The Effect of Matrix Composition and Geometrical Confinement on Engineered Myobundle Development

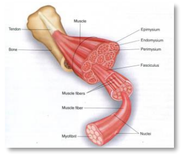
Sabina Yampolsky, Nethika Ariyasinghe, Megan L. McCain
Harvard Westlake, Class of 2020, SHINE 2017
Laboratory for Living Systems, Department of Biomedical Engineering

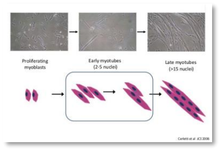
USC Viterbi School of Engineering

SHINE Summer High School Intensive in Next-Generation Engineering

Introduction

- Dr. Megan McCain's lab engineers striated muscle tissue models to study disease
- Skeletal muscle structure:
  - Myoblasts
    - Precursor to muscle fibers
    - Fuse together to form myotubes (myogenesis)
  - Extracellular Matrix (ECM)
    - Formulation of proteins and macromolecules
    - Provides structural and functional support
    - One of the most significant proteins is collagen (makes up 25%-35% of ECM)

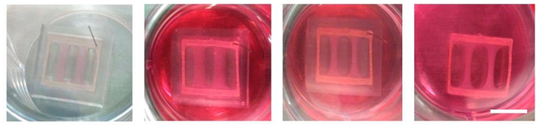




- Current research platforms such as animal models and traditional cell culture do not mimic skeletal muscle structure
- 3D cell culture allows us to create aligned muscle bundles (called myobundles) in an ECM environment similar to native muscle

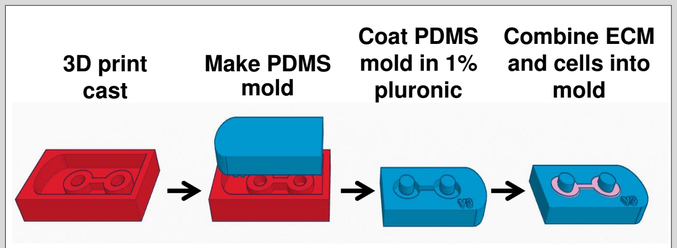
Objective & Hypothesis

- **Objective:** To determine how various ECM compositions and geometric confinement affect the development of myobundles
- **Hypothesis:**
  - We expected the ECM composition to affect myobundle formation
  - We did not expect geometric confinement (height) to affect myobundle formation



Example of Myobundles, Madden et al., eLife, 2015

Methods



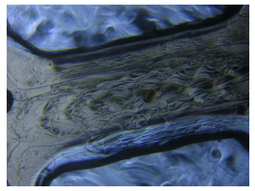
Variables Tested

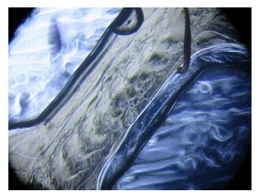
Height (mm)	ECM Compositions
• Short molds (2 mm)	• 0.15% Collagen (CN) & 10% Matrigel

• Tall molds (4 mm)	• 5% Gelatin (GN), 10% Matrigel, & MTG
	  • 4% MTG
	  • 2% MTG
	  • 1% MTG
	  • 0.5% MTG

	• 5% Gelatin (GN) & 10% Matrigel

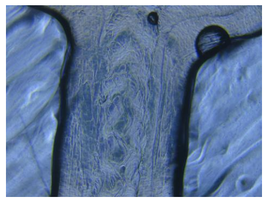
- Created cell-less bundles and bundles with cells
  - Cell-less bundles: observed bundle formation, bundle breakage, bundle dissolving, and change in bundle width

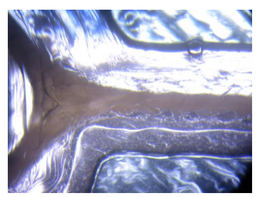




Cell-less GN Day 1 (Left) vs. Cell-less GN Day 15 (Right)

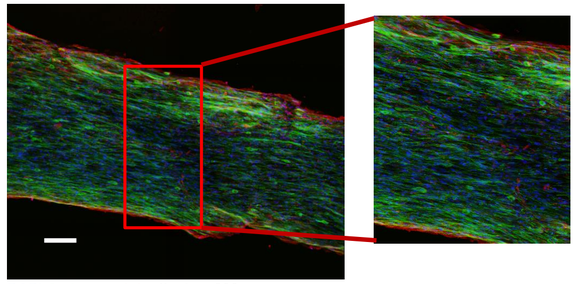
  - Bundles with cells: observed change in bundle width compared to cell-less bundles
    - Change in bundle width represents strain, which indicates bundle alignment
    - Alignment indicates cell development and native skeletal muscle structure





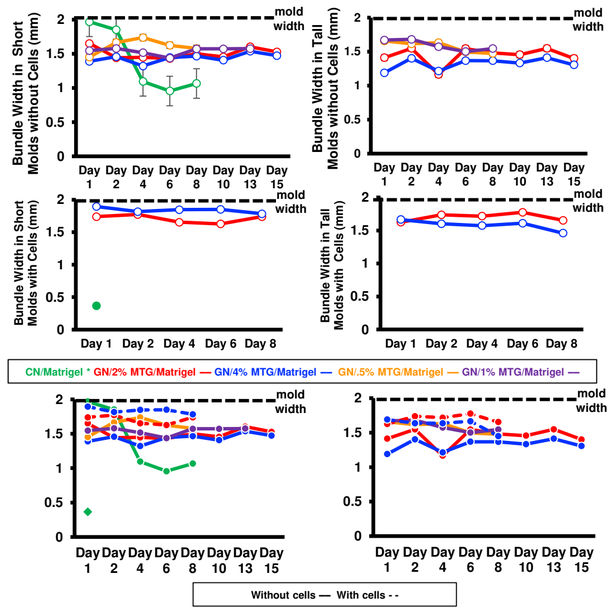
Cell-less CN Day 1 (Left) vs. CN with Cells Day 1 (Right)

Results



DAPI Actin α-actinin, Scale = 200 µm

Stained Collagen Bundle with Myotube Formation



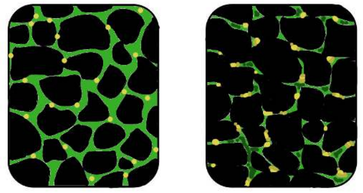
- Effects of Geometric confinement (height)
  - Little width variation between short and tall molds
  - Tall molds difficult to use and image
- Effects of ECM Composition
  - Collagen and Matrigel
    - Myotube formation occurred
    - Too weak– broke
  - GN, Matrigel, 2% and 4% MTG
    - Too strong, no bundle compaction
  - GN, Matrigel, .5% and 1% MTG
    - Cell-less bundles formed successfully but most dissolved after day 10; bundles with cells are currently in culture
  - GN and Matrigel
    - Did not form a stable bundle; melted away

Relation to my STEM coursework

Biomedical Engineering allows me to combine engineering techniques with biological knowledge and use them to create a more biomimetic experimental platform for skeletal muscle.

Future Steps

- Test GN with 0.5% MTG and 1% MTG with cells
- Create dystrophic tissue in ECM bundles using the results obtained from this experiment
- Use the muscle tissue bundles as an experimental platform for various drugs for muscular dystrophy



Normal

Muscular Dystrophy

Acknowledgements

- SHINE
  - Dr. Katie Mills
  - Emanuel Marquez
- Laboratory for Living Systems
  - Dr. Megan McCain
  - Nethika Ariyasinghe
  - Rest of McCain Lab
- SURE
  - Alyssa Arnheim

- Thank you for giving me such an amazing opportunity and providing me with newfound skills and knowledge!