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Perspective

Biohybrid Actuators for Soft Robotics: Challenges in Scaling Up

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Abstract: Living systems have evolved to survive in a wide range of environments and safely interact with other objects and organisms. Thus, living systems have been the source of inspiration for many researchers looking to apply their mechanics and unique characteristics in engineering robotics. Moving beyond bioinspiration, biohybrid actuators, with compliance and self-healing capabilities enabled by living cells or tissue interfaced with artificial structures, have drawn great interest as ways to address challenges in soft robotics, and in particular have seen success in small-scale robotic actuation. However, macro-scale biohybrid actuators beyond the centimeter scale currently face many practical obstacles. In this perspective, we discuss the challenges in scaling up biohybrid actuators and the path to realize large-scale biohybrid soft robotics.

Keywords: biohybrid actuators; soft robotics; biohybrid robots; tissue engineering

1. Introduction

Soft robotics, which has emerged as a rapidly growing research field in robotics in the last decade, now seeks to explore more organism-like characteristics beyond mimicking mechanics of natural locomotion by also capturing life-like properties in designing actuators [1,2]. Soft actuation may be the simplest but most critical function that drives soft-bodied robots to safely interact with other objects. To capture the soft actuation capabilities seen in animals, the majority of research in the soft robotics community employs synthetic-compliant materials for bioinspired and biomedical applications [1,3–5]. Soft actuators have been developed using a wide range of materials and techniques including pneumatics [6], shape-memory alloys [7] and shape-memory polymers [8], dielectric elastomers [9]. Although a complete review of soft actuator technologies is beyond the scope of this perspective, recent reviews on this subject are available [5,10,11]. Using these approaches, great advances have been made in the field of soft actuation. However, synthetic soft actuators fail to capture all the capabilities of living muscle in a single platform. Whereas each mode of soft actuation has its advantages [12], each similarly has its drawbacks. For example, there is the need for peripheral components or geometric limitations due to pressures in pneumatics; shape-memory materials require high temperatures, voltages, or currents; and thermo-responsive materials have slow actuation cycles. In contrast, living muscle, while not exceptional at any specific actuator metric, provides effective capabilities across all metrics [12]. To harness these abilities, such as self-healing, energy extraction from the environment, compliance, and differential force production, the field of biohybrid robotics seeks to use living muscle directly as an actuator in robotic systems [13–15].

By merging soft robotics and tissue engineering, technical challenges in actuation have been addressed across different length scales [2]. Progress towards living biohybrid actuators as an emerging class of alternative soft actuation have been conceptually demonstrated by recent works from biohybrid robotics at small-scales. Such small-scale robots, some as small as single-cell scale like bacteria, have been shown to be capable of manipulating micro-particles or structures for applications in targeted drug delivery [14]. Furthermore, biohybrid robots from the millimeter to centimeter scale have been demonstrated capable of crawling, rolling, swimming, and even simple object manipulation. These proof of concepts in small-scale robotics show promise for the field of biohybrid robotics [16–18]. However, although living tissue provides a renewable, environmentally friendly actuation solution, macro-scale biohybrid actuators beyond the centimeter scale have not been extensively explored by researchers due to many challenges in fabrication, vascularization, tissue maintenance, and tissue mechanical properties.

Even though many actuation approaches exist to drive robotic structures over the centimeter scale, such methods, including pneumatic, thermal and dielectric elastomer actuator (DEA) technologies, each face their own technical drawbacks in power consumption, response time, and output force [3]. Nevertheless, efforts in scaling up biohybrid actuators to build macroscopic biohybrid soft robots could transform soft robotics by providing actuation approaches that are renewable, environmentally friendly, adaptable, self-healing, and compliant (Figure 1A). Scaling up biohybrid actuators will allow expansion of the technology to macro-scale robotic applications and enable new design and control approaches for soft robotics. While most actuators rely on external power sources, biohybrid actuators could potentially extract energy from their environment, allowing tether-free devices to be designed without the need for heavy battery packs. The ability of biohybrid actuators to adapt to mechanical loading and self-heal will allow robotic devices to adjust to their environment and restore function after damage [1,2,19]. The ability to modulate actuator force through recruitment of additional muscle fibers will allow control approaches to tune the force of individual actuators in a compact package. By leveraging these capabilities, macro-scale biohybrid robots will enable safe interaction with a wide range of organisms, adaptation to mechanical loading and environmental conditions, and the possibility of energy extraction directly from the environment. By addressing the challenges highlighted here, biohybrid actuators will become another tool in the soft robotic toolkit. Addressing the current challenges in vascularization, interfaces, and activation will improve the accessibility of biohybrid technologies to roboticists across scales.

Although most biohybrid robot research has focuses on small length scales, biohybrid actuator-based soft robots have been developed across different length scales from microorganisms to microbots and millibots (Figure 1B). Key challenges that currently hinder scaling biohybrid actuators beyond these size ranges include (1) vascularization, (2) biotic/abiotic interfacial interactions, and (3) innervation and control methods [1,21,22]. The purpose of this perspective article is to highlight these challenges and discuss recent scientific and technological efforts that may enable scaling up biohybrid actuators. By addressing these challenges, biohybrid robotics researchers will take significant steps towards the creation of macro-scale biohybrid actuators for use in soft robotic devices with the goal of eventually matching the performance of natural muscles.

