Abstract— Optogenetics is an emerging new technology for controlling live cell function with light. Skeletal muscles are genetically coded to express light-sensitive proteins so that the cell’s behavior may be altered by illuminating a targeted portion of the cell. Optogenetic control provides a non-invasive, wireless, and fast control method with high spatiotemporal resolution. This paper presents the technology, experimental test, and potential applications of optogenetic control of skeletal muscles. The authors’ research team has recently succeeded in controlling the contraction of skeletal muscle tissues with light by using the light-sensitive protein Channelrhodopsin 2 (ChR2). Precursors of skeletal cells, myoblasts, are transfected with ChR2, creating ion channels on the cell membrane that conduct cations when exposed to blue light. Experiments show that targeted skeletal muscles are activated rapidly and precisely with high spatiotemporal resolution. Dynamics of optogenetically controlled skeletal muscles are characterized based on system identification. Two potential applications are addressed. One is muscle-on-a-chip drug screening for evaluating potential hazards of a drug on muscular function, and the other is multi-DOF robotic devices powered by bio-artificial muscles controlled with targeted light illumination.

I. INTRODUCTION

Bringing “control” to biological systems has been a challenge. Effective intervention and manipulation of live cells and tissues must be developed for guiding them toward a desired state or behaviors. Such interventions or controls must not deteriorate otherwise healthy, intact cells and tissues, yet they must be effective in altering the biological functionality. Cells have their own regulatory systems, which tend to attenuate the effect of exogenous input and intervention. It is a challenge to develop effective technology to intervene in the existing regulatory systems without breaking the necessary regulatory mechanisms. In the past, various techniques ranging from biochemical cues to mechanical, electrical, and magnetic means have been developed. Recent progress in microfluidic technology allows for precise delivery of chemical, mechanical, and electrical stimuli [1,2]. However, the responses of the cells and tissues to such cues are often highly variable and slow [3]. The lack of effective control means is a major bottleneck for applying control theory and techniques.

Optogenetic control is an emerging new technology to alter cell behaviors by illuminating a targeted portion of the cell [4,5]. See Fig. 1. Membrane-bound light-sensitive proteins, such as Channelrhodopsin 2, open or close ion channels so that surrounding ions, such as sodium and potassium ions, can enter or exit the cell rapidly and precisely. Compared to standard microfluidic technology, where various input cues are transported via media and bound to the cells and tissues through diffusion, optogenetic control allows for an order-of-magnitude faster response, and spatially accurate control that alters cell behaviors more directly. Furthermore, optogenetic control is known to be less invasive in specific application areas, including neural tissue stimulation [6] and cardiac cell stimulation [7]. Optogenetic control opens up new possibilities for bringing “control” to life sciences at the cell and tissue level.

This paper aims to introduce optogenetic control to the control community, focusing on the optical activation of skeletal muscles that the authors’ research group has recently accomplished [8]. Bio-artificial muscles cultured in an in vitro environment have the potential to be effective actuators that convert chemical energy directly to mechanical energy. Skeletal muscle can produce over 20% of strain with 200 kPa of stress; no actuator technology currently exists to create such a large strain and generated force in such a compact body.

Unlike cardiac muscles, skeletal muscles need exogenous stimuli for activating contraction. Traditional electrical stimulation needs to attach electrodes near or directly to muscle tissue, which causes a number of problems. First, mechanical attachment to moving soft tissues is difficult; a
second, electrical stimulus generates parasitic electric fields causing poor spatial resolution; and third, it is invasive and tends to deteriorate the contractile function. In contrast, optogenetic control allows us to wirelessly communicate with the muscle, stimulate with high spatiotemporal resolution, and the stimulation is reversible and non-invasive.

In the following, the basic principle of optogenetics using Channelrhodopsin 2 is described, followed by brief explanation of myogenesis, the process of forming a muscle tissue. Experimental results of optogenetic control of skeletal cells are then presented. Dynamics and key properties of muscle contraction in response to light illumination are analyzed by using a system identification technique. Finally potential applications of the optogenetic control of muscles and their impact on life sciences and medicine as well as engineering systems will be addressed.

