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Engineered Muscle Systems Having Individually Addressable Distributed Muscle Actuators Controlled by Optical Stimuli*

Devin Neal, H. Harry Asada, *Member, IEEE*

***Abstract*— A multi degree-of-freedom system using live skeletal muscles as actuators is presented. Millimeter-scale, optically excitable 3D skeletal muscle strips are created by culturing genetically coded precursory muscle cells that are activated with light: optogenetics. These muscle bio-actuators are networked together to create a distributed actuator system. Unlike traditional mechanical systems where fixed axis joints are rotated with electric motors, the new networked muscle bio-actuators can activate loads having no fixed joint. These types of loads include shoulders, the mouth, and the jaw. The optogenetic approach offers high spatiotemporal resolution for precise control of muscle activation, and opens up the possibility to activate hundreds of interconnected muscles in a spatiotemporally coordinated manner. In this work, we explore the design of robotic systems composed of multiple light-activated live muscular actuator units. We describe and compare massively parallel and highly serial/networked distributions of these building-block actuator units. We have built functional fundamental prototypes and present experimental results to demonstrate the feasibility of the construction of larger scale muscle systems.**

I. Introduction

Live cells and tissues cultured in microfabricated *in vitro* environments can be used as components for building robots [1-7]. Skeletal muscles, for example, can be actuators for powering a micro-robot or an artificial "animal". Muscle strips can be formed from their precursory cells, myoblasts, by guiding them through multi-stage myogenic process [8]. Muscle strips self-assembled within a mechanical structure have potential to activate a high degree of freedom micro-mechanism, which is a feature that is difficult to attain using current actuator technology [9]. Such live biological materials have the potential to be a significant paradigm changing technology in designing mechanical systems and extending their applications to broader fields.

Activation and control of skeletal muscles has been a challenging issue. Forming functional neuromuscular junctions remains a difficult task despite progress in recent years [11]. Electric stimuli using electrodes attached to skeletal muscles has been a standard technique [12]. However, electric stimulation is limited in performance due to several drawbacks. First, it is hard to secure electrodes to muscles that are contracting and are thus not stationary. Second, the spatial resolution of electrical stimulation is coarse since in wet environment it is hard to insulate a single muscle strip from others and, third, electrical stimulation is invasive, leading to rapid degradation of contraction.

Optogenetics is an emergent technology that genetically codes an excitable cell so that it becomes sensitive to an optical stimulus [13]. Recently, we have applied this optogenetic control technology to 3D engineered skeletal muscles microtissues [1]. Simply projecting a light beam on a muscle strip causes contraction of the muscle. No mechanical contact is involved; it allows for wireless control of each muscle strip. The temporal and spatial resolution of optical stimuli is significantly higher than electric stimulation, achieving millisecond-order temporal precision and 10 μm of spatial resolution. It is also less invasive than electric stimulation.

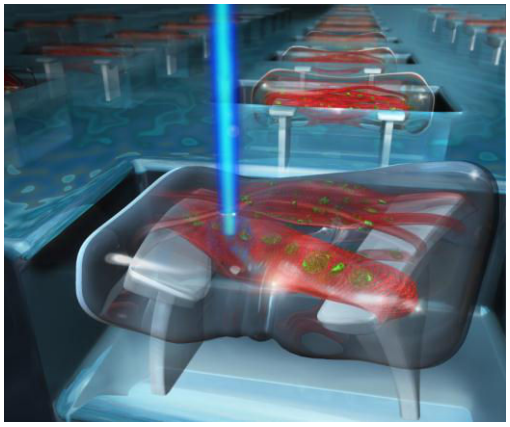

Figure 1. Optogenetic control of skeletal muscles [1]. Illumination of a skeletal muscle strip with blue light causes contraction of the muscle with high spatiotemporal resolution.

This optogenetic control of skeletal muscles opens up the possibility to activate hundreds of individual muscles in a manner that is spatiotemporally coordinated. This paper aims to explore novel actuation technology using *in vitro* skeletal muscles and their optogenetic control. Particularly unique is that live skeletal muscle actuators can activate degrees of freedom having no fixed axis. Examples of such systems include shoulder joints, the jaw, and the mouth. Fixed-axis-joints are conveniently activated by traditional actuators such as electromagnetic motors. However, those floating-axis-joints or floating loads are particularly difficult to activate. Live skeletal muscle actuators can conform to those floating

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D. Neal and H. H. Asada are with the d'Arbeloff Laboratory for Bio-Robotics in the Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge MA 02139 USA (phone: 617-256-9850; e-mail: dneal@mit.edu)

M. S. Sakar was with d'Arbeloff Laboratory for Bio-Robotics in the Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge MA 02139 USA. He is now with Institute of Robotics and Intelligent Systems, ETH Zurich, 8092-CH, Switzerland.

loads and create high DOF motion or even distributed DOF motion in a compact body. This high DOF, distributed actuation technology will be useful not only for medical devices, but also for creating and exploring entirely new robotics applications.