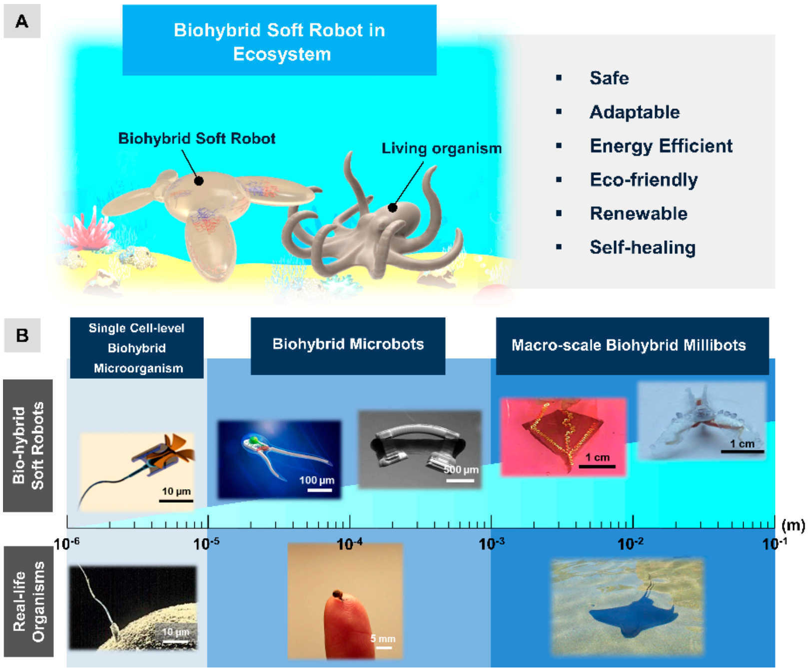

Figure 1. (**A**) Illustration of a biohybrid soft robot safely interacting with a living organism in an ecosystem (left) and a list of advantages of biohybrid soft robotics (right). (**B**) Increasing sizes for biohybrid soft robots from sub-micron scale to centimeter scale (top) and corresponding inspired real-life organisms (bottom). Biohybrid Soft Robotics: sperm-bot, [14] © Copyright 2017, ACS. Neuromuscular motile-bot, [16] © Copyright 2019, PNAS. Bio-bot, [17] © Copyright 2016, PNAS. Bat-ray inspired soft robot, [13] © Copyright 2018, Wiley. Living-machine with ganglia [20] © Copyright 2016, Springer Nature. Photo by Dr. Andrew Horchler. Real-life Organisms: sperm cell, sperm and egg fusing, public domain. Tiny snail on a finger-tip, "Tiny snail." by yomo_13 is licensed under CC BY 2.0. Bat-ray, "File:Bat Ray—Aquarium of the Pacific.jpg" by Nandaro is licensed under CC BY-SA 3.0.

2. Challenges in Scaling Up Biohybrid Actuators

Throughout this perspective article, we will discuss three key challenges for scaling up biohybrid actuators for future robotic applications. Although these are not the only challenges facing biohybrid robotics, we believe that addressing these key areas will substantially advance biohybrid actuation. This section discusses the state of the art in biohybrid actuation, current limitations in scaling up biohybrid actuators and possible solutions to these challenges from tissue engineering, advanced manufacturing, materials engineering and biology.

2.1. Vascularization

Vascularization is critical for keeping tissue alive; in native tissues, three dimensional (3D) networks of vessels within the tissue are necessary to supply nutrients and oxygen to the component cells (Figure 2A). Since living muscle cells form the functional elements of biohybrid actuators, it is critical to develop such a vascular network in engineered tissues, where oxygen, nutrient and waste can circulate and exchange [21]. However, a major limitation in achieving in vitro vasculature functionality in larger-scale tissues has been the lack of multi-scale 3D fabrication approaches needed to guide vascular patterning and self-assembly. Without this structural organization, achievable tissue sizes are limited. Many approaches from conventional tissue engineering may be adapted to vascularize biohybrid actuators on larger scales by guiding tissue organization and geometry.

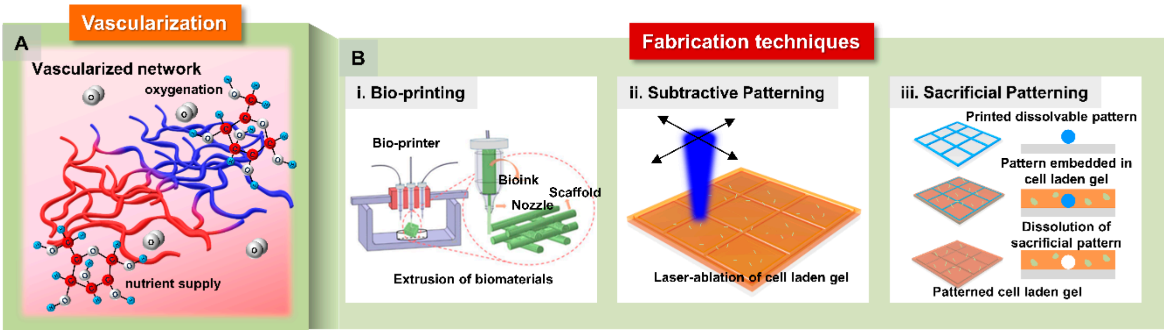

Figure 2. (**A**) Schematic illustration of vascularized network for efficient oxygenation and nutrient supply and removal of metabolic waste. (**B**) Fabrication-based approaches to engineering vascularized network: (**B**,**i**) bio-printing [23] © Copyright 2018, MDPI. (**B**,**ii**) subtractive patterning by laser ablation and (**B**,**iii**) sacrificial patterning using pre-defined dissolvable material.

