**Human plasma based composite muscle ECM directs adipose derived stem cells to vasculogenic and myogenic lineage**

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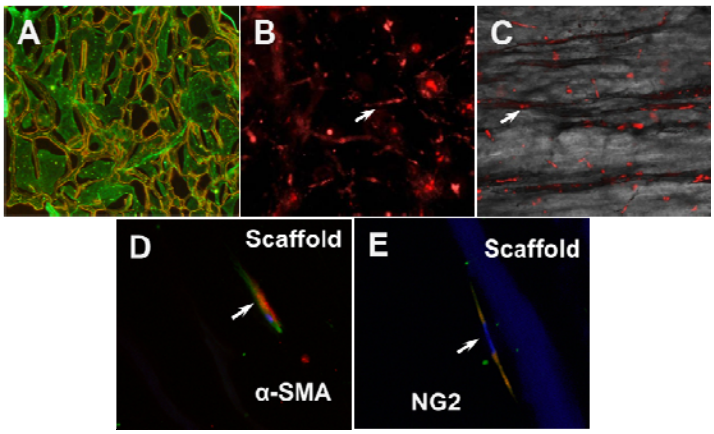
**Statement of Purpose:** Volumetric muscle loss (VML) is a debilitating injury with limited treatment options. Tissue engineered strategies for VML repair typically involves an acellular ECM scaffold (1). Unfortunately, ECM repair alone does not promote appreciable *de novo* muscle formation, but results in fibrosis with a marginal improvement in muscle function (2). Current evidence suggests that the addition of an exogenous cell source may be required to promote de novo muscle formation (1, 2). Another important consideration is establishment of a sufficient vascular supply for promoting skeletal muscle regeneration. The objective of this study was to develop a composite scaffold for the delivery of mesenchymal stem cells. Specifically, we have developed a cell-ECM composite construct by polymerizing human PEGylated plasma with adipose derived stem cells (ASCs) on sheets of decellularized porcine skeletal muscle. In the composite, the muscle ECM will provide a structural and biomechanical template, whereas the PEGylated plasma will enable cell proliferation, and provide a microenvironment for revascularization and myogenesis.

**Methods:** Porcine skeletal muscle was decellularized using an established protocol (3). Briefly, sheets of muscle (3mm) were washed in DMEM containing latrunculin B (50nm), subjected to hypotonic/hypertonic washes followed by DNAse treatment (5 KU/ml). ASCs ($1x10^5$) were suspended in platelet free plasma (PFP) containing succinimidyl glutarate PEG (0.4mg/ml). PEGylated plasma-cell solution was applied and allowed to infiltrate within sheets of decellularized muscle ECM. Subsequently, thrombin (12.5U) was added to initiate *in situ* gelation of PEGylated-plasma. The composite scaffold was maintained in culture in a 5% $CO_2$ humidified incubator at 37°C for 11 days using MesenPro complete growth media. Phenotypic and genotypic changes of ASCs within the composite scaffolds were analyzed using RT-PCR and immunofluorescence techniques. PFP and ASCs were obtained from the USAISR (human blood) and Brooke Army Medical Center (abdominoplasty) under IRB approved protocols H-10-023 and H-11-003.

**Results:** PEGylated plasma was incorporated in the muscle ECM and filled void spaces with in the ECM scaffold (Fig. 1A). ASCs appeared to differentiate into two distinct phenotypes. Within the gel, a tubular network-like structures (Fig. 1B) and, on the ECM, cells were elongated, directionally aligned (Fig. 1C). The cells within the gel also stain positive for vascular markers, αSMA and NG2 (Fig. 1D and E) and cells on the ECM stained positive for the myogenic marker, Troponin T (not shown). The differentiated ASCs within composite scaffold exhibited up-regulated level of both perivascular (*NG2, Ang1, αSMA*) and cell-derived extracellular matrix associated genes (*CO1A1, integrin β1, Fibronectin 1*).

**Figure 1:** Epifluorescent image of composite scaffold showing PEGylated plasma (green) integrated with the pores of muscle fibers (red) (**A**). ASCs on the composite




scaffold differentiated towards a tubular network within the gel (**B**) and elongated phenotype along the muscle fiber of the scaffold (**C**). Immunofluorescence showed differentiated ASC images staining positive for αSMA (D) and NG2 (E).

**Conclusions:** We describe a regenerative medicine approach of cell therapy for the repair of VML injuries. Here we demonstrate that the ASCs delivered via a PEGylated plasma onto decellularized muscle ECM scaffold differentiates into vasculogenic and myogenic lineages. This indicates that the composite scaffold may enhance cell migration, re-vascularize the injured muscle, and possibly promote muscle formation. Future studies are planned at investigating this hypothesis in a rodent VML injury model.

**References:**

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