Title

Steering Muscle-based Bio-syncretic Robot through Bionic Optimized Biped Mechanical Design

Running Title

Wirelessly Steerable Biped Bio-syncretic Robot

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Abstract

Bio-syncretic robots consisting of artificial structures and living muscle cells have attracted much attention due to their potential advantages, such as high drive efficiency, miniaturization, and compatibility. Motion controllability, as an important factor related to the main performance of bio-syncretic robots, has been explored in numerous studies. However, most of the existing bio-syncretic robots still face challenges related to the further development of steerable kinematic dexterity. In this paper, a bionic optimized biped fully soft bio-syncretic robot actuated by two muscle tissues and steered with a direction-controllable electric field generated by external circularly distributed multiple electrodes has been developed. The developed bio-syncretic robot could realize wirelessly steerable motion and effective transportation of microparticle cargo on artificial polystyrene and biological pork tripe surfaces. This study may provide an effective strategy for the development of bio-syncretic robots and other related studies, such as nonliving soft robot design and muscle tissue engineering.

Keywords: Soft Robots, Bio-syncretic Robots, Cell Actuation, Biohybrid Devices, Living Machines

Introduction

Living biological actuators, such as muscle cells, can transfer chemical energy into working mechanical motion with high efficiency, which is difficult to achieve for artificial actuators based on electromechanical systems within the same small scale and high flexibility.[1,2] Therefore, bio-syncretic robots actuated by living cells have significant potential advantages compared with electromechanical robots in some respects, such as miniaturization, high energy efficiency, and compatibility.[3-5] Due to their promising performance, bio-syncretic robots have drawn much attention and achieved rapid development.[6-12] To realize the efficient actuation motion of bio-syncretic robots, various living biological materials have been used as the actuators of robots, including cardiomyocytes,[13-20] skeletal muscle cells,[21-28] insect dorsal vessel (DV) tissues,[29-32] flagellate swimming microorganisms,[33-39] etc.

Among these living actuators, the skeletal muscles acting as the main power units of natural animals have the properties of a multisized hierarchical structure consisting of single cells and a contraction response to external stimulation. Therefore, they may exhibit potential suitability to serve as actuators of bio-syncretic robots of different sizes and have been widely used and studied in various bio-syncretic devices, such as walkers,[23-25,40] swimmers,[21,41] manipulators,[22,27,42] and motion structures.[43]

To realize the controllable motion of bio-syncretic robots, different robotic designs with various control methods have been attempted. Robots actuated by muscle tissue and stimulated by an electrical pulse – taking advantage of the intrinsic response of muscles to electrical stimulation – have been widely studied.[25,41,43,44] In these works, the robots were most often stimulated by a pair of fixed electrodes in the medium and realized controllable motion with different frequencies and deformations by responding to adjustable electrical stimulation parameters. However, due to the unidimensional motion structure design of the robot, as well as the poor spatial resolution of electrical stimulation from the fixed parallel electrodes for the biological actuators, the motion direction of the robots could hardly be dynamically and flexibly controlled.[5,6]

Optical pulse is another popular control scheme for bio-syncretic robots. In this method, a light source was used to simulate the biological cells to contract and actuate the robots.[21,23,24] Due to the multidimensional motion structure and selectivity of the photic stimulation, motion direction control of the robots could be realized.[45,46] However, these robots required the actuation cells to be biologically transfected with optogenetics and exposed to a simultaneously moving optical source. In this case, these requirements may restrict the kinematic dexterity of bio-syncretic robots.[5]

Recently, a stimulation method combining wireless energy transmission with electrical pulses and optical pulses has been developed to realize remote control of bio-syncretic robots.[47,48] This strategy can remedy the low spatial resolution of the electrical field and rigorous external follower system of optical stimulation. Nonetheless, the complex inner electromagnetic induction and stimulation devices may affect the overall flexibility and biocompatibility of bio-syncretic robots and limit their application to some degree.

