Tissue-Specific Extracellular Matrix Derived Coatings Promote Differentiation of Muscle Progenitor and Human Stem Cell Derived Cardiomyocytes In Vitro

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Statement of Purpose: The native extracellular matrix (ECM) is a complex tissue-specific network of proteins and polysaccharides, which regulate cell growth, survival and differentiation within various tissues. Despite the complex nature of the ECM, *in vitro* studies usually assess cell behavior on purified protein substrates such as collagen or laminin, which do not mimic the complex native ECM microenvironment, and to date there are a limited number of studies which have assessed cell behavior on the native ECM. We have developed a method to extract ECM from porcine skeletal muscle, cardiac, liver, kidney, lung and brain tissues, which we solubilized to retain a complex mixture of tissue-specific components. We hypothesize that this substrate microenvironment will provide the correct native cues for committed progenitor and stem cell differentiation, as the coatings can more appropriately emulate the adult ECM.

Methods: Porcine tissues were decellularized using detergents in PBS ranging from 0.01% to 1% SDS. Tissue was cut into pieces approximately 1 cm^3 and then stirred in SDS solution until decellularized. The decellularized tissue was then lyophilized, milled to form a fine particulate powder, and then lyophilized again. After enzymatic pepsin digestion, solubilized skeletal muscle and cardiac matrix (1 mg/mL) were coated onto tissue culture plastic by incubating at 37°C for 1h. To assess muscle progenitor differentiation on native tissue coatings, C2C12 skeletal myoblasts were plated on either collagen I or skeletal muscle matrix coated wells, and cultured in growth media consisting of DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were fixed and stained with Hoechst 33342 and anti-myosin heavy chain to assess differentiation into myotubes. Late stage stem cell differentiation was explored using HES2 that were differentiated into beating embryoid bodies using a protocol shown to effectively create cardiomyocytes[1]. Embryoid bodies were dissociated and then plated on either gelatin or cardiac muscle matrix coated plates. Cells were stained for titin M8, a late cardiac sarcomeric differentiation marker, DAPI for nuclei and desmoplakin, an intracellular junction protein.

Results: Skeletal muscle myoblasts on the skeletal muscle matrix coating displayed a significant increase in percent of differentiation ($p<0.05$), the number of nuclei per myotube ($p<0.01$) and myotube width ($p<0.01$) when compared to cells plated on traditional collagen substrates (Figure 1). Human embryonic stem cell (HES2) derived cardiomyocytes plated on the cardiac matrix coating displayed a significant increase in myofibrillar titin area ($p<0.01$), number of cardiomyocyte nuclei per area ($p<0.01$), and aggregation of the desmosomal cell-cell junction protein desmoplakin ($p<0.01$), when compared to cells plated on the traditional gelatin coating, indicating maturation of cardiomyocytes as desmoplakin localizes to the intercalated disc during maturation (Figure 1).

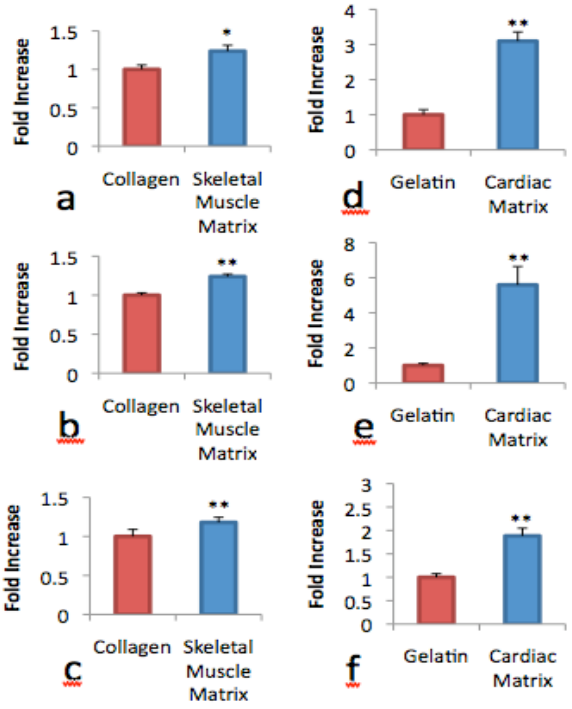

Figure 1: C2C12 results for % differentiation (a), myotube width (b) or nuclei per myotube (c). HES2 results for titin area (d), nuclei per cell area (e) or average desmoplakin size (f). * $p<0.05$ ** $p<0.01$

Conclusions: These studies illustrate that native ECM derived coatings provides a better milieu to promote differentiation *in vitro* when compared to traditional single component coatings. The ability to reconstitute the *in vivo* muscle ECM niche could have important applications for cell-mediated therapies and provide a platform for assays for drug development testing. There is also a potential application to use this technique with other non-cardiac and non-skeletal muscle cell types, as these tissue specific coatings can be created from several non-mineralized tissues. In conclusion, we have demonstrated that these tissue specific coatings increased cell differentiation of committed muscle progenitor cells, thus indicating the potential for this as a platform for improving *in vitro* cell culture.

References:

1. Yang L. Nature Letters. 2008; 453: 524-529.