**Human Skeletal Muscle Organoid Development: An Overview of Three-Dimensional Culture Construction**

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**Introduction:** Clinical and pre-clinical studies showed that Gestational Diabetes Mellitus is associated with Pregnancy Specific Urinary Incontinence increasing the risk of Urinary Incontinence and Pelvic Floor Muscle Dysfunction up 2 years post-cesarean delivery1. Translational studies in pregnant rats with moderate/severe induced diabetes showed the presence of a gestational diabetic myopathy (GDMy)2. Animal models and classical cell cultures have allowed important advances in the study of the pathophysiology of GDMy; however, there is limitations in their research potential. Three-dimensional cultures, such as organoids, emerge as a promising alternative as they enable the co-culture of different cell types that are structured in an analogous manner to the tissue, providing a relevant parallel model to human tissue *in vivo*. The aim of this project is to create skeletal muscle organoids from pregnant women as a 3D model for the pathophysiology study and biomarker’s identification of GDMy. **Methods:** Rectus abdominis muscle samples were collected at the cesarean section (Ethics Committee CAAE: 35971120.1.0000.5411). Progenitor muscle cells (PMCs) were isolated and cultivated following Carosio et al (2013) protocol3. After reach a cellular monolayer, differentiation culture media was used to induce cell’s maturation. The differentiated cellular monolayer was detached from the culture dish, transferred to a new one dish agarose-coated and pinned with stainless steel pins. Four days later, a self-organized tridimensional structure was formed. **Results:** The preliminary results of this ongoing study proved that protocol is effective for the isolation and cultivation of a heterogeneous cell population. After differentiation induction, it was possible to observe the spontaneous detachment of the cell monolayer at different points on the culture dish, as well as the beginning of aggregation of these cells. For this reason, it was not possible to perform the entire detachment of the monolayer, with only a piece of it being transferred to the agarose-coated dish. Four days after being pinned, the structure stretched between the anchor points. Inverted microscopy images were acquired and the organoid was harvest for histology. Hematoxylin & Eosin staining showed the formation of a cylindrical structure with highly compacted cells, but without the presence of elongated cells arranged in bundles, as observed *in vivo*. In these first days, organoid was unable to produce extracellular matrix, nor was the presence of endothelial cells observed. Furthermore, cell markers for fast, slow and intermediate myosin were not observed. **Conclusion:** The partial results showed that we were able to isolate and cultivate PMCs, as well as induct the tridimensional structuration. Although it’s still in the early stages it is a promising 3D model, suggesting the possibility to generate a skeletal muscle organoid for the pathophysiology study and biomarker’s identification of GDMy.

**References**

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