Development of an Electrical Current Stimulator for Activating Muscle Tissues in Biohybrid Machines

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***Abstract*—Soft and flexible robots are being developed as an alternative to traditional robotics. While they offer significant adaptability to the external environment, the integration of biological tissues as actuators presents several challenges. One of the critical challenges is the activation of the biological tissues to contract and move the robotic system's joints. In this paper, we discuss the development of an electrical current stimulator that can activate muscle tissues in soft robotic systems. The stimulator, realized with commercial components and designed using a stacked approach, combines a power supply board and an electrical stimulation front-end. A stacked-design approach allows to keep the device compact, with a total size of 59 mm x 28 mm x 25 mm (lxwxh). The stimulator, which has a power consumption of 1.3 W, can deliver up to 18 mA of stimulation current, and it has been verified to activate muscle tissues, demonstrating the ability to trigger muscle contraction by inducing up to 178 μN of contraction force.**

***Index Terms*—Current Stimulator, soft robotics, Biohybrid Machines, electrical stimulation, PCB, COTS, Muscle Tissue Activation;**

I. Introduction

Soft robots are attracting considerable interest for their unique mechanical properties, allowed by employing flexible, lightweight, and biocompatible materials. Biohybrid Machines (BHM) are a category of soft robots in which artificial materials are combined with living cells and tissues and employed as actuators to provide the movement of the robot [1], [2]. BHMs find their application in several fields, such as surgery and prosthetics, where typical soft robotic actuation methods (e.g., pneumatic) are not possible or inconvenient. Ricotti et al. identified two categories of Biohybrid actuators based on their scalability and use: 1) application-oriented actuators or non-scalable devices, based mainly on the use of bacteria, motile cells and devices based on explanted whole-muscle tissues; 2) general purpose actuators or scalable devices, based on the use of self-contractile tissues and engineered skeleton muscle tissues [2]. Although the first category shows slight dimensions, the second category guarantees larger actuation forces, even in the hundreds of μN range, which is suitable for soft robotic applications. In this sense, providing an effective actuation of tissue actuators becomes fundamental for the correct functionality of the soft robot [2]. Several approaches have been reported in the literature, including magnetic, optical, chemical, and electrical stimulation [3]. Among the forementioned excitation methods, the only two that guarantee rapid tissue response, the possibility of selective and parallel stimulation, and remote control are optical and electrical stimulation [3]. The main limitations of optical control lie in the need to genetically modify the biohybrid actuators to activate them through the light and a poor modulation of the contractile response. Moreover, light cannot penetrate the entire environment and is difficult to employ in several contexts, such as laparoscopic or surgical applications. Regarding electrical stimulation, the correct setting of the stimulation parameters is essential to prevent the formation of toxic reagents within the electrolyte solution of the bioactuator tissues [2], [3]. Electrical stimulation is often carried in voltage control mode [4], typically performed using bench instruments [5], [6]. However, this does not guarantee optimal control over the injected charge, making it difficult to maintain the electrode potential away from regions of corrosion that cause the formation of reagents harmful to the tissue [7]. In this regard, a programmable electrical current stimulation device is essential. In this paper, a circuit for the stimulation of biohybrid actuators through programmable current stimuli is reported.

The use of current stimulation allows precise control over the charge injected through the stimulation electrodes, allowing the variations in the potential of the electrodes to be effectively balanced and, consequently, limiting the onset of toxic reagents, which can affect the performance of the biohybrid actuators [8]. Although the development of electrical stimulators is well documented in sensory feedback and neural stimulation applications [9], [10], the development of ad hoc stimulators for soft robot applications still needs to be improved. The presented circuit is particularly conceived for the future stimulation of biohybrid actuators that can be integrated into endoscopic devices, such as catheters, for drug delivery. In the following, the design of the current stimulator is discussed, and its suitability as a programmable control system for 3D-bioengineered muscle cell actuators is demonstrated.

