# Anisotropy Increases Myogenic Regulatory Factors and Regenerates Skeletal Muscle

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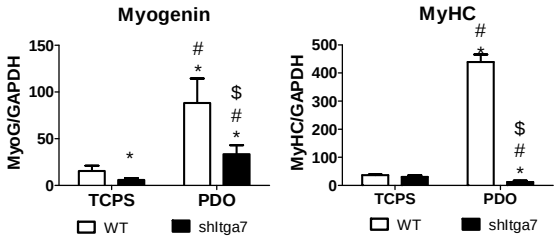
**Statement of Purpose:** Traumatic muscle injuries from crush, contusion, or blast can lead to volumetric muscle loss. These types of injuries often heal slowly and improperly, resulting in tissue fibrosis, denervation, and loss of function. Current approaches to regenerate skeletal muscle using stem cells have demonstrated limited success. This could be due to the mode of cell delivery. Stem cell injection is the most common, but the cells fail to engraft well [1]. This could be due to a lack of understanding about the unique, aligned extracellular matrix (ECM) structure present in muscle. The focus of this study was to determine the effect of anisotropy on myogenic regulatory factors and integrin expression, and how these effects would translate to a muscle defect model.

**Methods:** We first developed an in vitro model to determine the effect of anisotropy on myogenic regulatory factors (MRF) and integrin expression. Aligned and random polydioxanone (PDO) grafts were created using electrospinning. These grafts were seeded with murine myoblasts and cultured for 3, 6, and 12 days. Paired box 7 Pax7, Myod, Myog, Myf6, MyHC, integrin α7, integrin α5, and integrin β1 were measured at each time point. To determine the role of integrin α7 in myogenic differentiation, we silenced α7 using lentiviral particles. shItga7 myoblasts were seeded onto aligned PDO and TCPS and cultured for 6 days. MRFs and changes in integrin composition were measured with using real-time PCR and western blot. Working with Musculoskeletal Transplant Foundation, we developed a partially decellularized muscle matrix (PDM) that retained intact myofibers, preserving anisotropy. These grafts were seeded with adipose derived stem cells and implanted into a 1x1 cm defect in the lateral gastrocnemius of an athymic nude rat. Rats recovered for 8 weeks prior to histology.

**Results:** Anisotropy increased gene expression and protein levels for muscle cell markers, including Myog, Myod, and Myf6 in cells cultured on PDO-aligned surfaces compared to cells cultured on tissue culture polystyrene (TCPS) or PDO-random surfaces. These data correlated with the down-regulation of α5 and β1 integrin subunits and the up-regulation of integrin α7. shItga7 cells exhibited a significant decrease in gene expression and protein production for Myog, Myf6, and MyHC (Fig 1). The suppression of α7 also displayed increases in α5 and β1A. Moreover, anisotropy increased muscle markers when α7 was silenced. There was a significant increase in Pax7 expression in cells cultured on PDM v. PDO, but there was no difference between PDM and PDO-aligned substrates for Myod, Myog, and Myf6 expression. Alignment in PDM influenced myogenic gene expression. Tissue engrafted with PDM + cells or without cells had an




appearance similar to normal muscle. Hematoxylin and eosin sections Figure 1. Silencing α7 abrogates Myog and MyHC protein production, and anisotropy increases Myog protein over TCPS.

showed the presence of elongated nuclei along the myofibers, while Masson's trichrome showed small amounts of collagen between the myofibers. There was no discernable difference between ASC seeded and non-seeded PDM sections.

**Conclusion:** In the present study, we showed that alignment had a stimulatory effect on myogenesis using myoblasts, indicating that the innate alignment of PDM could have an effect. We also showed that integrin α7 is important in myogenic differentiation and directly influences MRF protein production, and that anisotropy enhanced MRF proteins in the absence of α7. This supports its role in muscle development and disease [2], and suggests that α7 and alignment are necessary for stem cells to create functional muscle. Our muscle defect model provided insight into this hypothesis by demonstrating that aligned structural properties promote muscle regeneration. This study demonstrated the advantages of anisotropy in biomaterials to regenerate skeletal muscle tissue and that its use could improve the lives of those affected by traumatic muscle injuries.

**References:**

1. Siegel AL. Stem Cells. 2009; 27(10):2527-38
2. Burkin DJ. Cell Tissue Res 1999(296):183-90