Spontaneous growth of vasculature in co-cultured tissues has been found to effectively generate small vascular networks in millimeter-scale tissues [24]. However, fabrication techniques must also be developed for scalable engineering of large vascularized networks in macro-scale tissues that will be required to power large soft robotic devices with living cells [21]. Such techniques include bio-printing, subtractive patterning and sacrificial patterning, which primarily rely on cell and scaffold patterning techniques [21,25,26]. In bio-printing, extrusion or droplet deposition of a variety of bio-inks using a bio-printer enables patterning of biomaterials to form the desired living tissue structure (Figure 2B(i)) [25,26]. In combination with bio-printing or as a separate process, laser ablation can be used to subtractively pattern pre-formed cell-laden gels to guide the growth of endothelial cells to generate functional vascularized vessels (Figure 2B(ii)) [27]. In addition to additive bio-printing and subtractive laser-based patterning, sacrificial patterning, where a biocompatible water-dissolvable material is extruded in a filamentary lattice structure along with cell-laden bio-inks, can be used to create an internal network of voids that can be populated with endothelial cells after dissolution of the sacrificial material (Figure 2B(iii)) [28]. Such patterning pathways offer a route to scaling up biohybrid structures in three dimensions by allowing nutrients and oxygen to penetrate into the tissue interior.

Each of the classes of techniques presented here have pros and cons for the creation of vascularized macroscale biohybrid actuators. Extrusion of biomaterials in bio-printing allows a wide range of extracellular matrix bio-inks to be deposited in 3D with or without cells [25,28]. However, these extrusion processes require precise control of ink viscosity, are time-intensive, and may result in shear and pressure on embedded cells which may trigger biological processes or differentiation changes. Alternatively, subtractive patterning enables relatively high-resolution patterning of channels, but is confined to the creation of 2D vasculature [28,29]. Lastly, sacrificial patterning can create the vasculature both in 2D and 3D within relatively faster time, but the resolution of the structure would be critically dependent with dissolvability of materials that requires capillary forces [30]. Moreover, as an alternative, biohybrid actuators can be fabricated to minimize the need for direct vascular patterning. For example, by stacking thin films seeded with the cells, a 3D laminar structure can be assembled to construct a bulk muscle [31]. This technique combines the ease of thin film manufacturing with 3D muscle culture techniques to create biohybrid actuators with increased contractile force. However, there is still an inherent size limitation with tissues produced with this approach.

Having artery and venous walls with capillary channels in between in 3-dimensional shapes would be an ideal structure of vasculature [32]. In the future, the combination of required resolution and 3D patterning suggests that fabrication approaches should be developed to combine the approaches currently reported in the literature. For example, systems capable of additive and subtractive patterning could be used for layer-by-layer patterning of multi-scale structures ranging from fine capillaries to larger vascular channels. Where the resolution limits of the fabrication approaches prevent scaling to sufficiently fine capillaries, vascular precursor cells could be patterned to promote vascularization during maturation. These approaches will need to balance the fabrication time with the loads experienced by the cells.

2.2. Interfacial Interaction

Living organisms have evolved and adapted to their environments. As a result of this adaptation, their structures have developed to withstand specific forces and loads, and these adaptations are clearly observed in how the musculature interfaces with the organism's structure. The musculature and skeletal system (or lack thereof) of organisms varies dramatically at different scales and speeds of behavior (Figure 3A). For example, the body structure of small-scale, relatively slow moving organisms such as sea-slugs and earth-worms consists of a muscular hydrostatic skeleton composed of only soft muscles with no skeletal support [33]. Conversely, humans or large animals are driven by forces transmitted from muscle to tendon and tendon to bone so that their hierarchically organized muscular and skeletal structures are engaged to produce macroscopic contractile forces by muscle tissues. Thus, the combination of physical, chemical and biological interactions in multi-scale schemes with components that have different moduli form a frame of the body that enables scalable control over a large-bodied organism's force and movements.

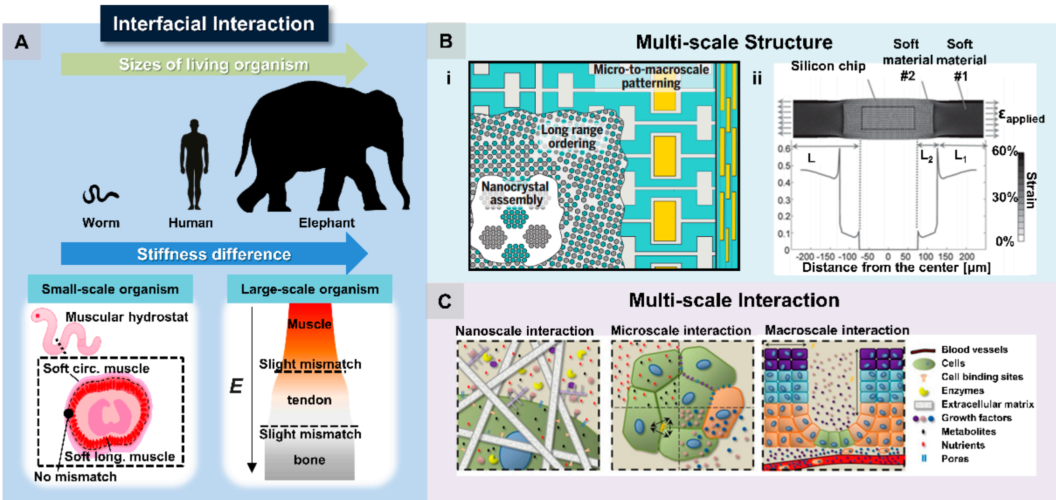

Figure 3. (**A**) Schematics of how the sizes matter interfacial interaction in living organisms. (**B**) Synthetic multi-scale structure for alleviating physical stress concentration due to mechanical mismatch. (**B**,**i**) Approach to bridging functional nanocomposites for constructing robust inorganic macroscale devices. [34] © Copyright 2019, AAAS. (**B**,**ii**) Stress gradient of materials for successful integration in hard to soft interfaces. [35] © Copyright 2017, Wiley. (**C**) Multi-scale interactions that take chemical and biological contributions inside tissue cells. [22] © Copyright 2016, Elsevier Inc.

