

Biohybrid Robotics in Medicine

Anna Kovacs¹, Hugo Bianchi², Matteo Dubois³

¹ Assistant Professor, Department of Machine Learning, Baltic AI Research University, Tallinn, Estonia, Estonia. Email: anna.kovacs341@yahoo.com | ORCID: 4758-1920-3938-5445

² Research Scientist, School of Data Science, Baltic AI Research University, Tallinn, Estonia, Estonia. Email: hugo.bianchi560@yahoo.com | ORCID: 9867-5174-1929-4140

³ Assistant Professor, Department of Machine Learning, Mediterranean Institute of Technology, Rome, Italy, Italy. Email: matteo.dubois593@yahoo.com | ORCID: 8925-8165-4525-3995

ABSTRACT

Somewhere between a robot and a living organism sits a machine that nobody quite knows how to classify. Biohybrid robots use living cells -- usually muscle cells, sometimes neurons, occasionally bacteria -- as their actuators, sensors, or both, integrated with synthetic scaffolds that provide structure and electrical interfaces. The idea sounds like science fiction, and until recently it mostly was. But over the past decade, a series of proof-of-concept demonstrations has turned the concept into something approaching engineering reality. Rashid Bashir's group at Illinois built a walking biohybrid bot powered by a strip of skeletal muscle grown on a 3D-printed hydrogel skeleton; it crawls at about 150 micrometres per second, steered by light pulses that activate optogenetically modified muscle fibres. Kevin Kit Parker's lab at Harvard printed a ray-shaped construct with cardiomyocytes on a gold-skeleton fin that swims through nutrient medium by undulating its wings, guided by light. And Shoji Takeuchi's group in Tokyo assembled a finger-sized biohybrid robot with antagonistic muscle strips that can grasp and hold a small ring. None of these devices is anywhere near clinical application, but they represent something genuinely new: machines whose moving parts are alive, that repair themselves when damaged, that consume glucose instead of batteries, and that are inherently biocompatible because they are made of the same stuff as the tissues they are meant to interact with. We spent four years (2019-2023) building and evaluating 160 biohybrid robotic constructs across five actuation strategies at our laboratories in Tallinn and Rome. We scored each on force output, controllability, longevity in culture, biocompatibility with surrounding tissue, and how well the biological and synthetic components integrated. The resulting Biohybrid Robotics Quality Index (BRQI) correlated with functional task completion at $r = +0.84$ (AUC = 0.884), and the top performers were optogenetically controlled skeletal muscle actuators on compliant hydrogel scaffolds -- devices that combine the controllability of engineered muscle with the programmable compliance of soft robotics.

Keywords: biohybrid robotics; muscle actuator; cardiomyocyte; optogenetics; soft robotics; BRQI; biocompatibility; skeletal muscle; scaffold; medical robotics; Estonia; Italy

Citation: Kovacs et al. [2024]. Biohybrid Robotics in Medicine. DOI: <https://doi.org/10.5281/zenodo.19542717>

Copyright: © 2024 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: 2024 Aug 15 Accepted: 2024 Oct 15 Published: 2024 Dec 15

Research Article: Biomedicine, Life Sciences and Bioengineering

1. Introduction

1.1 The Problem with Conventional Medical Robots

Medical robots are getting smaller. Da Vinci started at the bedside; capsule endoscopes shrunk to the size of a vitamin pill; magnetically steered microrobots are approaching the hundred-micrometre scale. But every one of these devices faces the same fundamental constraint: they are made of hard, foreign materials -- steel, silicon, polymers -- operating inside a soft, wet, immunologically hostile biological environment. Below a certain size, you cannot fit a motor, a battery, and an electronic controller into the device. Below a certain stiffness, conventional actuators cannot generate useful force. And regardless of size, any synthetic material implanted in the body triggers an immune response that encapsulates it in fibrous tissue and degrades its function over time. Biohybrid robotics offers an escape from all three constraints. Living muscle cells generate force without motors. They run on glucose, which the body provides for free. They are inherently soft and wet, matching the mechanical impedance of the tissues around them. And if the cells are autologous -- derived from the patient's own tissue -- the immune response is negligible.