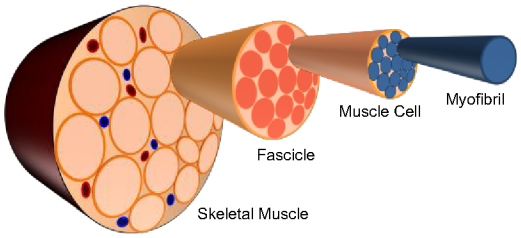


Figure 2. Skeletal muscle structure. Skeletal muscle contains many levels of hierarchical structure. The fascicle structure is what our building block muscle construct is inspired by.

II. Bio-Actuator Building Blocks

In order to construct a complex system of numerous degrees of freedom composed of multiple actuator units, we first established and characterized the fundamental building block unit. From this building block unit, numerous units may be combined in parallel and series producing redundant degrees of freedom and floating nodes.

The building block we have developed is similar to the fascicle level of naturally hierarchical muscle. Muscle is primarily composed of bundles of muscle fascicles as shown in Fig. 2. A muscle fascicle is a bundle of individual muscle cells that contract together. The form factor of a fascicle is significantly influenced by diffusion transport of chemicals. Essentially, it is an optimized building block that is robust against individual cell failure, and can be combined in a parallel arrangement allowing for scalability.

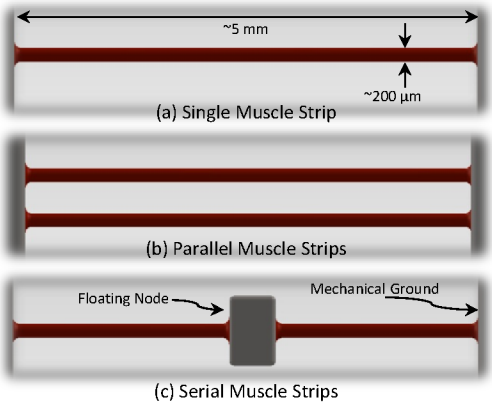


Figure 3. Fascicle-inspired building block muscle strip actuators (a). Basic scaling techniques include combining strips in (b) parallel and (c) series.

We have developed a muscle cell culturing technique for forming a fascicle-like muscle strip. See Fig.3(a). The self-assembling muscle strips can be arbitrary in length between 1 and 10 mm. Furthermore, we can vary nominal initial diameter between 250-500 µm. Maximal diameter is limited by chemical diffusion of nutrients and waste products into and out of the strip. Minimal diameter is limited by the size of contractile muscle cells within the strip. Based on data from our previous work [1], functional properties of the *in vitro* muscle tissues are estimated as passive force of 11 µN and optogenetically activated force 13 µN for a ~100 µm diameter muscle strip.

Beyond producing the building block actuator unit, our next objective was to produce two units in parallel (Fig. 3 (b)) and two units in series (Fig. 3 (c)). Note that the rectangular block in the middle is a floating node to which two muscle strip actuators are connected. These initial steps are vital to producing massively parallel and highly serial/networked units.

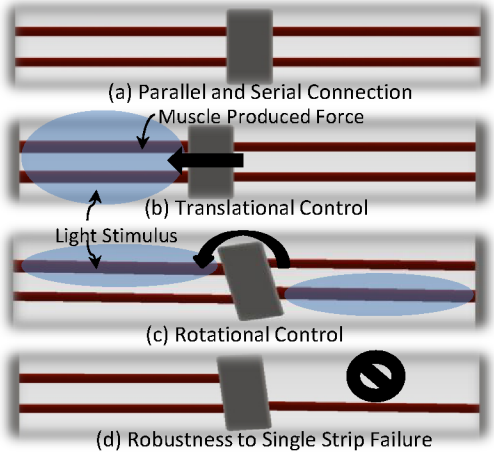


Figure 4. The ombination of parallel and serial connections of muscle strips (a). Such arrangement with light control enables translational (b), rotational (c), as well as failure robustness (d).

III. Scaling from Two Unit to Multi-Unit Systems

The next important milestone in producing a larger scale system is to combine parallel and series configurations into a single system.

Figure 4 illustrates a simple parallel and series arrangement: two parallel muscle strips in series with another two parallel muscle strips. This configuration combines the serial and parallel techniques of constructing a modular actuator and is sufficient proof that these techniques can be combined. Thus a system of arbitrary scale can be produced where further scaling is simply a matter of incorporating additional building block actuators in parallel or series.