Although when building small-scale biohybrid robots, multi-scale interaction between muscles and structure is less critical, when scaling up biohybrid actuators interfacial interactions and hierarchy become critically important to prevent tissue failure due to stress concentration. Hierarchical fabrication methods to produce patterned features of nanocomposites and micro- patterns in different moduli have been previously used to fabricate complex electronic devices (Figure 3B) [34,35], and such techniques could be translated to biohybrid actuator fabrication or used to inspire novel fabrication approaches. Hierarchical interfaces would enable the development of the bio-mechanical structures required for integration of biological tissues and inorganic heterogeneous structures with many mechanical mismatches.

Moreover, unlike traditional actuators, cells experience a multitude of multi-scale interactions with their environment through physical, chemical, and biological cues (Figure 3C). At the nanoscale to microscale, chemical and protein interactions play a crucial role in tissue organization and behavior. At slightly larger micro to macroscales, substrate mechanics, cell-to-cell communication and extracellular matrix composition determine tissue alignment, cellular differentiation, and systematic force generation [22]. Selection of the appropriate biomaterials and media to induce these multi-scale interactions will be crucial in realizing the biological and chemical functionality observed in native tissues. In addition, there are significant differences in the impact and types of multi-scale interaction on and experienced by cells, like muscle, in 2D vs. 3D culture [36]. Flat (2D) substrates have historically been used in cell culture to investigate the effect of biochemical factors, topography, stiffness, and mechanical load on cells in vitro [36,37]. However, these 2D environments do not capture the microenvironment that cells experience in vivo [37]. Even in in vitro cultures, marked differences between 2D and 3D culture have been observed in spatiotemporal distributions of oxygen, nutrients, and metabolic wastes thereby altering proliferation, migration, matrix production, and cell differentiation [37,38]. For biohybrid actuators, the creation of appropriate 3D scaffolds and culture conditions is critical as cultured muscle currently produces a fraction of the forces seen in vivo [39,40], and the use of 3D conditions is necessary for proper reproduction of neuromuscular junctions in vitro [41,42]. Although biohybrid roboticists can adapt the state-of-the-art in tissue engineering to biohybrid actuator fabrication, fundamental research on interfacial interactions is needed to identify effective synthetic compositions, integration techniques, and fabrication approaches for macro-scale biohybrid actuator production and integration with robotic structures.

2.3. Innervation: Control Methods

In native muscle tissues, neural innervation of muscle cells over a large area from a network of motor neurons provides a scalable control scheme for force generation. The ability to differentially activate and control engineered tissues will be critical in macro-scale biohybrid actuators (Figure 4A). This section addresses many proposed control methods that have been used to stimulate muscle cells in biohybrid robotics to date and the limitations thereof.

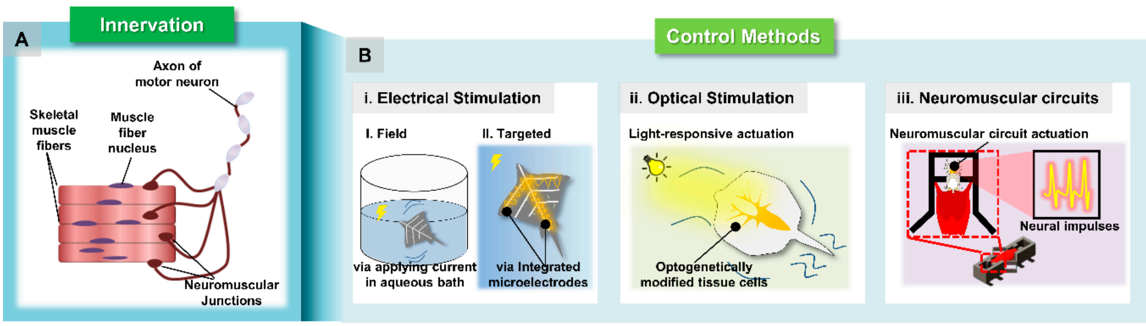

Figure 4. (**A**) Schematic illustration of muscle innervation. (**B**) Various control methods of centimeter-scale biohybrid soft robots (**B**,**i**) electrical stimulation, (**B**,**ii**) optical stimulation and (**B**,**iii**) neuromuscular co-culture. [43]

2.3.1. Electrical Stimulation

Electrical stimulation is the most common control method found in the biohybrid robotics literature [2,13,44,45]. This method is generally applied as either field or targeted stimulations. In field stimulation, electrical current is applied to the aqueous bath housing the robot (field, Figure 4B(i)) whereas in targeted stimulation, electrical current is selectively delivered through integrated electrodes embedded within a biohybrid robot (targeted stimulation, Figure 4B(ii)). Field stimulation is the most widely used electrical stimulation method to control biohybrid robots [1,2,46]. However, in direct comparison between field stimulation and targeted stimulation, integration of flexible electrodes with conductive gels and myotubes resulted in improved stimulation efficiency and controllability [13,47]. While field stimulation requires the presence of a bath and is essentially tethered to a dish, soft-electrode integration may also enable circuits to wirelessly control the robot and allow communication. However, electrical stimulation uses fundamentally different mechanisms of stimulation than natural neuromuscular control and may result in muscle fatigue when skeletal muscle cells are employed [48,49].

