**Design and realization of a 3D NMJ model as a novel tool for studying pathological alterations in neuromuscular diseases: preliminary results**

Forconi F1 \*, Cosentino M1, Caprini D2,Rizzuto E3,Casciola CM3, Del Prete Z3, Musarò A1

1DAHFMO – Unit of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy;

2Italian Institute of Technology, Centre for Life Nano Science, Rome, Italy;

3Department of Mechanical and Aerospace Engineering, Sapienza University of Rome, Rome, Italy.

The neuromuscular junction (NMJ) is a synaptic structure that plays a pivotal role in exchanging signals among lower motor neurons (LMNs) and skeletal muscles fibers. Investigating the functionality of neuron conduction and muscle contraction is pivotal in all those pathological conditions where NMJ is altered, such as aging, muscular dystrophy and Amyotrophic Lateral Sclerosis (ALS). Despite the NMJ has been widely investigated in animal models of neurodegenerative and muscular disorders (Fogarty et al., 2019), the mechanism behind the pathophysiology of these pathologies still needs to be clarified. Indeed, the development of a NMJ engineered model may represent an innovative tool to help pointing out the synaptic transmission alterations. In a previous work, we already proposed the generation of an in-vitro bioengineered three-dimensional vascularized skeletal muscle tissue (X-MET), able to recapitulate the architecture and the function of an in-vivo muscle (Carosio et al., 2013). Within this context, we aim at developing a 3D model of neuromuscular junction to enhance the fusion and the formation between the X-MET and motor neuron primary cultures. The resulting model is expected to allow improving the investigation of NMJ functionality in several pathological conditions. To do this, we initially characterize the bi-dimensional interaction between mouse spinal cord primary cultures and primary cultures obtained using muscle tissue harvested from mouse hind limbs, through a commercial 2D micro-fluidic device for NMJ formation. Subsequently, two different prototypes of a 3D micro-fluidic device were designed and realized to promote the formation of synaptic transmission between the X-MET and mouse spinal cord primary cultures. In the first one, the engineered muscle and the neurons are connected through a microgroove barrier, while in the second one they are connected by a single channel for the placement of a decellularized nerve. Preliminary results for the two devices are reported.

Name: Flavia Forconi

e-mail: flavia.forconi@uniroma1.it

Address and telephone number: Via Antonio Scarpa 16, 00161, Rome, Italy. Tel: +39 0649766607