The use of Skeletal Muscle Extracellular Matrix Extract to Study the Influence of an Aging Environment on the Regenerative Capacity of Skeletal Muscle Progenitor Cells

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Statement of Purpose: Sarcopenia, the loss of skeletal muscle mass and strength with age, represents one of the most prevalent and significant problems facing our rapidly growing elderly population. Muscle progenitor cells (MPCs), which are responsible for the maintenance and repair of skeletal muscle tissue through regenerative mechanisms, experience a reduction in regenerative capacity with age. A question that has not been adequately investigated is what roll factors extrinsic to MPCs play in this loss of regenerative capacity. The purpose of this study was to develop a model system to study the influence of skeletal muscle extracellular matrix (ECM) age on the regenerative capacity of MPCs. We hypothesized that age-related changes to skeletal muscle ECM lead to an impaired ability of MPCs to regenerate skeletal muscle tissue.

Methods: To investigate our hypothesis, we developed a cell culture model in which the interaction between MPCs and ECM is modeled by culturing rat MPCs on tissue culture dishes coated with rat skeletal muscle ECM extract. Young and old MPCs and ECM were obtained from < 7-month-old and > 24-month-old animals, respectively. ECM was extracted from decellularized quadriceps and hamstring muscle groups, and MPCs were obtained via explant culture methodology. The murine C_2C_{12} myoblast line was also employed. Statistical significance was determined by Student's T-test ($p < 0.05$)

Results: The model was used to demonstrate that skeletal muscle ECM extract influences the proliferation and differentiation of MPCs and myoblasts. Surprisingly, subsequent experiments demonstrated that the proliferation of MPCs is enhanced when grown on old ECM relative to young ECM.

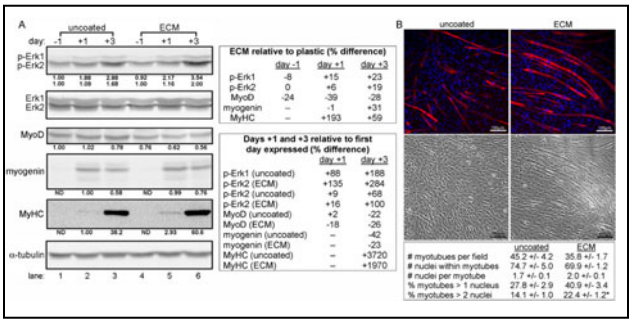

Figure 1: Cells grown on muscle ECM undergo enhanced Erk1/2 phosphorylation and myogenic differentiation over the course of 7 days. A) C_2C_{12} cells grown on uncoated or ECM extract-coated dishes were assayed for expression of phospho-Erk1/2 (p-Erk1/2) and markers of myogenic differentiation by Western analysis. Day numbers indicate the day of culture relative to the induction of differentiation on day 0. For densitometry, values obtained from cells grown on uncoated dishes on the first day of their expression were set to 1.00. A representative experiment is shown. **B)** C_2C_{12} cells grown on uncoated or ECM-coated surfaces were assayed for the formation of myotubes by staining for myosin heavy chain (red). * indicates a statistically different ($p < 0.05$) result compared to uncoated wells.

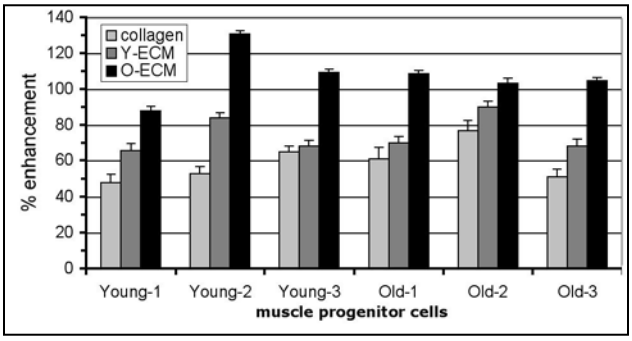

Figure 2: Old ECM extract enhances MPC proliferation better than young ECM extract when applied as a coating at equal protein concentrations. Skeletal muscle progenitor cells were obtained from three young and three old rats. Cells were seeded onto uncoated, collagen I coated, young ECM coated, or old ECM coated surfaces. Proliferation was measured by MTS assay, and the results were normalized to growth on an uncoated surface. Each of the six cell types proliferated better on old ECM extract than young ECM extract and on young ECM extract better than on collagen I. Note that the age of the MPCs did not influence the results

Conclusions: The use of ECM extract to study interactions between regenerative cells and ECM provides and simple and easily modified model system. Old skeletal muscle ECM extract enhances MPC proliferation better than young ECM extract. One possible explanation for this unexpected result is that collagen modifications, such as crosslinking, prevent efficient extraction of collagen from aged tissue and lead to a greater proportion of proliferation-enhancing, non-collagen molecules in old ECM extract. Future work will be directed towards comparing the effects of ECM age on MPC differentiation, characterizing the compositional differences between young and old ECM, and the development of additional models to study ECM-MPC interactions.