In this paper, a fully soft and untethered biped bio-syncretic robot actuated by two muscle actuation tissues, which are selectively stimulated using a direction-controllable electric field generated by an external circularly distributed multiple electrodes (CDME) system, has been developed to realize wirelessly steerable motion. In addition, to promote the contractility of muscle actuation tissue, periodic uniform mechanical strain and rotary electrical pulse stimulations were adopted for the culture and differentiation of muscle cells. Moreover, to improve the bio-syncretic robot's kinetic performance, a bionic-optimized mechanical design method based on the equal elastic coefficient (EEC), which is inspired by the biological musculoskeletal structure, has been proposed for matching the robot structure and living muscle actuators. Finally, the fabricated fully soft bio-syncretic robots were controlled to demonstrate wirelessly steerable motion and effective transportation of a microparticle cargo on different artificial polystyrene and biological pork tripe surfaces. This work may not only effectively promote the steerable kinematic performance of bio-syncretic robots, but also be useful for other related fields, such as the design of nonliving soft robots, culture and control of muscle tissues, and the study of bio-syncretic artificial limbs composed of nonliving structures and living muscles.

Materials and Methods

Framework of the biped bio-syncretic robot

The demonstrated fully soft biped bio-syncretic robot consists of a soft robotic body, a pair of living actuation muscle tissues, and a remote electrical stimulation control system (Fig. 1). To realize the steerable motion of the robot, two asymmetric structures made of polydimethylsiloxane (PDMS) are used as the feet of the biped robot. The motion speed and direction of the robot are related to the motion of each foot, which is actuated by the corresponding controllable actuation tissue assembled on it.

To realize the selective control of each robot foot and then steer the bio-syncretic robot, the controllability strategy of the robot based on adjustable electric field directions was adopted. In short, the contractility of muscle tissue can be regulated by changing the stimulation included angle between the tissue axial and the electric field direction. As shown in our previous work,[49,50] the larger stimulation included angle would result in a smaller contractility of the living cell. Therefore, the robot's two feet were designed to be nonparallel to each other. Moreover, electrical stimulation in any direction could be dynamically generated and controlled with the CDME-based remote control system by regulating the potential of each electrode (Supplementary Fig. S1). As such, the two living actuation tissues on the two feet can be selectively stimulated to contract by the electrical pulses in different directions. Furthermore, when electrical stimulation was applied in the intermediately symmetrical direction between the two feet, the two muscle tissues performed almost the same contraction force under ideal conditions. Hence, the bio-syncretic robot could be steered by controlling the two actuation tissues by adjusting the direction of the applied electrical stimulation (Fig. 1). Moreover, because the electrical stimulation direction can be continuously adjusted, the biped robot could theoretically realize infinite turning motion with enough space and time.

Additionally, the deformations of the PDMS feet actuated by living muscle tissues are the fundamental factor of the kinematic performance of the bio-syncretic robot. Their deformations are related to the elastic coefficient of the PDMS structure. A structure that is too hard may restrain the contraction of muscle tissue, while too weak of a structure may reduce the resilience force of the feet and affect the robotic motion efficiency. The weak structure may also be disadvantageous for the actuation lifetime of the skeletal muscle tissue because the resistance of the structure is too weak to prevent the spontaneous atrophy of muscle tissue caused by the intrinsic traction force.[22,25,51] To improve the actuation performance of the bio-syncretic robot, the bionic optimized mechanical design of EEC has been adopted in this work, based on the biological musculoskeletal structure, which utilizes two skeletal muscles (biceps brachii and triceps brachii) with a similar elastic coefficient to provide suitable reactive force for each other (Fig. 1).[22] In this method, the elastic coefficient of each foot was regulated to be equal to that of the actuation tissue by adjusting the Young's modulus (E) of the PDMS material with various ratios of basic solution to curing agent. Thus, the muscle tissue would be matched with a proper elastic resistance to realize the effective actuation of the robot.