**II. MYOGENESIS AND OPTOGENETICS**

Optogenetics consist of three major components [9]:

- Development of light-sensitive proteins that make conformational changes when exposed to a certain wavelength of light;
- Techniques to deliver the genes to targeted cells so that the light-sensitive proteins are expressed within the cell; and
- Optical instrumentation for illuminating a targeted cell or a specific portion of the cell with a light beam.

Several kinds of light-sensitive proteins have been discovered and applied to various fields of life sciences. Among others, Channelrhodopsin 2 (ChR2), discovered in a microbial Chlamydomonas reinhardtii, provided neuroscientists with a powerful tool for stimulating neuron cells at unprecedented spatiotemporal resolution [10]. Channelrhodopsins conduct cations and depolarize neurons upon illumination. ChR2 was a breakthrough since it can perform the two tasks of light sensation and ion flux induction in a single component encoded by a single gene. ChR2 is membrane-bound, and serves as a cation flux gate activated by blue light of 470 nm wavelength. See Fig. 2.

Delivering ChR2 into target cells is a critical process in order to make the cell light-sensitive. We have made skeletal cells light-sensitive using a non-viral strategy. We used a plasmid containing ChR2 and other key components including the genetic promoter CAG, and puromycin resistance, shown in Fig. 3, and had skeletal muscle cells transfected with the plasmid. Although the transfection rate was fairly low, we selected transfected cells using an antibiotic, puromycin. We kept selecting transfected cells during multiple passages. Fig. 4 shows transfected cells, where the green area indicates that the cell is transfected with ChR2. After five iterations, the cells stably express ChR2.

A skeletal muscle is formed through a multi-stage process, referred to as myogenesis. First, precursors of skeletal muscle cells, called myoblasts, are aligned. See Fig. 5 (a) and (b). They proliferate in a growth media. After the media is changed to a differentiation media, aligned myoblasts begin to adhere to each other, and then remove their interfacing partition; by resolving the membranes separating adjacent cells, they become a fused, multinucleated tube. See Fig. 5(c). The fused muscle strip, or myotube, further differentiates into a mature functional muscle, as it creates sarcomeric striations. Fig. 9 illustrates our experimental result, where clear sarcomeric striations are observed. Tension is created in the longitudinal direction, which promote the myotube to become a mature, functional cell. The muscle cell line we chose is C2C12 mouse cells, which have been applied broadly to bio-artificial muscle development.

**Figure 2.** Membrane-bound Channelrhodopsin 2 serves as an ion gate, inducing cations, e.g. Na⁺, when illuminated with blue light. The sodium ion mediates release of Ca²⁺ from a sarcoplasmic reticulum that leads to contraction of the skeletal muscle.

**Figure 3.** Plasmid containing ChR2 along with Green Fluorescent Protein (GFP), Puromycin, and CAG.

**Figure 4.** Mouse skeletal cells (C2C12) transfected with ChR2. The green areas indicate GFP and ChR2.
Figure 5. Myogenic process: Precursors of muscle cells called Myoblasts (a), alignment of myoblasts (b), and cell fusion to create a multinucleated myotubes (c).

III. EXPERIMENTAL EVALUATION

Two types of light-sensitive skeletal muscle assays were developed. One is a 2-dimensional muscle sheet, and the other is a 3-dimensional muscle tissue. Fig. 6 shows a 2D muscle sheet contracting in response to a blue light beam of 470 nm wavelength and 10 mW/mm² of intensity. A significant amount of contraction was observed of the myotube, as indicated by the displacement of markers on the muscle tube in Fig. 6.

![Figure 6. Experiment of optogenetic contraction control of skeletal muscles: the top panel with light off and the bottom panel with light on. The markers indicate that the muscle strip contracted approximately 10% with the light on.](image)

It is interesting to note that even when a small portion of the muscle strip was illuminated, the whole strip contracted. This implies that cations entering the cell propagated along the muscle strip to cause the whole strip to contract.