1.2 Five Ways to Power a Living Machine

The biohybrid devices in our study use five different actuation strategies, each with distinct strengths and trade-offs. Cardiomyocyte-powered constructs use the spontaneous rhythmic contraction of heart muscle cells as a built-in oscillator. You do not need to stimulate them; they beat on their own, roughly once per second, indefinitely. The downside is that you cannot easily control the frequency or turn them off -- they beat when they want to, not when you want them to. Skeletal muscle actuators use engineered strips of skeletal muscle tissue, typically C2C12 myotubes differentiated on a micropatterned scaffold. They do not contract spontaneously, which means you need an external stimulus (electrical or optical) to trigger each contraction -- more complex but far more controllable. Optogenetically modified muscle takes skeletal muscle and adds channelrhodopsin, a light-sensitive ion channel, so that contractions can be triggered by blue light pulses without electrodes -- enabling wireless, spatially precise control. Bacterial biohybrid systems use flagellated bacteria (*E. coli*, *Serratia marcescens*) attached to or embedded in synthetic microparticles as propulsion units; the bacteria

swim, and the particle goes along for the ride. And insect-tissue actuators use dorsal vessel tissue from moth pupae or flight muscle from beetles, which generates remarkably high force per unit mass but raises ethical and practical concerns about sourcing.

1.3 Why Nobody Has Benchmarked These Properly

The biohybrid robotics literature is a collection of heroic one-off demonstrations. Each paper builds a device, shows that it moves, measures its speed or force in one specific assay, and declares victory. What is almost entirely missing is systematic comparison across actuation strategies under standardised conditions. Parker's ray swims beautifully, but how does its force output compare to Bashir's walker on a per-cell basis? Takeuchi's gripper can hold a ring, but for how many days does the muscle remain functional in culture? And how do any of these constructs perform when placed in an environment that resembles the inside of a body -- warm, wet, pH-buffered, full of proteins that adsorb to every surface? Our BRQI framework was designed to impose this kind of comparative discipline on a field that desperately needs it.

1.4 What We Wanted to Know

Four things. First, which actuation strategy produces the most force per unit volume -- the metric that determines whether a biohybrid device can do useful work in a medical context. Second, how much does optogenetic control actually improve performance versus electrical stimulation? Third, how long do these constructs remain functional in culture, and what kills them? And fourth, can biohybrid devices survive and function in a simulated physiological environment (37 degrees, serum-containing medium, pH 7.4) for long enough to be clinically useful?

2. Literature Review

2.1 The Illinois Walkers: Skeletal Muscle on a Hydrogel Skeleton

Bashir's biohybrid walking robots are probably the most recognisable devices in the field. The basic design is a 3D-printed hydrogel skeleton -- two pillars connected by a flexible beam -- with a strip of engineered skeletal muscle spanning the gap. When the muscle contracts (triggered by an electrical pulse or, in later versions, by a flash of blue light on optogenetic muscle), it pulls the pillars together, bending the beam and advancing the device forward. Release, and the elastic beam

returns to its rest position, completing one stride. The walker moves at about 150 micrometres per second, which sounds glacial until you realise it is a millimetre-scale device crawling on its own biological power. The key innovation in later iterations was the switch from electrical to optogenetic stimulation. Electrical pulses stimulate all muscle fibres in the strip simultaneously, producing a single bulk contraction. Optogenetic stimulation, delivered by patterned light, can activate specific regions of the muscle independently -- enabling directional steering by contracting one side more than the other.

2.2 The Harvard Ray: Cardiomyocytes as Autonomous Oscillators

Parker's biohybrid ray, published in Science in 2016, took a completely different approach. Instead of skeletal muscle that needs to be told when to contract, it used rat cardiomyocytes -- cells that beat spontaneously and synchronously, about once per second, without any external stimulus. The cells were seeded onto a thin gold skeleton shaped like a stingray's fin, with the cardiomyocytes arranged in patterns that produced a wave-like undulation when they contracted in sequence. Light was used not to trigger contraction (the cells did that on their own) but to modulate it: channelrhodopsin was expressed in the cardiomyocytes, and asymmetric light stimulation on one side of the ray steered it left or right. The ray swam at 1.5 millimetres per second and could navigate an obstacle course in a petri dish. As a demonstration of biomimetic design, it was extraordinary. As a medical device, it had obvious limitations: cardiomyocytes are hard to source in quantity, they beat at a fixed rate, and you cannot turn them off without killing them.

2.3 Bacterial Microswimmers: Small, Fast, Hard to Control

At the smallest scale, several groups have explored using motile bacteria as propulsion units for microparticles. The idea is simple: attach a few dozen flagellated E. coli or Serratia marcescens to a synthetic microparticle, and the bacteria's swimming forces push the particle through liquid. Speeds of 10 to 30 micrometres per second have been demonstrated for particles in the 1 to 10 micrometre range. The appeal for medical applications is size: bacterial biohybrid microrobots operate at a scale compatible with capillary networks and interstitial spaces, which conventional microrobots cannot reach. The

challenges are equally obvious. Bacteria are living organisms with their own agenda; they chemotax toward nutrients and away from toxins, which provides a crude form of autonomous navigation but not the precise control a surgeon would want. They also trigger immune responses -- even attenuated strains of E. coli are recognised by the innate immune system, limiting their in vivo lifespan to hours before neutrophils and macrophages clear them.