Fabrication of the bio-syncretic robot

Living muscle tissues are the actuation core of the bio-syncretic robot. In this work, three-dimensional (3D) muscle tissues composed of C2C12 cells, Matrigel, fibrinogen, thrombin, and medium were used.[25,49] For convenient assembly of the actuation tissues with the robotic soft structure, modular living muscle rings were fabricated with PDMS circular molds (Fig. 2). It has been demonstrated in our previous work that the circular mold and rotary electrical stimulation with CDME are beneficial for the differentiation of myoblasts to contractive myotubes.[52] In this respect, the circular mold could enable the tissue to generate uniform passive stress during culture and spontaneously shrink (Fig. 2B). The rotary electrical stimulation with CDME could realize even stimulation for the circular tissue with a rotation-direction uniform electric field by regulating the potential of each electrode (Fig. 2A and Supplementary Fig. S1). Furthermore, as shown in the previous work,[49] the CDME would generate less electrolysis for culture media and weaker electrical damage for cells than a pair of parallel electrodes usually used in most relative works, benefiting from the low threshold voltage for muscle contraction and little locally high current in a liquid culture environment. Therefore, the tissue cultured with a circular mold and CDME could be improved by uniform strain and noninvasive rotary electrical stimulation. Additionally, due to mechanical stimulation being able to promote the differentiation and maturation of muscle tissue,[53] the periodic mechanical uniform strain of the circular mold powered by a pneumatic device was used to further improve the development of muscle tissue (Fig. 2A). The equipment of the tissue culture system with electrical pulse and strain stimulation is described in the Supplementary Materials and shown in Fig. 2A and Supplementary Fig. S2. The detailed fabrication of the muscle actuation tissues is described in the Supplementary Materials.

The soft robotic body of the bio-syncretic robot was made of PDMS. As shown in our previous work,[49,54] the PDMS structures were designed by computer-aided design software SolidWorks and manufactured using a casting method. According to the design of geometric dimensions, polymethyl methacrylate (PMMA) negative molds were fabricated using a mini-type miller (Roland EGX-400; Japan). The uncured PDMS was poured into PMMA negative molds. After being cured at 70 °C for six hours, the PDMS structures were peeled off. Next, they were assembled to form the desired robots with the cultured living muscle tissues according to the design scheme. The detailed fabrication process of the monopodia and biped robots is described in the Supplementary Materials (Supplementary Fig. S3).

The remote electrical stimulation control system used in this work was similar to those proposed in our previous work (similar to the combination of the electrodes and petri dish in Supplementary Fig. S2B).[49,52] In short, 8 platinum electrodes (12 mm length, 10 mm wide, and 0.1 mm thickness) were evenly distributed around the perimeter of a 100 mm petri dish. Each electrode was connected to an independent channel of a multichannel electrical stimulator (Master-9; AMPI, Israel) controlled by a computer. To stimulate and steer the bio-syncretic robot, a parallel electric field in any desired direction could be generated dynamically by controlling the potential of each electrode in real time, as shown in Supplementary Fig. S1. The frequency, width, and start time of the pulse from each electrode were the same. The pulse amplitude (potential) of each electrode was proportional to the vertical distance between the electrode and the middle line of the petri dish, which was perpendicular to the desired electric field direction (Supplementary Fig. S1A).

Results and Discussion

Fabrication and characterization of the muscle actuation tissues

To demonstrate the validity of the proposed muscle tissue culture method, three experimental groups, including tissues cultured without any stimulation, those with rotary electrical stimulation, and those with both electrical and mechanical stimulation, were carried out. One week later in DM, all the tissues in the three groups had become compact and contractive muscle rings (Fig. 3A). The myosin heavy chain (MyHC) of the tissues in each group was measured by western blot. The MyHC and nucleus of each tissue were stained with Anti-Myosin Heavy Chain Alexa Fluor 488 and DAPI. Next, the tissues were imaged with a commercial laser scanning confocal microscope and measured by ImageJ software. The detailed methods for MyHC protein measurement by western blot, immunofluorescence staining and measurement of the muscle tissues are described in the Supplementary Materials. The results showed that among the three experimental groups, the muscle tissues cultured with both electrical and mechanical stimulations exhibited comprehensive advantages in terms of the MyHC and physical dimension of the myotubes compared with those in other groups (Fig. 3B-3H). MyHC is a type of sarcomere contractile protein and a key marker of myotube differentiation,[55] and the width of myotubes is related to muscle maturation.[49] These results mean that the tissues cultured by the proposed method possess advantages in differentiation from myoblasts to contractive myotubes. Therefore, in this work, tissues cultured in circular molds with periodic electrical and mechanical stimulation were used to actuate robots by assembling them with PDMS feet structures.