2.3.2. Optical Stimulation

Whereas current targeted electrical stimulation requires the biohybrid robot be tethered, optical stimulation allows control of a robot without direct physical contact [15–17]. As such, optical stimulation via distributed optical electrodes may be an alternative solution for controlling macro-scale biohybrid soft robots. To implement the optical stimulation of biohybrid actuators, tissues must be fabricated using optogenetically modified muscle cells that respond to light. To do this, channel rhodopsins or other light-gated ion channels are used to transgenically modify the cells and enable light to control membrane depolarization. The use of targeted and field optical stimulation has been shown to enable phototactic guidance, steering, and turning maneuvers in small scale biohybrid robots [15]. In this example, optical stimulation induced real-time sequential muscle activation via serpentine-patterned muscle circuits, leading to coordinated undulatory swimming. Optical stimulation shows significant potential for tether-free biohybrid robot control; however, the dependence on genetically modified cells that respond to light limits the types of tissue that can be used.

2.3.3. Neuromuscular Co-Culture

In native tissues, muscle is stimulated by the nervous system through neuromuscular junctions. Webster et al. have demonstrated that stimulation via the natural neural circuitry improves biohybrid actuation force and reduces fatigue in comparison to electrical stimulation in neuromuscular tissue circuits isolated from *Aplysia californica* [43]. Additionally, the use of such intact tissue circuits allows the modulatory capabilities of the neuromuscular system to be leveraged. Recently, biohybrid robots with innervation from cultured neurospheres have shown the potential for neuromuscular co-culture in biohybrid actuator stimulation [16]. These neurospheres could be optically stimulated to induce muscle contraction. The use of neuromuscular circuits, whether explanted or cultured may allow the muscle to exert higher forces than electrical stimulation when the muscle is stimulated in macro-scale. However, to truly harness these capabilities, techniques are needed to controllably fabricate neuromuscular circuits for biohybrid robot control.

In translation of biohybrid actuators to the broader field of actuation, it will be important for future biohybrid actuator researchers to begin to report comparable metrics to those already reported in the soft actuator community. Such metrics include maximum strain, stress, work density, peak strain rate, life (cycles) at a given strain, efficiency, modulus, environmental operation range, fuel or energy source [12]. Although many biohybrid actuator papers report some of these metrics, none report all, making it difficult to position biohybrid actuators to engineering material. Although we cannot directly compare the actuation performance with quantitative measures to that of traditional actuators, the control methods presented here have clear advantages and disadvantages. Electrical stimulation utilizes applied voltages to actuate the cells either directly through patterned electrodes or indirectly through a bath providing direct control of muscle contraction. Both approaches require a tether or onsite power source, with indirect electrical stimulation further requiring a conductive liquid medium external electrical field which could damage or affect other robots or organisms in the environment [13,44]. Optical stimulation has great potential for untethered operation with the capability to shine the light over a wide area, but it is limited to applications with optically clear or shallow media with low refractive index such that light can reach the cells [15]. Neuromuscular stimulation is an emerging mode of biohybrid actuator control which may address challenges found in both electrical and optical stimulation by improving force, lowering fatigue, and enabling 'programming' of basic behaviors or control patterns by training the neural cultures [50–53]. However, direct head-to-head comparisons of these stimulation approaches are needed.

3. Perspectives and Future Directions

Biohybrid actuators have many desirable characteristics for soft actuation. However, to translate this technique from proof-of-concept small-scale robots to robotics at large, key challenges as presented here must be addressed. To address these challenges, a multidisciplinary approach bringing together collaborators from manufacturing, materials science, biology, tissue engineering, and robotics is needed. Future efforts towards macro-scale biohybrid actuators will need to focus initially on scalable fabrication approaches. Integrated multi-material fabrication approaches must be able to pattern muscle cells, guide the development of vascular networks, construct hierarchical interfacial structures and direct the organization of control circuits. Fabrication approaches should integrate micro-fabrication techniques to capture the small-scale architectures of the native muscle extracellular matrix with macroscale bio-patterning approaches for the extrusion of cellular bio-inks, composite reinforcements, and additive/subtractive patterning of vascular channels. Such fabrication approaches need to be broadly accessible for adoption by the larger robotics community. In parallel with development of fabrication approaches, direct comparisons of control approaches are needed to establish the impact of each approach on maximum actuation frequency, actuation force, life, and rate of fatigue across a wide range of biohybrid actuator cell sources to develop an engineering database for these systems. Furthermore, control approaches that improve actuator performance should be integrated into the multi-scale fabrication pipeline described above to ensure that distributed stimulation of macro-scale biohybrid actuators is possible. Control approaches can be further refined through collaboration with material scientists and neurobiologists to identify techniques to minimize cell fatigue while maximizing actuation force controllability. Ultimately, these approaches will need to be packaged in a tether-free system to allow autonomous or independent operation of the device.

Beyond the technical concerns of macro-scale biohybrid actuator fabrication and control, the development of these systems should be undertaken in partnership with bio- and robo-ethicists. Biohybrid robotics brings together ethical concerns from both biology and robotics. It will be important for the community of researchers to identify and adhere to ethical standards for this emerging field mirroring those of animal research. Where possible, bioactuators should be fabricated using commercially available, renewable cell lines, or using primary cells from invertebrates. For future applications where biohybrid robots will be deployed in the field, they should be developed from materials native to the local ecosystem and be biodegradable to minimize hazards to the environment.