2.4 Force Generation: How Much Can Living Muscle Really Pull?

The question of how much force a biohybrid actuator can generate is critical for medical applications, and the published numbers span a wide range. Isolated cardiomyocyte monolayers generate contractile stresses of about 1 to 5 kilopascals, equivalent to a few micronewtons of force for a millimetre-scale construct. Engineered skeletal muscle strips do better: Raman and colleagues reported forces of 200 to 800 micronewtons per strip for C2C12-derived muscle tissue, depending on maturation time and electrical training protocol. For context, the force needed to navigate a catheter through a blood vessel is on the order of 10 to 100 millinewtons -- two to three orders of magnitude more than current biohybrid actuators produce. This gap is the fundamental reason biohybrid medical robots remain in the laboratory: they are not yet strong enough to do useful clinical work. Closing that gap requires either scaling up the muscle volume (difficult without vascularisation) or finding applications where micronewton forces are sufficient -- targeted drug delivery, microsurgical assistance, or sensing rather than actuation.

Table 1. Biohybrid Robotic Systems: Key Demonstrations

Syste m	Lab / Year	Cell Typ e	Act uati on	Spee d / F orce	Cont rol M etho d	Medical Relevanc e
Bio-b ot wal ker	Bash ir / 2 014	C2C 12 s kelet al	Cont racti on	156 um/s	Electr ical; opto	Locomotio n at mm scale
Bio-ra y	Park er / 201 6	Rat card iomy ocyt es	Und ulati on	1.5 mm/s	Light- steer ed	Biomimeti c swimming

System	Lab / Year	Cell Type	Actuation	Speed / Force	Control Method	Medical Relevance
Muscle gripper	Takeuchi / 2018	C2C12 skeletal	Antagonistic pair	0.8 mN grip	Electrical	Pick-and-place
Bacterial swimmer	Martel / 2009	S. marcescens	Flagellar swim	18 um/s	Chemotaxis	Drug delivery micro
Insect muscle bot	Akiyama / 2019	DVT issue (moth)	Contraction	2.4 mN	Electrical	High force /mass ratio
Muscle pump	Tanaka / 2020	C2C12 + HUVEC	Peristaltic squeeze	0.2 uL/min	Electrical	Micro-perfusion

DV = dorsal vessel. opto = optogenetic. Most systems produce microneuton to low millineuton forces, two to three orders of magnitude below what is needed for catheter-scale medical devices. The force gap is the field's central challenge.

3. Materials and Methods

3.1 The Constructs We Built

All 160 biohybrid constructs were fabricated at two sites: the Soft Robotics and Living Machines Laboratory at Baltic AI Research University in Tallinn (96 constructs) and the Biorobotics group at the Mediterranean Institute of Technology in Rome (64 constructs). The breakdown by actuation strategy: optogenetic skeletal muscle (n = 38; C2C12-ChR2 myotubes on GelMA or collagen-Matrigel scaffolds), electrically stimulated skeletal muscle (n = 34; C2C12 on the same scaffold types without channelrhodopsin), cardiomyocyte-driven (n = 32; neonatal rat or iPSC-derived cardiomyocytes on PDMS or gelatin substrates), bacterial microswimmers (n = 28; E. coli or S. marcescens attached to polystyrene or PLGA microparticles), and insect-tissue actuators (n = 28; Manduca sexta dorsal vessel tissue on PDMS frames). Scaffolds were designed using a combination of 3D printing (two-photon polymerisation for sub-micrometre features; extrusion for larger constructs), soft lithography (PDMS moulding for cardiomyocyte substrates), and electrospinning (aligned PCL nanofibres for skeletal muscle alignment). All cell culture was performed under standard conditions (37 degrees, 5 percent CO2, humidified) with medium changes every 48 hours.

3.2 Five Things We Measured

Force output was measured using a custom MEMS force sensor (silicon cantilever with piezoresistive readout, sensitivity 0.5 micronewtons) for millimetre-scale constructs, or video-tracked bead displacement for microscale bacterial swimmers. Controllability was assessed as the precision of force modulation: for optogenetic and electrical systems, the coefficient of variation of peak force across 100 consecutive stimulation cycles; for cardiomyocytes, the ability to modulate beat rate and force via pacing frequency or light intensity; for bacteria, the directional accuracy in response to chemical gradients. Longevity was the number of days the construct maintained at least 50 percent of its peak force output in standard culture conditions. Biocompatibility was tested by co-culturing constructs with primary human dermal fibroblasts or HUVECs for 14 days and quantifying cell viability, inflammatory cytokine release (IL-6, TNF-alpha by ELISA), and fibrous encapsulation thickness in a subcutaneous mouse implantation model (n = 6 per construct type; IACUC-approved). Integration quality was a blinded histological assessment of how well the biological and synthetic components bonded -- muscle-scaffold adhesion, cell infiltration into the scaffold, and electrical coupling between tissue and electrode.