II. Materials and Methods

A. Stacked Electronic Stimulator

In order to keep dimensions feasible for a portable stimulator system, the developed circuit is configured as a 59 mm x 28 mm x 25 mm (lxwxh) stacked system, composed of

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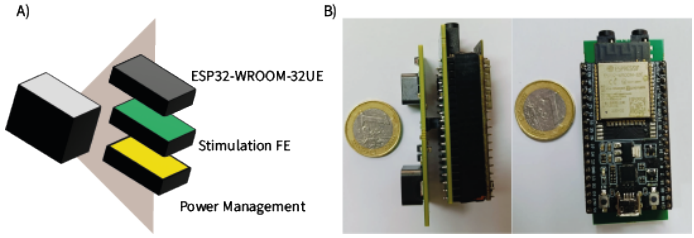

Fig. 1. The proposed stimulator. A) The stimulator has been designed using a stacked approach including two different custom PCBs for power supply and stimulation unit. B) The realized device in front and lateral view

two custom Printed Circuit Board (PCB) and a commercial microcontroller unit, as shown in Fig. 1. This approach separates the power management units and the stimulation Front-end (FE), resulting in a modular device that can be easily modified regarding voltage compliance and stimulation current characteristics. Each PCB is a 59 mm x 28 mm, two-layer board designed to be stackable (same size, same profile) on the commercial development microcontroller unit (MCU) board ESP32-WROOM-32UE, a dual-core 32-bit microprocessor with a built-in Bluetooth module and USB peripheral.

B. Power Management PCB

The power management unit PCB is located on the bottom of the stack. It contains two booster circuits based on the switching voltage regulators Analog Devices Inc. (Wilmington, MA, USA) LT8365, and a voltage inverting circuit based on Maxim Integrated (San Jose, CA, USA) MAX889RESA used to obtained the -5 V required by the stimulation FE. The LT8365 is a voltage converter employed in boosting or inverting voltages, guaranteeing a low quiescent current and low ripple on the output. It generates the ±90 V voltage compliance used in the stimulation output channel from the 5 V supply provided by the MCU.

C. Stimulation Front-end

The FE unit PCB is located on the middle of the stack, it takes the high voltage bipolar supply from the power management unit and the 5 V supply from the MCU. It hosts a 4 channels 16-bit bipolar digital-to-analog converter (DAC) ADC, Analog Devices LTC2664, connected to the four different stimulating channels. Each output channel is a voltage-programmable voltage-to-current converter that delivers the current to the load employing two amplifiers, a low voltage rail-to-rail amplifier Analog devices (Massachusetts, USA) AD8519ARTZ and high voltage Texas Instruments (Dallas, Texas, USA) OPA462IDDA, as reported in Fig. 2. The MCU uses the SPI protocol to program the DAC, allowing users to turn ON or OFF any four channels. The low voltage amplifier acts as a buffer to sink/source the stimulation current whose value is set by the DAC output voltage and resistor R_{stim}. The load requiring stimulation is connected in feedback to the high-voltage amplifier, ensuring the I_{stim} current flow as described in [11]:

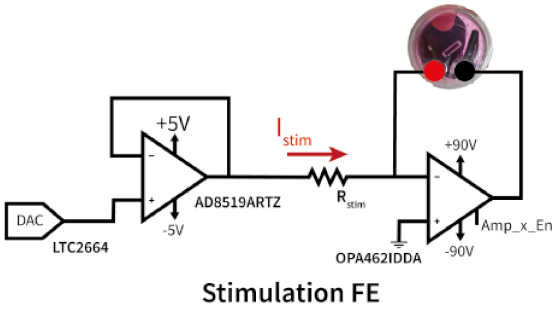

Fig. 2. Stimulation FE channel. The muscle tissue is connected in the amplifier feedback loop.

$$I_{stim} = \frac{V_{DAC}}{R_{stim}} \tag{1}$$

in this way, since the value of the applied current is independent from the impedance of the load, the total charge injected through monophasic or biphasic stimulation into the load is known:

$$Q = I_{stim} * PW \tag{2}$$

where Q is the injected charge, PW is the pulse width of the stimulation waveform. To ensure correct load stimulation, the OPA462IDDA amplifier adjusts its output voltage within the wide range provided by the ±90 V supply. In this way, it can guarantee stimulation currents of up to 18 mA for loads below 5 kΩ. The LTC2664 DAC is equipped with an internal reference voltage that can be programmed in the range of ±2.5 V and ±5 V, thus allowing 381 nA current resolution in the range of 0-12.5 mA and 762 nA from 12.5 mA to 18 mA. The DAC guarantees the generation of a maximum of 2ksps, thus allowing stimulation pattern shapes with frequencies higher than those required for tetanic contraction, typically lower than 100Hz [12], [13].

D. Electrical Stimulation

The full programmability of the proposed device allows for stimulation with arbitrary waveforms, including monophasic and biphasic stimuli. Continuous exposure to a sequence of monophasic pulses could potentially induce damage to the tissue due to irreversible chemical reactions near the electrodes, giving rise to toxic products for the tissues [14]. On the contrary, the employment of biphasic pulses may mitigate such risks, as the reverse electric field generated by the subsequent positive and negative phases of a biphasic pulse may impede the accumulation of cations/anions, thus preventing irreversible chemical reactions near electrodes [8], [14], [15].