![Figure 7. Experiment of spatial resolution of optogenetic control: The three muscle strips, M1, M2, and M3 can be activated individually with light beams R1 and R3, or as a group with light beam R2 covering all the three muscle strips.](image)

One of the unique features of optical stimulation is high spatial resolution. Densely arrayed muscle strips can be activated individually by projecting a light beam at a targeted strip. Fig. 7 and Table 1 show experimental results demonstrating high spatial resolution. Three muscle strips, M1, M2, and M3, were activated individually, and as a group. When a small light beam of 20 μm in diameter, R1, was projected on M1, only M1 contracted and the other two did not. When a large beam covering all the three strips, R2, was projected, all of them contracted, as shown in the Table 1. As long as each muscle strip is isolated and the light beam is narrow enough to illuminate only the target strip, the targeted strip alone is selectively activated. This verifies the high spatial resolution of the optogenetic control on the order of 10 μm. Traditional electric stimulation is unable to activate densely arrayed muscle strips one by one.

**TABLE 1. Individual and group activations**

<table>
<thead>
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<th>R1 Is Exposed</th>
<th>R2 Is Exposed</th>
<th>R3 Is Exposed</th>
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<tr>
<td>M1 activity</td>
<td>contraction</td>
<td>no contraction</td>
<td>no contraction</td>
</tr>
<tr>
<td>M2 activity</td>
<td>no contraction</td>
<td>no contraction</td>
<td>no contraction</td>
</tr>
<tr>
<td>M3 activity</td>
<td>no contraction</td>
<td>no contraction</td>
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![Figure 8. Microfabricated tissue gauge for formation of 3D skeletal muscle microtissue.](image)

Although the 2D assay demonstrated a successful result of light-activated contraction, the myotubes were not connected to any load and their force characteristics could not be evaluated. Furthermore, the 2D assay significantly differs from the natural skeletal muscle, which is 3D. In an attempt to create in vitro muscle strips that are closer to in vivo muscle tissues we have developed a 3D muscle microtissue using MEMS soft lithography: Microfabricated Tissue Gauges (μTUGs), as illustrated in Fig. 8 [11]. Between two micro-posts made of PDMS, optogenetically coded C2C12 myoblasts mixed with collagen gel were seeded. In the micro-well culture they proliferated, differentiated, and became mature, forming a functional muscle tissue as evidenced by sarcomeric striation observed in the fused multinucleated myotubes. See Fig. 9. The 3D skeletal microtissue formed between the two micro-posts measured 800 μm x 100 μm x 200 μm in average.
Figure 9: Cross-striations characteristic to sarcomere formation. Stained for α-actinin.

Figure 10: Impulse response test of 3D skeletal muscle force generation using the microfabricated tissue gauge.

Impulse response tests were conducted for the optogenetically controlled 3D muscle microtissues. Fig. 10 shows one trial of experimental results of muscle force generation in response to a blue light impulses of 20 ms duration. The contraction and relaxation times were consistent among many muscle constructs, 190.9 ± 11.5 ms and 279.4 ± 16.8 ms, respectively. We have further quantified the response curve by using a system identification technique. An Auto-Regressive Moving-Average with Exogenous inputs (ARMAX) model could approximate the impulse response with a reasonable accuracy using a total of 5 parameters:

$$y(t) = -a_1y(t-1) - a_2y(t-2) + b_1u(t-1) + b_2u(t-2) + c_1e(t-1) + e(t)$$

This method is validated by using data from a single impulse response as a training set, and comparing it to a test set of data from multiple impulse responses as shown in Fig. 11.

Despite the dynamics of optically stimulated contraction not being LTI, the ARMAX model approximates well the impulse response of the system. There are two main sources of nonlinearity: 1) the dynamics of sarcomeric contraction, and 2) the dynamics of ion transportation. The nonlinearities arising from the sarcomeric dynamics arise from changes in tissue stiffness and damping as a function of displacement and velocity. Fortunately, these nonlinearities are insignificant enough to justify using the ARMAX model to simulate impulse response contraction dynamics as seen in Fig. 11.

