Optimizing the Performance and Lifetime of Muscle-Powered Biological Machines

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Introduction: The combination of cells and tissues with soft robotics can enable the fabrication of biological machines with the ability to produce force and motion in response to controllable external signaling. The demand to respond to stimuli and exhibit controlled movement merit the use of skeletal muscle as a contractile power source for soft robots. The lack of significant spontaneous contractility, as well as the ability to interface with multiple other mammalian cell types, allows for more precise control over actuation via external input, and makes skeletal muscle an ideal platform for producing locomotion in 'living' cellular systems. We have developed a cell-extracellular matrix (ECM) muscle strip that, upon electrical stimulation, contracts with sufficient force to deform a 3D printed hydrogel structure and move the machine forward. However, cells within the muscle strip secrete proteases to remodel their immediate environment, leading to tissue breakdown and eventual device failure. Understanding matrix remodeling and characterizing the manner in which cells interact with their extracellular matrix and other cell types is necessary for machine design and preventing failure.

Materials and Methods: A Stereolithography Apparatus, a 3D printing-based bio-fabrication system, was used to fabricate the bio-bot structure with a pre-polymer solution of 20% poly(ethylene glycol) diacrylate of MW 700 g/mol and 0.5% Irgacure 2959 photoinitiator. A cell-matrix solution (fibrin, Matrigel™, and C2C12 murine myoblasts) compacted to form a solid muscle strip around the bio-bot structure in the presence of insulin-like growth factor (IGF-1) and with or without aminocaproic acid (ACA), a protease inhibitor (**Fig. 1A-B**). 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).

Results and Discussion: Applied electrical field stimulation triggered reproducible contraction of excitable myotubes in the muscle strip, which collectively generated sufficient force to deform the asymmetric hydrogel structure and produce net locomotion with a maximum velocity of 1.5 body lengths (BL) min^{-1} [1]. The addition of ACA increased the passive tension in the muscle strips as well as the lifetime before failure. Finally, we examined cysteine cathepsin proteases and matrix metalloproteinases (MMPs), two proteases involved in extracellular matrix remodeling and degradation, as model proteases (**Fig. 1C**).

Conclusions: We demonstrate an innovative and novel integration of advancements in biomaterials, tissue engineering, and 3D printing to forward engineer a controllable biological machine with a range of customizable and tunable functional properties. From a computational model, we plan to predict and control target substrate protein degradation for various combinations of cathepsins and use this in the development of long-lived biological machines. Building on this foundation will allow us to achieve higher-level functional control over biological machines by integrating multiple cell types with muscle.

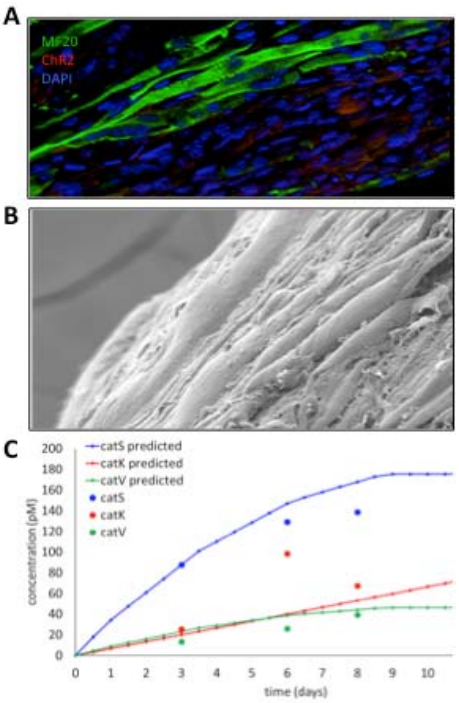

Figure 1. Immunostained (A) and electron scanning microscopy (B) images of the cell-matrix muscle strip. (C) Experimental zymography data predicted interactions between cathepsins K, S, V, and the collagen within the muscle strip.

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References: [1] C. Cvetkovic, R. Raman, et al. *PNAS* 111.28 (2014): 10125-10130