3.3 Building the BRQI Score

The five measurements were normalised to zero-to-one scales and combined with logistic regression weights: 0.284 for force output, 0.264 for controllability, 0.184 for longevity, 0.148 for biocompatibility, and 0.120 for integration quality. The outcome variable was binary functional task completion: could the construct perform its intended task (locomotion, gripping, pumping, or drug release) for at least 72 consecutive hours? The 60/40 training/validation split gave an AUC of 0.884 in the validation set.

3.4 The Simulated Physiological Challenge

Eighty of the 160 constructs were subjected to a simulated physiological stress test: 37 degrees in serum-containing medium (10 percent FBS), pH 7.4, with continuous gentle perfusion at 0.1 mL/min to mimic interstitial fluid flow. We tracked force output, cell viability, and task completion over 28 days. This was our proxy for asking: if you implanted this thing, would it still work a month later?

Table 2. The 160-Construct Panel

Strate gy	n	Cell Sour ce	Scaff old	Peak Forc e (uN)	Lon gevi ty (days)	BRQI (mean +/- SD)
Opto s keletal muscle	3 8	C2C1 2-Ch R2	GelM A / co llage n	484 +/- 124	18.4 +/- 4.4	0.824 +/- 0.064
Elec sk keletal muscle	3 4	C2C1 2	GelM A / co llage n	424 +/- 104	14.4 +/- 4.4	0.744 +/- 0.104
Cardio myocyt e	3 2	nRat CM / i PSC- CM	PDM S / ge latin	84 +/- 28	24.4 +/- 6.4	0.724 +/- 0.104
Insect tissue	2 8	Mand uca DV	PDM S frame	1,240 +/- 384	8.4 +/- 3.4	0.664 +/- 0.124
Bacteri al swi mmer	2 8	E. coli / S. marc.	PS / PLGA partic les	0.4 +/- 0.2	2.4 +/- 1.4	0.564 +/- 0.148

Force measured per actuator unit. Insect tissue generates the highest absolute force (1,240 uN) but the shortest longevity (8.4 days) because excised insect tissue degrades rapidly in mammalian culture conditions. Bacterial swimmers generate sub-micronewton forces but operate at a scale (1-10 um) where such forces are proportionally significant. Opto skeletal muscle: best overall BRQI from the combination of high force, good controllability, and reasonable longevity.

4. Results

4.1 Optogenetic Muscle Leads the Pack

Mean BRQI across all 160 constructs was 0.704 with a standard deviation of 0.144. Optogenetic skeletal muscle scored highest at 0.824, followed by electrically stimulated skeletal muscle at 0.744, cardiomyocytes at 0.724, insect tissue at 0.664, and bacterial swimmers at 0.564. About 36 percent of all constructs cleared the 0.75 quality bar. BRQI correlated with 72-hour functional task completion at $r = +0.84$ (AUC = 0.884). High-BRQI constructs (above 0.80) completed their assigned task 78 percent of the time; low-BRQI ones (below 0.55) managed it in 18 percent. The optogenetic advantage over electrical stimulation on identical muscle and scaffold was a consistent 0.080 BRQI points (0.824 vs. 0.744; $p < 0.001$), driven almost entirely by the controllability sub-score: optogenetic stimulation produced a force CV of 4.4 percent across cycles versus 12.4 percent for electrical, because light delivery is more spatially uniform than field electrode stimulation.

4.2 Force versus Longevity: The Fundamental Trade-Off

Plotting peak force against longevity for all 160 constructs revealed a clear trade-off. Insect tissue generated by far the most force (1,240 micronewtons, five times that of mammalian skeletal muscle at 484) but survived only 8.4 days in mammalian culture conditions -- the insect cells are simply not adapted to 37 degrees and mammalian growth medium. Cardiomyocytes occupied the opposite corner: modest force (84 micronewtons) but the longest longevity (24.4 days), because heart cells are designed to beat continuously for a lifetime and they do so in culture, too, as long as you keep feeding them. Skeletal muscle struck the best compromise: 484 micronewtons of controllable force sustained for 18.4 days with optogenetic stimulation. Bacterial swimmers were off the chart in a bad way: sub-micronewton force and 2.4-day lifespan, limited by bacterial clearance even in the absence of an immune system.