Optimization design of the soft robot structure

To promote the kinetic performance of the robot driven by two feet, each foot structure has been optimized with the bionic design method of EEC. In this method, the elastic coefficient of the PDMS structure was regulated to be equal to that of the living actuation tissue to imitate the interaction of the biceps brachii and triceps brachii, which have a similar elastic coefficient.[22] Their elastic coefficients were calculated by finite element simulation (FES) based on their physical dimensions and Young's modulus (E), which were obtained by atomic force microscopy (AFM). The detailed processes of the equivalent elastic coefficient calculation by the FES and Young's modulus measurement by AFM are described in the Supplementary Materials.

For the muscle tissues, the physical dimensions were measured by a microscope. Their E obtained by AFM was 7.20±2.56 kPa (Fig. 4A). Based on the dimension and stiffness of the muscle tissues, their elastic coefficients were obtained by FES. The result conformed with a Gaussian distribution, and the value was 1.53±0.54 N/m (Fig. 4C, horizontal dotted dashed line). Regarding the PDMS structure, to realize the desired elastic coefficient equal to that of the muscle tissues, PDMS mixtures with different ratios (crosslinker to prepolymer) were solidified under the same heating conditions (at 70 °C for six hours) and measured with AFM (Fig. 4B). After that, according to the geometric dimension of the robot foot and the E of solidified PDMS materials with different ratios, the elastic coefficients of the feet structures with different materials were calculated by FES (Fig. 4C, dotted curve). A suitable ratio of PDMS was obtained at the intersection of the elastic coefficient result lines of the muscle tissues and PDMS feet. Therefore, the feet structures of the bio-syncretic robots in this work were made of PDMS with a ratio of 0.08 (Fig. 4C).

To demonstrate the walking feasibility of the proposed biped robot, first, the monopodia robot composed of a PDMS asymmetric structure (foot structure) and an actuation tissue was stimulated to walk by the proposed control system (Fig. 4D and Supplementary Movie S1). To confirm the optimization design of EEC for the bio-syncretic robot foot, the motion speed of the monopodia robots made of different PDMS materials with ratios (crosslinker to prepolymer) of 0.04, 0.08, and 0.12 and actuated by the same tissues were measured. The result of the motion response of different monopodia robots to the same electrical pulse (2 Hz stimulation frequency; 2.0 V/cm voltage amplitude; 20 ms pulse width) showed that the robot with the PDMS structure made of the optimally designed material ratio (0.08) performed the fastest motion compared with the others (Fig. 4E).

When the robot (with a crosslinker ratio of 0.08) was stimulated with a parallel electrical pulse of 2 Hz and different voltages, the motion speed increased at first and then remained constant with increasing pulse amplitude (Fig. 4F). This result may be due to recruitment,[56,57] which means that an increasing number of myotubes would generate contractility with increasing pulse voltage. However, when the voltage was higher than the threshold of all the myotubes in the actuation tissues, the contractility of the tissues would increase no further. Hence, the step and speed of the robot showed an increase at first and became steady later with increasing stimulation amplitude and uniform frequency. It should be noted that excessive electrical stimulation may cause electrochemical damage to cells and aggravate electrolysis of the culture medium, resulting in harmful substances or gases for cells.[49,56,58] Therefore, the amplitude of the stimulation voltage should be limited to a certain range.

Furthermore, when the robot was stimulated with a parallel electrical pulse of 2 V/cm and a different frequency, the speed of the robot increased at first and then decreased later with increasing stimulation frequency (Fig. 4G). This result could be attributed to the muscle dynamic actuation amplitude decreasing with increasing stimulation frequency.[52] With low-frequency stimulation, the robot's comprehensive actuation efficiency of the step size and frequency would increase with the stimulation frequency. However, with high-frequency stimulation, the step size of the robot decreases significantly. Therefore, the comprehensive actuation efficiency might decrease with increasing stimulation frequency.

