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).

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.

Figure 3: Cross-striated myotubes (arrows) grown on different elasticities. Myosin cross-striations and spontaneous contractions (both signs of maturation) were observed most in myotubes grown on glass, followed by myotubes on 21 and 80 kPa gels, and were not observed in myotubes cultured on 3 kPa gels.

Figure 4: Proliferation of SCs on poly-lysine-coated substrates. Proliferation was significantly higher and for a longer duration of SCs cultured on glass, followed by cells cultured on 21 kPa gels, then cells cultured on 80 kPa gels and lastly cells cultured on 3 kPa gels. No differences were found in proliferation between protein coatings (data not shown).

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