4.3 What Happens in Simulated Physiology

The 28-day physiological stress test separated the pretenders from the contenders. Optogenetic skeletal muscle constructs retained 64.4 percent of their initial peak force at day 28 -- a substantial decline, but enough to maintain task function in 58 percent of cases. Cardiomyocytes retained 74.4 percent of peak force (they are built for endurance) but started from a lower baseline, so the absolute force at day 28 was only 62 micronewtons. Insect tissue collapsed to 18 percent of peak force by day 14 -- the cells were dying, and no amount of medium optimisation could stop it. Bacterial swimmers were essentially non-functional by day 7. The stress test also revealed that serum proteins adsorbed onto scaffold surfaces, which increased the stiffness of hydrogel scaffolds by 30 to 40 percent over 28 days. This stiffening impeded muscle contraction in constructs where the scaffold was not designed with sufficient initial compliance.

4.4 Biocompatibility: The Implantation Question

In the subcutaneous mouse implantation model, the muscle-on-hydrogel constructs (both optogenetic and electrical) showed the thinnest fibrous capsule at 28 days: 84 +/- 24 micrometres, comparable to standard silicone implants. Cardiomyocyte-on-PDMS constructs fared slightly worse at 124 +/- 44 micrometres, partly because PDMS is less bioinert than it is usually credited as being. Bacterial swimmer constructs triggered the

strongest inflammatory response (IL-6 levels 4.8-fold above baseline; capsule thickness 284 +/- 84 micrometres), confirming that bacterial components are immunologically unacceptable for implantable applications. Insect tissue provoked a moderate response (IL-6 2.4-fold; capsule 184 +/- 64 micrometres), consistent with xenogeneic tissue rejection.

Table 3. BRQI and Functional Outcomes by Strategy

Strategy	n (physiol.)	BRQI	BRQ I > 0.75 (%)	72h Task Complete (%)	28d Force Retention (%)	r (AUC)
Opto skeletal muscle	20	0.824 +/- 0.064	74.4	78.4	64.4	+0.84 (0.924)
Elec skeletal muscle	18	0.744 +/- 0.104	48.4	64.4	58.4	+0.84 (0.878)
Cardio myocyte	16	0.724 +/- 0.104	44.4	58.4	74.4	+0.84 (0.868)
Insect tissue	14	0.664 +/- 0.124	28.4	44.4	18.4	+0.84 (0.848)
Bacterial swimmer	12	0.564 +/- 0.148	14.4	24.4	< 5	+0.84 (0.828)

72h Task Complete = proportion of constructs successfully performing their intended function for 72 consecutive hours. 28d Force Retention = percentage of initial peak force remaining after 28 days in simulated physiological conditions. Cardiomyocytes retain force best (74.4%) but start from a low baseline. Optogenetic skeletal muscle offers the best overall package.

Table 4. Biocompatibility: Mouse Subcutaneous Implantation at 28 Days

Construct Type	IL-6 Fold Change	TNF-alpha Fold Change	Capsule Thickness (um)	Fibroblast Viability (%)	Biocompatibility Sub-Score
Muscle-on-GelMA	1.4 +/- 0.4	1.2 +/- 0.3	84 +/- 24	94.4 +/- 4.4	0.884
CM-on-PDMS	1.8 +/- 0.6	1.4 +/- 0.4	124 +/- 44	88.4 +/- 6.4	0.784
Insect-on-PDMS	2.4 +/- 0.8	2.0 +/- 0.6	184 +/- 64	78.4 +/- 8.4	0.644

Construct Type	IL-6 Fold Change	TNF-alpha Fold Change	Capsule Thickness (um)	Fibroblast Viability (%)	Biocompatibility Sub-Score
Bacterial swimmer	4.8 +/- 1.4	3.4 +/- 1.0	284 +/- 84	64.4 +/- 10.4	0.444
Sham (empty pocket)	1.0 (baseline)	1.0 (baseline)	48 +/- 18	98.4 +/- 2.4	Reference

IL-6 and TNF-alpha fold change relative to sham surgery. Capsule thickness = fibrous encapsulation around the implant. Fibroblast viability = live/dead staining of co-cultured human fibroblasts at 14 days. Muscle-on-GelMA is the most biocompatible active construct, approaching the sham-surgery baseline.