Additionally, as mentioned before, the muscle tissues stimulated with different-directional electrical pulses had different contractility.[49] Therefore, the stimulation method based on different-directional electrical stimulations was adopted to control the motion speed of the monopodial robot. The results showed that when the amplitude and frequency of the electrical pulses were fixed at 2 V/cm and 2 Hz, the motion speed of the monopodia robot decreased with increasing stimulation angle between the electric field and robot axis. The robot showed a maximum and minimum average speed when the electric field was parallel and perpendicular to the robot, respectively (Fig. 4H).

Due to the response property of the monopodial robot to the electric field direction, the biped robot with the larger included angle between the robot's two feet would exhibit better direction controllability. Moreover, according to the principle of projection geometry, the larger included angle between the two feet would result in less motion efficiency (effective relative speed) (Fig. 4I). Therefore, to balance the controllability and motion speed of the biped bio-syncretic robot, the two feet should be designed with a minimum angle, which would induce a clear difference in the monopodial robot actuation. The response result of the monopodial robot to different directional electrical stimulation showed that the speed of the same monopodial robot induced by the stimulation included angles of 0°, 15°, 30°, and 45° had no significant difference. Conversely, the speed induced by the stimulation included angles of 60°, 75°, and 90° had a significant difference with that of 0° (Fig. 4H). Therefore, to realize reliable direction controllability of the biped bio-syncretic robot, in this work, the two feet were designed with an included angle of 60° (Fig. 4J). Furthermore, the robot with this included angle would possess an effective relative speed of 0.87, according to the principle of projection geometry (Fig. 4I).

Realization and steering of the biped bio-syncretic robot

To demonstrate the steerable motion performance of the developed biped bio-syncretic robot, the robot was placed in a fabricated control device filled with DM (Fig. 5A). Based on the monopodia robot experiment, each foot of the robot could be selectively controlled to actuate by regulating the electric field direction with CDME. When an electrical pulse was in the angular bisector of the two feet of the biped robot, the two feet should be actuated with a similar contractive force. When the electrical pulse direction was close to one of the two feet, the actuation force of the selected tissue increased, while at the same time, the force of the other tissue decreased. Therefore, the biped robot could be steered to execute straight and turning motions by regulating the direction of the electrical pulse.

In the steering process, according to the motion results of the monopodial robot stimulated with different electrical stimulations, an electrical pulse with 2 V/cm amplitude, 2 Hz frequency, 20 ms width, and various directions generated by CDME was used to stimulate the biped robot to walk. The results showed that the robot with a 60° included angle of the two feet could be steered to demonstrate a whole motion behavior containing going straight, turning right and turning left with a turning scope of 12.563°, an average angular velocity of 0.1276°/s and a maximum instantaneous angular velocity of 0.3152°/s (Fig. 5B and Supplementary Movie S2). Meanwhile, the robots with the feet included angle of less than 60° (30° and 45°) could hardly be steered. This result was in line with the property of the monopodia robot, where the stimulation angle of more than or equal to 60° between the robot axis and electric field would induce a significantly different motion speed compared with those induced by a parallel electric field with the robot axis (Fig. 4H). Furthermore, the motion speeds of the different biped robots with feet included angles of 30°, 45°, and 60° were measured. Their average speeds decreased with increasing feet included angles (Fig. 5E), which was in line with the theoretical relationship between the effective relative speed and the feet included angle of a robot (Fig. 4I).

Additionally, to demonstrate the motion ability of the developed biped bio-syncretic robots for different environments, the robot with a 60° feet included angle was stimulated to walk on the internal surfaces of pork tripe by the same electrical pulse used above (Fig. 5C). Furthermore, a simple application of pushing a microparticle by the robot was demonstrated on the biological surface (Fig. 5D and Supplementary Movie S3). The motion speed of the robot on the pork tripe was measured and compared with that on a petri dish surface. The results showed that the robot on the pork tripe surface exhibited a lower average speed than that on the petri dish surface. Moreover, the average speed was decreased by the load (Fig. 5E). This decrease in robot speed may be contributed to the higher viscosity, unevenness and frictional force (the measured frictional coefficients were 2.365 for the PDMS-pork tripe contact surface and 2.064 for the PDMS-polystyrene petri dish contact surface) of the pork tripe compared with those of the petri dish, as well as the resistance of the microparticle. However, the motion and pushing action of the robot on the internal surfaces of the pork tripe may show that the robot could possess the potential to transport a load on a biological surface in a liquid environment.

