functional and metabolic response. A proper myofiber type differentiation requires specific pattern of stimulation frequencies, which are delivered on optogenetic transfected stable clones directly in the incubator as led light pulses. At regular time, differentiation state is monitored, by means of expression of specific myogenesis genes, in parallel with key contractile and metabolism related proteins. In parallel, electrophysiology experiments, allow to characterize the development of ion channels typical of adult differentiated myofibers. This first step paves the way to identify the best protocol in term of intensity, frequency and duration of the light stimulus to use for the 3D scaffold. After achieving a good level of muscle maturation, mitochondrial biology and in vitro 3D muscle contraction ability will then be assessed. On top of that, iPS cells from patients will be used as second step.

Results: Preliminary results indicate that the hChR2expression is stable with an efficiency of transfection of about 30% and that almost all transfected cells properly integrate in the scaffold. Parallel experiments in SF-1cell line from the dystrophic mdx mouse suggest a similar transfection efficiency. Different training protocol are under validation on cells monolayer to understand which frequency works better to induce a complete maturation of the muscle culture to be then applied to the 3D structure, as well as the maintenance of dystrophic phenotype upon in vitro differentiation.

Conclusions: The early results encourage to further proceed for assessing proper differentiation of the optogenetic driven 3D muscle structure by means of electrophysiological and biochemical assessments. The following step will be to verify the suitability of the 3D structure for pharmacological studies before moving toward the use of patients-derived pluripotent stem cells and to approach of personalized medicine (supported by NL-DPP and Brains to the South).