Forward Engineering the Functionality of 3D Printed Skeletal Muscle-Powered Biological Machines

Caroline Cvetkovic[1], Meghan Ferrall-Fairbanks[2], Ritu Raman[3], Manu Platt[2], and Rashid Bashir[1]
[1]Department of Bioengineering, University of Illinois at Urbana-Champaign, Urbana IL 61801 [2]Wallace H. Coulter Department of Biological Engineering, Georgia Institute of Technology and Emory University, Atlanta GA 30332 [3]Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana IL 61801

Introduction: Biological building blocks – different cell types or tissues in an instructive environment – can be assembled in various ways to promote the emergence of the cells in a system with well-defined functionality, allowing for the realization of dynamic cellular machines with the ability to interface with the environment and other living systems[1]. We reported the design and fabrication of a skeletal muscle powered biological robot ('bio-bot') that could be reliably controlled and paced via applied external signaling[2] while permitting control over physical, mechanical, biological, and biochemical cues[3]. Skeletal muscle can interface with multiple other mammalian cell types, such as neurons and endothelial cells, making it an ideal actuator for producing force and locomotion in living systems, as well as a platform for drug screening probing heterotypic cell interactions.

Materials and Methods: An additive manufacturing technology (stereolithography) was used to fabricate the bio-bot skeleton from a pre-polymer solution of poly(ethylene glycol) diacrylate. A cell-matrix solution (fibrin, Matrigel™, and C2C12 murine myoblasts) compacted to form a fibrous muscle tissue around the bio-bot structure in the presence of insulin-like growth factor (IGF-1). Muscle tissues were electrically[2] or optically[4] stimulated with a custom-designed setup. For lifetime and protease studies, muscle strips were removed from the hydrogel structures and snap-frozen in liquid nitrogen at different time points in preparation for lysing and *in situ* gelatin zymography of cysteine cathepsin proteases and matrix metalloproteinases (MMPs). For neuromuscular junction studies, motor neurons were differentiated from mouse embryonic stem cells through the formation of embyroid bodies (EBs) before being added to muscle tissues differentiated in parallel.[5]

Results and Discussion: Maintaining consistent results becomes challenging when sustaining a living cellular system for weeks or months. Skeletal muscle must be preserved in a differentiated state at environmental conditions[3]. Moreover, the system is subject to degradation by cell-secreted proteases that can break down the mechanical integrity of the ECM in the tissue and thus eventually lead to device failure, despite the presence of protease inhibitors[2]. Muscle strips demonstrated tissue breakdown and loss of tension over time. Multiplex zymography was used to examine the activity of cell-secreted proteases (MMPs and cathepsins) in muscle strips. Preliminary results indicate that cathepsin L and MMP-2 activity increased over time, while MMP-9 activity decreased. Furthermore, electrical activity resulted in ~25% reduced expression of cystatin C, an endogenous cysteine protease inhibitor, corroborated qualitatively by decreased lifetime of bio-bots subjected to stimulation. These results indicated the need for exogenous factors to control muscle strips' lifetime and slow their degradation. Finally, the engineered hydrogel-muscle platform is ideal for introducing different cell types and biomaterials. The "biological parts" comprising the majority of higher-level systems have been assembled to date as individual components with one cell type. We hypothesized that a biological machine with greater functionality would require the integration and coordination of multiple cell types; i.e., moving from a homotypic cell cluster, such as a muscle strip, towards a heterotypic co-culture, such as a neuromuscular junction (NMJ)[1]. We demonstrate preliminary methods for integrating motor neuron-containing embryoid bodies (EBs) onto the skeletal muscle bio-bots, an important first step and a major challenge in the innervation of 3D muscle.

Conclusions: With a bio-fabricated system supporting the integration of a variety of scaffolding materials and cell types, our system can be utilized as platform for studies integrating different biomaterials and cell types. Additionally, this research allows for the understanding of how natural biological systems can be engineered to achieve new ends, such as drug screening in an autonomous platform and organ-on-chip mimics.

Acknowledgements: NSF STC Emergent Behavior of Integrated Cellular Systems (Grant CBET-0939511).

References: 1. Kamm, R. D. & Bashir, R. Creating Living Cellular Machines. *Ann. Biomed. Eng.* (2013). 2. Cvetkovic, C. *et al.* Three-dimensionally printed biological machines powered by skeletal muscle. *PNAS* **111,** 10125–10130 (2014). 3. Duffy, R. M. & Feinberg, A. W. Engineered skeletal muscle tissue for soft robotics: fabrication strategies, current applications, and future challenges. *WIREs Nanomed Nanobiotechnol* **6,** 178–195 (2014). 4. Raman, R. *et al.* Optogenetic skeletal muscle-powered adaptive biological machines. *PNAS* (2016). 5. Wichterle, H. & Peljto, M. in *Curr. Protoc. Stem Cell Biol.* Ch. 1 (2008).