E. Muscle cell Culture

The fabrication of the skeletal muscle-based actuators was done following the protocol reported in [16]. Briefly, C2C12 mouse myoblasts were purchased from ATCC (CRL-1772) and cultured in flasks until 80% confluence, at 37ºC and 5%

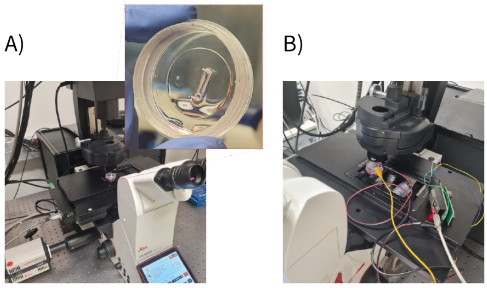

Fig. 3. Measurement Setup. A) The muscular tissue's bending induced by the stimulator was recorded using the Leica Thunder Imaging system. B) The proposed stimulator was connected to the tissue, while contraction was recorded using the Leica system.

CO2, in growth media (GM), which consist of high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (Gibco), 200 nM l-glutamine (Gibco), and 1% penicillin-streptomycin (Gibco). Once cells reached confluency, they were trypsinized and mixed with the hydrogel mix composed of 30% v/v of Matrigel (Corning), 4 U/mL of thrombin (Sigma-Aldrich), 4 mg/mL of fibrinogen (Sigma-Aldrich) and 10 million/ml of C2C12 cells. The 3D cell-laden hydrogel was casted in a circular mould made of PDMS, which was previously 3D printed, cured and sterilized under UV-light. Cells were cultured in the hydrogel mix for 2 days in GM supplemented with 1 mg/ml 6-aminocaproic acid (ACA, Sigma-Aldrich) to avoid hydrogel degradation by cell protease. After this culture time, cell-laden hydrogels were gently removed from the circular moulds and transferred to a flexible PDMS-based two-post system, where they were cultured in differentiation media (DM) until the day of experiments (day 10-11 of differentiation). DM consist of DMEM containing 10% horse serum (Gibco), 200 nM l-glutamine (Gibco), 1% penicillin-streptomycin (Gibco), 50 ng/ml IGF-1 (Sigma-Aldrich), and 1 mg/ml ACA.

F. Bending recording Contraction Force Measurement

The muscular tissue's bending induced by the stimulator was recorded using the Leica Thunder Imaging system, as reported in Fig. 3. The contraction force of the muscle actuators was analyzed following the protocol reported in [17]. Briefly, actuators were stimulated at 1 Hz and 15 mA with monophasic and biphasic stimulation waveforms. Contractile actuators exert a force towards the two-post system, generating a bending. The cell activity is video recorded and then analyzed using a homemade Python script that calculates the post displacement related to the cell contraction. Euler–Bernoulli's beam bending equation was used to estimate the contractile force from the post displacement:

$$P = \frac{3EI_z y(a)}{a^3} \tag{3}$$

where P is the applied force, E is the Young's modulus of the PDMS post (206 kPa), a is the height where the force is being measured (being the z-axis position of the tissue in the post under evaluation, a value that can be taken from the metadata of a z-stack taken from the tissue to the bottom of the post), I_z is the second moment of area of the post around the z-axis, and y(a) is the displacement of the post obtained with Python. The second moment of area (I_z) around the z-axis of the post is a constant and follows the following equation:

$$I_z = \frac{w^3 L}{12} \tag{4}$$

where w = 0.6 mm and L = 2 mm are the lateral dimensions of the post base.

III. Results and Discussion

The power consumption of the device has been measured to be 1.3W. The stimulator can properly deliver up to 18 mA in biphasic stimulation for a load with a total impedance of less than 5 kΩ. Fig. 4A shows the test using a resistive load of 4.6 kΩ. Stimulation on muscle tissue was delivered at 1 Hz with an amplitude of 15 mA, collecting 10 seconds of stimulation through the Leica Thunder Imager. Results are reported in Fig. 4B-C. The stimulation pulse width was initially set to 2 ms, showing the possibility of eliciting moderate muscle contraction forces (27.02 µN ± 2.56 µN for biphasic and 22.44 µN ± 2.94 µN for monophasic). The pulse width was then changed between 20 ms and 50 ms. In these conditions, the contraction force raised to 163.19 µN ± 4.41 µN and 178.12 µN ±3.58 µN for biphasic pulses at 20 ms and 50 ms, respectively, and of 130.27 µN ± 5.79 µN and 133.74 µN ± 5.74 µN, for monophasic 20 ms and 50 ms pulses, respectively. The results agree with what is indicated in [18] and show how symmetric biphasic stimulation can more remarkably recruit muscle fibers and induce a stronger muscle contraction. The use of a double polarity makes the biphasic symmetrical waveform the best solution for this application thanks to its ability to improve the contraction of muscle tissues and its ability to be safer than the monophasic waveform [8], [19], [20].

