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Real Time Analyses of Myogenesis in Behaving Myoblasts

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INTRODUCTION: Effective Tissue engineering ultimately depends on understanding the interplay between the proliferation and differentiation stages of development. One of the best systems to examine these processes is myogenesis. Activated myoblasts first undergo many rounds of cell division. Depending on the needs of the cells, myoblasts may next withdraw from the cell cycle to fuse to form myotubes.[1] Division and fusion are differentially modulated by mechanical stimuli transmitted to the cell's biosynthetic mechanisms via the actin-based cytoskeleton; Specifically cyclic stretch promotes proliferation and inhibits fusion.[2] By inference, the nature of cell attachment to the substrate should also influence transition through myogenesis. We therefore investigated the effect of the extracellular matrix on behaving cells undergoing either cytokinesis or fusion using atomic force microscopy (AFM) as well as confocal laser scanning microscopy (CLSM) in fluorescent mode.

METHODS: C2C12 muscle cells were seeded (5,000 cells/cm^2) onto glass coverslips which had been coated with either Fibronectin or Laminin and maintained in growth media (20% Fetal Bovine Serum (FBS)) for two days in order to favour proliferation. Fusion was induced two days later with a switch to differentiation media (10% Horse Serum (HS)). Contractile competent myotubes were apparent 9 days after plating when grown on glass coverslips that had been patterned with lanes of extracellular matrix proteins. The topography of living myoblasts was analysed with an AFM (from JPK Instruments) in CO_2 independent media supplemented with 0.13% Glutamine and 20% FBS, or 10% HS, for cytokinesis and fusion studies respectively. The 2D organization of the actin-based cytoskeletal network was examined by CLSM after staining myoblasts with Rhodamine labelled Phalloidin. To distinguish between individual cells and to quantify the size of the myotubes, nuclei were stained with DAPI.

RESULTS: When compared to conventional confocal microscopy, our AFM analysis rendered more information concerning myoblast-myoblast and myoblast-substrate attachments. Whereas laminin promoted proliferation and cell migration, fibronectin was more permissive for tight myoblast-myoblast contacts. Myoblast-myoblast adhesion was characterized by the expression of interdigitating membranous processes uniting groups of fusing myoblasts. The precise topography of the membrane tubules was most evident with the AFM. The same structures were also observed with CLSM and revealed that these membrane tubules were built upon a scaffold of actin filaments. Similar membrane processes were less evident during cytokinesis.

DISCUSSION & CONCLUSIONS: These results suggest that the substrate is of importance for myoblast behaviour during myogenesis. Since myoblasts interact with laminin and fibronectin via distinct integral membrane protein complexes (dystroglycan and integrins, respectively) these results suggest that distinct extracellular matrix-binding complexes differentially govern transition through myogenesis. Membrane tubules were observed in the fusion process and therefore we believe that, in accordance with recent findings in the drosophila system[3], the nucleation of actin filaments initiates the fusion process. Actin could then in myoblast fusion, as in the drosophila system, be responsible for a transportation of vesicles to the cell membrane. These would then in turn constitute the active part of the fusion process.

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