Essential environmental cues from the satellite cell niche: optimizing proliferation and differentiation
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Essential environmental cues from the satellite cell niche

Optimizing proliferation and differentiation


Introduction
Satellite cells (SCs) are very efficient in regenerating muscle defects in vivo. However, their functionality in vitro has been disappointing. We hypothesized this is due to loss of the natural niche and anticipated that this niche needs to be mimicked in culture conditions. Therefore, we explored the influence of substrate elasticity and protein coating on differentiation and proliferation capacity of SCs.

Material and Methods

Cell isolation and culture: Single fibers were isolated from muscles of C57BL/6 mice, and SCs were liberated with a 19G needle. Passage 0 cells were plated (1000 cells/cm²) on coated coverslips or polyacrylamide (PA) gels (figure 1).

Differentiation: MyoD/Myosin/DAPI immunocytochemical stainings were performed to evaluate differentiation and maturation.

Proliferation: SCs were exposed to BrdU for 16 hours, after which BrdU incorporation was detected with a microscope.

Results

Differentiation & Maturation

Figure 2: Differentiation on coated coverslips after 11 days. Timing of differentiation did not depend on elasticity or protein coating. However, more and thinner myotubes were formed on laminin and poly-lysine, compared to Matrigel (not shown), ECL-gel and Collagen IV.

Conclusions

Elasticity of the substrate influenced proliferation and maturation: Cells grown on coverslips and substrates of 21 kPa (close to physiological elasticity of skeletal muscle), proliferated most and for the longest duration. In addition, maturation (evaluated by cross-striations and spontaneous contractions), was best on coverslips, followed by 21 and 80 kPa gels and lastly 3 kPa gels. The extracellular matrix proteins used for coating of the substrate only influenced differentiation of the cells: more and thinner myotubes were found on poly-lysine and laminin, compared to Matrigel, ECL-gel and Collagen IV.

References