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## Engineering skeletal muscle using PLGA scaffolds with oriented tubular microstructure

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**INTRODUCTION:** Engineering skeletal muscle still remains a major challenge. Skeletal muscle comprises a highly organized structure containing cells arranged in parallel in order to contract as a syncytium. Previous work has shown that porous scaffolds with uncontrolled structure allow 3D growth of myoblasts [1]. However, random structures are ineffective for proper differentiation of the cells into elongated myotubes. An oriented scaffold providing the necessary contact guidance and topographical cues for the cells is therefore desired [2]. Biodegradable microtubular oriented PLGA scaffolds seem to be well suited for this purpose [3]. In this study, we studied the differentiation of myoblasts cultured on porous PLGA scaffolds with the goal of creating 3D muscle constructs for the treatment of musculoskeletal diseases.

**METHODS:** PLGA scaffolds (70/30) were fabricated by the thermal-induced phase separation technique, according to established methods [3]. Samples were coated either with type I collagen (50 µg/ml in 0.02M acetic acid) or Matrigel (1:50 in serum free medium). Samples with the desired size and shape were cut using a biopsy punch (diameter = 3 mm) and seeded with murine myoblasts (C2C12) at an approximate density of  $3 \times 10^3$  cells/mm<sup>3</sup> of sample. Growth medium consisted of DMEM with fetal calf serum (10%) and antibiotics. Proliferation was measured at 24, 48, and 96 hours using a fluorescence-based assay. After 96 h, medium was replaced by differentiation medium (DMEM with horse serum (2%) and antibiotics), and cells were further cultured for two weeks. After 14 days cells were fixed and stained using Hoechst 33342, Bodipy 558/568 Phalloidin (actin) and an anti-myosin heavy chain antibody conjugated with an Alexa Fluor 488 secondary antibody. Differentiation was assessed by wide-field fluorescence microscopy.

**RESULTS:** Cell numbers displayed a steady increase in all samples during the whole observation period. Growth rates were similar and were not influenced neither by coatings nor pore structure. Multinucleated myosin-positive cells

were found in all samples. However, elongated myotubes, arranged in parallel, were only observed in the microtubular oriented scaffolds. As shown in Fig.1, these myotubes followed the orientation of the microtubules. The quality of myotubes was essentially similar in type I collagen and Matrigel-coated scaffolds.

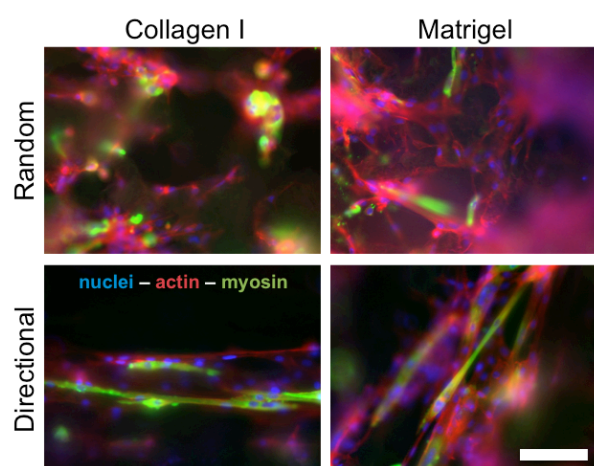


Fig. 1: Morphology of myoblasts and myosin-positive myotubes in different scaffolds at day 14 after induction. Scale bar = 100 µm.

**DISCUSSION & CONCLUSIONS:** These results indicate that PLGA scaffolds coated with ECM molecules can sustain proliferation of myoblasts. However, only those with microtubular oriented structure support the proper alignment required to form muscle fibers. In perspective, this tubular structure may also serve for the development of vascularization inside of the constructs. These types of scaffolds possess a strong potential for the development of 3D tissue constructs for the treatment of muscle diseases.

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