3D Printed Optogenetic Skeletal Muscle-Powered Biological Machines

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Introduction: Cell-based soft robots, or "bio-bots," that can accomplish such objectives of robotics as sensing, storage and processing of signals, and a resultant response such as actuation can address many engineering challenges. Perhaps the most intuitive demonstration of a biological machine is one that can generate force and produce motion. Skeletal muscle, a natural actuator, is designed to efficiently generate force and, when coupled to an appropriate mechanical design, generate motion. We have developed a skeletal muscle based millimeter-scale bio-bot in which muscle cells seeded in a synthetic extracellular matrix apply traction forces to compact over time into a "strip" that is capable of contractility and force generation. Electrical and optical signals can drive contraction of the muscle and change the conformation of the hydrogel backbone. The resulting flexion causes the bio-bot to crawl across surfaces, demonstrating net locomotion.

Materials and Methods: Hydrogel bio-bots consisting of two capped pillars connected by a flexible backbone were fabricated using a stereolithographic 3D printer (SLA) (Fig 1A). The material properties of the patterned polymer, 20% (v/v) poly (ethylene glycol) diacrylate (PEGDA) 700 g/mol (Sigma-Aldrich) with 1% (v/v) Irgacure 2959 photoinitiator, were determined using a tensile test apparatus. C2C12 murine myoblasts transduced with pLenti2-EF1a-ChR2-tdTomato-WPRE plasmid to express a light-sensitive ion channel, Channelrhodopsin (ChR2), were cultured in DMEM (Corning) with 10% FBS (Sigma-Aldrich). Myoblasts were embedded in a cell-matrix solution containing 4-10 mg/ml fibrinogen (Sigma-Aldrich), 0.5-4 units/ml thrombin (Sigma Aldrich), and 0-30% Matrigel (BD Biosciences) and applied traction forces to compact into a strip of engineered muscle and cultured in media containing 10% horse serum (Sigma-Aldrich), 1 mg/ml 6-aminocaproic acid (Sigma-Aldirch), and 50 ng/mL human insulin-like growth factor-1 (Sigma-Aldrich) (Fig 1B, C). Custom-built electrical and optical stimulation apparatus were used to trigger contractility of bio-bot muscle strips.

Results and Discussion: Given the measured material properties of the hydrogel structure, we developed a relationship between the measured deflection of the hydrogel and the passive and active tension forces generated by the engineered skeletal muscle strip. Modeling and simulation was used to optimize geometric and material design parameters of the bio-bots to understand the force-stimulation frequency relationship, maximize deflection in response to generated force, and drive net locomotion across a substrate. Contractility of bio-bots was triggered via electrical stimulation, resulting in net locomotion of the bio-bot with a maximum velocity of ~156 μm s^{-1}, which is over 1.5 body lengths (BL) min^{-1} (Fig 1D). Contractility triggered via optical stimulation (490 nm) generated active tension actuation forces of the same magnitude as via electrical stimulation (Fig 1E). A custom-built stimulation apparatus that can precisely control the spatiotemporal optical excitation of muscle strips is currently being used to drive complex directional locomotive behaviors of optogenetic bio-bots (Fig 1F).

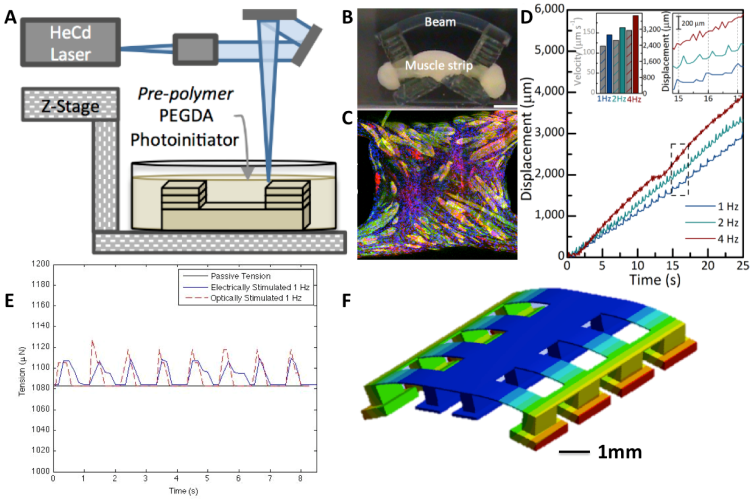

Figure 1: A) Fabrication of hydrogel structure via stereolithographic 3D printing; B) Engineered muscle strip integrated with flexible hydrogel backbone; C) Differentiated contractile myotubes shown via immunofluorescence staining; D) Force-stimulation frequency relationships showing net locomotion of bio-bots in response to electrical stimulation[1]; E) Optically stimulated actuation of engineered muscle strips; F) Simulation of complex biomimetic design and directional locomotive behavior enabled by precise spatiotemporal optogenetic stimulation.

Conclusions: We have developed a microfabricated soft robotic device capable of locomotion powered by the contraction of tissue engineered skeletal muscle. Contractility triggered by both electrical and optical stimulation has been demonstrated, setting the stage for developing machines capable of complex controlled actuation behaviors. This demonstration advances the goal of realizing forward-engineered integrated cellular machines and systems, which can have a myriad array of applications in high-throughput drug screening, programmable tissue engineering, noninvasive drug delivery, and biomimetic machine design.

Acknowledgements: Project funded by NSF Graduate Research Fellowship (Grant DGE-1144245), NSF IGERT (Grant 0965918), and NSF STC Emergent Behavior of Integrated Cellular Systems (Grant CBET-0939511).

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