Biohybrid actuators provide another material option to the soft robotics community that is self-healing, reasonably efficient, adaptive, and renewable. These actuators have broad potential for applications in medicine, agriculture, environmental monitoring, or anywhere that biodegradable devices are needed. Of course, biohybrid actuators require long-term maintenance, may have limited environmental conditions under which they can operate, and likely will result in more stochastic performance than traditional actuators. However, animals clearly demonstrate the ability of these actuators to enable meaningful behaviors. By developing the fabrication and control approaches outlined here, we will move towards capturing these capabilities for robotic systems and as platforms for testing theories in tissue engineering and neuromuscular control. Finally the characteristics, mechanics, and performance of the biohybrid actuators of the future must be reported using metrics common to soft robotics. This will help ensure that biohybrid actuators can transition to being seen as engineering materials.

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References

1. Ricotti, L.; Trimmer, B.; Feinberg, A.W.; Raman, R.; Parker, K.K.; Bashir, R.; Sitti, M.; Martel, S.; Dario, P.; Menciassi, A. Biohybrid actuators for robotics: A review of devices actuated by living cells. *Sci. Robot.* **2017**, *2*, eaaq0495. [CrossRef]
2. Sun, L.; Yu, Y.; Chen, Z.; Bian, F.; Ye, F.; Sun, L.; Zhao, Y. Biohybrid robotics with living cell actuation. *Chem. Soc. Rev.* **2020**, *49*, 4043–4069. [CrossRef]
3. Rich, S.I.; Wood, R.J.; Majidi, C. Untethered soft robotics. *Nat. Electron.* **2018**, *1*, 102–112. [CrossRef]
4. Cianchetti, M.; Laschi, C.; Menciassi, A.; Dario, P. Biomedical applications of soft robotics. *Nat. Rev. Mater.* **2018**, *3*, 143–153. [CrossRef]
5. Kim, S.; Laschi, C.; Trimmer, B. Soft robotics: A bioinspired evolution in robotics. *Trends Biotechnol.* **2013**, *31*, 287–294. [CrossRef]
6. Shepherd, R.F.; Ilievski, F.; Choi, W.; Morin, S.A.; Stokes, A.A.; Mazzeo, A.D.; Chen, X.; Wang, M.; Whitesides, G.M. Multigait soft robot. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20400–20403. [CrossRef]
7. Kheirikhah, M.M.; Rabiee, S.; Edalat, M.E. A review of shape memory alloy actuators in robotics. In *Proceedings of Robot Soccer World Cup*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 206–217.
8. Lendlein, A.; Gould, O.E. Reprogrammable recovery and actuation behaviour of shape-memory polymers. *Nat. Rev. Mater.* **2019**, *4*, 116–133. [CrossRef]
9. Youn, J.-H.; Jeong, S.M.; Hwang, G.; Kim, H.; Hyeon, K.; Park, J.; Kyung, K.-U. Dielectric elastomer actuator for soft robotics applications and challenges. *Appl. Sci.* **2020**, *10*, 640. [CrossRef]
10. Polygerinos, P.; Correll, N.; Morin, S.A.; Mosadegh, B.; Onal, C.D.; Petersen, K.; Cianchetti, M.; Tolley, M.T.; Shepherd, R.F. Soft robotics: Review of fluid-driven intrinsically soft devices; manufacturing, sensing, control, and applications in human-robot interaction. *Adv. Eng. Mater.* **2017**, *19*, 1700016. [CrossRef]
11. Hughes, J.; Culha, U.; Giardina, F.; Guenther, F.; Rosendo, A.; Iida, F. Soft manipulators and grippers: A review. *Front. Robot. AI* **2016**, *3*, 69. [CrossRef]
12. Madden, J.D.; Vandesteeg, N.A.; Anquetil, P.A.; Madden, P.G.; Takshi, A.; Pytel, R.Z.; Lafontaine, S.R.; Wieringa, P.A.; Hunter, I.W. Artificial muscle technology: Physical principles and naval prospects. *IEEE J. Ocean. Eng.* **2004**, *29*, 706–728. [CrossRef]
13. Shin, S.R.; Migliori, B.; Miccoli, B.; Li, Y.C.; Mostafalu, P.; Seo, J.; Mandla, S.; Enrico, A.; Antona, S.; Sabarish, R. Electrically driven microengineered bioinspired soft robots. *Adv. Mater.* **2018**, *30*, 1704189.
14. Xu, H.; Medina-Sánchez, M.; Magdanz, V.; Schwarz, L.; Hebenstreit, F.; Schmidt, O.G. Sperm-hybrid micromotor for targeted drug delivery. *ACS Nano* **2018**, *12*, 327–337. [CrossRef] [PubMed]
15. Park, S.-J.; Gazzola, M.; Park, K.S.; Park, S.; Di Santo, V.; Blevins, E.L.; Lind, J.U.; Campbell, P.H.; Dauth, S.; Capulli, A.K. Phototactic guidance of a tissue-engineered soft-robotic ray. *Science* **2016**, *353*, 158–162. [CrossRef] [PubMed]
16. Aydin, O.; Zhang, X.; Nuethong, S.; Pagan-Diaz, G.J.; Bashir, R.; Gazzola, M.; Saif, M.T.A. Neuromuscular actuation of biohybrid motile bots. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 19841–19847. [CrossRef] [PubMed]
17. Raman, R.; Cvetkovic, C.; Uzel, S.G.; Platt, R.J.; Sengupta, P.; Kamm, R.D.; Bashir, R. Optogenetic skeletal muscle-powered adaptive biological machines. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3497–3502. [CrossRef]
18. Feinberg, A.W.; Feigel, A.; Shevkoplyas, S.S.; Sheehy, S.; Whitesides, G.M.; Parker, K.K. Muscular thin films for building actuators and powering devices. *Science* **2007**, *317*, 1366–1370. [CrossRef]
19. Appiah, C.; Arndt, C.; Siemsen, K.; Heitmann, A.; Staubitz, A.; Selhuber-Unkel, C. Living Materials Herald a New Era in Soft Robotics. *Adv. Mater.* **2019**, *31*, 1807747. [CrossRef]
20. Webster, V.A.; Chapin, K.J.; Hawley, E.L.; Patel, J.M.; Akkus, O.; Chiel, H.J.; Quinn, R.D. *Aplysia californica* as a novel source of material for biohybrid robots and organic machines. In Proceedings of the Conference on Biomimetic and Biohybrid Systems, Living Machines 2016, Edinburgh, Scotland, 18–22 July 2016; pp. 365–374.
21. Grebenyuk, S.; Ranga, A. Engineering organoid vascularization. *Front. Bioeng. Biotechnol.* **2019**, *7*, 39. [CrossRef]
22. Leijten, J.; Khademhosseini, A. From nano to macro: Multiscale materials for improved stem cell culturing and analysis. *Cell Stem Cell* **2016**, *18*, 20–24. [CrossRef]
23. Naghieh, S.; Sarker, M.; Karamooz-Ravari, M.R.; McInnes, A.D.; Chen, X. Modeling of the mechanical behavior of 3D bioplotted scaffolds considering the penetration in interlocked strands. *Appl. Sci.* **2018**, *8*, 1422. [CrossRef]