Recall that each muscle strip is individually addressable with optogenetic stimulation. In the system shown in Fig. 4 (a) there are 4 controllable inputs. The central floating node has 2 controlled degrees of freedom; one translational, and one rotational. When 2 parallel muscle strips are contracted, the floating node translates as shown in Fig. 4 (b). When a pair of muscle strips located at diagonal positions is contracted, the floating node rotates as shown in Fig. 4 (c).

Note that the degrees of freedom of the floating node within the plane are three, while the number of individually addressable actuators is four. This redundancy yields robustness against individual unit failure. If a single unit fails, as shown in Fig. 4 (e), the control is still maintained over the 2 degrees of freedom of the floating node.

The above example is the simplest case of parallel and serial arrangement. This can be scaled-up to massively parallel arrangements as well as to massively serial/networked system.

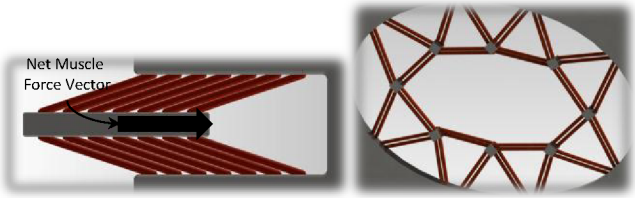
A. *Massively Parallel/Networked Systems*

A massively parallel system is composed of many individual units acting together to apply force to a single point as shown in Fig. 5 (a). Skeletal muscle examples from the human body include the pectoralis major (chest muscle), or the deltoid (shoulder muscle). Both of these are composed of a great number of individual fascicles in bundled structures that originate from different parts of the skeletal frame, and converge on a single area. In both of these examples, a small number of degrees of freedom are controlled, but the strength is tremendous due to their parallel architecture.

With the use of optogenetic control, individual muscle strips may be addressed in an ON-OFF manner. Because of the plurality of building block actuators applying force redundantly, the total applied force may be finely discretized to facilitate a smoother, more continuous, range of applicable force.

A massively serial or networked system is composed of many individual units acting independently to apply force to numerous floating nodes as shown in Fig 5 (b). An example from the human body is the mouth and lips. This structure contains numerous interconnected nodes that have numerous degrees of freedom as a system. The mouth is a highly controllable orifice with functions including a controllable nozzle, a fine manipulator, and effective social communication tool.

With the use of optogenetic control, controlled individual muscle strips grant control of each degree of freedom. This is in contrast to the parasitic electric field generation from electrical stimulation that may limit the specificity of control of individual units in a wet environment.



(a) Massively Parallel Bipennate Structure

(b) Highly Networked Mouth-Like Muscle System

Figure 5. Numerous parallel muscle strips concentrating force generation via a bipennate configuration (a). A mouth-inspired highly networked system of floating loads controlled by individually addressable muscle strips (b).

IV. Experimental Materials and Methods

C2C12 mouse myoblasts (Ameican Type Culture Collection) are cultured in growth medium (GM) containing DMEM (American Type Culture Collection), supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), 1% penicillin-streptomycin 100X (Invitrogen), and 0.1 mg/ml Normacin (Invivogen). These cells are transfected with optogenetic DNA as described in [1].

Cell/gel suspension is molded such that a uniform cylinder of controlled dimensions is suspended in medium anchored at both ends to the walls of a well. The cell/gel suspension contains GM with 20% Matrigel (BD Biociences), with C2C12 cells at a concentration of 15e6 cells/ml, fibrinogen (Sigma-Aldrich) at a concentration of 5 mg/ml, and 6-aminocaproic acid (Sigma-Aldrich) at a concentration of 0.5 mg/ml. The initial fluid surrounding the construct contains thrombin (Sigma-Aldrich) at a concentration of 5 U/ml. The wells are 1 to 6 mm in length, with walls made of polydimethylsiloxane (PDMS, Dow Corning) plasma bonded to a glass coverslip (VWR) which constitutes the bottom of the well. The length of the molded gels are nominally 1-6 mm, and the initial nominal diameters of the molded gels range from 250 μm to 500 μm.

After 24 hours, the initial fluid is changed to GM supplemented with 0.5 mg/ml 6-aminocaproic acid. This medium is replaced daily for 2 days, then switched to differentiation medium, DM, containing DMEM supplemented with 10% horse serum (Sigma-Aldrich) 1% pen-strep, 0.1 mg/ml Normacin, and 0.5 mg/ml 6-aminocaproic acid. DM is changed daily.

Optical stimulation is accomplished using one of two methods depending upon the degree of spatial specificity required. For less specific, broadcasts illumination, light from a mercury lamp is passed through a blue filter and controlled with a mechanical shutter. For greater spatial control, an Epson PowerLite 1965 LCD projector is used as the light source granting XGA resolution to the microscope field of view.

