Skeletal muscle stem cells (MuSCs) are an adult stem cell population essential for muscle homeostasis and regeneration throughout adulthood. Clinical muscle cell therapies are limited by the rarity of MuSCs and the lack of bona fide self-renewal in myogenic progenitors. Thus, long-term adult MuSC expansion to a clinical-scale yield is a critical unmet need for cell-therapeutic approaches to treat acute and chronic muscle defects.

To address this challenge, we used high-throughput microprinted arrays on tunable stiffness hydrogels for assessing combinatorial effects of mechanical and chemical signals on MuSC and myoblast phenotype. We examined combinatorial cytokine stimulation of murine myoblasts, which revealed time-dependent synergisms in phosphoprotein signaling dynamics. Partial least-squares regression modeling using a cue-signal-response paradigm accurately predicted phenotypic response outcomes. For example, immediate p38 pathway upregulation was anticorrelated with differentiation, but the correlation flipped if p38 was upregulated in extended culture.

Using these insights, long-term MuSC culture environments were fabricated with hydrogels at varying rigidities conjugated with MuSC niche proteins to facilitate MuSC adhesion. FACS-isolated MuSCs were cultured on the gels for 5 weeks and stimulated with soluble growth factors and inflammatory cytokines transiently upregulated during the in vivo muscle repair process. Proliferation on laminin-coated 12 kPa hydrogels was enhanced by stimulation with a cocktail of bFGF, IL-1α, IL-13, TNFα, and IFNγ via activation of the JNK and p38 pathways. Combined cocktail stimulation and late-stage p38 pathway inhibition yielded >10^7-fold expansion and maintained MuSC transplantation potential for 5 weeks of culture.

These findings suggest that large pools of MuSCs can be obtained through long-term self-renewal expansion in designed microenvironments while maintaining therapeutic potential and minimizing loss of phenotype.