**Role of electro-mechanical stress on the switch proliferation/differentiation on C2C12 cells.**

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Mechanical signals are important regulators of cellular proliferation and differentiation. Two transcriptional co-activators, YAP and TAZ, mediate cellular response to mechanical stress. These proteins are regulated by phosphorylation and shuttle between the cytoplasm and the nucleus, where they interact with TEAD transcription factors that in turn activate proliferation. It has been shown that in human mammary hepitelial cells (MEC), growing on soft matrix, the YAP/TAZ proteins are predominantly located in the cytosol. Instead, when cells were grown on stiff material these proteins migrate in nucleus and became active [1]. It has been also demonstrated that YAP phosphorylation is required for differentiation of mouse myoblast cell line (C2C12) [2]. The aim of this study is to test the role of mechanical stress on the switch proliferation/differentiation mediated by YAP/TAZ proteins on myogenic cells grown in a three dimensional matrix, using a semi-synthetic hydrogel made from polyethylene glycol (PEG) and fibrinogen [3] with different rigidity. A comparison of the degree of YAP phosphorilation of C2C12 cells grown in a bidimensional environment or in a tridimensional matrix indicates that YAP is phosphorilated at a shorter time in the 3D matrix. This result correlates with the morphological features as detected by immunofluorescence following the myosin heavy chain. These results confirm the importance of the environment in modulating cellular differentiation. If compared with the classic cell culture growth on two-dimensional substrates, the three-dimensional hydrogel represented the ideal medium to transmit the external mechanical signals to cells and allowed us to obtain experimental results closer to reality: in fact, the cells grow three-dimensionally in living organisms. In addition, the hydrogel has provided an environment easily deformable and permitted us to differentiate the physical stress applied to the cells (compression, elongation, periodic oscillations, ...) not applicable instead with standard two-dimensional growth. We have designed systems that are capable of transmitting stable, controlled and repeatable mechanical signals to the cells: for example, cells are compressed with a homemade setup with the capabilities to measure the instantaneous pressure applied and optionally to vary the intensity over time. We observed the cells at various stages of their growth and we have gathered important information that reflect the state of the cells with respect to their microenvironment changes.

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