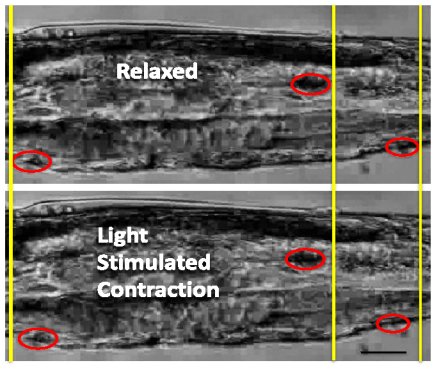


Figure 7. Individual building block actuator contracting with 4.3% measured strain. Vertical lines are constant horizontal position across both images. Ovals are identifiable marks within the tissue, that translate upon contraction. 50 μm scale bar.

V. Experimental Results

A. *Single Building-Block Muscle Actuator*

Within a single muscle strip, multinucleated contractile muscle cells form in parallel with others suspended in fibrin gel. Each muscle cell extends ~ 2 mm in length along the axis of the strip. These cells populate the full length of the fascicle-like muscle construct and have generated 4.3% strain.

B. *Serial and Parallel Prototypes*

Multiple building block actuators have been combined in series and parallel. Parallel muscle strips behave identically to individual muscle strips. Our initial prototype demonstrates that parallel arrangements diagramed in Fig. 3 (b) are possible as shown in Fig. 8 (a).

A serial connection between multiple muscle strips as diagramed in Fig. 3 (c) is demonstrated in functional prototypes as well (Fig. 8 (b)). In this figure, muscle actuators are anchored to a PDMS block that functions as a floating series node. Fibrin gel and non-functional cells occupy and anchor to the hollow cylinder in the center of the PDMS block. Light stimulation of the left or right muscle actuators moves the floating series node left or right, respectively.

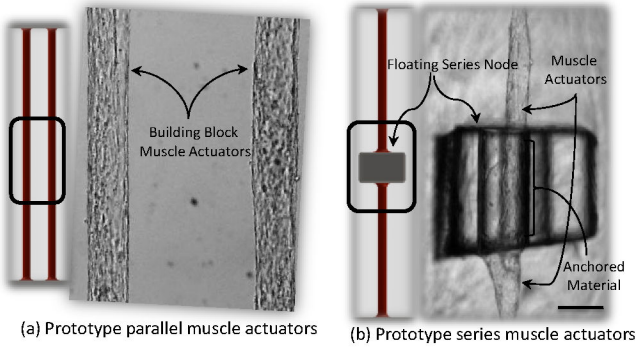


Figure 8. Prototype muscle actuator systems consisting of fascicle-like muscle actuators in parallel (a), and series (b). Scale bar is 350 μm.

C. *Multi-Unit Actuator System Prototypes*

We have produced functional serial/parallel systems using our building block actuators as components as shown in Fig. 9. The unforced system in Fig. 9 (a) contains four muscle actuators anchored two hollow cylinders in a transparent PDMS block.

The un-actuated young's modulus has been measured using our custom-built force probe to be 210 kPa as shown in Fig. 9 (b). In the loaded configuration, the geometric structure is bipennate further showing the potential concentration of generated forces.

VI. Conclusion

We have generated functional prototypes demonstrating the first initial steps necessary for producing large scale muscle actuators based on optimized building block muscle units. Our basic units produce force and displacement upon optical stimulation. These quantities along with material stiffness have been measured using custom force sensing equipment. These bio-actuators allow us to build unique distributed actuator system having no fixed axis; floating loads.

We are advancing our characterization and control techniques to keep up with the development of our actuator systems. We are developing a feedback controlled light stimulation system to track complex actuators and maintain tight control of their numerous degrees of freedom.

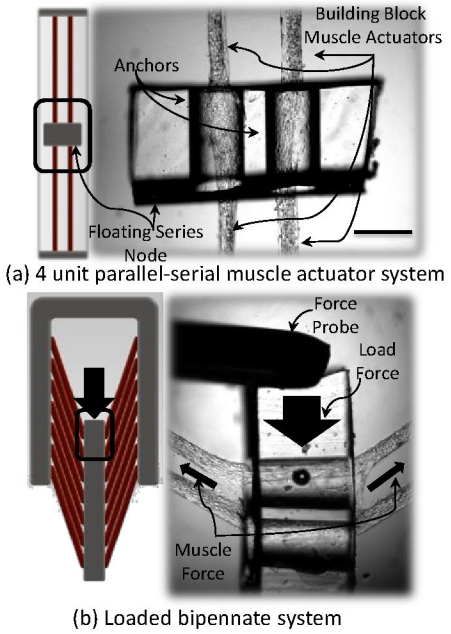


Figure 9. Prototype muscle parallel and series actuator system consisting of four fascicle-like muscle actuators and a PDMS node. Force displaces the central node downwards generating bipennate geometry (b). Scale bar is 350 μm.

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