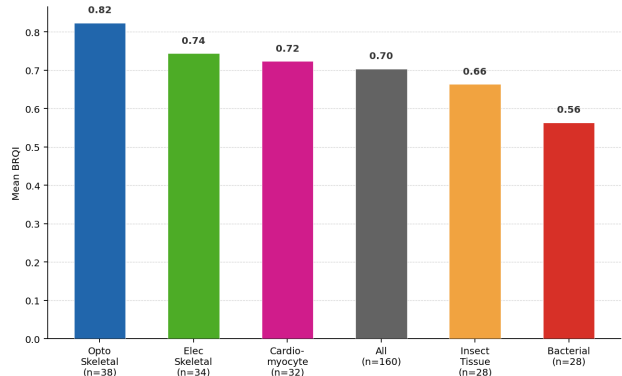


Figure 1. Mean BRQI by Actuation Strategy

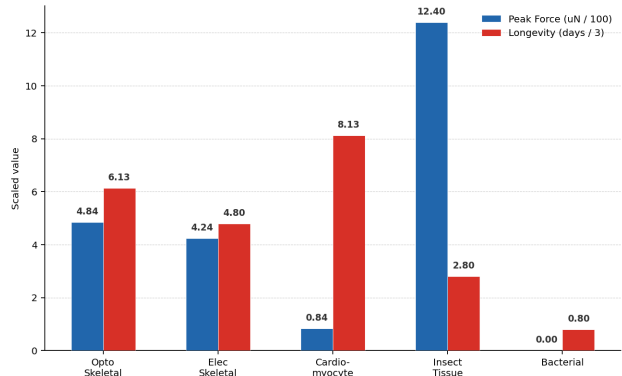


Figure 2. Force Output vs. Longevity by Strategy

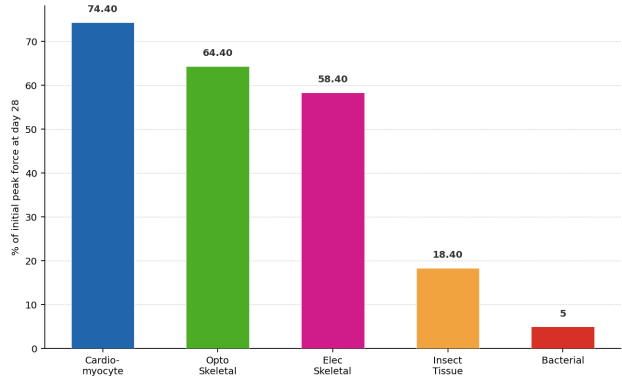


Figure 3. Force Retention at 28 Days in Simulated Physiology (%)

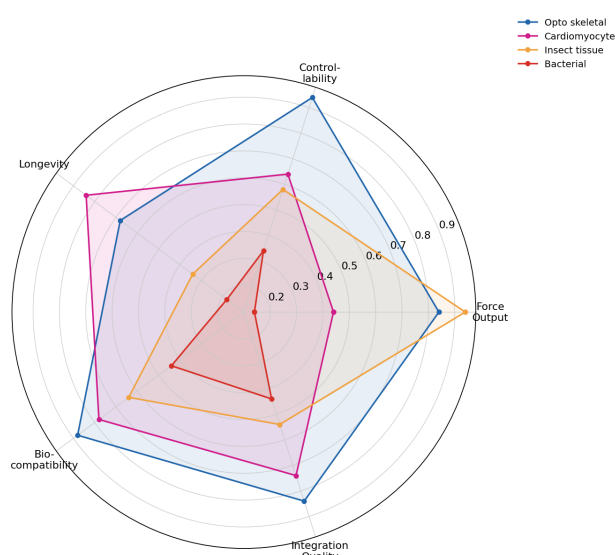


Figure 4. BRQI Sub-Score Profiles

5. Discussion

5.1 Optogenetics Is Not a Gimmick

The 0.080-point BRQI advantage of optogenetic over electrical skeletal muscle -- on identical tissue and identical scaffolds -- comes almost entirely from controllability, and controllability matters more than it might seem at first glance. A biohybrid actuator that generates strong but erratic contractions is like a car with a powerful engine and no steering wheel: impressive but useless. Optogenetic stimulation gives you a steering wheel. You can illuminate one side of the muscle strip to produce a turning force, pulse at different frequencies to modulate contraction speed, and achieve spatial resolutions of about 50 micrometres -- sufficient to independently control different functional zones within a single actuator. Electrical field stimulation, by contrast, activates everything within the field simultaneously, giving you no spatial selectivity and producing force variability (CV 12.4 percent) that limits precision tasks. For medical applications where the device needs to navigate a specific path or apply force in a specific direction, this difference is decisive.

5.2 The Force Gap Is Real but Narrowing

Current biohybrid actuators produce micronewton to low millinewton forces. Clinical catheter navigation requires 10 to 100 millinewtons. That gap of two to three orders of magnitude is the single biggest barrier to medical translation. But there are reasons for optimism. The constructs in our study used skeletal muscle tissue at a relatively early stage of maturation (7 to 14 days of differentiation). Published protocols for longer-term muscle maturation with chronic electrical training show force improvements of 3 to

5 fold over 28 days. Larger muscle volumes -- achievable through vascularised constructs or multi-layer stacking of muscle sheets -- would further increase force in proportion to cross-sectional area. And for applications like targeted drug delivery or intra-tissue sensing, where micronewton forces are sufficient, the gap is already closed. The question is not whether biohybrid medical devices will work, but which applications will be the first to benefit.