From the data, it can be seen that the stimulator can induce controlled and reproducible contractions in the biohybrid actuator. The programmability of the device allows the modulation of muscle contraction not only based on the amplitude of the stimulus but also through variations in pulse width and shape of the stimulus to explore new strategies for controlling the actuator.

IV. Conclusions

This paper reports a prototype of a current stimulator for 3D-bioengineered muscle tissue biohybrid actuators, suitable for the development of BHM-based endoscopic devices. The system shows programmability in current pulse features, low power consumption, and adaptability enabled by the modular design. The circuit has been fabricated and tested in a laboratory environment, showing the possibility of modulating the contraction force of the biohybrid actuators by playing on the current pulse width and amplitude. Although this result is promising, a future study on the device's ability to control the biohybrid actuators' tetanic contraction is still essential, an

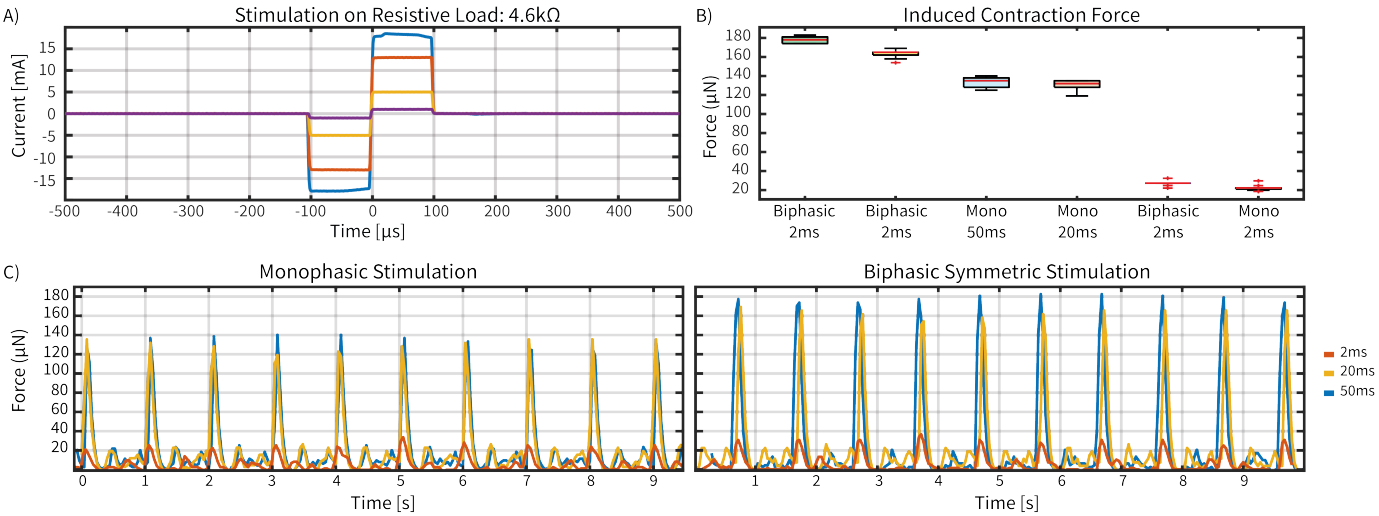

Fig. 4. A) Stimulation on a resistive load of 4.6 kΩ with biphasic symmetric waveform. B) Muscle tissue contraction force. Muscle tissue was stimulated using monophasic and biphasic stimulation at 1 Hz with a pulse width of 2 ms, 20 ms and 50 ms the peak contraction was collected and reported on the boxplot. C) Stimulation was performed during a session of 10 seconds at 1 Hz, 15 mA.

indispensable phenomenon for the direction of a robotic joint or a laparoscopic probe. Even though performances still need to be assessed in stimulating small BHM with miniaturized electrodes, the reported results are a step forward in developing a reliable control strategy for BHMs that can be adapted to different applications and robot dimensions.

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