24. Clevers, H. Modeling development and disease with organoids. *Cell* **2016**, *165*, 1586–1597. [CrossRef] [PubMed]
25. Lee, A.; Hudson, A.; Shiwarski, D.; Tashman, J.; Hinton, T.; Yerneni, S.; Bliley, J.; Campbell, P.; Feinberg, A. 3D bioprinting of collagen to rebuild components of the human heart. *Science* **2019**, *365*, 482–487. [CrossRef] [PubMed]
26. Kalmykov, A.; Huang, C.; Bliley, J.; Shiwarski, D.; Tashman, J.; Abdullah, A.; Rastogi, S.K.; Shukla, S.; Mataev, E.; Feinberg, A.W. Organ-on-e-chip: Three-dimensional self-rolled biosensor array for electrical interrogations of human electrogenic spheroids. *Sci. Adv.* **2019**, *5*, eaax0729. [CrossRef]
27. Applegate, M.B.; Coburn, J.; Partlow, B.P.; Moreau, J.E.; Mondia, J.P.; Marelli, B.; Kaplan, D.L.; Omenetto, F.G. Laser-based three-dimensional multiscale micropatterning of biocompatible hydrogels for customized tissue engineering scaffolds. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 12052–12057. [CrossRef]
28. Kolesky, D.B.; Homan, K.A.; Skylar-Scott, M.A.; Lewis, J.A. Three-dimensional bioprinting of thick vascularized tissues. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 3179–3184. [CrossRef]
29. Okano, K.; Hsu, H.-Y.; Li, Y.-K.; Masuhara, H. In situ patterning and controlling living cells by utilizing femtosecond laser. *J. Photochem. Photobiol. C Photochem. Rev.* **2016**, *28*, 1–28. [CrossRef]
30. Cerchiari, A.; Garbe, J.C.; Todhunter, M.E.; Jee, N.Y.; Pinney, J.R.; LaBarge, M.A.; Desai, T.A.; Gartner, Z.J. Formation of spatially and geometrically controlled three-dimensional tissues in soft gels by sacrificial micromolding. *Tissue Eng. Part C Methods* **2015**, *21*, 541–547. [CrossRef]
31. Morimoto, Y.; Onoe, H.; Takeuchi, S. Biohybrid robot powered by an antagonistic pair of skeletal muscle tissues. *Sci. Robot.* **2018**, *3*, eaat4440. [CrossRef]
32. Pollet, A.M.; den Toonder, J.M. Recapitulating the Vasculature using organ-on-chip technology. *Bioengineering* **2020**, *7*, 17. [CrossRef]
33. Kier, W.M.; Smith, K.K. Tongues, tentacles and trunks: The biomechanics of movement in muscular-hydrostats. *Zool. J. Linn. Soc.* **1985**, *83*, 307–324. [CrossRef]
34. Begley, M.R.; Gianola, D.S.; Ray, T.R. Bridging functional nanocomposites to robust macroscale devices. *Science* **2019**, *364*, eaav4299. [CrossRef] [PubMed]
35. Naserifar, N.; LeDuc, P.R.; Fedder, G.K. Material gradients in stretchable substrates toward integrated electronic functionality. *Adv. Mater.* **2016**, *28*, 3584–3591. [CrossRef] [PubMed]
36. Duval, K.; Grover, H.; Han, L.-H.; Mou, Y.; Pegoraro, A.F.; Fredberg, J.; Chen, Z. Modeling physiological events in 2D vs. 3D cell culture. *Physiology* **2017**, *32*, 266–277. [CrossRef] [PubMed]
37. Fontoura, J.C.; Viezzer, C.; dos Santos, F.G.; Ligabue, R.A.; Weinlich, R.; Puga, R.D.; Antonow, D.; Severino, P.; Bonorino, C. Comparison of 2D and 3D cell culture models for cell growth, gene expression and drug resistance. *Mater. Sci. Eng. C* **2020**, *107*, 110264. [CrossRef] [PubMed]
38. Ravi, M.; Paramesh, V.; Kaviya, S.; Anuradha, E.; Solomon, F.P. 3D cell culture systems: Advantages and applications. *J. Cell. Physiol.* **2015**, *230*, 16–26. [CrossRef]
39. Bono, N.; Pezzoli, D.; Levesque, L.; Loy, C.; Candiani, G.; Fiore, G.B.; Mantovani, D. Unraveling the role of mechanical stimulation on smooth muscle cells: A comparative study between 2D and 3D models. *Biotechnol. Bioeng.* **2016**, *113*, 2254–2263. [CrossRef]
40. Urciuolo, A.; Serena, E.; Ghua, R.; Zatti, S.; Giomo, M.; Mattei, N.; Vetralla, M.; Selmin, G.; Luni, C.; Vitulo, N. Engineering a 3D in vitro model of human skeletal muscle at the single fiber scale. *PLoS ONE* **2020**, *15*, e0232081. [CrossRef]
41. Dixon, T.A.; Cohen, E.; Cairns, D.M.; Rodriguez, M.; Mathews, J.; Jose, R.R.; Kaplan, D.L. Bioinspired three-dimensional human neuromuscular junction development in suspended hydrogel arrays. *Tissue Eng. Part C Methods* **2018**, *24*, 346–359. [CrossRef]
42. Krieger, J.; Park, B.-W.; Lambert, C.R.; Malcuit, C. 3D skeletal muscle fascicle engineering is improved with TGF-β1 treatment of myogenic cells and their co-culture with myofibroblasts. *PeerJ* **2018**, *6*, e4939. [CrossRef]
43. Webster, V.A.; Young, F.R.; Patel, J.M.; Scariano, G.N.; Akkus, O.; Gurkan, U.A.; Chiel, H.J.; Quinn, R.D. 3D-printed biohybrid robots powered by neuromuscular tissue circuits from *Aplysia californica*. In Proceedings of the Conference on Biomimetic and Biohybrid Systems, Living Machines 2017, San Francisco, CA, USA, 25–28 July 2017; pp. 475–486.
44. Wang, T.; Migliori, B.; Miccoli, B.; Shin, S.R. Bioinspired Soft Robot with Incorporated Microelectrodes. *JoVE (J. Vis. Exp.)* **2020**, *156*, e60717. [CrossRef]