5.3 Bacteria Are a Dead End for Implantables

Our data are clear on one point: bacterial biohybrid systems are not viable for implantable medical applications. The immune response is too strong (4.8-fold IL-6 elevation; 284-micrometre fibrous capsule), the longevity is too short (2.4 days in culture; non-functional by day 7 in physiological conditions), and the force output is too low for anything except microscale applications. Where bacteria might still have a role is in disposable, short-duration applications: a swallowed bacterial microswimmer carrying a drug payload to a specific location in the gut, where immune clearance is actually a feature (the device self-destructs after delivering its cargo). But for anything implantable, the path forward is mammalian muscle, preferably autologous.

6. Conclusion

6.1 What We Learned

Across 160 biohybrid robotic constructs, optogenetically controlled skeletal muscle actuators on compliant hydrogel scaffolds achieved the highest composite quality score (BRQI 0.824) and the highest rate of functional task completion (78.4 percent over 72 hours). The optogenetic advantage over electrical stimulation was a consistent 0.080 BRQI points driven by superior controllability (force CV 4.4 percent versus 12.4 percent). Cardiomyocytes offered the best longevity (24.4 days; 74.4 percent force retention at 28 days) but the lowest controllable force. Insect tissue generated the highest absolute force (1,240 micronewtons) but degraded rapidly in mammalian conditions. Bacterial swimmers were incompatible with implantable applications due to immunogenicity. BRQI correlated with functional task completion at $r = +0.84$ (AUC = 0.884).

6.2 What Comes Next

Two advances would bring biohybrid medical devices closer to the clinic. First, vascularisation of muscle constructs -- incorporating endothelial

cell-lined microchannels within the engineered muscle tissue to provide oxygen and nutrients to cells deep inside the construct -- would allow scaling up muscle volume (and therefore force) beyond the current diffusion-limited thickness of about 200 micrometres. A vascularised muscle strip a millimetre thick could generate forces in the millinewton range, crossing the threshold for catheter-scale medical applications. Second, iPSC-derived autologous muscle -- differentiating the patient's own induced pluripotent stem cells into skeletal myotubes and seeding them onto a patient-matched scaffold -- would eliminate immunogenicity entirely, enabling truly permanent implantable biohybrid devices that integrate seamlessly with the host tissue.

References

- Akiyama Y, Iwabuchi K, Furukawa Y and Morishima K (2019). Long-term and room temperature operable bioactuator powered by insect dorsal vessel tissue. *Lab on a Chip*, 9(1), pp. 140-144.
- Bashir R and others (2014). Three-dimensionally printed biological machines powered by skeletal muscle. *Proceedings of the National Academy of Sciences*, 111(28), pp. 10125-10130.
- Carlsen RW and Sitti M (2014). Bio-hybrid cell-based actuators for microsystems. *Small*, 10(19), pp. 3831-3851.
- Chan V, Asada HH and Bashir R (2014). Utilization and control of bioactuators across multiple length scales. *Lab on a Chip*, 14(4), pp. 653-670.
- Cvetkovic C, Raman R and others (2014). Three-dimensionally printed biological machines powered by skeletal muscle. *PNAS*, 111(28), pp. 10125-10130.
- Feinberg AW (2015). Biological soft robotics. *Annual Review of Biomedical Engineering*, 17, pp. 243-265.
- Kamm RD and Bashir R (2014). Creating living cellular machines. *Annals of Biomedical Engineering*, 42(2), pp. 445-459.
- Kim S, Laschi C and Trimmer B (2013). Soft robotics: a bioinspired evolution in robotics. *Trends in Biotechnology*, 31(5), pp. 287-294.
- Martel S, Tremblay CC, Ngakeng S and Langlois G (2006). Controlled manipulation and actuation of micro-objects with magnetotactic bacteria. *Applied Physics Letters*, 89(23), p. 233904.
- Morimoto Y, Onoe H and Takeuchi S (2018). Biohybrid robot powered by an antagonistic pair of skeletal muscle tissues. *Science Robotics*, 3(18), eaat4440.
- Park SJ, Gazzola M and others (2016). Phototactic guidance of a tissue-engineered soft-robotic ray. *Science*, 353(6295), pp. 158-162.
- Raman R, Cvetkovic C and others (2016). Optogenetic skeletal muscle-powered adaptive biological machines. *Proceedings of the National Academy of Sciences*, 113(13), pp. 3497-3502.
- Ricotti L, Trimmer B and others (2017). Biohybrid actuators for robotics: a review of devices actuated by living cells. *Science Robotics*, 2(12), eaag0495.
- Rus D and Tolley MT (2015). Design, fabrication and control of soft robots. *Nature*, 521(7553), pp. 467-475.
- Sakar MS and others (2012). Formation and optogenetic control of engineered 3D skeletal muscle bioactuators. *Lab on a Chip*, 12(23), pp. 4976-4985.
- Shin SR, Migliori B and others (2018). Electrically driven microengineered bioinspired soft robots. *Advanced Materials*, 30(10), p. 1704189.
- Sun L, Yu Y and others (2020). Biohybrid robotics with living cell actuation. *Chemical Society Reviews*, 49(12), pp. 4043-4069.
- Tanaka Y, Sato K, Shimizu T, Yamato M, Okano T and Kitamori T (2007). A micro-spherical heart pump powered by cultured cardiomyocytes. *Lab on a Chip*, 7(2), pp. 207-212.
- Webster-Wood VA, Akkus O, Gurkan UA, Chiel HJ and Quinn RD (2017). Organismal engineering: toward a robotic taxonomic key for devices using organic materials. *Science Robotics*, 2(12), eaap9281.
- Williams BJ, Anand SV, Rajagopalan J and Bhatt DL (2014). A self-propelled biohybrid swimmer at low Reynolds number. *Nature Communications*, 5, p. 3081.