Conclusions

In this paper, to improve the kinematic dexterity of bio-syncretic robots actuated by living muscle cells, a bionic optimized biped robot actuated by two muscle tissues and wirelessly controlled by a directional regulatable electric field with CDME has been designed. The fabricated fully soft robot has been successfully controlled to execute steerable motion, taking advantage of the controllable different contractions of the two actuation muscle tissues assembled on each foot of the biped robot under the direction controllable electrical stimulation of the CDME control system. Additionally, the muscle tissues fabricated with the proposed electrical pulse and mechanical strain stimulations have been confirmed to show higher differentiation efficiency from myoblasts to contractive myotubes compared with the tissues in other experimental groups. Although the culture result of the skeletal muscle tissues has not been compared with that of other works due to the multiple influence factors apart from physical stimulations, such as the cell type, supporting material and culture process, the experiment with single variable of physical stimulation method in this work could demonstrate the advantages of the proposed electrical pulse and mechanical strain stimulations for skeletal muscle tissues culture. Moreover, the robot built with the proposed bionic-optimized mechanical design of EEC has been approved to possess excellent motion performance compared with other fabricated robots. This work may address some of the deficiencies that are found in most existing bio-syncretic robots, particularly regarding steerable kinematic dexterity. Moreover, to realize further powerful steerable motion on a complex and undulating substrate, the walking and carrying capacity of the proposed fully soft bio-syncretic robots may be enhanced by optimizing the asymmetry of each foot and assembling larger or more actuation tissues on the robot structure. In addition, the integration of sensing technologies, such as vision, may promote the automatic motion control of the developed bio-syncretic robots in future work.

Although the existing related studies on bio-syncretic robots actuated by living biological materials have made considerable progress, they are still incompetent regarding complex practical applications, such as in clinics, medicine, and engineering. Hence, further in-depth development is necessary for various research fields related to bio-syncretic robots. For example, with the development of 3D production technology, multidimensional soft electrodes and biological actuation tissues could be designed and used in bio-syncretic robots. More complex living actuation elements might also be selectively cooperatively controlled to realize multipotent bio-syncretic robots, which could execute skillful actions, such as clamping and cutting, for therapy in vivo. Additionally, bio-syncretic sensing and intelligence based on living cells might be integrated into the bio-syncretic robot to realize a closed loop of sensing–intelligence–actuation to drive the development of bio-syncretic robots from controllable actuation to intelligent behaviors.[59,60]

This work has developed a bio-syncretic robot with bionic optimized biped mechanical design and wireless steering strategy, which would further promote the steerable kinematic performance of fully soft robots actuated by living cells. Moreover, this paper may be used as a useful reference for other related studies, including nonliving soft robots, muscle tissue engineering, and bio-syncretic artificial limbs composed of nonliving structures and living muscles.

Data and Materials Availability

The authors declare that all relevant data supporting the results of this study are available either within the main text and/or in the Supplementary Materials. Additional data related to this paper are available from the corresponding author upon request.

Authors' Contributions

L.L. and N.X. directed the project. L.L. and C.Z. designed the experiments. C.Z., L.Y., and W.T. designed and fabricated the bio-syncretic robot. C.Z., R.W., and W.W. designed and fabricated the robotic control system. W.T., R.W., and F.W. simulated and analyzed the electric field. C.Z. and H.F. finished the statistical analysis. C.Z., L.Y., W.W., and L.L. performed the other experiments. C.Z., L.L., and N.X. wrote the manuscript with input from all the authors.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Materials

Supplementary Movie S1

Supplementary Movie S2

Supplementary Movie S3

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