45. Pagan-Diaz, G.J.; Zhang, X.; Grant, L.; Kim, Y.; Aydin, O.; Cvetkovic, C.; Ko, E.; Solomon, E.; Hollis, J.; Kong, H. Simulation and fabrication of stronger, larger, and faster walking biohybrid machines. *Adv. Funct. Mater.* **2018**, *28*, 1801145. [CrossRef]
46. Webster-Wood, V.A.; Akkus, O.; Gurkan, U.A.; Chiel, H.J.; Quinn, R.D. Organismal engineering: Toward a robotic taxonomic key for devices using organic materials. *Sci. Robot.* **2017**, *2*, eaap9281. [CrossRef] [PubMed]
47. Kim, Y.; Pagan-Diaz, G.; Gapinske, L.; Kim, Y.; Suh, J.; Solomon, E.; Harris, J.F.; Nam, S.; Bashir, R. Integration of Graphene Electrodes with 3D Skeletal Muscle Tissue Models. *Adv. Healthc. Mater.* **2020**, *9*, 1901137. [CrossRef] [PubMed]
48. Mizrahi, J.; Levin, O.; Aviram, A.; Isakov, E.; Susak, Z. Muscle fatigue in interrupted stimulation: Effect of partial recovery on force and EMG dynamics. *J. Electromyogr. Kinesiol.* **1997**, *7*, 51–65. [CrossRef]
49. Ibitoye, M.O.; Hamzaid, N.A.; Hasnan, N.; Abdul Wahab, A.K.; Davis, G.M. Strategies for rapid muscle fatigue reduction during FES exercise in individuals with spinal cord injury: A systematic review. *PLoS ONE* **2016**, *11*, e0149024. [CrossRef] [PubMed]
50. Cvetkovic, C.; Rich, M.H.; Raman, R.; Kong, H.; Bashir, R. A 3D-printed platform for modular neuromuscular motor units. *Microsyst. Nanoeng.* **2017**, *3*, 17015. [CrossRef] [PubMed]
51. Natarajan, A.; Sethumadhavan, A.; Krishnan, U.M. Toward building the neuromuscular junction: In vitro models to study synaptogenesis and neurodegeneration. *ACS Omega* **2019**, *4*, 12969–12977. [CrossRef]
52. Bakooshli, M.A.; Lippmann, E.S.; Mulcahy, B.; Iyer, N.; Nguyen, C.T.; Tung, K.; Stewart, B.A.; van den Dorpel, H.; Fuehrmann, T.; Shoichet, M. A 3D culture model of innervated human skeletal muscle enables studies of the adult neuromuscular junction. *Elife* **2019**, *8*, e44530. [CrossRef]
53. Pimashkin, A.; Gladkov, A.; Mukhina, I.; Kazantsev, V. Adaptive enhancement of learning protocol in hippocampal cultured networks grown on multielectrode arrays. *Front. Neural Circuits* **2013**, *7*, 87. [CrossRef]