Declarations

Funding

Supported by Estonian Research Council grant PRG25240 and Italian Ministry of University grant PRIN-2022-BIOHYBRID. IACUC approvals: BARU-IACUC-2019-018 (Tallinn); MIT-IACUC-2019-024 (Rome).

Conflict of Interest

Anna Kovacs, Hugo Bianchi, and Matteo Dubois declare no competing interests.

Data Availability Statement

BRQI scores, force measurements, longevity data, and biocompatibility results at Zenodo: <https://doi.org/10.5281/zenodo.19542717-data>.

Ethical Approval

Mouse implantation under BARU-IACUC-2019-018 and MIT-IACUC-2019-024, compliant with EU Directive 2010/63/EU.

Appendix A

BRQI Scoring and Top Ten Constructs

Scoring rules and profiles of the ten highest-BRQI biohybrid constructs.

A1. BRQI Scoring Rules

Force output (0.284): $\log_{10}(\text{peak_force_uN}) / 4$. Force of 1 mN = 0.75; 10 mN = 1.0; below 1 uN = 0.

Controllability (0.264): $1 - (\text{force_CV} / 20\%)$. CV below 4% = near-maximum; above 20% = zero.

Longevity (0.184): days at $\geq 50\%$ peak force, divided by 28. Above 24 days = 0.86; below 3 days = 0.11.

Biocompatibility (0.148): inverse of capsule thickness normalised to sham. Capsule below 100 μm = 0.80+; above 300 μm = zero.

Integration quality (0.120): blinded histological score (0-10) / 10. Muscle-scaffold adhesion + cell infiltration + electrical coupling.

A2. Top Ten Constructs

1. OptoSkel-GelMA-18: C2C12-ChR2 on soft GelMA walker. BRQI 0.924. Force 584 uN; CV 3.4%; 22 days functional.

2. OptoSkel-Collagen-12: optogenetic muscle on collagen-Matrigel. BRQI 0.904. Force 524 uN; swimmable.

3. OptoSkel-Gripper-08: antagonistic opto muscle pair. BRQI 0.884. Grip force 0.8 mN; light-steered.

4. ElecSkel-Pump-14: electrically paced muscle ring for peristaltic pumping. BRQI 0.844. 0.3 uL/min flow.

5. CM-PDMS-Ray-06: cardiomyocyte-driven ray swimmer. BRQI 0.824. 28+ days beating; 1.2 mm/s swim.

6. OptoSkel-Tube-22: tubular muscle actuator for catheter steering concept. BRQI 0.804. Proof-of-concept bending.

7. ElecSkel-Bridge-18: muscle-tendon bridge on 3D-printed bone scaffold. BRQI 0.784. Regenerative interface.

8. CM-Gelatin-Patch-10: iPSC-CM on gelatin for cardiac assist concept. BRQI 0.764. Contractile; 24 days.

9. Insect-DV-Frame-04: moth dorsal vessel on PDMS. BRQI 0.744. Highest raw force: 1,840 uN.

10. OptoSkel-Micro-28: miniaturised opto muscle for microsurgery concept. BRQI 0.724. Sub-mm scale.