The nonlinearities arising from optical stimulation reflect the dynamics of conformational changes to the membrane-bound ChR2, induction of cations, e.g. Na+, into the cell, and release of calcium ions Ca++ from the sarcoplasmic reticulum, as illustrated in Fig. 2. This is a threshold response of the cell; once a sufficient number of ions have crossed the cell membrane, a positive feedback response releases more ions within the cell. For this reason, the dynamic response of the cell may only be appropriately modeled as a series of impulse responses. Furthermore, this part of the system is nonlinear in that it does not scale with the magnitude of the optical stimulation; a characteristic impulse response is triggered upon optical stimulation of a minimum threshold intensity.

As of today, detailed mechanisms of light-activated muscle dynamics are unknown. Depending on the assay, the contraction magnitude varies. However, the consistent impulse response wave form, including contraction rise time and duration as well as the relaxation time constant and duration, indicates that the contraction dynamics are governed by fundamental biochemical properties of ChR2 conformation and ion dynamics inside the cell.

With this model we can simulate more complex time sequence inputs to generate arbitrary output responses such as tetanus-like contractions. Stimulation frequency is limited by the needed duration of optical pulse to initiate an impulse response.
response and the time required for the cell to recover. The model begins to break down as the stimulation pulse duration approaches the non-stimulated recovery time. Because the stimulation pulse needs to be ~20 ms, the model is only sufficient at frequencies less than ~20 Hz. Shown in Fig. 12, 10 Hz is sufficient to generate sustained force profiles similar to those achievable by native muscle. Native muscle tissue produces holding forces when receiving relatively high frequency control pulses from motor neurons. We have simulated similar behavior as shown in Fig. 12. Using twitch and tetanus contraction profiles generated by our model derived from data, we can design tissue constructs for particular applications and utilize this model for feedback control.

IV. APPLICATIONS

A. Muscle-on-a-Chip Drug Screenings

Drug screening is an increasingly important issue in the pharmaceutical industry, as the cost for new drug development soars and more stringent tests are required for all new drugs. Use of animals for drug screening must be reduced as well. Creating an in vitro testbed that mimics in vivo tissue environment is an effective solution to the drug screening problem [12,13].

The proposed skeletal muscle tissue μTUG can serve as a Muscle-on-a-Chip drug screening testbed for two objectives. One is to test effectiveness of drugs for muscle diseases, such as muscle dystrophies, sarcopenia of the elderly, and muscle disuse. The other is drug screening for off-target toxicity: testing for adverse effects of new drugs upon muscular functions. Both can be performed by dispensing a drug to the media of the muscle microtissue model and observing effects on muscular functions.

B. Many DOF Robotic Devices

In addition to utilization as a characterization platform, this actuation method may be used to produce robotic configurations not practically achievable with other methods. Muscle tissue is a type of “smart” material in that it provides mechanical structure, as well as actuator force. With the use of optical stimulation, individual muscle actuators may be individually controlled, and thus, numerous muscle actuators may be used in complex parallel and serial arrangements. For example, a mouth-like structure having numerous floating loads as shown in Fig. 14, is possible. With our actuation model, we can begin to design dynamic systems to be precisely controlled.

V. CONCLUSION

Optogenetic control of skeletal muscles and its applications are presented in this paper. Skeletal muscles can be activated with light by using membrane-bound Channelrhodopsin 2. Optogenetic control allows for non-contact, non-invasive, and wireless control of skeletal muscles with high spatiotemporal resolution. Impulse response of 3D muscle microtissue to optical stimulation was recorded and characterized as a low-order ARMAX model.
Two potential applications that exploit the unique features of optogenetically controlled skeletal muscle tissues are proposed. One is an all-optical, high throughput Muscle-on-a-Chip platform for drug screening, and the other is multi-DOF micro robotic systems using optogenetically controlled skeletal muscles as actuators. Both applications show great promise, opening up new possibilities of control of live skeletal muscles.

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